TISSUE ENGINEERING: Part B
Volume 20, Number 4, 2014

DOI: 10.1089/ten.teb.2013.0227

Toward 3D Biomimetic Models to Understand the Behavior of Glioblastoma Multiforme Cells

Shreyas S. Rao, PhD,[1] John J. Lannutti, PhD,[2] Mariano S. Viapiano, PhD,[3] Atom Sarkar, MD, PhD,[4] and Jessica O. Winter, PhD[1,5]

Glioblastoma multiforme (GBM) tumors are one of the most deadly forms of human cancer and despite improved treatments, median survival time for the majority of patients is a dismal 12–15 months. A hallmark of these aggressive tumors is their unique ability to diffusively infiltrate normal brain tissue. To understand this behavior and successfully target the mechanisms underlying tumor progression, it is crucial to develop robust experimental *ex vivo* disease models. This review discusses current two-dimensional (2D) experimental models, as well as animal-based models used to examine GBM cell migration, including their advantages and disadvantages. Recent attempts to develop three-dimensional (3D) tissue engineering-inspired models and their utility in unraveling the role of microenvironment on tumor cell behaviors are also highlighted. Further, the use of 3D models to bridge the gap between 2D and animal models is explored. Finally, the broad utility of such models in the context of brain cancer research is examined.

Introduction

Glioblastoma multiforme (GBM), a central nervous system tumor derived from glial or glial-precursor cells, accounts for ~15% of intracranial tumors and affects over 20,000 individuals annually in the United States.[1–4] While their frequency is relatively low, these are among the most malignant of human cancers, and prognoses associated with this lesion are bleak.[1,3,5] Despite dramatic improvements in micro-neurosurgical techniques, neuro-imaging, chemotherapy, and radiation therapy, the outcomes for patients with aggressively managed tumors still remains dismal.[6] Further, it has been shown that migrating GBM cells at the leading front divide more slowly than those in the core, rendering cytotoxic chemotherapies ineffective.[7,8] As a consequence of their highly infiltrative nature, recurrence can occur both locally and distantly within the brain.[9] Given these factors, median survival for a patient with optimal care is ~14 months, with many patients succumbing to their illnesses precipitously.[1,3,10]

Most therapeutic strategies aimed at GBMs target rapidly proliferating cells through a combination of cytotoxic therapies.[11–13] Fewer attempts have been made to target GBM migration, although targeting cell migration could provide significant benefits.[11] Understanding the aggressive, invasive behavior of GBMs is therefore, crucial to the development of new, precisely targeted therapeutics.[14,15] A major limitation in new anti-invasive treatments is the lack of powerful *in vitro* experimental models predicting migration in the brain. Current models, specifically two-dimensional (2D) culture on tissue culture polystyrene (TCPS), do not adequately reproduce the complex *in vivo* tumor microenvironment and therefore, are poor predictors of tumor cell behavior *in vivo*. To gain detailed insight into GBM dispersion in brain tissue, experimental models that recapitulate both the *in vivo* niche and provide highly reproducible, tunable microenvironments are needed. These models would allow identification of factors that play a pivotal role in disease progression, eventually leading to novel therapeutic options with implications for cancer treatment *in vivo*.

This review examines recent developments in models used to study GBM migration, especially those that incorporate elements from the field of tissue engineering to approximate the tumor niche. Changes occurring in tumor versus normal brain extracellular matrix (ECM) compositions are reviewed. Then, state of the art experimental models to study GBM migration *in vitro* and the limitations of those models in providing reproducible, *in vivo*-like behaviors are presented. Further, recent attempts to develop three-dimensional (3D) models that mimic several aspects of the *in vivo* environment are highlighted. Finally, the potential of improved 3D tissue analogs to impact brain cancer research, as well as that of other cancers, is discussed.

[1]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, Ohio.
[2]Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio.
[3]Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.
[4]Laboratory for Nanomedicine, Department of Neurosurgery, Geisinger Health System, Danville, Pennsylvania.
[5]Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio.

The Tumor Niche: Extracellular Matrix in Glioma Versus Normal Brain

The neural ECM is the macromolecular scaffold surrounding neurons and glial cells, and is comprised of free glycosoaminoglycans (GAG), proteoglycans (PG), and glycoproteins that tether the cells. The ECM has long been recognized as an important contributor in tumorigenesis and tumor cell migration.[16,17] The normal central nervous system exhibits a substantially different ECM composition compared to that of other organs. The normal human brain contains ~20% ECM by volume (Fig. 1), which is comprised predominantly of hyaluronic acid or hyaluronan (HA), a hydrophilic, anionic glycosaminoglycan. HA interacts noncovalently with ECM PGs of the lectican family, HA-binding proteins, and tenascins[18] to form the basic ECM scaffold. The primary fibrillar ECM components found in other tissues (e.g., collagens, laminin, and fibronectin) are not found in the brain parenchyma; they are instead restricted to the basal lamina of blood vessels and the subpial surface.[19]

The composition of the ECM changes dramatically in gliomas. Free GAG production increases threefold,[20] leading to a significant increase in the volume, tortuosity, and interstitial pressure of the extracellular space, which facilitates cell dispersion and at the same time hinders efficient drug delivery.[21] Total PG composition is also altered, with significant up regulation of PGs secreted by glioma cells, such as brevican and neurocan, and marked decrease in the neuronal PG aggrecan resulting from neuronal death during tumor growth.[22] Since brevican and versican are HA-binding PGs of the lectican family, their increase in glioma leads to structural changes in the ECM that favor the opening of interstitial spaces for cell motility.[19] More importantly, both PGs have been shown to act as signal-triggering molecules, activating EGFR signaling, increasing integrin binding to the ECM, and promoting cell adhesion and motility.[23,24] Other ECM molecules in the blood vessels and neuropil are also up- or downregulated compared to normal brain, as summarized in Table 1.[18,25] It is worth noting that the mechanisms by which the structure and composition of the ECM change in gliomas compared to normal brain are very poorly understood since the regulation of ECM genes is still poorly defined. However, current evidence suggests that glioma cells themselves introduce most of the changes by secreting large amounts of neural ECM molecules, as well as other molecules that are present in mesenchymal but not in neural ECM;[11,19] therefore, creating an environment with novel properties that favors tumor cell adherence, growth, and dispersion.

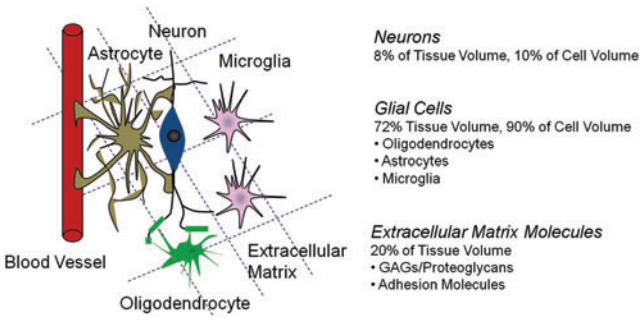

FIG. 1. Schematic of the brain microenvironment. Color images available online at www.liebertpub.com/teb

In addition to chemical alterations in the tumor microenvironment, mechanical properties are altered. For example, clinical observations using magnetic resonance elastography have revealed that cancerous brain tissue displays different mechanical properties as compared to normal brain tissue.[26–29] However, it has been difficult to conclusively determine whether the tissue becomes stiffer or softer when compared to normal tissue, which likely has repercussions in the ability of cells to metastasize away from the tumor origin. These varied observations result from a number of factors, including the heterogeneity of these tumors, changes in interstitial pressure, and secretion of several ECM components. Nonetheless, recent evidence suggests that the mechanical properties of the microenvironment can strongly influence the migration capabilities of GBM cells.[30–32]

Finally, the brain has a unique microanatomy that provides "migration highways" that promote tumor dissemination[15] (Fig. 2). The most significant of these "highways" are the white matter tracts formed by long axons aligned and organized into myelinated bundles by oligodendrocytes. These fibrillar structures have individual fiber diameters ranging from 0.5 to 3 μm and fiber densities of ~10,000–30,000 fibers/mm^2.[33,34] The largest white matter track in the human brain, the corpus callosum, connects the brain hemispheres,[35] and constitutes a cellular highway for contralateral tumor dispersion.[36] In addition to white matter tracts, GBM cells can also migrate along the surface of brain blood vessels and in the subpial space.[9,18,37–39] Recent studies have reinforced the importance of microanatomy in GBM migration, demonstrating that GBM cells respond strongly to topographical cues.[40–42] Thus, biochemical, biomechanical, and unique architectural features presented in the brain could potentially contribute to tumor cell migration. Furthermore, certain microanatomies also likely limit invasion into specific regions.

Modeling Cell Migration in 2D

Invasive cell dispersion through the brain parenchyma is the hallmark of malignant gliomas, and one of their most unique properties. Malignant tumors that metastasize to the brain almost never invade soft neural tissue and grow instead as focal metastases.[43] Conversely, glioma cells can disperse long distances in the central nervous system without causing clinical symptoms, but almost never metastasize to other tissues and, when implanted peripherally, form compact masses that replicate poorly the phenotype of the original tumor.[44] Glioma cell migration is therefore, a complex process that requires a concerted interaction of the tumor cells with their native microenvironment, and is perhaps the most defining phenotypical property of these tumors when compared to other solid cancers. Understanding glioma dispersion provides a critical insight into the biology of these tumors and the key aspects that drive them in the neural microenvironment.

To better understand tumor cell migration, controlled environments that can recapitulate specific physical and chemical features of the tumor microenvironment are required. Unfortunately, the standard model of inquiry—2D culture on TCPS—fails rather dramatically in this regard. Here we provide specific examples of standard 2D culture models used to examine GBM cell migration, and highlight

Table 1. Composition of the Brain ECM: Major Components Identified in Normal Brain Versus Glioma

	Major proteins	*GAGs*	*PGs*
Blood vessels (tumor associated)	Fibronectin, collagen, laminin, osteopontin, tenascin-C, thrombospondin-1, sparc[a]		CSPGs[b] Versican
Blood vessels (normal)	Fibronectin, collagen, laminin, vitronectin, entactin	HS[c]	
Neuropil (tumor associated)	Vitronectin, osteopontin, tenascin-C, sparc	HA[d] ↑ CS[e], HS ↑ KS[f], DS[g] ↑	Brevican ↑ Versican ↑ Aggrecan ↓
Neuropil (normal)	No vitronectin, no tenascin-C	HA GAGs (three times lower than tumor associated neuropil)	Phosphacan Neurocan Brevican (most abundant CSPG) Versican

[a]Secreted protein acidic and rich in cysteine.
[b]Chondroitin sulfate PG.
[c]Heparan sulfate.
[d]Hyaluronan/hyaluronic acid.
[e]Chondroitin sulfate.
[f]Keratin sulfate.
[g]Dermatan sulfate.
ECM, extracellular matrix; GAG, glycosoaminoglycans; PG, proteoglycan.

inadequacies in their ability to mimic native tumor cell behavior *in vivo*.

The monolayer wound-healing assay (scratch assay)

The monolayer wound-healing assay was one of the earliest methods employed to investigate GBM cell migration. In this technique,[45] GBM cells are cultured until they form a confluent monolayer (Fig. 3A). A scratch is then made in the monolayer, and the time required for cells to fill the voided gap is measured. The migration rate is calculated by dividing the distance traveled by the cells over time. These assays have been routinely used to investigate the signaling pathways associated with GBM migration (e.g., PTEN, a tumor suppressor protein was shown to inhibit GBM migration through its C2 domain).[46] It is important to note that the width of the "wound" often determines the migration rate. For example, cells fill larger wounds more slowly than smaller wounds.[45] Although calculation of migration rates is straightforward, this assay examines GBM cell migration on 2D, rigid substrates (e.g., plastic, glass with elastic modulus (E) > ~100 kPa[47]), culture conditions that do not replicate the *in vivo* mechanical environment ($E \sim 0.1$–1 kPa,[47] a 2–3 order of magnitude difference), and are less physiologically relevant. Further, this model provides no topographical cues, similar to those presented by white matter tracts. Chemical cues, however, can be added by coating the TCPS with adherent molecules or by adding soluble factors to the culture medium.

The microliter scale migration assay (radial migration assay)

Microliter-scale migration assays[45] examine radial migration of GBM cells, usually in presence of ECM molecule(s) (Fig. 3B) (However, the assay can also be performed without ECM molecules on 2D TCPS.). Selected molecules are deposited on a substrate (e.g., 10-well Teflon printed microscopic slides), and the cell-containing solution is placed at the center of these wells. Radial migration of cells is then monitored by quantifying the increase in area of the circle that

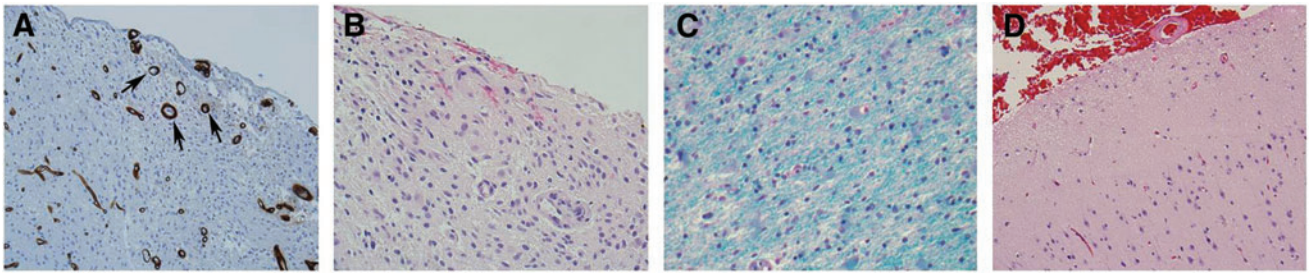

FIG. 2. Clinical presentation of glioblastoma multiforme (GBM) tumors. Histology images of brain tissue stained using hematoxylin and eosin: **(A)** GBM cells seen around blood vessels (labeled brown (collagen IV) via immunoperoxidase staining and indicated by black arrows). **(B)** GBM cells seen below the surface of pia mater (sub-pial spread). **(C)** GBM cells migrating along white matter tracts (labeled blue via Luxol fast blue). **(D)** Histology image of a normal brain cortical tissue showing sparsely populated neurons, preserved cellularity and architectures as opposed to **(A–C)** that show extensive tumor cell infiltration, disruption of normal architectures, and hypercellularity. Color images available online at www.liebertpub.com/teb

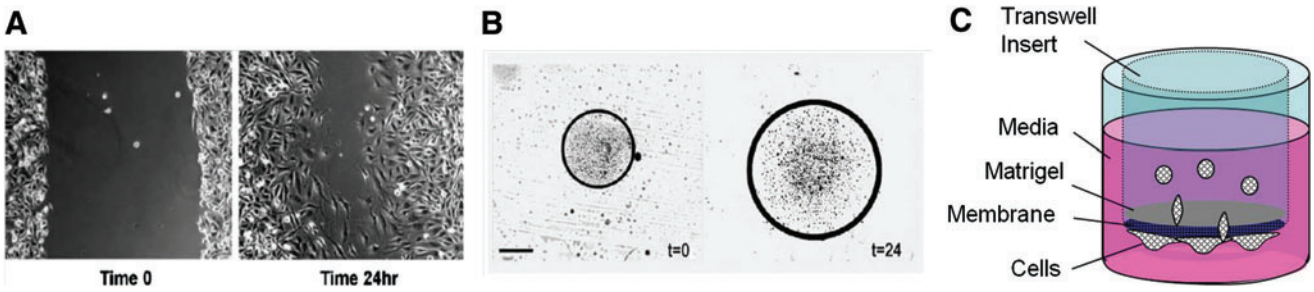

FIG. 3. State of the art cell culture models. **(A)** two-dimensional (2D) gap assay. **(B)** 2D microliter scale migration assay. **(C)** Transwell™ insert assay or chamber assay. Both **(A, B)** taken from[45] are reprinted with permission from Elsevier, Inc. Copyright © 2005 Elsevier, Inc. Color images available online at www.liebertpub.com/teb

encompasses the cells over a stipulated time period. This assay has been used to study tumor cell migration with a variety of ECM molecules found in the brain parenchyma or blood vessel basal lamina, such as collagen type IV,[48–50] laminin,[48,50] vitronectin,[48–51] fibronectin,[48–50] merosin,[49,51] tenascin,[49,52] HA,[49] and myelin extracts.[53,54] These assays permit investigation of the potential influence of chemical cues and have allowed screening and identification of several ECM components that are permissive or nonpermissive for GBM migration, such as those listed in Table 1. However, quantification of migration area or perimeter may be biased by the presence of outlier cells that make image analysis challenging. Also, similar to the scratch assay, the Microliter scale migration assay examines migration on 2D rigid substrates that do not provide mechanical environments equivalent to those found *in vivo*.

Boyden chamber assays

The Boyden chamber assay examines migration of cells through a porous insert (Transwell™) in response to specific attractant or repellent cues. Cells are seeded on the top of the insert and the number of cells crossing through the membrane pores is counted. Migration can be promoted with a gradient of culture serum or other chemoattractants. The requirement for myosin II in glioma invasion was demonstrated using these assays utilizing inserts with different pore sizes. In particular, for glioma cells to squeeze through pores smaller than their nuclear diameter, both A and B isoforms of myosin-II were required.[55] These results were in contrast to cells migrating on a bare surface, wherein migration proceeded without the involvement of myosin-II. However, it is important to note that the rigidity of the plastic membrane (typically made of poly(ethylene terephthalate) [PET]) may dramatically influence migration capacity.

Matrigel-modified chamber assays overcome some of the shortcomings of the "naked" Boyden chamber assay. Matrigel is an self-gelling suspension of ECM molecules derived from the Engelbreth-Holm-Swarm mouse sarcoma (a connective tissue tumor) and provides a less rigid microenvironment than TCPS.[56] In this assay,[45,57–61] culture inserts are first coated with Matrigel, forcing the cells to pass through the gellified Matrigel before reaching the underside of the insert (Fig. 3C). Because Matrigel forms a natural gel, it can be employed to explore the role of physiologically relevant ECM molecules (e.g., unmodified HA[58,59,61]), by incorporating them as an additive or as a chemoattractant in the lower chamber (e.g., type I and IV collagen, laminin, fibronectin[60]). Although this assay permits some modification of the mechanical environment with the addition of Matrigel and other additives, migrating cells still contact the synthetic membrane, which can influence results substantially. Furthermore, consisting primarily of laminin, entactin, and collagen-IV,[62] Matrigel does not adequately recapitulate the composition of brain ECM, which lacks these proteins. In addition, Boyden chamber assays are end point-based, and therefore, fail to provide dynamic information on migration patterns of tumor cells. For example, one has to incorporate additional methods to distinguish cell proliferation from cell migration in static end-point assays that rely on counting cell numbers to calculate migration parameters.

Animal and Animal-Derived Models

Given the deficiencies of 2D culture and the ultimate goal of understanding tumor behavior *in vivo*, several animal-derived models for studying GBM migration *ex vivo* have been developed. While these assays are arguably the closest possible approximation to the conditions found in patients, disadvantages may include substantial animal to animal variation, inability to control the tumor microenvironment, and the costs and scalability issues involved with animal studies. Here we discuss the most commonly used models and their applications, as well as their limitations.

Brain slice assays

In this method,[37,45,63–65] brain tissue slices (100–300 μm thick) are obtained using a vibratome and cultured on porous inserts. Fluorescent glioma cells are then placed on slices, and their dispersion is observed using wide-field or confocal fluorescence microscopy. Cell invasion is quantified as a function of distance from the initial seeding point over time. Using dynamic time lapse microscopy, brain slice assays have demonstrated that glioma cells migrate along blood vessels within slices *in vitro*.[66] Further, these assays have been applied for identification of genes differentially regulated in glioma invasion.[67]

Brain slice assays have many positive features, most notably the fact that they model the tissue microenvironment closely and tumor cell invasion in slices largely reproduces the behavior of the cells *in vivo*.[68] However, they also have limitations. These models are time consuming, requiring 3–7 days for completion, and are impractical to scale up as screening assays. In addition, the viability of normal brain

cells decays rapidly over time, while some resident cell populations (such as microglia) can become highly proliferative, affecting tumor cell behavior. Additionally, animal to animal variation may necessitate a considerable number of replicates to overcome experimental variability. The major limitation of these assays, however, is that they fail to provide fine investigator control of the local environment for systematic study of the influence of chemistry and mechanics on cell behaviors.

Confrontational tissue assays

Confrontational assays[69–73] assess the migration of tumor cells in the interfacial environment between tumor and normal brain by culturing normal and cancerous brain tissue in close proximity (e.g., one tissue on top of the other or side by side). Other versions of this assay employ tumor spheroids in confrontation with normal brain tissue or use normal brain cell aggregates in confrontation with tumor cells. The infiltration of tumor cells into normal tissue is then investigated via microscopy. Confrontational assays have declined in popularity because of their limited utility and present shortcomings similar to those of the brain slice assay.

Tumor xenograft models

Examination of tumor invasion directly in animal models is the most physiologically relevant standard because cells can be embedded in their native microenvironment. In these models, tumor cells are implanted into the brain and their progression over time is monitored by imaging techniques or longitudinal histology.[74–78] Although quantification of invasion in tissue sections is usually not straightforward, there are several standards that can be applied in these models, such as quantification of "islets" of cells away from the core mass and measurement of the distance of these islets to the central tumor core.[79,80]

These models are assumed to reproduce adequately the tumor microenvironment and can be employed to study the spatiotemporal tumor distribution (i.e., the different anatomical structures that favor tumor cell dispersion *in vivo*) and even dynamics of tumor cell migration (e.g., intracellular cytoskeletal organization[81]). These models have enabled identification of human brain tumor initiating cells for the first time.[82] Moreover, xenograft models are routinely utilized to investigate the *in vivo* efficacy of individual targeted therapies (e.g., antivascular endothelial growth factor [VEGF] treatment[83]), chemotherapies (e.g., Carmustine or *bis*-chloroethylnitrosourea [BCNU]),[84] as well as combination therapies (e.g., angiostatin, an angiogenesis inhibitor and ionizing radiation[85]).

Unfortunately, animal studies present significant downsides, such as animal-to-animal variability, high cost, challenges in live imaging, and impractical scale up for screening assays. Animal studies also fail to provide control of the local environment where the tumor grows and invades, thereby making reliable studies of specific interactions (e.g., tumor cell response to modulus or soluble factors) impossible.

Genetically engineered models

In these models, the tumor is formed spontaneously in the brain of the animal (i.e., mouse).[86] This is achieved by inducing genetic alterations seen in human tumors into mice (either via germ-line or somatic modifications[87–89]). One of first genetically engineered models for glioblastomas involved mutations in two tumor-suppressor genes: Nf1 and Trp53.[90] Since then, several models have been developed and are reviewed in detail elsewhere.[86–88] Genetically engineered models offer several advantages, including the ability to model key early events in the evolution of the tumor, as well as identification of molecular pathways and oncogenes involved in tumor initiation and progression.[86,91] However, the relatively long time period needed for tumors to develop combined with tumor heterogeneity in terms of low tumor penetrance, location, and growth rate complicates the use of such models.[92,93]

Making the Transition to 3D: Bridging the Gap Between 2D and Animal Models

While most 2D models can be executed rapidly and easily, they fail to recapitulate the highly complex, 3D environment of *in vivo* niches. Further, it is now widely recognized that 2D cell culture can produce substantially different results in tumor cell behavior and signaling cascades than 3D culture.[94–96] Animal models offer one possible solution, but procedures are technically complex, costly, and include additional levels of variability. Thus, researchers have begun to leverage lessons learned from tissue engineering to create well-defined 3D niches for a variety of cell types,[97–100] including GBMs. These environments are created using either naturally derived or synthetic biomaterials, can recapitulate several aspects of the *in vivo* environment, and provide important material cues to tumor cells. Most importantly, engineered materials permit investigator control of the microenvironment, a critical feature for dissecting the multitude of factors—mechanics, chemistry, topography—which govern the biology of tumor cell migration. Not all materials are suitable for such investigations; however, the application of tissue engineering to *in vitro* models offers the potential to advance these studies. Here we discuss biomaterial development for recreating GBM tumor niches, the application of tissue engineering concepts to these environments, and the degree of engineering control that can be achieved.

Tissue engineering inspired models

Biomaterial platforms inspired from tissue engineering have now begun to be utilized as engineered models of cell behavior to elucidate mechanisms of tumor progression. Hydrogels and electrospun fibers, biomaterials commonly employed in tissue engineering,[101,102] are the two most valuable *in vitro* disease models currently in use. Both models are attractive in terms of their mechanical and chemical tunability and ability to incorporate a number of cell responsive cues into the material (e.g., adhesion molecules, growth factors). Specifically, hydrogels, cross-linked polymeric biomaterials, can be engineered to mimic structural and mechanical features of brain tissue because of their similarity to GAG and PGs found in the native brain ECM. Electrospun fibers mimic fibrous structures (e.g., white matter, blood vessels) that act as highways for GBM dispersion *in vivo*. These materials can be composed of either natural or synthetic materials designed to closely mimic specific features of the 3D tumor microenvironment.

Interactions of GBMs with several biomaterial systems are summarized in Table 2.

Synthetic biomaterials. Several synthetic biomaterials have been used to study GBM cell behavior. For example, sheets of silicone rubber (poly(methylphenyl)siloxane)[103] and poly(acrylamide)-based hydrogels[30] have been used to elucidate the role of mechanics on glioma cell migration. These studies showed that migration is proportional to the rigidity of the underlying substrate. In particular, with poly(acrylamide)-based hydrogels,[30] it was observed that as the rigidity ($E \sim 0.8$ kPa) approached that of the brain tissue, migration was drastically reduced compared to that observed on stiff substrates ($E \sim 119$ kPa), demonstrating the strong role played by the mechanical environment in guiding migration (e.g., Fig. 4). In these investigations, the primary goal was to modulate mechanical properties, and thus, synthetic materials were used as they offer more flexibility in the range of mechanical properties that can be obtained.

Similarly, to investigate the role of topography on GBM behaviors, synthetic electrospun fibers derived from poly(ε-caprolactone) (PCL) have been used. In particular, glioma migration was observed to be a strong function of substrate topography as GBM cells migrated much faster on aligned PCL fibers than on random PCL fibers[40] (Fig. 5). In a separate study, the sensitivity of glioma cell migration to low concentrations of signal transducer and activator of transcription-3 (STAT-3) inhibitors was observed in cells dispersing in cultured brain slices and on aligned nanofibers, but not on TCPS,[41] demonstrating the unique sensitivity to topography provided by tissue-engineering inspired materials. Furthermore, inhibition of STAT-3 did not reduce cell translocation in a Transwell migration assay at low concentrations of inhibitors, in contrast to observations on both nanofibers and brain slices. This system also recapitulated *in vivo*-like migratory morphologies with migration correlating to STAT-3 signaling, a known driver of cell migration *in vivo*.[40,41,104] More recently, using aligned core-shell nanofibers (i.e., Gelatin-PCL, poly(dimethylsiloxane) [PDMS]-PCL, poly(ethersulfone) [PES]-PCL) and aligned PCL nanofibers mimicking white matter tract topography, GBM migration was shown to be a strong function of nanofiber mechanics, with a peak in migration speed seen on aligned PCL nanofibers of intermediate modulus ($\sim$8 MPa).[42] The few reports that have investigated the mechanical properties of white matter tracts primarily use atomic force microscopy techniques.[105] Properties measured using this technique display values that have a similar order of magnitude to the electrospun nanofibers reported in the study. Additional studies to further understand changes in the mechanical modulus of white matter tracts in response to cancer are currently underway and could increase the physiological relevance of electrospun nanofibers studies.

Synthetic materials provide user control over material properties, but unfortunately cannot fully recapitulate the complex, time-varying chemistry, including chemical and mechanical changes, of the *in vivo* microenvironment. In addition, although cell motility largely reproduces the cellular and molecular parameters of *in vivo* migration, the cells are usually not challenged by a true, 3D ECM-like system that provides a platform for cell invasion. Therefore, these materials represent an intermediate environment between 3D and 2D cultures (a "2.5D" environment). The lack of a true ECM-like barrier hampers the ability to examine the role of tumor-secreted proteases and glycanases on tumor cell migration. While there have been few studies with these materials, a dramatic increase in their use as 3D tissue analogs with the development of new biomaterial combinations is expected in the coming years primarily because of their simple preparation procedures, tunability, and reproducibility.

Natural biomaterials. Naturally derived scaffold materials are also ideal candidates for developing 3D models for tumor cell migration investigations. Standard models employed include Matrigel[31,106–108] and collagen[109–112] assays. These assays investigate invasion into the gel by seeding cells on the gel surface or may utilize tumor cell spheroids to examine radial migration of cells away from the tumor core. While these assays are good starting points for studying cell migration in 3D environments and continue to be used, they usually access a limited range of physicochemical properties (e.g., stiffness, ligand density) making certain tumor cell characteristics difficult to capture.

To circumvent these issues, several studies have focused on developing more advanced biomaterial models, including multicomponent and tunable systems, to isolate the role of specific factors on cell behaviors in 3D. For example, using hybrid hydrogels of collagen and agarose, GBM migration was shown to be inversely related to matrix stiffness in 3D. Further, a change in migration pattern from mesenchymal to amoeboid was also observed.[113] Similarly, by exploiting the gelation dynamics of collagen gels, GBM migration was examined as a function of pore size in 3D networks.[114] Migration was hindered in constructs with a small pore size (i.e., 2 μm); however, the invasion distance was not very sensitive in the pore size range from $\sim$5 to 12 μm. Using chitosan-alginate composite scaffolds, factors promoting tumor malignancy (i.e., VEGF, matrix metalloproteinase-2 [MMP-2]) were shown to increase in human GBM lines compared to 2D cultures.[115]

To increase physiological relevance, investigators have also employed HA as a hydrogel biomaterial, singly or with other biomolecules to create multicomponent systems. These biomaterials have been used to investigate migration capacity of GBM cells in both 2.5D and 3D cultures.[32,116–121] Specifically, using gels containing HA and the RGD peptide, the migration capacity of GBM cells was shown to be a strong function of stiffness and ligand density (i.e., RGD concentrations of 0–5 mg/mL) in 2.5D culture.[32] In contrast, in 3D cultures, migration was permitted only in less dense HA cultures and strongly inhibited by highly dense HA-based hydrogel cultures[32,118,121] (Fig. 6). This behavior was also observed with chondroitin sulfate addition to collagen-based gels wherein migration of GBM spheroids was inhibited.[122] Similarly, HA also reduced GBM migration when presented as a "shell" on a PCL "core" nanofiber versus bare PCL nanofiber in an aligned white matter topography mimetic setting.[42] In addition to matrix parameters, several other factors, such as elastin-derived peptides (i.e., kappa-elastin)[117] and growth factors (i.e., stromal cell-derived factor-1α and basic fibroblast growth factor),[116] have been shown to increase migratory capacities of GBMs in HA-based hydrogel systems. Studies have pointed toward cell-specific differences in invading HA-based hydrogels, indicating inherent differences in cell type.[119] However, it

Table 2. Interactions of GBMs with Natural and Synthetic Biomaterials

Biomaterial	*Cell type*	*Tumor cell Model*	*Culture type and dimensionality*	*Assay type*	*Observations*	*Refs.*
Poly (methylphenyl) siloxane)	SNB-19	S	H, 2.5D	D	Migration=*f* (stiffness)	103
Poly (acryl amide) coated with fibronectin	U-373 U-87 U-251 SNB-19 C6 (R)	S	H, 2.5D	D	Migration=*f* (stiffness)	30
PCL coated with fibronectin	X-12 U-251 U-87 G-8, G-9 (M)[a]	S, A	F, 2.5D	S, D	Migration=*f* (topography)	40,41
Gelatin-PCL PCL PDMS-PCL PES-PCL PCL-collagen PCL-matrigel PCL-HA	OSU-2	S	F, 2.5D	D	Migration=*f* (nanofiber mechanics) HA reduced migration on aligned nanofibers	42
Matrigel	U-87	A	H, 3D	D	Tumor exerts mechanical stress and traction on its surrounding	108
Collagen	U-87	A	H, 3D	S	Invasion=*f* (collagen concentration at early time points)	109
Collagen	U-87 U-373 GBM 1 GBM 2 GBM 3 GBM 4	A	H, 3D	S	Invasion was influenced by tissue cohesion and N-cadherin expression	111
Collagen	U-87	S	H, 3D	D	Migration=*f* (epidermal growth factor stimulation)	110
Collagen	C6 (R)	S, A	H, 3D	S	Migration=*f* (pore size)	114
Collagen-tenascin-C	U-251 U-178	S	H, 3D	S	Presence of tenascin-C increases invasiveness	112
Chitosan-alginate	U-87 U-118 C6 (R)	S	H, 2.5D	S	Provided an environment leading to formation of solid tumor-like cells VEGF and MMP-2 secretion↑for human cell lines	115
Collagen-agarose	U-373	S, A	H, 3D	S, D	With increasing agarose, migration mechanism is altered and eventually abrogated	113
HA	U-87 U-251 U-343 U-373	S	H, 2.5D	S	The extent of invasion was cell type dependent	119
HA	CB-191 CB-193	S	H, 2.5D	S	Invasion=*f* (hyaluronidase activity)	120
HA	CB-191	S	H, 2.5D	S	Invasion was influenced by adhesion molecules (collagens) and growth factors (stromal cell-derived factor-1α and basic fibroblast growth factor)	116
HA-κE	CB-74 CB-109 CB-191	S	H, 2.5D	S	Invasion increased in the presence of κE, MMP-2 ↑	117
HA-RGD	U-373 U-87 C6 (R)	S A	H, 2.5D H, 3D	S, D	Migration=*f* (stiffness and ligand density)	32
HA-collagen	OSU-2	S	H, 3D	D	Migration=*f* (HA density)	118,121
Collagen-HA Collagen-CS	C6 (R)	A	H, 3D	S	Migration=*f* (CS concentration) Effect of HA not significant	122

Cell source is Hu unless otherwise noted in cell type column in parentheses.
Species: Hu, human; M, mice; R, rat.
Tumor cell model: S, dissociated single cells; A, tumor aggregates/spheroids.
Culture type: H, hydrogel; F, electrospun fiber.
Culture dimensionality: 2D, cells cultured on tissue culture plastic/glass; 2.5D, cells cultured on top of hydrogels/electrospun fibers; 3D, cells encapsulated in hydrogels.
Assay type: S, static, end point based; D, dynamic, time lapse imaging based.
[a]Tumor initiating cells implanted into mice to generate tumors and these tumor explants are cultured on biomaterial surfaces.
f, functional dependence; κE, kappa-elastin; GBM, glioblastoma multiforme; VEGF, vascular endothelial growth factor; PCL, poly (ε-caprolactone); PDMS, poly(dimethylsiloxane); PES, poly(ethersulfone); MMP-2, matrix metalloproteinase-2.

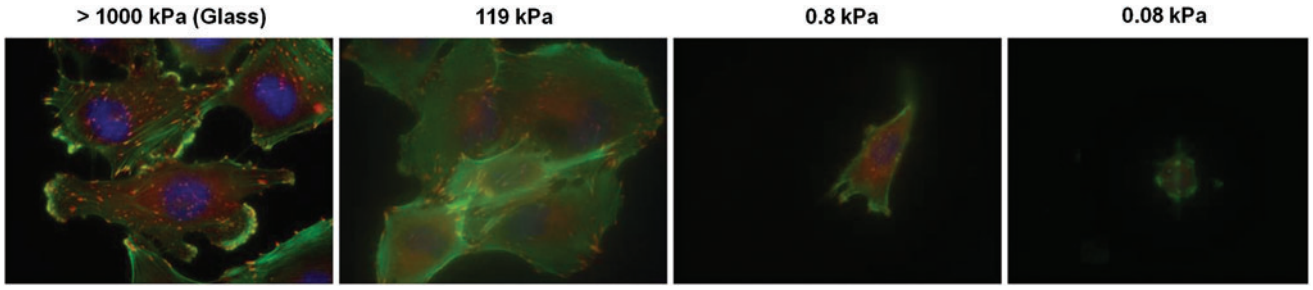

FIG. 4. Morphology and cytoskeletal organization of U373-MG human glioblastoma tumor cells on laminin-coated glass and laminin-coated polyacrylamide hydrogels of varying stiffness. Cells were stained for F-actin (green), nuclear DNA (blue), and vinculin (red). Figure courtesy of Sophie Wong, Dr. Theresa Ulrich, and Dr. Sanjay Kumar (University of California, Berkeley; Dr. Ulrich is currently at the Massachusetts Institute of Technology). Color images available online at www.liebertpub.com/teb

should be noted that HA by itself usually does not support cell migration. Thus, modification with additional materials is required to generate *in vivo*-like migration behaviors. This most likely results from a number of factors, including the dense nature of HA hydrogel with few open pores to support migration and also chemical factors (i.e., HA hydrogels do not promote cell attachment on their surfaces).

Studies with naturally derived biomaterials offer several advantages.[123] For example, with an increase in the development of systems that offer tunability, it is becoming easier to assess the role of competing cues (e.g., chemistry, stiffness) on tumor cell behavior. While this is also possible with synthetic biomaterials, the importance of translating these findings to the clinic using natural biomaterial systems

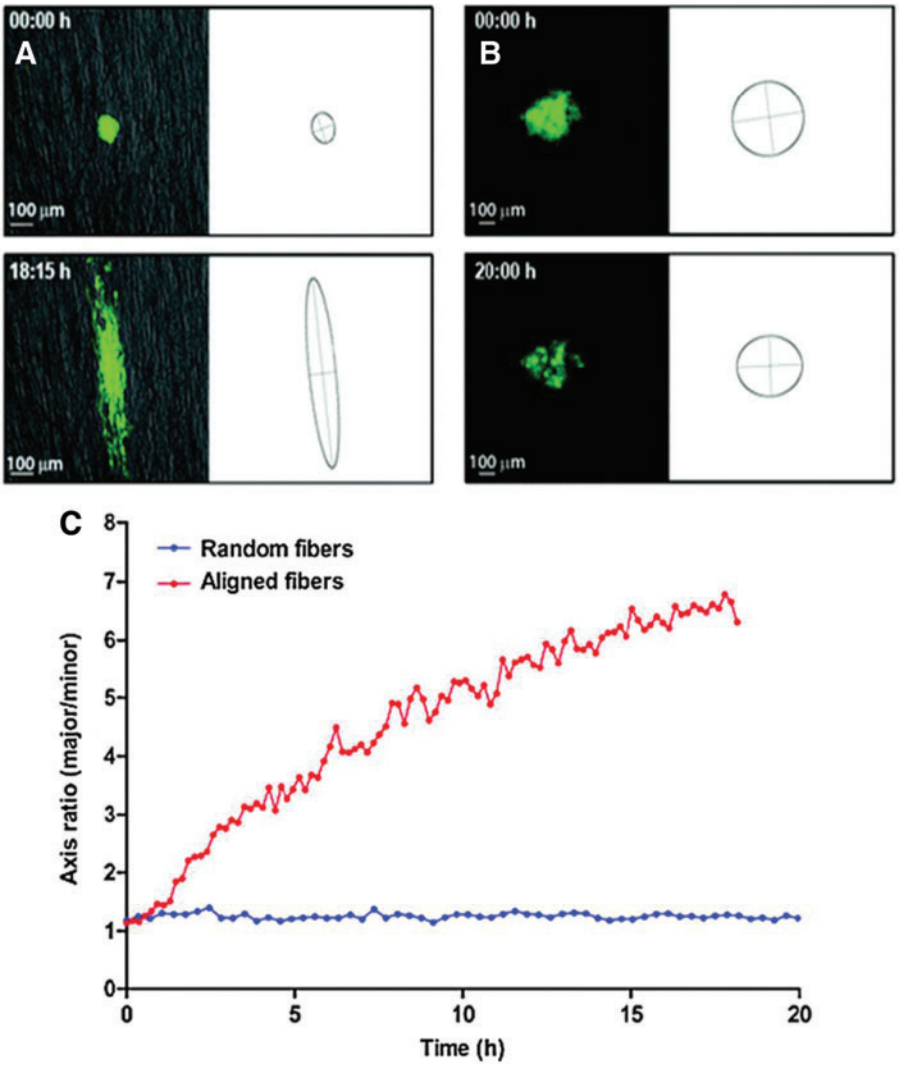

FIG. 5. GBM tumor cell dispersion from neurospheres on **(A)** random and **(B)** aligned poly(ε-caprolactone) (PCL) electrospun nanofibers mimicking white matter tract topography. **(C)** Quantification of cell dispersion as measured by a change in the ratio of elliptic axes over time. Figure taken from[40] reprinted with permission from Mary Ann Liebert, Inc. Copyright © Mary Ann Liebert, Inc. Color images available online at www.liebertpub.com/teb

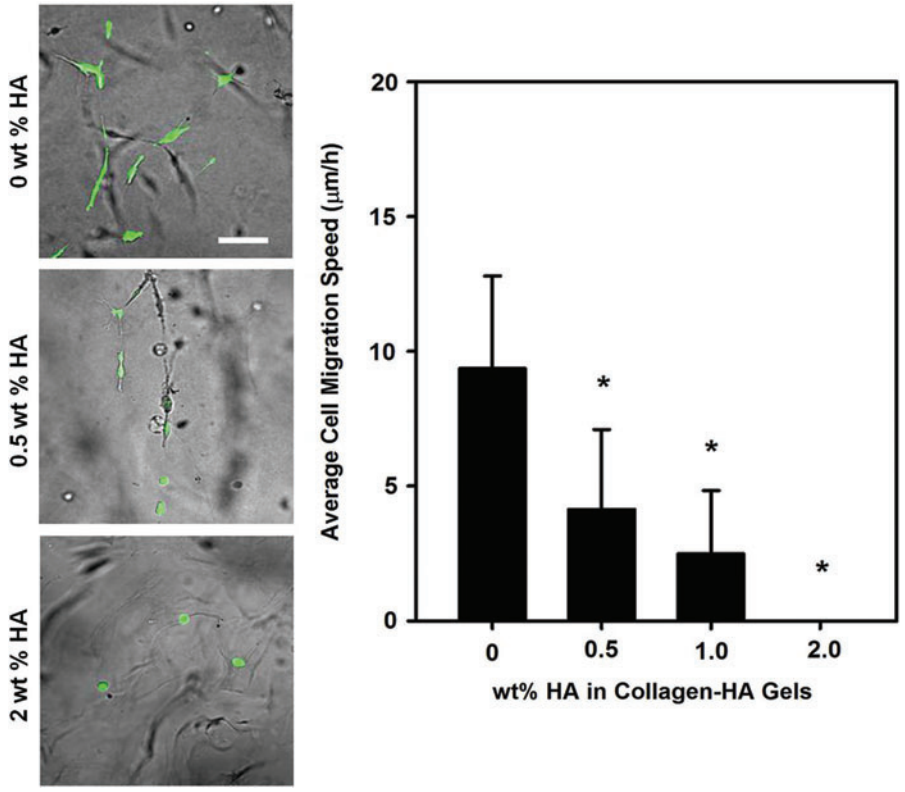

FIG. 6. Morphology and cell migration speed of patient derived OSU-2 glioblastoma tumor cells within three-dimensional (3D) Collagen-hyaluronic acid or hyaluronan (HA) composite hydrogels of varying HA density. Cells were stained with Cell tracker Green. Scale bar indicates 100 μm. *$p < 0.0001$ compared to 0 wt% HA (i.e., pure collagen gels). Color images available online at www.liebertpub.com/teb

cannot be underscored. Also, naturally derived materials can elicit specific signaling responses that can influence cell behaviors similar to those observed *in vivo*. However, it is also important to realize that, for natural systems, variations in composition can strongly influence experimental findings and only certain ranges of engineered mechanical and topographical properties may be accessible. In addition to natural and synthetic biomaterials, peptide and protein-based engineered ECMs[124] are gaining relevance as they can combine native architectural features with physiologically relevant stiffness and could become a useful tool to investigate GBM behaviors. Similarly, microfluidic devices have begun to be utilized in both 2D and 3D culture microenvironments to investigate GBM behaviors, as they can maintain stable gradients, can incorporate flow, and provide time-sensitive resolution of migration.[125–127]

Compared to animal tissue-based models, results obtained using 3D *in vitro* biomaterial assays are more reproducible. Furthermore, these assays are inexpensive, quicker, and provide ease of execution compared to brain slice models or confrontational assays. However, recognizing that GBM behavior is extremely complex, it is likely that a combination of *in vitro* assays (e.g., brain slice, hydrogel, and/or fiber-based assays) will be required to fully understand tumor cell behavior. For instance, 3D cell culture models could be used to identify specific effects via high-throughput screening, which can be further explored using animal models. Detailed studies of intracellular signaling cascades and other migration regulatory pathways using existing, as well as forthcoming physiological biomaterial platforms should enable development of new therapeutic targets.

A Look to the Future: How Will We Benefit from 3D Brain Tissue Models?

2D cell culture models (e.g., monolayer cultures) are currently used to test efficacy of anticancer drugs and evaluate their potential before clinical trials.[128] Despite rapid advances in high-throughput drug screening procedures, 2D assays have largely failed to effectively predict the efficacy of anti-invasive drugs, and several studies have indicated that 2D assays provide only marginal benefit in evaluating anticancer drugs.[129,130] This is mainly because cells grown on 2D TCPS adapt to this artificial environment and may no longer display characteristics of the original tumor. Classical human cell lines commonly used to study glioblastoma behavior, such as U-87, U-118, and U-138, were established in the late 1960s and have been subjected to extensive adaptation to conventional culture conditions. Therefore, it is no surprise that models using these cells do not adequately predict tumor *in vivo* response, creating a huge scientific and financial challenge.

Biomimetic 3D models could become powerful predictors in high-throughput drug screening and drug discovery by uniquely bridging the gap between 2D and animal models in a cost effective manner,[96,131] potentially reducing time to market. Further, 3D brain tissue models will also be of immense use to clinicians to investigate tumor migration rate *in vitro*, thereby guiding patient care and treatment decisions. For example, improved 3D tissue analogs could serve as tailored bioassays for patient-derived tumor biopsies and for testing different drug combinations for personalized medicine approaches. Further, as compared to well established cell lines, GBM tumor stem cells could become a valuable

and robust model cell system as they have been demonstrated to closely recapitulate the genotype and phenotype of primary human tumors.[132,133]

However, there are several significant challenges remaining for 3D cultures. For example, oxygen diffusion in 3D cultures is a substantial concern, especially for long-term cultures. In addition, downstream assays, such as western blots and immunofluorescence assays are difficult to perform in 3D culture. Some of these challenges can be partially overcome. For example, to increase oxygen transport in 3D cultures, bioreactors may be employed; leveraging lessons from the tissue engineering community.[134,135] Similarly, downstream assays can be performed on thin 3D slices taken from the samples via histological sectioning.

Future models will increase in complexity. While these early models are mainly designed based on the ECM of the tumor, a variety of cues found in the *in vivo* microenvironment can be added. For example, cell–cell interactions are also important. Investigating interaction of brain tumor cells with other resident cell types in the brain (e.g., oligodendrocytes, astrocytes, endothelium[136,137]) through coculture will improve understanding of the influence of these interactions on tumor progression. Further, the role of several growth factors in combination with ECM cues on tumor cell behaviors can be explored. Increasing model complexity is not without challenges; understanding the nutritional requirements and culture conditions for each specific cell type and their cocultures will be crucial.

Tissue engineering-inspired 3D biomaterial models are poised to make substantial contributions to the field of cancer biology and patient care. Brain mimetic 3D models can be used to not only predict tumor cell behaviors, but also provide a powerful tool to understand and evaluate fundamental questions in neuroscience (e.g., migration of other cell types in the brain) that are yet unexplored. For example, crucial information gained from these models may be applicable as a guide in designing scaffolds for neural tissue engineering and creating stable nerve tissue-electrode interfaces; thus, demonstrating the broad applicability of this research.

Acknowledgments

The authors would like to acknowledge Sophie Wong and Dr. Sanjay Kumar (University of California, Berkeley) and Dr. Theresa Ulrich (Massachusetts Institute of Technology) for providing Figure 4. The authors would also like to acknowledge financial support from the National Science Foundation (CBET BME 0854015, to J.O.W. and A.S. and EEC 0425626 to J.J.L.), Women in Philanthropy, OSU (to J.O.W.), the H.C. "Slip" Slider Professorship (to J.O.W.), and a Pelotonia Graduate Fellowship (to S.S.R.). Any opinions, findings, and conclusions expressed in this material are those of the author(s) and do not necessarily reflect those of the Pelotonia Fellowship Program.

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Jessica O. Winter, PhD
William G. Lowrie Department of Chemical and Biomolecular Engineering
The Ohio State University
140 W 19th Ave.
Columbus, OH 43210

E-mail: winter.63@osu.edu

Received: April 9, 2013
Accepted: September 6, 2013
Online Publication Date: October 30, 2013