Engineered 3D matrices to study regulation of glioblastoma cell malignancy

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Statement of Purpose: Patients with glioblastoma multiforme (GBM), the most aggressive form of primary brain tumor, have a poor prognosis. Due to rapid, diffuse infiltration of tumor cells into normal parenchyma, these tumors have high recurrence rates and traditional therapies - surgery, radiation and chemotherapy - provide only palliation. A major obstacle to understanding the role of extracellular matrix in the regulation of GBM invasion and malignancy is the absence of model biomaterial systems to recapitulate the heterogeneous compositional and biophysical features of gliomas. Such culture systems may facilitate improved *in vitro* analyses to contribute to the understanding of cancer cell dependency on microenvironmental conditions. In order to study glioma cell behavior in a 3D physiologically-relevant context, we have developed a methacrylated gelatin (GelMA) hydrogel platform to recreate physiochemical properties of native gliomas. This allows us to precisely control diffusion-mediated oxygen and nutrient transport, cell-cell interactions, as well as other key biophysical properties such as matrix density and biodegradability to selectively impact glioma cell morphology and malignant phenotype.

Methods: We have designed a hydrogel platform based on natural GelMA, hyaluronic acid (HAMA), and synthetic poly(ethylene glycol) (PEG) materials, to form homologous series of stable networks with variable mesh sizes depending on the concentration and meth(acrylate) molarity. Network elasticity and diffusivity are tuned via network crosslinking density, readily modified by the bulk concentration or degree of methacrylation of the macromer. FRAP was used to quantify the diffusion coefficients of molecules within each 3D network. A microfluidic mixing platform was used to form composite hydrogel matrices containing overlapping gradients of biophysical and cellular cues. Gene expression profiles (VEGF, MMP-2, MMP-9, fibronectin) were analyzed for U87MG and +EGFR glioma cells via RT-PCR. Cocultures of GL261 glioma cells and activated 2C T cells were used to assess immunotherapy targeting of glioma cells in culture. ELISA was used to quantify the protein secretion while confocal microscopy and fluorescent antibodies were used to monitor cell morphology. Cell viability and proliferation was assessed by Live/Dead staining and MTT assay. Cellular hypoxia was quantified via HIF-1 immunofluorescence.

Results: We noted key biophysical properties, namely matrix density, crosslinking density, and biodegradability, significantly impact glioma cell morphology, proliferation, and motility. Deficits in diffusion-mediated oxygen and nutrient transport significantly increased angiogenic signaling. GelMA bulk mechanical properties were primarily impacted by the density of the network, while change in both bulk wt% polymer and macromer DOF had significant impacts on microstructure and small molecule diffusive transport. HIF-1 and VEGF production were significantly upregulated in GelMA constructs relative to 2D cultures where nutrient and oxygen biotransport concerns are negligible. Due to increased diffusion path lengths, construct bulk dimensions were tuned to induce spatial gradients of hypoxia within diffusion restrictive environments characteristic of nascent tumor microenvironments *in vivo*.

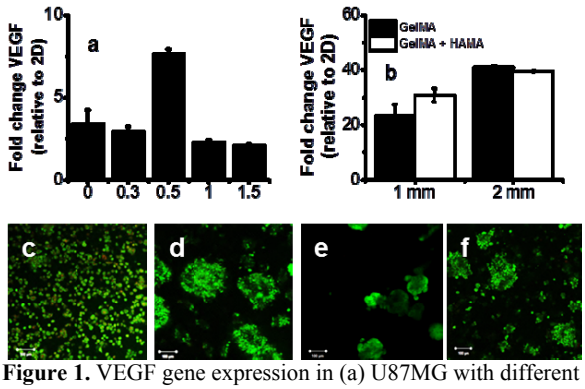

Figure 1. VEGF gene expression in (a) U87MG with different concentrations of HA and (b) U87 +EGFR with 0.5 wt% HAMA in two substrate geometries. (c) U87MG cells encapsulated within PEG hydrogels, (d) with 0.5 wt% HAMA and (e) 1 wt% HAMA; (f) U87 +EGFR in GelMA with 0.5 wt% HAMA. Scale bar 100 μm.

A biphasic relationship was observed between HA (a main ECM constituent of the brain) concentration and malignant profiles. Notably, significant changes in VEGF and MMP expression level, discrete morphological patterns and formation of glioma cell aggregates, and differential levels of glioma cell invasion into the surrounding matrix. Glioma cell aggregation was observed exclusively in HA containing gels, with colony size dependent on HAMA concentration (Fig. 1 c-f). Distinct from traditional 2D culture, U87MG and +EGFR lines showed similar proliferation profiles, with proliferation restricted in the presence of sequestered HA.

Conclusion: This study provides new information regarding GBM malignancy as a function of local biophysical properties using a model GelMA-HAMA biomaterial platform. We believe that these gels provide physico-chemical flexibility to mimic brain-tumor microenvironment and study key factors in tumor formation and progression. After identifying ranges of biophysical environments impacting glioma malignancy, ongoing work is evaluating glioma cell malignancy as a function of gradients of cues created within small-volume matrices by using microfluidic mixers. Ongoing work is also exploring the use of gradients of activated T, stromal, and glioma cells within the matrix to explore tumor-induced immunosuppression and immunotherapy.

References:

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