Development of *in vitro* brain tumor analogs to investigate glioma cell malignancy

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Introduction. Patients with glioblastoma multiforme (GBM), the most aggressive form of primary brain tumor, have a poor prognosis due to a rapid diffuse infiltration of tumor cells into normal parenchyma. The biochemical and biophysical interactions between tumor cells and brain extracellular matrix play an important role in the rapid progression of the tumor. Platforms to replicate the tumor microenvironment are a critical topic in the field of cancer research, and offer unique opportunities to engage next generation genomic tools. These technologies may serve as diagnostic platforms for clinical assessment of therapeutic strategies using patient-specific biopsies. As a result, they will turn into a powerful clinical tool to characterize the tumor microenvironment, and the associated intercellular signaling network in individual patients, enabling personalized therapy.

Methods: We have designed a hydrogel platform based on gelatin (GelMA) and hyaluronic acid (HAMA) that forms a stable network with variable mesh sizes depending on methacrylate molarity. The elasticity and diffusion can be controlled by changes to the network crosslinking density, modified by varying the functionality of the macromer (i.e. 25 to 85%). The microfluidic diffusive mixer contains channels with a 200 µm (wide) x 100 µm (tall) cross-section with 50 µm high staggered herringbone features. Cells were encapsulated by suspending the cells in the prepolymer mixture and followed by UV photopolymerization reaction. Expression profiles associated to bad cancer prognosis (VEGF, MMP-2, MMP-9 and fibronectin) were analyzed for U87MG and +EGFR cell lines and patient-derived glioma cells by RT-PCR under different conditions. ELISA was used to quantify the secretion of proteins and actin staining to evaluate differences in cell morphology. Cell viability and proliferation was assessed by Live/Dead staining and PicoGreen® assay. We identified cellular hypoxia by immunofluorescence of HIF-1 in the hydrogel platforms.

Results: We have developed a versatile gelatin-based biomaterial platform to present combinations of mechanical, structural, and cellular cues inspired by the native glioblastoma microenvironment. Strategies to decorate these biomaterials with biomolecular cues (e.g. hyaluronic acid), common glioma mutations (e.g. EGFR) and chemokines that regulate cell motility, proliferation and survival (e.g. CXCL12) demonstrate an impact in their response to microenvironment. Moreover, spatial and temporal gradients regulate the cell proliferation, migration, and differentiation during cancer. Therefore, we use a microfluidic approach to fabricate patterned biomaterials that have the ability to examine transitions between defined environments (e.g., glioma core, periphery and neural tissue). We developed a series of gelatin and hyaluronic acid (HA) macromers in order to create libraries of composite hydrogel structures. Hydrogels containing brain-mimetic HA show significant impact on GBM malignancy metrics in comparison to 2D culture or through the use of 3D GelMA hydrogels. Glioma cell clusters were observed exclusively in HA containing gels as well as HA-dose dependent gene expression patterns (Figure 1). Cell morphology evolved to a more spread shape from low to high crosslinking density of gelatin and hypoxic levels depended on cell concentration. Gene expression and MMP secretion levels of encapsulated glioma cells correlated with changes in matrix biophysical properties and biomolecule presentation.

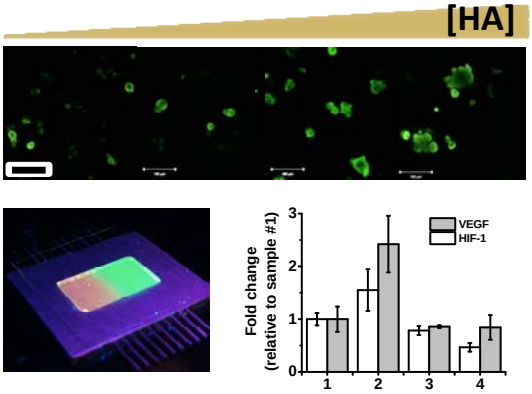

Figure 1. Top image represents live/dead images of U87MG cells along a gradient of hyaluronic acid concentration in a gelatin hydrogel. Images show an increase in cell aggregation with increasing HA concentration up to 1wt%. Bottom left image displays a gradient hydrogel platform after fabrication, from 85% (red) to 25% (green) degree of functionalization. Bottom right graph shows VEGF and HIF-1 gene expression in U87MG cells along the HA concentration gradient represented above (1 corresponds to GelMA and 4 to HAMA 1wt% side).

Conclusions: Using this tool we aim to generate a brain tumor biochip to examine how the heterogeneities within the tumor microenvironment impact glioma malignant transformation, growth, and the conditions that limit therapeutic efficacy. We combined this technology with clinical specimens and data obtained from diagnosis of patients in order to prognosticate the cell dynamics in tumor progression and lead, as a result, to the design of personalized therapy.

References: Pedron S, Harley BA. J Biomed Mater Res. A. 2013; 101:3404-3415; Pedron S, Harley BA. Biomaterials. 2013;34:7408-7417; Carlson BL, Sarkaria JN. Curr Protoc Pharmacol. 2011; 14.16.1-14.16.23.

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