Brain Mimetic Hydrogels for Investigating Glioblastoma Multiforme Migration in 3D

Shreyas S. Rao[1], Jessica DeJesus[2], Atom Sarkar[4], Jessica O. Winter[1, 3]
[1]William G. Lowrie Department of Chemical & Biomolecular Engineering, [2]Neurological Surgery,[3]Biomedical Engineering, The Ohio State University, Columbus, OH, USA, [4]Neurosurgery, University of Arkansas, Little Rock, AR, USA.

Statement of Purpose: Glioblastoma multiforme (GBM), a tumor of central nervous system astrocytes, affects ~ 22500 individuals in the US annually [1]. Unfortunately, median survival time is extremely low (~12-15 months) [1] because of GBM's high infiltration capacity and resistance to current treatment options, including radiation, chemotherapy, and surgery. Consequently, to develop robust treatment methods and expand existing therapeutic options, it is crucial to understand the complex behavior of GBMs. Most existing models of tumor cell migration utilize two dimensional (2D) substrates [2] that fail to recapitulate the complex *in vivo* three dimensional (3D) microenvironment. Here, we present one of the first physiologically relevant and highly tunable 3D brain mimetic biomaterial platforms to investigate GBM migration. The specific tissue mimetics used consist of hyaluronan (HA) and collagen blend hydrogels. HA was chosen because it is one of the primary components in both normal and cancer brain extracellular matrix (up to ~ 50%) [3]; collagen is found in cancer brain (localized to the blood vessel and glial limitans externa) [4]. Blend hydrogels were characterized using compression testing. GBM morphology, spreading, and migration were then examined in 3D culture. In addition to GBMs, behavior of non-cancerous human derived astrocytes was also investigated for comparison. **Methods**: GBMs were procured from brain tumor patients at OSU and developed into a cell line (OSU-2) for experimental use. Non cancerous human astrocytes were obtained from Invitrogen. Hydrogel blends were created using thiolated HA and collagen (types I and III). Thiolated HA was sterilized using UV illumination (~30 min) and placed in a 96 well plate. Pre-labeled OSU-2 cells or non-cancerous astrocytes at ~ 175,000 cells/ml were then mixed with a diluted collagen stock solution and directly added to thiolated HA followed by thorough mixing to create cell-laden 3D hydrogel constructs with constant collagen concentration of 1 mg/ml and HA concentrations ranging from 0-2 wt %. Cell-laden blend hydrogels were incubated at 37ºC, 5% CO_2 for ~ 1h to permit crosslinking prior to addition of cell culture media. Blend hydrogels were evaluated for their elastic modulus using unconfined compression testing. OSU-2 cell migration in 3D hydrogels was observed using time-lapse confocal microscopy. Normal cells in hydrogels were imaged using epifluorescence microscopy. **Results:** Hydrogels compression testing showed that increasing HA concentration to 2wt% increased the gel modulus, with compositions containing 1 and 2 wt% HA being statistically significant compared to collagen gel controls. GBMs adhered to and migrated in brain mimetic hydrogels only at lower HA concentrations exhibiting spindle shaped morphologies; at higher HA concentrations, cells exhibited rounded morphologies (Fig 1). This was further confirmed by quantification of morphology (cell area and circularity index) and migration behavior (individual cell velocity) (Fig 2) using Image J. Normal astrocytes primarily showed rounded morphologies, with a few displaying small

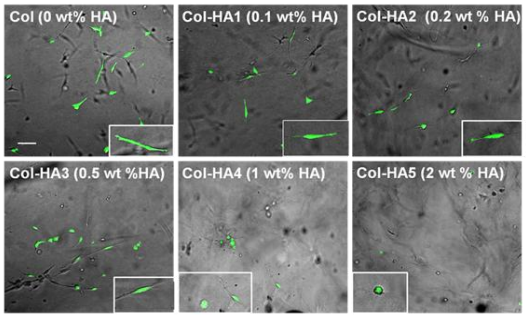

Fig. 1: GBM Morphology in Blend Hydrogels.

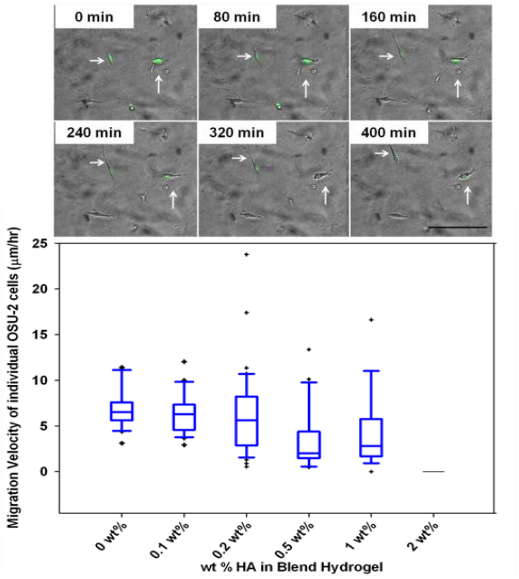

Fig. 2: (top) GBM migration stills in Col-HA2 (0.2 wt %) at different times; (bottom) GBM migration velocity in 3D blend hydrogels (Box & Whisker Plots). Scale = 100 µm.

processes. Interestingly, these hydrogels also allow us to morphologically distinguish normal cells (mostly rounded) from cancer cells (spindle) at lower HA concentrations. **Conclusions and Future Work:** A novel brain mimetic hydrogel-based 3D platform for investigating GBM migration was developed. GBMs show *in vivo* like (spindle) morphologies and migrate at lower HA concentrations, whereas at higher HA values cells were rounded, thus allowing investigator control of cell function in a 3D platform. Future work will investigate the effects of topography (using electrospun fibers) on GBM behavior, which will complement the mechanical and chemical cues provided by hydrogels. **References:** [1] P.Y. Wen et al., *N Engl J Med*. 2008. **359** (5); 49. [2] A. Valster et al., *Methods*. 2005. **37** (2); 208. [3] H.B. Newton. Expert Rev Anticancer Ther, 2004. **4**(5): 803. [4] Giese & Westphal. *Neurosurgery*. 1996 **39**: 235.