Inducing Tumor Cell Migration to Apoptotic Hydrogels using Topographical Cues

Anjana Jain, Debayan Bhaumik, and Ravi V. Bellamkonda
Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

Statement of Purpose: Malignant gliomas account for 15,000 deaths in the United States annually, and are the most common form of tumor originating in the central nervous system. The standard treatments for patients with malignant gliomas are surgical resection, chemotherapy, and radiation. However, these treatments do not allow for the complete termination of all the tumor cells in the area, leading to a high recurrence rate of the glioma.

It has been shown that the invasion of malignant gliomas has predominantly occurred along the white matter fiber tracts, as well as blood vessels. Studies have shown that myelin along the white matter fiber tracts aids with the adhesion and migration of the glioma cells. Other proteins in the basement membrane have been implicated in association with migration along the blood vessels.

We propose to exploit the invasive nature of tumors to '*exvade*' tumors out of the brain along an engineered path of 'least resistance' and direct them to a cytotoxic 'sink'. The design of our 'path of least resistance' to tumors is implemented using aligned nanofiber based films to provide the topographical cues, and coat these 10 micron thin-films with laminin to mimic the ECM cues of the leptomeningeal pathway to provide the biochemical cues. As a destination for the *exvading* cells, we engineer an apoptotic hydrogel 'sink'.

Methods: We used an *in vitro* model that has the following elements- an *exvasion* facilitating thin-film and a tumor 'sink' (Figure 1).

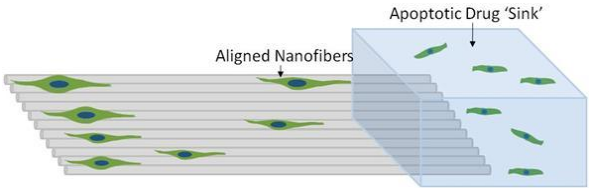

Figure 1. Schematic demonstrating the tumor cells migrating on the aligned nanofibers into the apoptotic drug conjugated hydrogel.

Fabrication of nanofibers: The aligned thin films present oriented topographical cues to facilitate tumor cell migration along with biochemical cues that are substrate bound –collagen type 1– to mimic leptomeningeal structural and chemical cues. Nanofibers that are 600-800nm in diameter are fabricated using well-established methodologies involving electrospinning. Although nanofibers can be fabricated in a random or aligned configuration, it has been shown that directed cell migration occurs only when the fibers are aligned.

Fabrication of hydrogel 'sink': A collagen 'sink' hydrogel was engineered by covalently coupling pro-apoptotic triggers. Covalent coupling ensures that the apoptotic agents do not leach into surrounding neural tissue. Smoothened inhibitor, cyclopamine, which has been shown to effectively regress high grade brain tumors through programmed cell death or apoptosis. Covalent coupling of the cyclopamine to the collagen hydrogel was performed using the crosslinker N,N'-carbnyldiimidazole.

Results: To test whether tumor cells migrate on the nanofiber scaffold, U87mg cells, a human glioblastoma cell line were cultured on the scaffold and it we demonstrate their migration along the fibers (Figure 2).

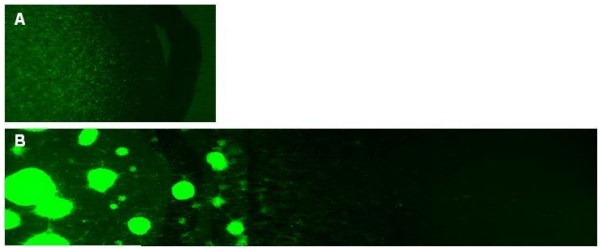

Figure 2. Tumor cell migration on aligned nanofibers. A. Fluorescent image of tumor cells after seeding. The black line depicts the end of the cells. B. Tumor cell migration along the aligned nanofiber scaffold after 1 week.

For the second element, the hydrogel 'sink', cyclopamine conjugated to the collagen hydrogel, as well as cyclopamine mixed with the collagen hydrogel (data not shown) was analyzed for the presence of cyclopamine using NMR. It can be seen in Figure 3 that the cyclopamine was conjugated to the hydrogel. We also saw that the various cancer cell lines underwent cell death when cultured in the cyclopamine conjugated hydrogel.

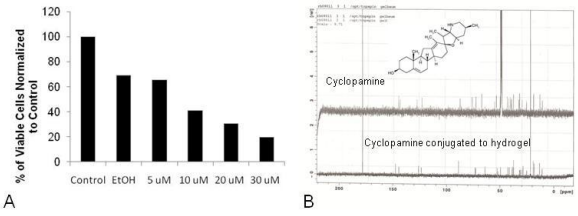

Figure 3. Toxicity and Conjugation of Cyclopamine. A. Cytotoxicity assay to determine cell viability after treatment with different dosages of cyclopamine. B. NMR of cyclopamine alone and with cyclopamine conjugated to collagen hydrogel.

Conclusions: In our approach, the tumor cells are directed to the drug, rather than the current strategy of delivering the drug to the tumor, which is problematic due to the irregular vasculature and poor diffusivity of the tumor tissue. The data demonstrates the ability for the tumor cells to migrate on the nanofiber scaffold. Also, the apoptotic drug is conjugated to the hydrogel to prevent leaching into the healthy tissue and inducing programmed death in neurons. Our future studies include conducting *in vivo* studies to characterize the change in tumor migration and growth.

Acknowledgements: We would like to acknowledge funding by NIH EUREKA (R.V.B. R01CA153229-01).