Investigating mechanisms of glioma cell migration within 3D biomimetic microenvironment

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Statement of Purpose: Despite advances in medical practices and technologies, glioblastoma multiform continues to present as one of the most malignant forms of cancerous cerebral tumors. A rapid, infiltrative cellular phenotype characterizes glioblastoma tumors, often leading to tumor necrosis and uncontrollable vascular proliferation reflected through a malignant morphology. Histologically defined by numerous multinucleated giant cells with various morphological features, glioblastoma presents with numerous proliferative, migration, and varying other physiological features. These morphological features have shown to be influenced by the structure of the surround extracellular matrix (ECM), as well as by the presence of gradients imposed by nutrients and other components necessary for cell vitality. Little research, however, has gone into studying the effects of diffusible gradients mediating the migration of tumor masses. Thus, we evaluate the role of ECM proteins on glioblastoma cell migration, proliferation, and metastasis in response to diffusive chemogradients, done primarily using a microfluidic platform.

Methods: A microfluidic device able to integrate multiple laboratory conditions upon a single chip a few millimeters in size was fabricated using a silicon wafer developed using photolithographic processes (**Fig. 1A**). Microdevices fashioned from the designed mold were made with polydimethylsiloxane (PDMS), as this medium is easily fabricated, translucent in appearance, and offers little resistance to alterations in physical properties such as elasticity, gas permeability, biological inertness, etc. Concentrations of 1, 2, and 3 mg/ml of type I collagen were injected into the proposed microfluidic device, along with 0.1, 1, and 10 μM concentrations of either vascular epidermal growth factor (VEGF) or epidermal growth factor (EGF). A finite element analysis and simulation packaged software was used to properly understand the rate of diffusion of our studied growth factors through the various concentrations of collagen matrices before any actual laboratory experiments were performed. COMSOL Multiphysics software offers a microfluidic module able to integrate every aspect of our experimental design, including matrix and growth factor concentration, with the actual device proposed for our studies (**Fig. 1B**). Time-dependent gradient profiles were generated using this software and later used to determine the concentration of growth factors within designated locations in the microdevice at specific times. The cell line chosen for our specific study was adult human U-87 glioblastoma cells. Upon seeding these cells into the microfluidic platform infused with the designated concentrations of collagen matrices and growth factors, time-lapsed imaging was done at regular intervals over a 48 hour time period.

Results: All images taken were analyzed for the number of cells migrated, distance and angle of migration, rate of migration, and cell shape index. Results indicated that the composition of matrix along with the introduction of increasing concentrations of growth factors have a direct influence upon the mobility of cancerous cell types (**Fig. 1C-D**). Cellular phenotype is significantly affected, with increasing matrix stiffness and decreasing pore sizes lengthening cell bodies, arguably by increasing the available binding sites for cell attachment. The number of cells migrated into the scaffolds, including velocity and distance moved steadily increased with the amplification of growth factor concentration. Definite quantitative relationships between concentrations, cell migration, and cell surface marker expression levels have been found.

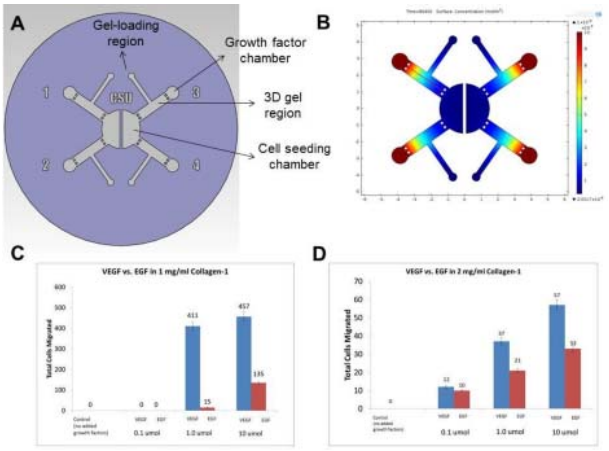

Figure 1. (A) Schematic of the microfluidic device developed for the cancer cell chemotaxis study. The device has designated chambers for cell seeding, seperate gel-filling ports for injecting collagen gel into the designated gel chambers, and separate growth factor loading chamber to create diffusive gradients across the scaffold. **(B)** COMSOL snapshot of the diffusion of VEGF chemogradients through 2 mg/ml collagen scaffold at 24 hr time point. Quantification of the total number of cells migrated through the 1 mg/ml **(C)** and 2 mg/ml **(D)** scaffolds under VEGF and EGF gradients.

Invasion of cancerous cell types into non-diseased tissue is influenced by multiple factors, including cell interactions with ECM, as well as with biochemical processes supportive of active cell migration and proliferation. Alterations to cell morphology are predisposed by the stiffness of the surrounding scaffold, as well as by apparent nutrients and growth factors. Our microfluidic device, capable of mimicking *in vivo* cellular micro-environment, allowed for the control of diffusible gradients imposed through the addition of various concentrations of growth factors and collagen matrices. Further research into the motility of glioblastoma cells can profoundly impact the development of migration-target approaches seeking to treat both adult and pediatric glioblastoma.