Migration of MPIO-labeled glioma cells in the rat brain: validation with histology and fluorescence microscopy

D. Raman[1], A. Krishnan[2], S. Kennedy[3], J. Olschowka[4], S. N'dive[2], D. Davis[5], and W. G. O'Dell[2]

[1]Biomedical Engineering, University of Rochester, Rochester, NY, United States, [2]Radiation Oncology, University of Rochester, Rochester, NY, United States, [3]Biophysics, University of Rochester, Rochester, NY, United States, [4]Neurobiology and Anatomy, University of Rochester, Rochester, NY, United States, [5]Imaging Sciences, University of Rochester, Rochester, NY, United States

Purpose: Brain Tumor recurrence in humans has been shown to occur more frequently along white matter tracts. We have developed a computational model for migration of cancer cells that is influenced by the underlying brain fiber architecture measured from Magnetic Resonance Diffusion Tensor Imaging (MR-DTI). The model thus attempts to predict the location of human brain tumor recurrence following radiation treatment. MPIO labeling has been used by others to detect single progenitor cells and macrophages, but not previously applied directly to monitor the migration patterns of native cancer cells. This study aims at validating the existing computational model by quantifying the migration of individual glioma cells that are dual-labeled with Micrometer sized Super Paramagnetic Iron Oxide (MPIO) particles and GFP.

Methodology: MR-DTI data of an *ex vivo* control rat brain was acquired and tractography was performed to identify the paths of the major fiber bundles. The constrained random walk model was run with a tumor seed location near the corpus callosum. The simulated cells were allowed to walk for 3000 steps with a step size of ¼th the voxel size. Approximately 20,000 GFP labeled CNS-1 rat glioma cells, genetically modified to enhance infiltration, were labeled *in vitro* with BioMag® MPIO particles (Bangs Laboratories, Inc., IN) of a mean diameter of ~1.63μm. The MPIO particles themselves were labeled by the manufacturer with fluorescent microphores emitting at 600nm. The MPIO-labeled cells were mixed with another 80,000 GFP-labeled cells and injected into a Lewis rat brain at a location approximating the seed location in the model. The animal was sacrificed at 20 days post-engraftment and the brain was fixed. The brain was then transferred to PBS containing Gd contrast agent in the ratio 300:1. The fixed brains were immersed in a test tube filled with fomblin and 2 echos were obtained at 3 and 10s to generate proton and T2* weighted images at 141x117x117μm and 70x58x58μm spatial resolution; TR 100ms; flip angle 45^{o}; and 8 averages. The brains were then histologically sectioned coronally at 40μm intervals. Fluorescence images were obtained using FITC and Rhodamine filters for GFP and MPIO labeled tumor cells, respectively.

Results: The distribution of migrating cells predicted from the model showed that most cells remain close to a major fiber bundle. The fluorescence microscopy images show a central tumor mass of 4.2mm in diameter (Fig 1) on the left hemisphere, but infiltrating cells labeled with both GFP and MPIO were observed throughout both hemispheres, with the largest migratory distance of more than 10mm. Few labeled cells were observed ventrally in the rat brain, suggesting that spread of cancer cells occurred primarily along the corpus callosum. In contrast, the computational model predicted some spread laterally along the corpus callosum to the ventral regions. Some dark pixels observed in the MR images were visually correlated with aggregates of MPIO-labeled glioma cells in the corresponding histological sections, however single MPIO-labeled cells observed histologically with the rhodamine filter were not typically matched with a corresponding darkened MR voxel, suggesting that smaller MR voxel sizes are needed.

Conclusion: The fluorescence microscopy images show a glioma cell spread pattern that suggests far-field migration along the corpus callosum that is qualitatively in good agreement with the predictions of the computational model. MPIO-labeled cells were observed at great distances from the site of engraftment at 20-days post-injection, demonstrating that labeled CNS-1 cells are viable long-term and retain the ability to infiltrate. This work suggests that MPIO labeling can be used in future studies to track *in vivo* cell migration in real time with MRI, the goal being to quantify critical physiologic cell migration parameters such as in-vivo migration velocity, persistence in direction, and strength of affinity for fiber bundles. This knowledge can then be used to improve the computational migration model for clinical applications.

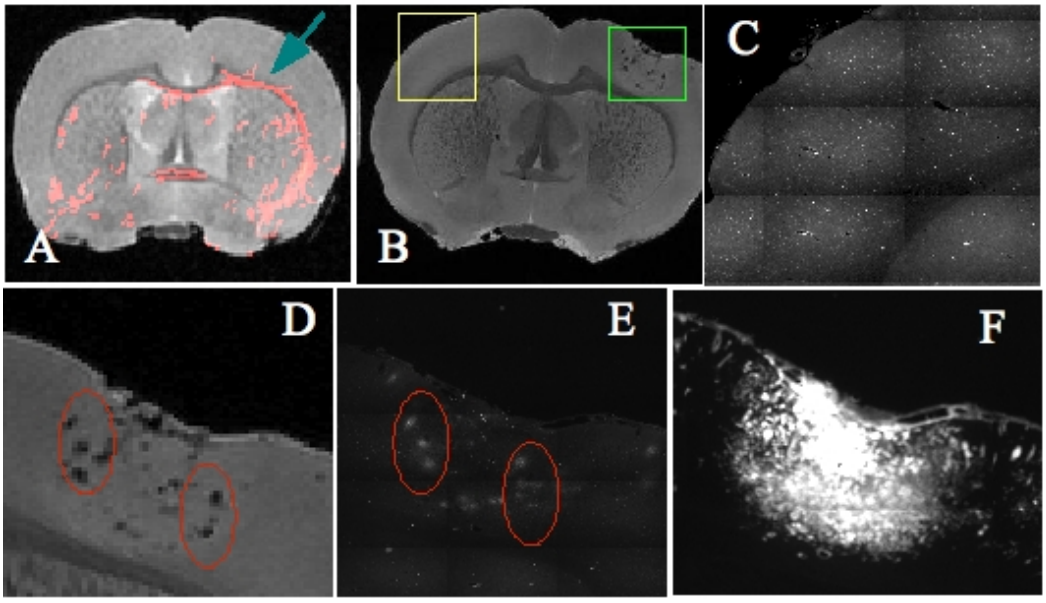

Figure 1. (A)Representative Rat Brain *ex vivo* image overlaid with predicted cell spread from the computational model showing dominant spread along major fibers. Arrow shows site of engraftment. (B) T2* weighted MR Image showing tumor [green box]. (C)Magnified red fluorescence image of Yellow boxed area in [B] showing far-field migration to the contralateral hemisphere. (D,E,F) Magnified Images of Green boxed area in Image B. (D) MRI showing loss of signal due to MPIO. (E) Corresponding red fluorescence image showing MPIO. (F) Corresponding GFP Image.