Convection-enhanced Delivery of Cancer-targeting Biomacromolecules

Michael R. Caplan[1], Jill M. Stukel[1], Alessio Gizzi[2], Christian Cherubini[2], Simonetta Filippi[2].
[1]Arizona State University, [2]Università Campus Bio-Medico di Roma

Statement of Purpose: Poor prognosis for glioblastoma multiforme patients (<10% 5-year survival) is due primarily to cells having invaded other parts of the brain and formed nascent secondary tumors by the time the primary tumor is detected and removed. We and others have developed bio-macromolecular targeting constructs designed to target these secondary tumors by intracranial injection and perfusion through brain tissue. The very small size (~6kDa) of these constructs allows them to perfuse freely in tissue although they are orders-of-magnitude too large to pass through intact blood-brain barrier (nascent secondary tumors have intact BBB and are not compatible with nano-particles dependent on the enhanced permeability and retention, EPR, effect for delivery). Here we show *in vitro* proof-of-principle that convection-enhanced delivery (CED) has potential to successfully and specifically deliver bio-macromolecular targeting constructs to secondary tumors. Finite element modeling (FEM) predicts that clinically accepted drug delivery methods can achieve such targeting throughout an entire hemisphere of a human patient's brain.

Methods: Mathematical modeling was performed using a finite element method (COMSOL Multiphysics) to simulate Darcy's Law and mass transport equations in a porous medium. Isotropic medium was modeled with constant diffusion coefficient and hydraulic permeability with binding to cells modeled as in Stukel (2008). Constant pressure was set at the outer boundary, and injection was simulated by a constant flow boundary (5 µl/min) at the catheter (0.1 cm diameter circle at center). Constant concentration boundaries were set for the injection ($t<t_c$: $C=C_0$; $t>t_c$: $C=0$) and outer boundaries ($C=0$), where t_c is the time at which injection switches to saline to simulate convective wash. Simulation in brain tissue is identical except the injection sites are modeled as spheres and diffusion coefficients and permeabilities are position dependent as defined by a matrix based on an apparent diffusion coefficient map generated by MRI.

Bio-macromolecular constructs are made as described in Rosca (2007). Briefly, three TWYKIAFQRNRK peptides are each linked by three poly(ethylene glycol) oligomers using standard peptide synthesis chemistry. Fluorescein isothiocyanate is added at the amine terminus. Normal human astrocytes (NHAs) and a glioblastoma cell line (SF767s) were encapsulated in regions of an agar/collagen (0.3%/0.3%) gel equidistant from an injection site (5 µL/min, C=50µM until convective wash, t_c=10min). Images are taken with inverted fluorescence microscopy (20X).

Results: FEM results predict that no contrast between tumor (circle to left of injection) and non-tumor (circle to right of injection) should be observed until after the injection bolus has been pushed past the tumor region (no contrast in Fig. 1A or 1D, contrast in Fig. 1G and 1J). Experimental results show no contrast at 1hr (Fig 1B vs. 1C), little contrast at 2hr (Fig 1E vs. 1F), good contrast at 4hr (Fig 1H vs. 1I), and best contrast at 14hr (Fig 1K vs. 1L). However, intensity of fluorescence decreases with time indicating a tradeoff between contrast and intensity.

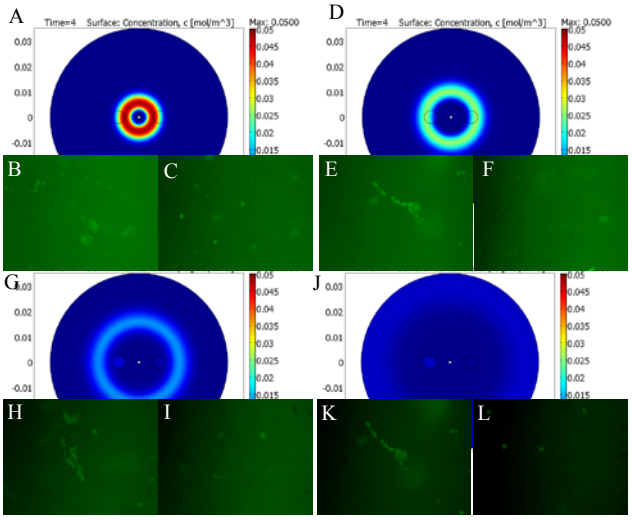

Figure 1. FEM (A,D,G,J) and experiment (B,C,E,F,H,I,K, L) of injection and wash at 1 hr (A-C), 2 hr (D-F), 4 hr (G-I), and 14 hr (J-L). Microscopy of SF767 (B,E,H,K) and NHA regions (C,F,I,L) are shown for each time.

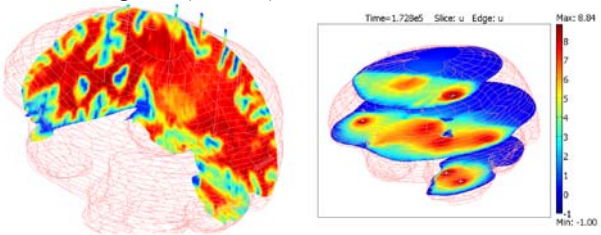

Figure 2. (Left) Position-dependent diffusion coefficients defined for each finite element, red=high blue=low. (Right) Concentration profile, red=8.8µM blue=0µM, after CED for 48 hr from 5 catheters with multiple ports.

Figure 2 predicts that five catheters, with 2-3 ports each, injecting constructs for 48 hrs can distribute efficacious concentrations to an entire hemisphere of the brain.

Conclusions: Delivery of bio-macromolecular via CED does allow specific targeting of glioma cells but contrast is only observed after a convective wash. This confirms earlier results showing that specificity is only enhanced at low concentration (Rosca, 2009) and predictions of a tradeoff between contrast and intensity as time increases (Stukel, 2008). Modeling realistic geometry and anisotropy of brain tissue predicts that it is possible to achieve targeting throughout the entire hemisphere of the human brain to specifically target migratory glioma cells.

References: Rosca EV. *Biomacromolecules*. 2007; 8: 3830-3835. Stukel JM. *Ann Biomed Eng*. 2008; 36: 1291-1304. Rosca EV, *Biotech & Bioeng*. 2009; 104: 408-417.