# Bioactive Hydrogel Microspheres for Support and Delivery of Neural Stem Cell-based Therapeutics

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**Statement of Purpose:** Although neural stem cell (NSC) transplantation has shown significant promise as a stroke therapy in animal models, graft mortality is typically high and tissue regeneration is negligible.[1] This outcome is likely caused by the hostile environment within the stroke lesion, where neural tissue, vasculature, and matrix can be completely disrupted.[2] In order for a therapeutic approach to be successful, the cells must be provided with both structural support and a favorable microenvironment. This research investigates the use of a biocompatible, bioactive poly(ethylene glycol) or PEG-based hydrogel as a biomimetic scaffold for neural stem cell-based therapy. The hydrogel material was covalently modified to present bioactive peptides and protease sensitive sequences for cell mediated degradation.[3] Endothelial cells were encapsulated along with neural stem cells in order to enhance vascularization of the graft and to provide similar cell interactions to those observed in the endogenous NSC niche.[4] BrdU labeling was used to track cell fate following implantation. The microspheres were implanted by injection into the lesion of a rat stroke model and outcomes were evaluated by MRI and histology.

**Methods:** Succinimidyl carboxymethyl PEG monoacrylate was covalently bound to the cell adhesive peptide, RGDS. Similar chemistry was utilized to embed a collagenase sensitive peptide, GGGPQGIWGQGK between two acrylated PEG spacers to form the backbone of the MMP-sensitive hydrogel. A 10% solution in PBS of this polymer was cross-linked into a hydrogel by addition of 100 µmole/mL Eosin Y photoinitiator, 3.4 µL/mL N-vinylpyrrolidone, 1.5% v/v triethanolamine and exposure to light from a metal halide lamp. Microspheres were produced by cross-linking the polymer solution after generation of an emulsion in sterile mineral oil containing 3 µL/mL 2-2-dimethoxy-2-phenyl acetophenone in N-vinylpyrrolidone (300 mg/mL). A co-culture of conditionally immortalized neural stem cells (MHP36) and immortalized murine brain endothelial cells (bEnd.3, ATCC) were encapsulated within the hydrogel and cultured overnight *in vitro* before transplantation. The particles were delivered via stereotactic injection into the lesion as defined by MRI two weeks following middle cerebral artery occlusion (MCAO). Graft success was evaluated through MRI analysis of lesion volume and histology one week post-transplantation.

**Results:** The microsphere encapsulation procedure was optimized to preserve high viability for both cell types. (representative live/dead stain shown in Figure 1-A) With *in vitro* culture, these hydrogel particles supported both spreading and proliferation of the cells in the 3D environment. A co-culture encapsulation of MHP36 cells and bEnd.3 cells were evaluated at multiple time points with immunostaining to identify expression of phenotypic markers such as nestin (neural stem cells) and CD31 (endothelial cells). (Figure 1-B)

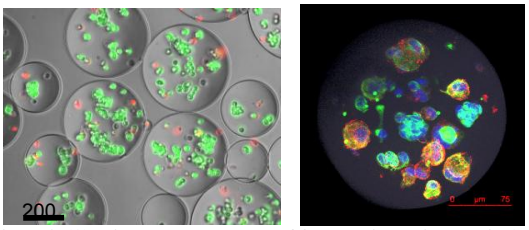
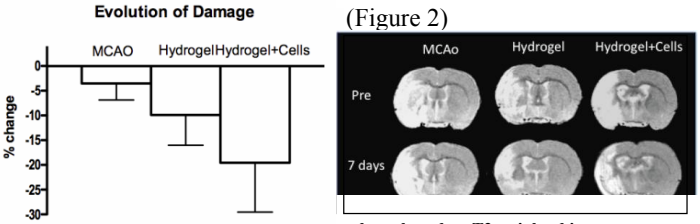



**Figure 1. (A) Microencapsulated MHP36 cells stained with calcein AM (live) and ethidium homodimer (dead). (B) Confocal projection of micro-encapsulated co-culture of MHP36 (nestin) bEnd.3 cells (CD31).**

The co-culture was additionally tested *in vivo* as a cell based therapeutic in a rat model for stroke. MRI images at the one week time point showed a clear trend of decreased lesion volume with implantation of the microencapsulated cells, however the group size was not sufficient for (Figure 2)




**.. volume based on T2 weighted image taken 7 days post-implantation shows trend of reduced size with treatment.**

BrdU labeling of implanted cells allowed for their later identification during histological analysis of tissue from the 7 day time point. It was confirmed that both cell types survived delivery and were localized to the injection site. The implanted neural stem cells were observed to have undergone differentiation down neural and glial lineages especially when in close proximity to endothelial cells. Finally immunohistochemistry showed minimal immune reaction to the implanted microspheres.

**Conclusions:** This research introduces highly tunable, hydrogel microspheres as a platform technology for neural tissue engineering. The particles are demonstrated to effectively support cells *in vitro* and to function as a supportive carrier for implantation of cells *in vivo*. Both hydrogel and transplanted cells were identified after one week post-implantation, and differentiation of the neural stem cells was observed in some cases. In addition to the RGDS peptide used here, the material can be precisely modified through the covalent attachment of multiple peptides or whole proteins in order to further mimic the micro-environment of the endogenous niche. Providing an appropriate surrogate niche for implanted NSCs may be the key to promoting successful survival and engraftment with the host tissue. Finally, the microsphere formulation which can be achieved through a simple oil emulsion provides structural support to the cells while still allowing delivery via controlled injection.

**References:**

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