Bioactive Hydrogel Microspheres as a Versatile Support and Delivery System for Stem Cell-Based Therapies

Christy L Franco, Jennifer L West
Rice University, Houston

Statement of Purpose: Neural stem cells (NSCs) have shown significant promise for treating a variety of central nervous system disorders.[1] For a therapeutic approach to be successful, however, the cells must be provided with both structural support and a favorable microenvironment. Mimicking the adult NSC niche, where proliferating neural precursors are observed near sites of angiogenesis, may be one solution.[3] 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 specific bioactive peptides and protease sensitive sequences for cell mediated degradation.[2] Additionally, a method was developed to allow rapid microencapsulation of cells within this material while preserving high viability. The microsphere formulation was demonstrated to sustain complex cell interactions *in vitro* and could potentially allow injectable delivery of these prestarted tissue constructs for therapeutic applications.

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). Control over cell behavior was demonstrated by evaluating the adhesion and spreading of NSCs on the surface of hydrogels containing varying concentrations of the RGDS adhesive peptide. Encapsulated cell viability was assessed with calcein AM and ethidium homodimer staining. Versatility of the polymer microspheres to support niche-like interactions was demonstrated with a conditionally immortalized neural stem cell line (MHP36) and an immortalized murine brain endothelial cell line (bEnd.3, ATCC). Potential for prevascularization was investigated with a co-culture of human umbilical vein endothelial cells (HUVEC, Lonza) and smooth muscle progenitor cells (10T1/2, ATCC). Immunostaining was performed to monitor these co-culture interactions within the particles over time.

Results: The microsphere encapsulation procedure was optimized to preserve high viability for multiple cell types. (Figure 1) With *in vitro* culture, these hydrogel particles were observed to support long term survival, proliferation and spreading, of cells in a 3D environment. As seen in Figure 2, a 1 to 1 co-culture of bEnd.3 and MHP36 cells developed extensive interactions between endothelial processes and proliferating NSC clusters which were similar to those observed in the *in vivo* niche.[3]

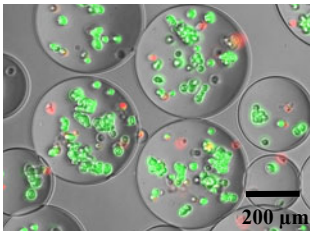

Figure 1. Calcein AM, ethidium homodimer staining of microencapsulated MHP36 cells

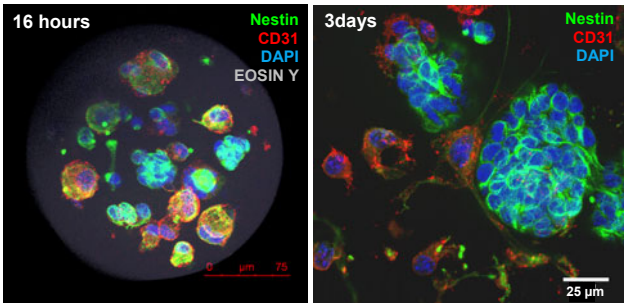

Figure 2. 50/50 co-culture microencapsulation of MHP36 and bEnd.3 cells observed after 16 hours and 3 days in culture

A co-culture system of HUVECs and 10T1/2 cells provides a more complex model of angiogenesis with which the NSCs can interact. In Figure 3, DAPI and phalloidin staining are used to visualize a 4 to 1 co-culture microencapsulation of HUVEC and 10T1/2 cells.

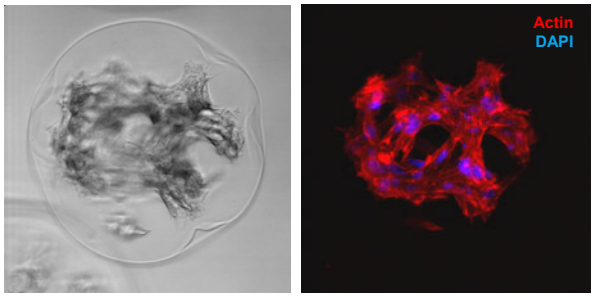

Figure 3. 4 to 1 co-culture microencapsulation of HUVEC and 10T1/2 cells stained with DAPI and rhodamine-phalloidin after 7 days in culture

By 7 days the cells had organized into a primitive vascular plexus within the particles which was stable for over 14 days in culture. NSC behavior will also be evaluated within this system.

Conclusions: This research introduces highly tunable, hydrogel microspheres as a platform technology for neural tissue engineering. The particles are demonstrated to support multiple cell types as well as complex co-cultures interactions *in vitro*. 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 mimic the microenvironment of the *in vivo* 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:

1. Lundberg C. Exp Neuro. 1997;145:342-360.
2. West JL. Macromol. 1999;32:242-244.
3. Palmer TD. J Comp Neurol. 2000;425:479-494.