Development of a Bioactive Hydrogel Scaffold as a Simulated Niche for Investigating Neural Stem Cell Behavior *In Vitro*

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Statement of Purpose: Neural stem cells (NSCs) have shown considerable promise as a cell-based therapy for the treatment of neurological disease and injury.[1] There is, however, a continued need to better understand the regulatory mechanisms that control their behavior and lineage specification. *In vivo*, these cells are primarily supported by a unique microenvironment or niche.[2] Previous research has produced an extensive map of the niche composition, however functional roles are not fully understood. This work proposes the use of a modular biomimetic scaffold as a tool for probing the response of a clinically relevant, human cortical NSC line (CTX0E03) to various bioactive components of the native niche in a controlled, *in vitro* system. Several extracellular matrix mimicking peptides as well as fibroblast growth factor (FGF) were covalently linked to the scaffold and shown to support 2D culture of the cell line. Furthermore, an enzyme cleavable peptide embedded within the backbone of the polymer chains allowed encapsulation of cells within the matrix and translation of 2D results into a comparable 3D culture system.

Methods: Succinimidyl carboxymethyl PEG mono-acrylate was covalently bound to the fibronectin derived RGDS or laminin derived IKVAV, and YIGSR peptides as well as a cyclic form of RGD, c(RGDfK). Similar chemistry was utilized to embed a collagenase sensitive peptide, GGGPQGIWGQGK (PQ) between two acrylated PEG spacers to form the backbone of an MMP-sensitive hydrogel.[3] Finally a modified version of this protocol was utilized to link FGF basic protein to an acrylated PEG spacer for incorporation into the hydrogel. PEG-FGF bioactivity was compared to unmodified growth factor through a proliferation assay with CTX0E03 cells. For hydrogel generation, a 10% solution in PBS of PEG diacrylate or degradable polymer was cross-linked into a solid scaffold by addition of 10 µM Eosin Y photoinitiator, 3.4 µL/mL N-vinylpyrrolidone, 1.5% v/v triethanolamine and exposure to white light. Cell response to adhesive peptides was investigated by addition of 0 to 6 mM PEG-peptide to the pre-polymer solution. PEG-FGF was also studied at a concentration of 40 ng/ml in the hydrogel solution.

Results: PEG-FGF retained equivalent bioactivity to the unmodified growth factor as shown with a Celltiter96 proliferation assay. (Figure 1)

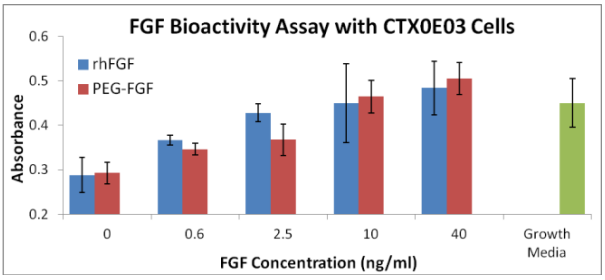

Figure 1. Cell number after 72 hrs culture in the presence of varying concentrations of FGF or PEG-FGF as compared to growth media containing 10 ng/ml FGF

Surface seeded CTX0E03 cells showed significantly enhanced adhesion and spreading on hydrogels modified with the cyclic form of RGD as compared to linear RGDS or laminin peptides. (Figure 2 shows RGD comparison only)

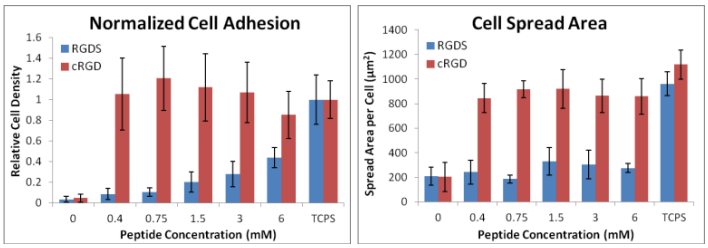

Figure 2. Quantification of NSC adhesion and spreading on linear vs. cyclic RGD presenting hydrogels

The hydrogel matrix was shown to maintain undifferentiated NSC growth (determined by expression of stem cell markers such as nestin) both when cells were cultured on the surface of cRGD presenting scaffolds and encapsulated within the bulk of the degradable material. (Figure 3)

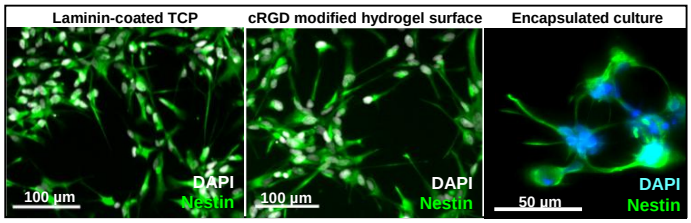

Figure 3. Immunostaining for DAPI and nestin of CTX0E03 NSCs grown on laminin compared to surface seeded and 3D encapsulated hydrogel cultures.

Finally, in the presence of differentiating media, the hydrogel was found to support NSC differentiation along neuronal and glial lineages based on immunostaining for βIII tubulin and GFAP respectively. (Figure 4)

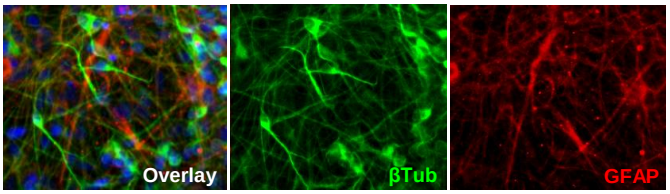

Figure 4. Differentiated CTX0E03 cells on the surface of a cRGD presenting hydrogel show development of neural (βTub) and astrocyte (GFAP) phenotype.

Conclusions: This research presents a highly tunable, hydrogel scaffold as a platform technology to isolate and explore the influence of various matrix components and growth factor signals on NSC behavior and fate. Due to mild cross-linking conditions, cells can be encapsulated within the hydrogel to investigate the effects of a more natural, 3D environment. Ultimately, since the material is highly biocompatible, these hydrogels could be designed to provide a surrogate niche for protection and delivery of NSCs-based therapies *in vivo*.

References:

1. Burns TC, et al. J Comp Neurol. 2009; 515:125-144.
2. Conover JC, et al. Cell and Tissue Res. 2008; 331:211-224.
3. Moon JJ, et al. Biomat. 2010; 31(14):3840-38477.