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SPINAL CORD RESEARCH

Final Narrative Report

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1. Original aims of the project.

The long-term objective of this research is to develop a tissue engineering strategy for spinal cord regeneration. One of the hurdles is identifying a source of cells to replace damaged neurons. Embryonic stem cells offer great potential for spinal cord regeneration and other pathologies where cell sources are limited. The key then becomes controlling the differentiation of the stem cells to generate a population of specific cells. Traditional 2D tissue culture methods have demonstrated that adhesive cues from extra cellular matrix (ECM) molecules significantly influence phenotypic behavior of cells and stem cell differentiation [1]. Other experiments indicate that the mechanical properties of the 2D substrate can also influence these phenomena [2]. However, 2D tissue culture methods do not comprehensively replicate the 3D environment experienced during development in vivo. Three-dimensional tissue cultures provide significantly different cell-cell interaction and cell-matrix cues that we hypothesize influence stem cell differentiation efficiency and cell signaling. The primary focus of this research is to investigate the influence of these cues on stem cell differentiation into neuronal lineages, by using microfluidics to manipulate the biomaterial mechanical and adhesion properties within a 3-dimensioned tissue engineered construct.

Specific Aim 1: Elucidate the relationship between stem cell differentiation and the mechanical properties of a biomaterial scaffold.

Specific Aim 2 Evaluate the relationship between stem cell differentiation and the gradient of cell adhesion sites on a substrate.

Specific Aim 3: Evaluate the effects of substrates with both mechanical and adhesion gradients on stem cell differentiation.

2. Project successes.

Thus far, we have made significant strides towards the completion of our goal. We have developed protocols to manipulate the bioactivity of collagen by grafting peptides on to the collagen backbone with carbodiimde chemistry, and we have extensively characterized changes in mechanical properties due to genipin, which is a naturally derived cell-tolerated crosslinking agent. We have also adapted these protocols for use in microfluidic networks that create 3D microenvironments with spatial control of cell adhesion bioactivity and mechanical properties. We believe that we will be able to use this technology to create engineered tissues with regionally varying properties that are optimal for control of heterotypic cell behavior. We are currently using this system to assay the differentiation of stem cells in these environments.

Encouraged by our previous results we have included the use of additional adhesion/signaling molecule in our collagen gels to direct differentiation. In the original proposal we proposed using RGD to create cell-adhesive gradients. However, as reflected in our no cost extension, we have revised our original plans to include the use of a

peptide mimic to the HNK-1 carbohydrate epitope as well. The HNK-1 carbohydrate epitope (HNK) is recognized by several neural recognition molecules as well as integrins and is highly expressed on the surface of cells within the neural crest. The recognition of HNK epitope by integrins functionally links it to the extracellular matrix. The availability of a peptide mimic to this carbohydrate makes it inexpensive to use and the flexibility of our system allows it to be easily included in our protocols. The inclusion of HNK mimic on the collagen backbone allows us to target more specifically the differentiation of stem cells into neuronal lineages. We believe the grafting of the HNK mimic will yield a much higher percentage of nestin positive cells that in turn can differentiate to neurons. Our continued efforts have validated this finding and we are currently in process of preparing a manuscript to report these findings.

In addition to HNK-1, with newer findings published each year in the literature, we have extended our repertoire of peptides to include signaling molecules that are known to guide differentiation of stem cells into neural lineage precursors. The new peptides were easily incorporate into our experimental designs given the flexibility of our system. These molecules include a peptide mimics to polysialic acid and Wnt antagonist peptides. Results from the inclusion of these peptides have been very positive and additional experiments are ongoing to test combinations of peptides presented together.

Following completion of these studies, we will have determined how heterotypic cell adhesion bioactivity in a 3D collagen gel can be optimized for specific differentiation into neural lineages. This will provide a significant step forward towards ours long-term objective of developing an implantable biomaterial to generate an optimal environment for restoration of function following spinal cord injury.

3. Project challenges.

The biggest challenge faced during this project was identifying an adhesive molecule that would consistently differentiate stem cells into neural lineages. Selected candidates would often perform better than control experiments but the variability in the extent of differentiation was significant. A major contributor to this variability was the presence of serum in our system. Serum is essential for cell viability but also allows for spontaneous differentiation. Serum free media was one possible solution to this challenge; however, it is expensive and requires the use of highly regulative growth factors that are also involved in stem cell differentiation. The inclusion of antagonist signaling peptides to our system helped minimize this challenge. Adding antagonist peptides to other wise strongly regulating pathways allowed for less variable results.

The development of microfluidic technology in our lab provided another set of challenges. Traditional microfluidics is performed with liquid or gas filled microchannels that hold picoliter volumes. In our application, we fill the channels with a fibrillar gel – collagen – that is porous, which allows flow to permeate through the gel. In traditional microfluidics, the introduction of a bubble into a microchannel can be problematic, in that it disrupts flow patterns that may have been specifically engineered, but the patterns generally return after the bubble has traversed out of the network. However, in our application, the pore size of the collagen gel is much smaller than any bubble. The introduction of any bubbles subsequently destroys the gel, deforming it

beyond use. Our protocols called for several switches of inlet solutions, and each step increased the risk that air would be introduced to the system. A significant amount of time was spent developing a troubleshooting a protocol that has virtually eliminated bubbles. A second major challenge involved overcoming diffusive limitations within the networks. Although the channels were only several millimeters in length, this was long enough to limit the availability of oxygen and nutrients from the inlet solutions, and cell viability suffered. Dissolving more oxygen into the medium was not an option because it would have dramatically increased the likelihood of introducing bubbles. We ultimately designed a multi-layered microfluidics assay that provided additional inlet and outlet ports for medium and oxygen.

4. Implications for future research and/or clinical treatment.

The promise of stem cells therapies have opened innumerable treatment possibilities for severe degenerative diseases and disorders that are otherwise obdurate to today's therapies. Its practice however, relies on the successful transplantation of healthy functional stem cell derived cells into injured sites with appropriate vehicles. Tissue engineering approaches provide one such vehicle for delivery. Tailoring implantable biomaterials to provide appropriate cues to guide the differentiation of stem cells may afford a means to implant stem cells within injured sites and gain functional recovery.

5. Plans to continue this research, including applications submitted to other sources for ongoing support.

An application is pending as part of a Center Proposal for Stem Cell Engineering to the New Jersey Commission on Science and Technology. Another is planned for an October submission to the NIH (R01).

6. List and include a copy of all publications emerging from this research, including those in preparation.

Publications

Monteiro, G.A., et al., *Positively and Negatively Modulating Cell Adhesion to Type I Collagen Via Peptide Grafting.* Tissue Eng Part A, 2009.

Sundararaghavan, H.G., et al., *Neurite growth in 3D collagen gels with gradients of mechanical properties.* Biotechnol Bioeng, 2009. **102**(2): p. 632-43.

Sundararaghavan, H.G., et al., *Genipin-induced changes in collagen gels: correlation of mechanical properties to fluorescence.* J Biomed Mater Res A, 2008. **87**(2): p. 308-20.

Planned Publications

Monteiro, G.A., Schachner, M. and Shreiber, D.I. Collagen grafted with eptide glycomimetics induce neural differentiation of embryonic stem cells. Stem Cells. Expected submission, May, 2009.

Monteiro, G.A., Schachner, M. and Shreiber, D.I. Functionalizing biomaterials with signal transduction factors for improved stem cell differentiation.. Stem Cells. Expected submission, Oct, 2009.

Conference Presentations and Posters

Monteiro, G.A., Sundararaghavan, H.G., Fernandes, A. and Shreiber, D.I. *Optimizing Adhesion to Enhance Cell Migration in Collagen Gels.* NJCBM. New Brunswick, NJ. 2008.

Monteiro, G.A., Sundararaghavan, H.G. and Shreiber, D.I. *Stem Cell Differentiation in HNK-1 Modified Collagen Gels.* NJCST. Bridgewater, NJ. 2008.

Monteiro, G.A., Sundararaghavan, H.G., Fernandes, A. and Shreiber, D.I. *Enhancing cell migration in collagen gels by modulating collagen adhesivity.* ASME Summer Bioengineering Conference. Marco Island, FL. 2008.

Sundararaghavan, H.G., Monteiro, G.A.. and Shreiber, D.I. *Microfluidic Generation Of Adhesion Gradients Through 3D Collagen Gels: Implications For Neural Tissue Engineering* ASME Summer Bioengineering Conference. Marco Island, FL. 2008.

Monteiro, G.A. and Shreiber, D.I., *Three Dimensional Scaffolds for Embryonic Stem Cell Differentiation and Delivery,* The 8th New Jersey Symposium on Biomaterials Science. New Brunswick, NJ. 2006.

Monteiro, G.A. and Shreiber, D.I., *Regulation Of Embryoid Body Germ Layer Formation in Tunable Collagen Scaffolds*, 2006 Annual Fall Meeting of the Biomedical Engineering Society. Chicago, IL. 2006.

Monteiro, G.A. and Shreiber, D.I., *2D Vs 3D Collagen Environment for Embryoid Body Formation.* BMES, Annual Fall Meeting. Baltimore, MD. 2005.