Final Narrative Report

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1. Original aims of the project.

The broad, long-term objective of this research is to develop a novel biomaterial that spurs regeneration of the spinal cord. *The goal of this proposal* is to manufacture a designer protein-based hydrogel that can have its mechanical properties and porosity tuned for neuronal growth. It has been shown that substrate rigidity and porosity have profound effects on neurite outgrowth. By controlling the stiffness and porosity of the biomaterial, we believe we can selectively promote the growth of regenerating neurites (which prefer porous, softer substrates) while limiting invasion by astrocytes (which prefer more rigid substrates and have been shown to be inhibitory to neuronal regeneration in the spinal cord). Our design comprises four oligopeptide subunits that will self-assemble into a fibrous network at physiological temperature and pH.

The hydrogel we propose to fabricate is comprised of four peptide subunits. Two of these subunits are 28 amino alpha helices that dimerize to form a coiled coil with exposed hydrophobic ends. These peptides, denoted Saf1 and Saf2, have been shown to form long fibers when placed in Phosphate Buffered Saline. The remaining two oligopeptide subunits, denoted Saf1L and saf2L, are two Saf1 or Saf2 unlinked units joined by a large hydrophilic linker. The function of these subunits is to assist the forming gel network in the uptake of water. This function is necessary to form a hydrogel, rather than a water-insoluble network of fibers.

The *ultimate* use of this hydrogel is for transplantation into an injured spinal cord, where it would coax regeneration of damaged or axotomized neurons to grow into the hydrogel at the proximal site of injury, traverse the length of the implant, and then fuse with axons at the distal site of injury. By sealing this breach, we feel that there is a possibility that we might eventually be able to restore neural transmission to the damaged cord.

This Project has three Specific Aims:
Specific Aim 1: To fabricate a hydrogel composed of four oligopeptide subunits that self assembles at physiological temperature and pH and supports the attachment and viability of dissociated chick spinal cord neurons.

Specific Aim 2: To alter the pore size and substrate rigidity of the assembled hydrogel by modulating the peptide formulation of the gel.

Specific Aim 3: To optimize the substrate rigidity and pore size of the assembled hydrogel to maximize the rate of outgrowth of dissociated chick neurons seeded into the gel.

2. Project successes.

Use of bioinspired, genetically engineered proteins in tissue engineering scaffolds represents a new opportunity for engineering these constructs. However, the production and rational modification of new, artificial proteins is hindered by significant gaps in knowledge

regarding expression of artificial gene constructs in *E. coli* and their molecular modeling. This project focused on the production of a novel hydrogel scaffold composed of four self-assembling protein modules and their rational modification using Molecular Dynamics (MD) simulations. Two of the modules were based on the ABA triblock copolymer design. In this triblock, a hydrophilic, random coiled region is flanked by 28 amino-acid α−helical endblocks. The purpose of these endblocks is to function as virtual crosslinkers and support network formation. The length of the endblocks can be changed by the addition of two unlinked, fiber-forming peptides and thus potentially alter the gelation and melting points of the hydrogel.

We evaluated the efficacy of production of these endblocks by two separate expression strategies in *E. coli* and demonstrate their ability to form hydrogels. We were able to show that all four units were required to form a gel, and we characterized the rheological properties of a preliminary sample of gels.

We also extended the project to an in silico domain. We analyzed the Gibbs free energy of formation of oligomeric intermediates that arise early on during fibrillogenesis from the unlinked peptides using Molecular Dynamics simulations via the MM/PBSA module of Amber 9. Thermodynamic data demonstrated changes in the primary structure of these peptides affect the stability of the intermediate that seeds fiber formation. This analysis also suggests a shift in the fiber forming mechanism from monomer addition to protofibril addition. We show howed this data can be used to improve interhelical interactions between endblocks and unlinked peptides and how to develop coarse-grain models of fiber formation.

3. Project challenges.

Production and purification proved to be the greatest challenge for this project. The scaffold is comprised of 4 peptides: 2 short (28AA) 'fiber forming peptides' and 2 longer (173AA) 'water attracting' peptides. Because the scaffolds would ultimately require milligram quantities of these peptides, our goal was to produce them in bacteria and then purify. This required design of the DNA and inclusion of a purification system(s). Unfortunately, we were unable to produce and purify the two small peptides in bacteria, as they appeared to immediately degraded. This is not uncommon for small peptides, which may be viewed as unwanted protein fragments. Thus, we were forced to pay for direct synthesis of the two 28AA peptides.

For the larger peptides, we designed DNA to express these peptides in bacteria along with a fusion protein for a purification scheme – the inclusion of maltose binding protein (MBP), which would allow the peptides to be purified on an amylose column. We did successfully express these peptides with fusion proteins as measured with MALDI-TOF mass spectroscopy. However, when we attempted to cleave the MBP fusion protein off of our peptide, the enzyme, geninase, also degraded our peptide. We were therefore forced to abandon this approach and redesign the DNA with another purification scheme. We used the 6x HIS tag that was in a flanking region of our large peptides as a binding site for a Nickel column for purification, and successfully expressed and purified enough of the two longer peptides to form a few hydrogels for mechanical testing. However, the delay in our failed attempts with the MBP system, combined with the high costs of the synthesized peptides and molecular biology reagents prevented us from pursuing to research further.

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

The most intriguing and promising aspect of the research was the results of the molecular modeling, which point to the mechanism of early aggregation events that drive fiber (and therefore scaffold) formation, and which may be re-designed in silico prior to generation in the lab to specifically pre-engineer desired properties, such as mechanical compliance or gelation time and temperature.

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

Unfortunately, the molecular benchtop work is too expensive for us to continue, and, frankly, too expensive for long-term clinical feasibility. Designer scaffolds from self-assembling peptides does hold promise, but the peptides meed tp be smaller and easier to purify. We did receive to Supercomputing Awards for CPU time to perform our Molecular Dynamics simulations from the NCSA,and an internal award from the Charles and Johanna Foundation for Biomedical Research ($20,000).

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

Publications

In preparation:

Gaughan CL and Shreiber DI. Molecular dynamics simulation of an artificial self-assembling peptide system. Journal of Biological Chemistry. Submission Expected Mar, 2009.

Conference Presentations and Posters (1 page abstracts)

Gaughan CL and Shreiber DI. Modulating Fiber Diameter Formed from Self Assembling Peptides. 2006 Biomedical Engineering Society Meeting, Chicago, IL.

Gaughan CL, Chiew Y, and Shreiber DI. Simulation of fiber formation from alpha helical peptides. 2005 BMES, Baltimore, MD, September, 2005.