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Final Narrative Report
"Potential Application of Peptide/Nanofiber Technology for Neuronal Regeneration"
Grant Number 03-3022-SCR-E-0
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1. Original aims of the project

The original aims of the project were to (1) covalently modify electrospun nanofibers with neuroactive peptides derived from the extracellular matrix molecule tenascin-C, and to (2) surgically implant the peptide-functionalized nanofibers into a spinal cord lesion in the adult rat so as to provide a fibrous scaffolding for guided axonal regrowth.

2. Project successes

Electrospun nanofibers have a geometry that is reminiscent of the fibrillar organization and the three-dimensional structure of the extracellular matrix on which neurons normally grow. The purpose of this study was to investigate the hypothesis that nanofibers comprised of polyamide can provide a biomimetic surface for neuronal growth *in vitro* and neuronal regeneration *in vivo*. *In vitro*, cultured rat cerebellar granule neurons adhered to polyamide nanofibers and extended neurites. *In vivo*, polyamide nanofibers implanted into the hemisected adult rat spinal cord promoted axonal regrowth onto the fibers and across the lesion while excluding reactive astrocytes. These findings provide evidence that polyamide nanofibers may serve as an important new, completely synthetic material for the repair of spinal cord injury. Moreover, they may offer a unique surface for more physiologically relevant culture based assays of neuronal function and pathology.

3. Project challenges

We originally planned to use oriented electrospun nanofibers in this project to direct axons across the lesion. However, we found that the oriented nanofibers were very rigid and caused trauma to the delicate spinal cord tissue. To circumvent this problem, we used a random array of nanofibers that more closely resembles the structure of the extracellular matrix. We found that axons made their way across the lesion on this random array. This suggests that intrinsic environmental cues in the spinal cord apparently direct appropriate axonal topography and that oriented nanofibers are not needed.

We originally proposed to use nanofibers comprised of poly-ε-caprolactone polymers because this material is biodegradable and FDA-approved for human use. However, we found cultured neurons and axons in the spinal cord preferred to grow on polyamide nanofibers in comparison to poly-ε-caprolactone nanofibers. Polyamide nanofibers are not degradable in the short term. Despite this fact, we have speculated since the original application that a non-degrading material like polyamide might be equally or better suited than a biodegradable material to the task of maintaining and reinforcing the reformed neuronal circuitry in the spine, especially long-term. Moreover, breakdown of biodegradable materials such as polyglycolate or polylactate can result in the release of monomers (e.g., lactate) that can significantly lower the local pH and negatively effect the viability of regenerating neurons. In addition, recent studies have highlighted the utility of non-degrading materials in general, and polyamide microfilaments in particular, in the promotion of axonal regrowth in the PNS. For all of these reasons, we believe that non-degrading scaffolds for neuronal regeneration in the spine necessitate closer scrutiny.

Thus far, axonal growth in our studies has been assessed using antibodies against neurofilament, which non-specifically stain all axons, and CGRP, which stain a subpopulation of sensory axons. Although the data collected using these antibodies is very promising, we need to conduct track tracing studies to help ensure that the axons in fact represent regenerating axons, as opposed to axons spared in the lesion process.

Because our original grant was funded for only one year, all of our work thus far has been done with underivatized nanofibers. This work is in progress.

4. Implications for future research and/or clinical treatment

In our studies, polyamide nanofibers exhibited good integration with host tissue, exclusion of reactive astrocytes, and little or no inhibition of axonal egress. Notably, these neuronal activities were induced as a consequence of the surface nanotopography and physical properties of a non-biological material. Given the uniformity of these manufactured materials, we propose that polyamide nanofibers can play an important role in therapies directed at SCI repair and could be the subject of clinical treatments.

5. Plans to continue this research

We have applied for and been awarded a continuation grant from the New Jersey Commission of Spinal Cord Research entitled "Engineering Nanofibrillar Surfaces for Spinal Cord Repair" (Grant Number 04-3034-SCR-E-O, June 15, 2004-June 30, 2006). During this time, we will compare the growth of neurites *in vitro* and damaged axons *in vivo* on peptide-derivatized vs. non-derivatized fibers. We will investigate whether the histological results correlate with behavioral results by assessing the functional recovery of the rats. We will also further characterize the glial scarring response and begin to investigate the immune response to nanofibers.

6. Manuscripts and abstracts emerging from this research

Manuscripts
1. Liu, H.-Y., Mercado, M.L.T., Nur-e-Kamal, A., Schachner, M., and Meiners, S. Neurite guidance by the fnC repeat of human tenascin-C: development of an "attractive" peptide for use in strategies to improve directed axonal regeneration. In preparation.

2. Ahmed, I.*, Amor, N.*, Voura, E.B., Chung, H.Y., Grafe, T.H., Graham, K.M., Schindler, M., and Meiners, S. Polyamide nanofibers support the growth of axons in the injured rat spinal cord by polyamide nanofibers. In preparation.

Copies of these manuscripts will be provided following their completion.

Abstracts
1. Ahmed, I.*, Amor, N.*, Voura, E.B., Chung, H.Y., Grafe, T.H., Graham, K.M., Schindler, M., and Meiners, S. Polyamide nanofibers promote axonal regeneration and diminish glial scarring

in the injured rat spinal cord. Soc. Neurosci. Abstr. (2004).
*Equal contributors

See attached.

2. Liu, H.-Y., Schachner, M., and Meiners, S. Neurite Attraction and Neurite Retention are Mediated by Distinct Sites in the FnC Domain of Human Tenascin-C. Amer. Soc. Cell Biol. Abstr. (2004).
> *This abstract was chosen as one of 16 out of 1,200 by the ASCB with the strongest news value for the general public. It is included in the ASCB Press Book, "Cell Biology 2004".*

See attached.

Polyamide nanofibers promote axonal regeneration and diminish glial scarring in the injured rat spinal cord

Ahmed, I.*, Amor, N.*, Voura, E.B., Chung, H.Y., Grafe, T.H., Graham, K.M., Schindler, M., and Meiners, S.
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Electrospun polyamide nanofibers have a geometry that is reminiscent of the fibrillar organization and the three-dimensional structure of the extracellular matrix on which neurons normally grow. The purpose of this study was to investigate the hypothesis that polyamide nanofibers can provide a biomimetic surface for neuronal growth *in vitro* and neuronal regeneration *in vivo*. *In vitro*, cultured rat cerebellar granule neurons adhered to polyamide nanofibers and extended neurites. *In vivo*, polyamide nanofibers implanted into the hemisected adult rat spinal cord promoted axonal regrowth onto the fibers and across the lesion while excluding reactive astrocytes. These findings provide evidence that polyamide nanofibers may serve as an important new, completely synthetic material for the repair of spinal cord injury. Moreover, they may offer a unique surface for more physiologically relevant culture based assays of neuronal function and pathology.

Neurite Attraction and Neurite Retention are Mediated by Distinct Sites in the FnC Domain of Human Tenascin-C

Liu, H.-Y., Schachner, M., and Meiners, S.

Extracellular matrix-derived neuroactive sequences have the potential to function as specific growth cues to promote axonal regeneration following central nervous system (CNS) injury. Such cues, when incorporated into grafts or scaffolds that traverse the injury site, could function either to facilitate axonal elongation or to orient axonal migration across the wound. This study demonstrates that the alternatively spliced fibronectin type-III repeat C of human tenascin-C, fnC, provides directional cues to elongating neurites *in vitro*. When given a choice at an interface with poly-L-lysine (PLL), neurites preferentially crossed onto fnC, while neurites originating on fnC preferentially remained on fnC. Therefore, fnC functions to both "attract" and "retain" neurites. Guidance motifs were further refined using synthetic peptides spanning the sequence of fnC. We found that a peptide with amino acid sequence DINPYGFTVSWMASE was sufficient to attract and retain neurites. Within this sequence, NPYG and ASE are sufficiently exposed in predicted extended loop regions of fnC for interactions with neurons. To investigate the hypothesis that NPYG and ASE are critical for neurite guidance, we tested recombinant fnC proteins and peptides with alterations in NPYG or ASE. Molecules with alterations in NPYG facilitated neurite retention but not attraction, and conversely, molecules with alterations in ASE facilitated neurite attraction but not retention. Since a major concern with grafts in the CNS is inhibition of axonal egress, these results suggest that a novel therapeutic strategy might be designed which incorporates "attractive" fnC-derived peptides with minimal retention activity to facilitate guided axonal regrowth following CNS injury.