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Sally Meiners, Ph.D.
Department of Pharmacology
UMDNJ-Robert Wood Johnson Medical School
675 Hoes Lane
Piscatway, New Jersey 08854
(732) 235-2890
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Final Narrative Report
Engineering Nanofibrillar Surfaces for Spinal Cord Repair
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06-3058

1. Original aims of the project

1. Evaluate the hypothesis that peptide-modified nanofibers implanted into the injured adult rat spinal cord can increase the growth and functional recovery of severed axons.
2. Evaluate the hypothesis that peptide-modified nanofibers can attenuate the pro-inflammatory tissue response and glial/fibrotic scarring following spinal cord injury.

2. Project successes

We developed a novel spinal cord prosthetic comprised of longitudinally bundled strips of a nonwoven fabric comprised of randomly deposited nanofibers. The nanofibrillar fabric was first covalently modified with a peptide derived from the extracellular matrix molecule tenascin-C, termed the D5' peptide. The strips were dipped one by one into SeaPrep® agarose and stacked next to and on top of each other. SeaPrep® agarose has a gelling temperature of 8-17° C, and, without special preparation remains semi-liquid at room temperature. Thus it provides a liquid or soft-gel medium at body temperature to allow for ready infiltration of cells and fasciculation of axons in between nanofiber layers.

Neurofilament-M-labeled axons were observed within the peptide-modified nanofibrillar implant 3 wk after injury, growing both rostrally and caudally. Calcitonin gene-related peptide (CGRP)-labeled axons were also observed within the implant. The nanofibrillar strips appeared to allow a good amount of longitudinal growth of regenerating axons, despite the random orientation of the electrospun nanofibers. Since we have demonstrated that randomly deposited nanofibers allow neurite outgrowth in any direction, this suggests that intrinsic spinal cord cues may help to guide axonal growth.

We conducted behavioral studies using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale and have noted earlier improvements in the first wk-10 days for animals that received peptide-modified implants in comparison to injury only control animals. However, this probably reflects, for example, earlier recovery from shock rather than axonal regeneration, since significant axonal regrowth in the treated animals has not been observed after such a short period of time. Behavioral studies are still ongoing.

Glial fibrillary acid protein (GFAP) labeling was done to evaluate the presence of reactive astrocytes in and around the peptide-modified nanofibrillar implant. Very few GFAP-positive astrocytes were seen within ~200 μm of the host-implant interface or within the implant itself. These results, as proposed in the original application, support the hypothesis that the nanofibrillar implants (along with their agarose and peptide components) do not provoke glial scarring. This may be due at least in part to a decrease in inflammation (see below). However, since early postnatal cerebral cortical and adult spinal cord astrocytes grew on unmodified and peptide modified nanofibers *in vitro*, it is unclear why resting state astrocytes were not seen within the implant.

We originally suggested that interruption of the inflammatory cascade in spinal cords that received nanofibrillar implants might have contributed to the attenuation of the scarring response. This hypothesis was based on the reduced levels of interleukin 1β (IL-1β) mRNA

observed in tissue removed from the lesion site for implanted spinal cords in comparison to injury only controls 3 days after injury. The results of more recent experiments indicated that the inflammatory response was, for the most part, similar in all groups as determined by similar levels of IL-1β and TNF-α mRNA 3 h after injury in animals receiving unmodified implants, peptide-modified implants, and injury only controls. The discrepancy could be due to animal-to-animal variation, or quite likely, to the 3 day time point in the original study vs. the 3 h time point in the recent study. That is, cytokine mRNA levels at the injury site may have been initially elevated in spinal cords receiving implants to the same extent as in injury only controls, but then decreased more rapidly in the implanted spinal cords.

Support for the hypothesis that inflammation is more rapidly attenuated in implanted spinal cords comes from ED1 labeling for activated macrophages and microglia, which was performed 3 h, 6h, 24 h, 1 wk, and 2 wk following injury. Very little ED1 labeling was observed in or around the lesion site 3 h after injury for injury only controls or animals that received a peptide-modified implant, suggesting that macrophages/microglia were not the source of the elevated levels of IL-1β and TNF-α mRNA observed at this time point. The greatest degree of ED1 labeling was observed 1-2 wk after injury, and it was clearly reduced in the implanted spinal cords in comparison to injury only controls.

3. Project challenges

In the original application, we proposed to use a complete transection model and a multi-layered nanofibrillar device incorporating parallel layers of polyamide nanofibers separated by spacers (beads). However, we found that the spacers in between the nanofibrillar layers resulted in a rather inflexible material in comparison to nanofibers alone, with potential to cause further damage to the spinal cord upon implantation. As such, we created the alternative multi-layered nanofibrillar device described above using oriented strips of a nanofibrillar fabric comprised of randomly deposited nanofibers. Randomly deposited nanofibers were employed instead of the parallel, aligned nanofibers that we originally proposed to use for two reasons. First, aligned polyamide nanofibers are considerably stiffer than randomly oriented nanofibers, a property incompatible with spinal cord tissue. Second, aligned polyamide nanofibers have proven to be more difficult to electrospin with reproducibility than random polyamide nanofibers.

We also modified the spinal cord injury model from the complete transection originally proposed to an over-hemisection injury performed at T8-9. Irridectomy scissors were used to make a transverse cut 2 mm deep. Another transverse cut was made 2 mm caudal to the first, and the tissue in between was removed with scissors. Our observations have indicated that this injury is severe enough to allow reproducible behavioral deficits and therefore represents a compromise between the simple over-hemisection model originally employed and a complete transection, which results in great trauma to the animals.

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 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 spinal cord injury repair and could be the subject of clinical treatments. Moreover, we have recently extended our studies to include evaluation of FGF-2-modified nanofibrillar devices as well as D5' peptide-modified devices. FGF-2-modified nanofibers can be stored in their dry state for 6 months at 4° C and still retain significant biological activity. In contrast, FGF-2 in solution is notoriously unstable. This is important given the clear advantage of a grafting material that can be prepared ahead of time and ready for use as the need arises. We have found that devices that incorporated nanofibers modified with FGF-2 encouraged substantially more axonal regeneration and better functional recovery (assessed using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale) than did devices that incorporated unmodified nanofibers. Furthermore, an FGF-2-modified device also encouraged revascularization. Neither type of device promoted glial scarring or induced an apparent foreign body or inflammatory response.

5. Plans to continue this research

We applied for a continuation grant from the New Jersey Commission of Spinal Cord Research entitled "FGF-2-modified nanofiber prosthetics for spinal cord repair" but were not awarded the grant. We plan to submit a modified version of this grant to the Christopher and Dana Reeve Foundation in December, 2008 and possibly to the National Institutes of Health as well.

6. Publications emerging from this research

Ahmed, I., Ponery, A.S., Nur-E-Kamal, A., Kamal, J., Meshel, A., Sheetz, M., Schindler, M., and **Meiners, S.** Mouse embryonic fibroblasts cultured on synthetic three dimensional nanofibrillar surfaces demonstrate altered morphology, cytoskeleton organization, and myosin dynamics. Mol. Cell. Biochem. 301, 241-249 (2007)

Meiners, S., Ahmed, I., Ponery, A.S., Amor, N., Harris, S.L., Ayres, V., Fan, Y., Chen, Q., Delgado-Rivera, R., and Babu, A.N. Engineering electrospun nanofibers for spinal cord repair: A discussion. Polymer Internat. 56, 1340-1348 (2007)
Invited manuscript for In Focus issue

Delgado-Rivera, R.*, Ahmed, I.*, Harris, S.L.*, Babu, A.N., Patel, R., Kamal, J., Ayres, V.A., Flowers, D., and **Meiners, S.** Increased FGF-2 secretion and ability to support neurite outgrowth by astrocytes cultured on polyamide nanofibrillar matrices. Submitted.
* Equal contributors

Harris, S.L., Delgado-Rivera, R., Crockett, D.P., and **Meiners, S.** An FGF-2-modified nanofibrillar prosthetic for spinal cord repair. In preparation.