**Aligned Electrospun Fibers Foster Axonal Regeneration in a Complete Transection Model of Spinal Cord Injury**

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**Statement of Purpose:** Aligned polymeric fibers have emerged as a promising engineered platform for neural tissue repair. Several groups investigating aligned fiber matrices have demonstrated contact-mediated guidance of axons and alignment of glial cells in culture.[1,2] Additionally, neural conduits containing aligned electrospun fibers have fostered regeneration across large sciatic nerve defects (≥ 15 mm in rat models).[2,3] The purpose of this study was to investigate the ability of aligned fiber matrices to promote axonal regeneration in a complete transection model of spinal cord injury.

**Methods:** Thin polymeric films were generated by solution casting poly-L-lactic acid (Natureworks, Cargill-Dow LCC, Minnetonka, MN) solution onto glass coverslips. Fiber matrices were applied to films by electrospinning a poly-L-lactic acid solution through 15kV field onto glass coverslips/films attached to a grounded rotating disc for aligned fibers (AF), or a grounded stationary target for randomly oriented fibers (RF).[1] Dorsal root ganglia (DRG)isolated from P4 rat pups were cultured on film, film + RF, and film + AF scaffolding materials in serum free media with NGF (Roche Applied Science) and B-27 (Gibco) to evaluate in vitro neural response. At 5 days, DRG were fixed and stained for neurofilament (NF145). Neural conduits were developed by using a mandrel to roll film, film + RF, or film + AF into guidance channels. Conduits of each type were implanted into a 3 mm spinal cord gap created by excision of 2 mm of spinal cord tissue at the T8/T9 level. Animals (Female, Sprague-Dawley, Harlan Laboratories) were perfused 1 week (n = 3 for each type of conduit), 2 weeks (n = 4), and 4 weeks (n = 6) after implantation. Tissue was sectioned into 20 um thick sections in 10 series, and sections were stained for tissue architecture (cresyl violet), axons (RT-97), astrocytes (GFAP), chondroitin sulfate proteoglycan (CS-56), Schwann cells (P75), blood vessels (RECA), macrophages/monocytes (ED1), and myelin (MBP). Tissue volume within conduit lumen and the number of axons penetrating 1.5 mm into conduits were evaluated using stereological methods.

**Results:** Using an optimized electrospinning protocol, highly aligned fiber matrices (1-1.5 microns in diameter, Figure 1A) were created. Axonal growth from DRG cultured on AF scaffolds sprouted axons parallel to the direction of fibers (Figure 1C), whereas film (Figure 1B) and RF scaffolds did not specify the majority direction of axonal growth. Quantitative analysis indicates axons emanating from dorsal root ganglion grow significantly longer than axons on either film or RF scaffolds. Conduits containing AF scaffolds fostered robust aligned regeneration of axons into conduit lumen (Figure 1D), as compared with film (Figure 1E) or RF controls. Interestingly, astrocytes migrated into the lumen of AF conduits in close proximity to axonal extension. Tissue volume within conduits containing either RF or AF scaffolding was significantly greater than film controls at 1, 2, and 4 weeks post-injury/ implantation.

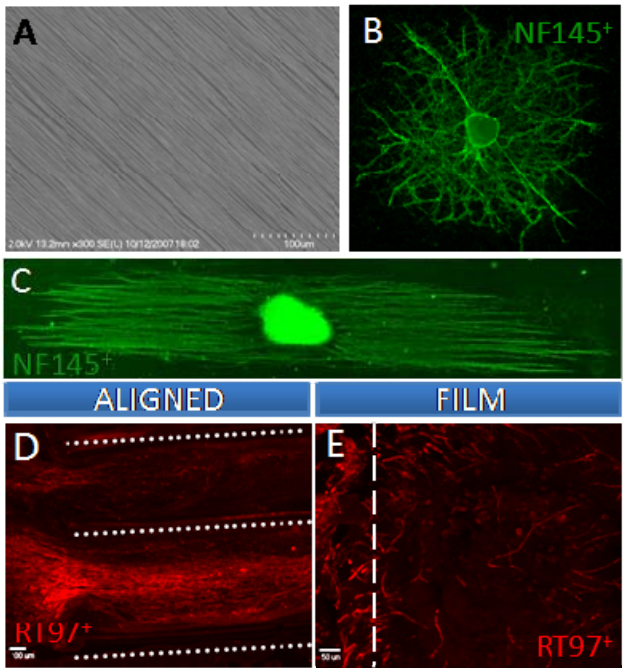


**Figure 1.** A) Aligned electrospun fibers. B) Dorsal root ganglion cultured on film. C) Dorsal root ganglion cultured on aligned fibers. D) Conduits containing AF scaffolding promote extensive axonal regeneration into conduit lumen after 4 weeks. E) Conduits containing no scaffolding did not promote axonal regeneration.

**Conclusions:** From this data, aligned fiber matrices act to enhance axonal regeneration in cell culture systems and an animal model of spinal cord injury. We suggest that the material may act to promote regeneration by modulating the response of astrocytes after injury. A critical question unanswered by the current data is whether or not axons are able to enter the distal stump over a longer period of time. We are currently pursuing studies with long survival times (16 weeks) in order to address this question. Together, we suggest that aligned fiber matrices are a promising substrate for axonal regeneration within the central nervous system.

**References:**

[1]Wang HB, et al. J Neural Eng. 2009;6(1):016001.
[2] Kim YT, et al. Biomaterials. 2008;29(21):3117-27.
[3]Chew SY, et al. Adv Funct Mater. 2007;17(8):1288-96.