Aligned Laminin-Polycaprolactone Blends Promote Regeneration in Tibial Nerve Defect Model

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Statement of Purpose: Peripheral nerve transection occurs commonly in traumatic injury, causing deficits distal to the injury site. One option for surgical repair is the nerve conduit. Conduits currently on the market are hollow tubes; however, they often fail due to the slow rate of regeneration over long gaps. To facilitate increased regeneration speed and greater functional recovery, the ideal conduit should provide biochemically relevant signals and physical guidance cues, thus playing an active role in regeneration. We have previously shown electrospun laminin nanofibers can function as a basement membrane mimetic material, both in terms of geometry and composition, driving attachment, differentiation, and process extension of neuron-like or neuronal precursor cells (Neal RA Tissue Eng Part C Methods. 2009;15:11-21). Electrospinning can produce both random and aligned nanofiber meshes which provide the necessary structural support and high surface area to volume ratios to facilitate cell migrations required to bridge peripheral nerve gaps. To that end, laminin-polycaprolactone (PCL) blend nanofibers were fabricated to mimic peripheral nerve basement membrane in both composition and structure and examined for regenerative capacity in a rat model of tibial nerve transection.

Methods: Laminin was isolated from the murine EHS tumor and used in lyophilized form for these studies. PCL was purchased from Sigma. To fabricate blend nanofibers, polymer was dissolved 1,1,1,3,3,3-hexafluoro-2-propanol (HFP). Electrospinning parameters were determined for individual solutions within the following ranges: 0.5 and 3mL/hr flow rate; 10-15cm distance to collector; 10-30kV driving voltage. Nanofiber samples where characterized for composition using Fourier transform infrared (FT-IR) spectroscopy, for degradation using gel permeation chromatography (GPC), and for geometry and surface structure using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). An incision along the length of the tibial axis was made in female Sprague Dawley rats (250g), the muscles were separated, and the tibial nerve was gently freed from surrounding tissue. The nerve was transected with removal of a 5mm segment. 15mm conduits containing various compositions and orientations of nanofibers were inserted into the gap and sutured in place to leave a 10mm nerve gap. The incision was closed and animals were tested for motor and sensory function recovery weekly for six weeks. At six weeks, the surgical site was reopened and nerve conduction velocity was measured. Animas were euthanized and tissue was harvested for histology.

Results: Blend laminin-PCL nanofiber meshes were successfully fabricated and characterized for physical and biological properties. Fourier transform infrared spectroscopy confirmed the presence of both polymers in the meshes. Neither tensile strength nor degradation in aqueous solution was significantly affected by laminin content within the meshes. *In vitro* assays such as cell attachment and neurite extension from both PC12 cells and murine dorsal root ganglia (DRG) established 10% (wt) laminin content as the maximum required to retain the bioactivity of laminin. In addition, modified collector plate design to introduce an insulating gap enabled the fabrication of aligned nanofibers. Aligned fibers produced significant cellular alignment and created a pattern of directional guidance to regenerating axons. In the *in vivo* tibial defect model, animals receiving nanofibers within the lumen of the conduit performed significantly better in motor, sensory, and nerve conduction studies than animals that received hollow conduits. Aligned PCL nanofibers significantly improved motor function; aligned laminin blend nanofibers yielded the best sensory function recovery. Retrograde nerve conduction speed at six weeks was significantly faster in animals receiving aligned nanofiber-filled conduits than in those receiving random nanofiber-filled conduits.

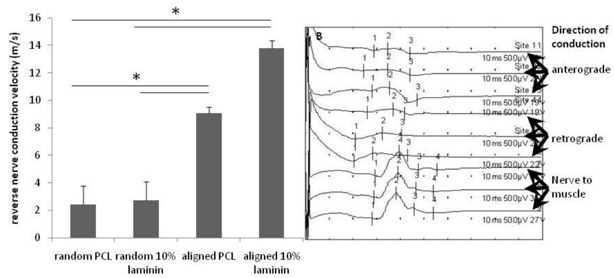

Figure 1. Reverse conduction indicates stimulation distal to injury and recording proximal to injury site. (A) Conduction velocities were significantly greater when animals received conduits containing aligned nanofibers ($p < 0.01$), regardless of composition. Sample electrode traces demonstrate electrophysiological response (B) conduits containing nanofibers after six weeks. Direction of stimulation is indicated on the plots.

Conclusions: In conclusion, laminin-PCL blend nanofibers containing 10% by weight laminin maintain the bioactivity of pure laminin nanofibers in terms of cell adhesion and neurite extension. Alignment of nanofibers further increases the length of process extension. When used in the lumen of a conduit for peripheral nerve regeneration, the nanofiber structure provides significant benefit over a hollow conduit, with even greater benefit and faster healing seen with laminin content, and the fastest healing with alignment along the length of the conduit. These studies provide a firm foundation for the use of natural-synthetic blend electrospun nanofibers to enhance existing hollow nerve guidance conduits. The similarity in surgical technique and obvious benefit to the patient should lead to faster translation into clinical application.