Enhancing Peripheral Nerve Regeneration through Nanofibers, Microspheres and Physical Therapy.

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Statement of Purpose: Peripheral nerve injuries are estimated to affect 20 million people in the United States. The current gold standard for critical size defects (>5mm) is an autologous nerve graft. Commercially available nerve growth conduits (NGC) consist of hollow tubes that lack directional cues for nerve regeneration. Several research strategies have shown that incorporating cell guidance cues, including topographical and chemical cues, can direct neurons and support cells both *in vitro* and *in vivo* [1]. The goal of this project was to develop a NGC with aligned hyaluronic acid (HA, topographical cue) nanofibers electropun with glial cell-line derived neurotrophic factor (GDNF, chemical cue) loaded Poly(lactic-co-glycolic acid) (PLGA) microspheres encased in a polycaprolactone (PCL) hollow conduit. We included a physical therapy (PT) regimen because it has been shown to upregulate neurotrophins and increase myelination in developing axons after injury [2]. We hypothesized that combining PT with a conduit that incorporates cell guidance cues would improve function recovery in peripheral nerves.

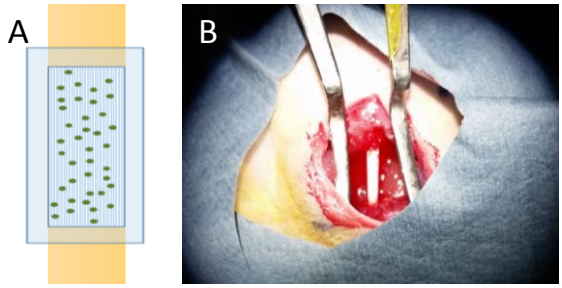

Figure 1: (A) Schematic of the engineering conduit with aligned hyaluronic acid nanofibers and GDNF loaded microspheres. (B) Image of a transplanted conduit in the rat sciatic nerve.

Methods: Evaluation of the NGC was done using a sciatic nerve injury model in 45 female Lewis rats. Circumferentially aligned nanofibers of PCL were layered with longitudinally aligned electrospun HA fibers and rolled into conduits with an inner diameter of 1.25mm and length of 10mm. The conduits were implanted into an 8mm sciatic nerve gap in female Lewis rats (Figure 1). The animals were divided into five groups: aligned fibers (Fibers) and aligned fibers with GDNF microspheres (Fibers+GF) both with and without PT and autograft control. The physical therapy group walked on treadmills at 5m/min for two sessions of 30 minutes a day for 30 days post-surgery. Animals were pre-exposed to functional testing and underwent weekly functional testing post-surgery for 60 days. Functional testing included: Static Sciatic Index, Ladder Walking Test, and Von Frey Filament Mechanical Sensory Test. At the end of the study each animal had Compound Muscle Action Potentials (CMAP) and muscle contraction forces recorded. A stimulation electrode was placed around the sciatic nerve above the injury, stimulation voltage was systematically increased while CMAP and force of the gastrocnemius muscle was measured. All surgical procedures and animal testing was approved by the Institutional Animal Care and Use Committee.

Results: We found that animals exposed to the growth factor (GF) and PT had the best results and recovered to the level of the autograft control in 4 weeks. This was seen in both motor skill tests (Ladder walking) and sensory tests (Von Frey). The Fiber+GF+PT group animals traversed the ladder missing less rungs than the other groups, indicating better muscular control. The Fiber+GF+PT group also required less force to elicit a response on the Von Frey test, indicating improved sensory perception. Endpoint CMAP and gastrocnemius muscle weight showed increased stimulation voltage was required for rats with PT coupled with a decrease in muscle atrophy. Ongoing histological analysis will be used to further evaluate regeneration.

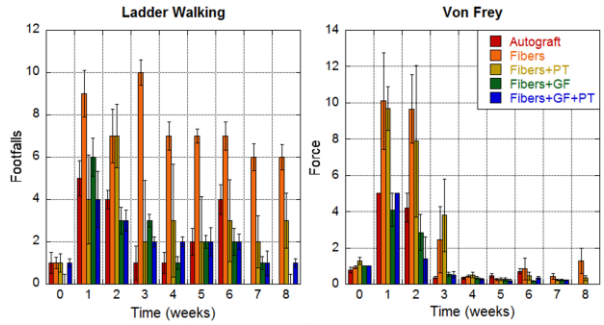

Figure 2: Results from all 5 groups show a decreasing number of footfalls by week for all conditions with the least recovery in conditions that do not have growth factor and/or PT. Similar results were seen for Von Frey Analysis. Fibers+GF+PT group recovers close to the level of the autograft control by 4 weeks.

Conclusions: This study shows that including a combinatorial approach can be the key to improved functional recovery following peripheral nerve injury. Important components include aligned fibers, growth factors and a physical therapy regimen. Future studies will incorporate additional cell signaling cues to further enhance peripheral nerve repair including adhesive and electrical signals. Additionally, similar materials are being used in a spinal cord injury study informed by this work.

References:

1. Wrobel, M.R. and H.G. Sundararaghavan, Tissue Eng Part B Rev, 2014. **20**(2): p. 93-105.
2. Goulart, C.O., et al., PLoS One, 2014. **9**(10): p. e110090.