# Enhanced Nerve Regeneration by Minimizing Intraneural Scarring using a Semi-Permeable Nanofiber Wrap.

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## Abstract

**Background:** Despite great advances in microsurgery, functional outcomes following nerve repair remain suboptimal. Scar formation at the repair site is recognized as a major impediment to regenerating axons. In this regard, an inert barrier around the coaptation site that prevents inflammatory cells infiltration while still allowing the diffusion of nutrients and nerve growth factors holds great potential in promoting nerve regeneration and functional return. In this study, we examined the efficacy of a novel semi-permeable nanofiber construct, prepared from FDA approved biomaterials, to be used as a wrap around the repair site to promote nerve regeneration and functional recovery.

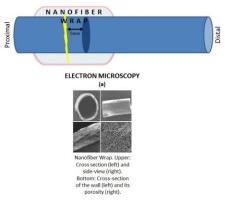
**Methods:** Nerve wraps comprised of nonwoven electrospun poly (ε-caprolactone) nanofibers with pores smaller than 10 μm were synthesized (Fig. 1a). They were wrapped around the repair site in a sciatic transection/repair model in Thy-1 GFP rats. At 5 weeks, their neuro-protective and neuro-regenerative potentials were assessed. At 16 weeks, functional recovery was evaluated.

**Results:** At 5 weeks, the nanofiber wraps resulted in significantly decreased collagen deposition and inflammation/macrophage invasion at the repair site (Fig. 1b). The total number of myelinated axons was significantly increased (Fig. 1d), and there was a trend towards a higher number of regenerated dorsal root ganglion sensory neurons. Mechanistically, these outcomes were correlated to an up-regulation of the anti-inflammatory cytokine (IL-10) and down-regulation of the pro-inflammatory cytokine (TNF-α) (Fig. 1e). In addition, at 16 weeks, the nerve wrap group showed enhanced functional recovery as demonstrated by electrophysiology (Fig. 1f), gait analysis, neuromuscular junction re-innervation (Fig. 1g), and gastrocnemius muscle weight and histology.

**Conclusions:** Our results demonstrate favorable outcomes of a novel semi-permeable and clinically translatable nanofiber nerve wrap in protecting the coaptation site and enhancing axonal regeneration through scar-free nerve repair, resulting in optimal functional recovery.

Disclosure/Financial Support None

#### SCIATIC NERVE TRANSECTION MODEL



### 5 WEEKS:

HISTOMORPHOMETRY

(c)

ne Blue st ining

#### Early measures of neuroregeneration

HISTOLOGY (b)

Masson's Trichrome staining





26% 24% 10% 8% 6% 4%

2%

\* p<0.05

Control (n=5) Experimen (n=5)

The percentage area of Masson's Trichrome blue staining for collagen was quantified at the coaptation site (10 µm sections, 100x mag).



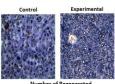
nce co-staining





(n=5) Intraneural macrophage (positive for ED-1) were counted at the coaptation site, and results are coaptation site, and results are expressed as cells/mm<sup>2</sup> (10 μm sections, 100x mag). ED-1 (CD68): Macrophage marker TUJ1 (Neuronal Class III β-Tubulin): Neurofilament marker

HI/HH







axons were counted at 5 mm distal to the repair site (ultra-thin sections, 1000x mag).



RETROGRADE LABELING

orsal Root Ga

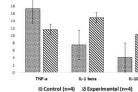
(d)



Control (n=8) Experimental (n=8) The number of labeled Dorsal Root

Ganglion neurons (L4-L6) was counted using a fluorescent microscope (20 µm sections, 100x mag).

(e) ne Expression at the Repair Site Infla



qRT-PCR

Up-regulation of anti-inflammatory cytokine IL-10 and down-regulation of pro-inflammatory cytokine TNF-a were detected at the nerve repair site in the experimental group.

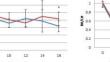
16 WEEKS:

#### Late measures of neuroregeneration

#### ADVANCED GAIT ANALYSIS (CATWALK)

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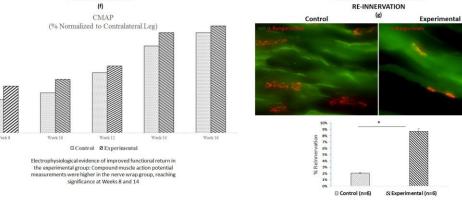
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**Run Duration** 





Histological evidence of improved functional return in the experimental group: Neuromuscular junction staining showed increased % reinnervation in the nerve wrap group

## Figure 1. Analysis of Early and Late Measures of Nerve Regeneration Using the Nanofiber Nerve Wrap.