Anisotropic Hydrogels for Peripheral Nerve Regeneration Across Long Nerve Gaps

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Introduction: Peripheral nerve (PN) regeneration across long nerve gaps (> 15mm) remains a challenging problem. To date, most scaffolds for PN regeneration have been *isotropic*, in that no directional cues are provided to the regenerating nerve. However, nature uses haptotactic and chemotactic patterns, involving gradients of extracellular matrix (ECM) proteins and neurotrophic factors to guided cell migration or neurite extension *in vivo*. Our laboratory has developed biomimetic, *anisotropic* scaffolds that present progressively greater growth stimulatory cues along the direction of regeneration to facilitate regeneration across long nerve gaps. We have developed a perfluoroaryl azide based photochemical method to couple gradients of large proteins to hydrogel scaffolds. In this study we report fabrication of novel three-dimensional scaffolds with immobilized and diffusive gradients of Laminin-1 (LN-1) and nerve growth factor (NGF) respectively, for regeneration of transected sciatic nerves across a 20mm nerve gaps in adult rats.

Materials and Methods:
Synthesis of tubular scaffolds: Semi-permeable Polysulphone tubes, 22 mm long, filled with 0.5% (w/v) SeaPrep® agarose hydrogel were used as tubular scaffolds for nerve regeneration. Gradients of LN-1 and NGF were designed in the polysulphone tubes such that concentrations increased from the proximal end of the tube to the distal. Slow-release lipid microcylinders, loaded with NGF, were embedded in the agarose hydrogel in a graded manner so as to create diffuse gradients of NGF. Gradients of LN-1 were made by allowing LN-1 to diffuse into the tube from one end, and immobilizing the LN-1 to the hydrogel using a photocrosslinker conjugated to it. Tubular scaffolds with uniform concentrations of LN-1 and NGF, and nerve autografts were used as controls.
Animal surgery: Male Fisher inbred rats weighing 300-320g were used for studying peripheral nerve regeneration after injury. The sciatic nerve on right hind limb was exposed and a transection nerve injury was made. The two ends of the severed nerve were sutured to the two opposite ends of the polysulphone tube such that a) there was a nerve gap of 20mm and b) concentrations of LN-1 and NGF increased from the proximal nerve end to the distal nerve end. The rats were sacrificed 4 months post-surgery.
Evaluation of regeneration: Nerve regeneration was evaluated by behavioral analysis (sciatic functional index, i.e. toe spread before and after regeneration) and immunohistochemistry of explanted tubular scaffolds for comparing axonal regeneration. Regeneration was also evaluated by electrically stimulating the nerve proximal to the implant, and measuring the amplitude and latency of compound action potential distal to the implant. Transport of retrograde dye from the gastrocnemius muscle to the spinal cord by the regenerated nerve, and relative gastrocnemius muscle weight were also used to evaluate regeneration.

Results / Discussion: Behavioral tests (Sciatic Functional Index) show that rats implanted with *anisotropic* scaffolds of LN-1 and NGF, have better toe spread than animals with *isotropic* scaffolds of LN-1 and NGF. Also the relative gastrocnemius muscle weight was significantly better for animals with *anisotropic* scaffolds than for rats with *isotropic* scaffolds. Work is in progress to determine the axonal density of regenerated nerves by sectioning the explanted scaffolds, analyzing compound action potentials across tubular scaffolds and staining for newly synthesized neuromuscular junctions at the target muscle.

Conclusions: Anisotropic scaffolds have performed better than isotropic scaffolds at promoting nerve regeneration.

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