Sustained Delivery of GDNF for Improved Peripheral Nerve Regeneration in a Sciatic Nerve Injury

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Statement of Purpose: The most severe form of peripheral nerve injury is complete transection, whereby not only the connective tissue, but also the continuity of axons is lost. Though most surgeons prefer to use a nerve autograft for bridging nerve defects, there are obvious disadvantages to their use. Commercially available nerve guides are an alternative to nerve grafts and can be used to repair small (< 3 cm) nerve gaps. These guides aim to realign the severed nerve stumps and contain the endogenous fluids; however, the scaffold is lacking agents to promote nerve growth across the defect. In this study, it was hypothesized that a nerve guide which delivered neurotrophic factors in a sustained manner to the injured nerve would improve functional recovery following injury. To evaluate this hypothesis, we have constructed a biodegradable polymer nerve guide which releases bioactive Glial Cell Line-Derived Neurotrophic Factor (GDNF) for over 60 days. This nerve guide was implanted into a critical size (1.5 cm) sciatic nerve defect in the Lewis rat model.

Methods: Double-walled microspheres were fabricated using an oil-in-oil emulsion technique with poly(lactic-co-glycolic acid) (PLGA) and poly(lactide) (PLA). GDNF was encapsulated within the PLGA core and was protected by a PLA shell, thus slowing the protein release from microspheres. Poly(caprolactone) nerve guides incorporating microspheres were prepared using a novel rolling technique. 15 mg of microspheres were distributed evenly on a grid drawn on parchment paper. Glass mandrels were immersed into a PCL slurry-containing polymer dissolved in ethyl acetate and NaCl as a porogen. After the polymer solution was allowed to partially harden, the mandrels were smoothly rolled across the microspheres, embedding them into the nerve guide wall. These nerve guides were then sterilized with ethanol and implanted across a large (1.5 cm) sciatic nerve defect. 58 Lewis rats were randomly divided into three groups, those which received isografts (positive control), those which received nerve guides releasing GDNF and those which received nerve guides with non-encapsulating microspheres. Animals were sacrificed after 16 weeks and nerve regeneration was evaluated both histologically with Masson's Trichrome stain and toluidine blue, and functionally with video assessment of gait kinematics and an analysis of sensory recovery.

Results: In vitro release studies of GDNF from nerve guides with double-walled microspheres showed GDNF was liberated from the guides for over 100 days (Figure 1). Upon nerve guide retrieval at 16 weeks, the implanted guides were observed as well vascularized and sheathed in a soft fibrous coating. Results from a preliminary pinch test indicated that lower limb re-innveration was seen in 100% of the animals treated with GDNF, but in only 70% of the negative control animals.

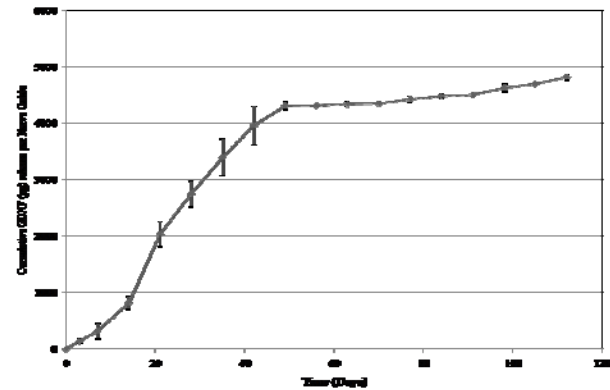

Figure 1. Cumulative Release of GDNF from PCL guides

have some muscle reinnervation.

Analysis of gastrocnemius contraction force showed that muscles innervated by nerves repaired with GDNF had an average contraction force of 0.43N which was not statistically different from isograft controls. Negative control empty guides resulted in a mean gastroc contraction force of 0.07N, which was significantly less than isograft and GDNF treatment groups. High magnification histomorphometry revealed higher g ratios and increased axon counts in transverse segments of the distal portion of the nerve conduits and the distal nerve stump (Figure 2).

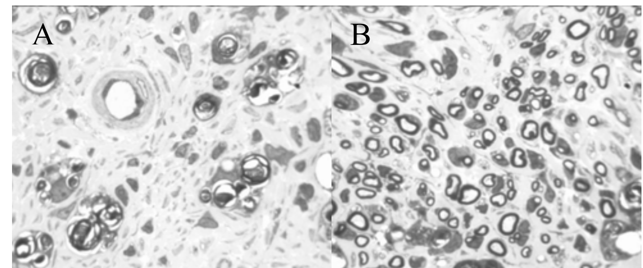

Figure 2. High magnification semi-thin transverse sections of the distal nerve stump from empty control PCL guides (A) and GDNF-releasing guides (B).

Conclusions: GDNF release was detected from nerve guides for over 100 days. Visual inspection of guides during extraction showed denser, more robust nerves within GDNF releasing conduits. Functional assessment of nerve regeneration measuring gastrocnemius contraction force after stimulation of the regenerated nerves showed statistically equivalent contraction forces between positive control and GDNF experimental group. Evaluation of paw retraction from hot water (an indication of sensory recovery) showed increased withdraw rate in GDNF animals. Histological assessment of distal regions of conduits showed nerve fibers in animals treated with GDNF while negative control guides resulted in very few fibers regenerating across the long nerve gaps.

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