Sustained Release of VEGF and Pleiotrophin Stimulates Nerve Regeneration across Long Gap Peripheral Nerve Defects using a Biodegradable Nerve Guide

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Statement of Purpose: Peripheral nerve injuries resulting in extensive loss in nerve continuity pose a challenge in reconstructive surgery. Autografts still remain the treatment of choice for nerve defects despite the need of donor nerve harvest and the associated morbidity of this procedure. In contrast to short gap injuries, isografts achieve minimal functional recovery for gaps longer than a critical 30 mm length, and simple tubularization methods fail completely [1].The regenerative failure of peripheral nerves through long gaps can be attributed to the lack of appropriate growth substrate and trophic support [1-3]. We hypothesize that for successful nerve regeneration across long-gap nerve defects, neurotrophic support and early vascularization of the regenerated nerve are critical factors. Vascular endothelial growth factor (VEGF) and Pleiotrophin (PTN) have growth promoting effects on neurons in the central and peripheral nervous systems [4-6], hence are suitable candidates for long gap repair.

Methods: We recently developed simple and reproducible method for the fabrication of multi-luminal Biosynthetic Nerve Implant (BNI) using a transparent biodegradable Nerve Guide (NG) made of Cross-linked Urethane doped Polyester Elastomer (CUPE). PLGA nanospheres were used for sustained delivery of growth factors. A small segment of the peroneal nerve in rabbits was excised and the 30mm BNI was implanted. Testing for the return of the toe-spreading reflex assessed motor function recovery after injury. Compound Muscle Action Potential (CMAP) was recorded from the peroneal muscle by stimulating the distal side of the implant. Immunohistochemistry was done for Neurofilament Protein (axons) and S-100 (Schwann cells). For testing the biodegradable CUPE-NG a 15mm rat sciatic nerve injury model was used.

Results: The CUPE-NG allowed proper visualization of the multi-luminal hydrogel loaded with nanospheres. The CUPE-elicited foreign body response was controlled and specifically directed towards the NG with sparing of adjacent cells. In the rabbit peroneal nerve injury model, quantification of the number of NFP+ axons in the regenerated tissue area showed that multi-luminal BNIs had a tenfold increase in the number of regenerated axons compared to the simple tubularization repair. VEGF and PTN further enhanced nerve regeneration by as much as threefold. Similarly, in the distal sections we saw that the total area containing the regenerated axons was significantly higher in the animals treated with VEGF and PTN. The VEGF and PTN groups demonstrated higher values of CMAP than BNI collagen. After nine weeks the toe spread was positive in all the BNI treated animals and higher in the PTN and VEGF groups indicating motor function recovery.

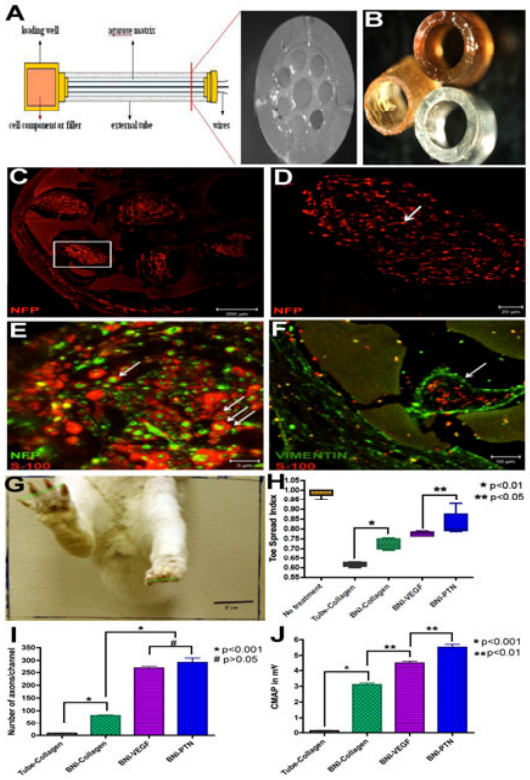

Figure1: (A) Casting device for BNI fabrication, (B) Transparent CUPE-NG of two different thicknesses (top, ambar) compared to a Polyurethane-NG. (bottom, clear), (C,D) NFP-200+ staining confirmed axonal regeneration across the CUPE-BNI, (E) S-100+ Schwann cells wrapping regenerated axons, and (F) Vimentin+ fibroblast in the periphery of the regenerated nerve. (G) Toe spread assessment method, (H, I, J) Comparison of Toe spread index, axonal number and CMAP among the treatment groups.

Conclusions: Our study suggests that incorporating both nerve and vascular growth factor in a biodegradable NG seems necessary to overcome the lack of nerve regeneration in long-gap peripheral nerve defects.

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