Synergistic Effects of Neurotrophins and Pleiotrophins in Stimulating Nerve Regeneration across Long Gap Peripheral Nerve Defects

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Introduction: Peripheral nerve injuries resulting in the extensive loss of nerve continuity pose a challenge in reconstructive surgery. Current treatments, such as autograft, isografts and simple tubularization, are limited by the need for donor nerve harvest, minimal functional recovery, and gap restrictions(< 3cm). These limitations can be attributed to the lack of appropriate growth substrate and trophic support. Growth factors such as brain-derived growth factor (BDNF), glial-derived nerve factor (GDNF) and pleiotrophin (PTN) have been demonstrated to induce axonal growth of spinal cord motor neurons, as well as the proliferation and migration of Schwann cells, fibroblasts and endothelial cells. Previously, a systematic approach to find a suitable combination of growth factors was tested in vitro to provide growth factor specific regenerative potency. Further, we tested whether multiluminal biosynthetic nerve implants (BNI) loaded with a combination of PTN and GDNF in the microchannels provide a synergistic effect to promote regenration across a critical long gap (4 cm) in a rabbit model.

Materials and Methods: Twenty-four rabbits were anaesthetized and 4 cm of the peroneal nerve was transected and replaced with a BNI conduit containing BSA, GDNF, PTN, PTN and GDNF, or an autograft. The animals were subjected to functional recovery tests every 3 weeks post injury which included a toe spread index and ankle angle measurement. The toe spread index was measured by the maximum distance between the first and forth toe created by the startle response using a custom-made apparatus. The ankle angle was calculated for different rabbit hopping phases by gait analysis using the CinePlex Studio (Plexon Inc.,). Immunocytochemistry was performed twenty weeks post implantation. The harvested tissues were assessed for the neuronal and myelination markers β-Tubulin and P0.

Results and Discussion: The toe spread index, measured at 19 weeks post implantation, increased by 25% with PTN and GDNF treatment compared to 15% for the BSA group when compared to those at 4 weeks post injury. As expected, the autograft group shows maximal functional recovery, with a 38% increase between the two time points (Fig. 1). Similarly, the ankle angle revealed that, from 4 weeks to 20 weeks post injury, the GDNF group (14° decrease) had the highest recovery when compared to BSA (2.5°), PTN (0°), and PTN+GDNF (3°), while the autograft treatment (24°) still remained the best . In addition, immunohistochemistry of the distal tissue sections showed that the total area containing regenerated axons was higher in the animals treated with PTN+GDNF than BSA, PTN, and GDNF.

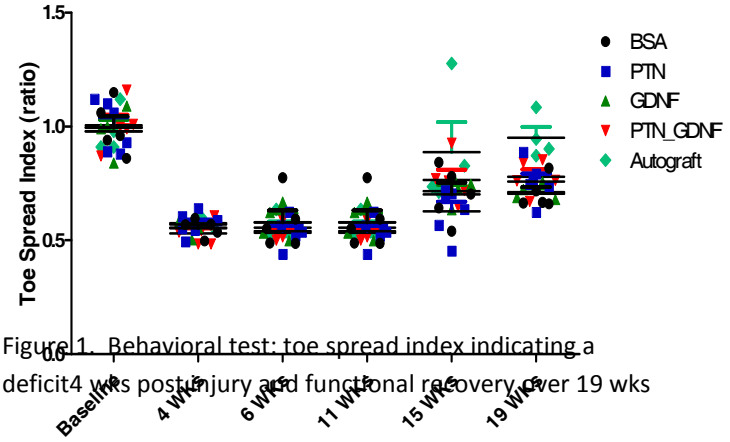

Figure 1. Behavioral test: toe spread index indicating a deficit4 wks post injury and functional recovery over 19 wks

Conclusion: Our results demonstrated that PTN and GDNF act synergistically to bridge a long gap. The functional recovery assessed by toe spread index and ankle angle measurement indicate successful motor neuron regeneration. In addition, immunohistochemistry showed positive staining for β-tubulin and P0 indicating neural regeneration across the 4 cm gap.