

Neurotrophic factors release from nerve conduits for peripheral axonal regeneration

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INTRODUCTION: Axonal regeneration after peripheral nerve injury is insufficient for functional recovery and is therefore a significant clinical problem. Neurotrophic factors (NTFs) play crucial role in neuronal survival and axonal regeneration, and nerve conduits (NC) provides support and protection to the regenerating axons. NC combined with NTFs have been used in several studies to improve nerve regeneration, but the clinical outcome remained unsatisfactory¹. This may be attributed to several poorly known or understood parameters such as NTF dose, temporal and spatial requirements (i. e., release kinetics), and need for multiple factors rather than single factor mostly applied so far. In the present study, we have developed collagen nerve conduits for the co-delivery of glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) to exploit the synergistic effects of these two factors on axonal regeneration. The release kinetics was controlled by physical cross-linking of collagen and by an integrated delivery system.

METHODS: Collagen NCs were produced by spinning mandrel technology. NTFs were loaded into NC and the tubes were coated with poly (lactide-co-glycolide). *In vitro* release of GDNF and NGF was determined by incubating the NC in citrate buffer (pH 5.0); the released NTFs were assayed by ELISA and a bioassay. For the latter, DRGs were isolated from E9-chicken embryos (9 days old), and the explants were cultured as described previously².

RESULTS: The *in vitro* release of GDNF and NGF from the NC was sustained over 30 days. The main parameter that influenced the NTFs release was the dehydro-thermal (DHT) treatment of the NC. During the initial 2-3 days of release, the DHT-treated NCs released the NTFs at significantly lower rates, whereas this difference vanished after 3 days. The total release of NGF and GDNF amounted to, respectively, 56 and 68% of the total dose for DHT-treated NC; for not DHT-treated NC, these values were 83 and 78%, respectively. In both NC types, the total amount of each growth factor was 80 ng.

The bioactivity of released GDNF and NGF was assessed in an *in vitro* bioassay. Incubation of

DRG explants with release medium containing NGF and GDNF released from NCs resulted in axonal outgrowth (Fig. 1). These results show combined release of biologically active GDNF and NGF from collagen nerve conduits over 30 days.

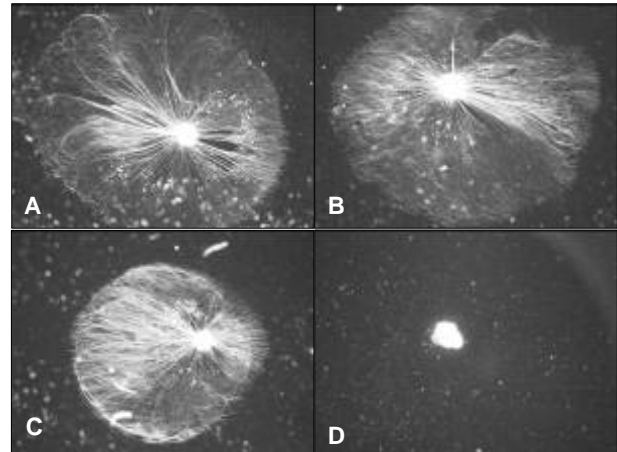


Fig. 1: Bioactivity of neurotrophic factors released from nerve conduits, as determined using DRG explants culture. (A) GDNF+NGF; total concentration: 1 ng/ml (positive control); (B) 1st day release medium with GDNF+NGF released from DHT-NC; (C) 30th day release medium with GDNF+NGF released from DHT-NC; (D) DRG treated with buffer (negative control).

DISCUSSION&CONCLUSIONS: Collagen nerve conduits were successfully developed with integrated delivery system for combined release of GDNF and NGF. Our results prove the feasibility of developing nerve conduits for co-delivery of NTFs. Future studies will assess the potential benefit of combined GDNF+NGF over single factor delivery in a nerve gap model in the rat.

REFERENCES: ¹L. B. Moran, et al (2004) *Brain Res Rev* **44**:154-78. ²E. Stoeckli et al (1991) *J Cell Biol* **112**:449-55

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