Novel conduits for Schwann Cell Induced Spinal Cord Repair

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Statement of Purpose: Suitable conduits are needed for effective axonal regeneration using Schwann cell (SC) transplant approaches in completely transected spinal cords. SCs introduced into the cord form an irregular cord/SC interface due to the extension of astrocyte processes which have been shown to enhance brainstem axon regeneration. A combination therapy of SCs with brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) has demonstrated robust regeneration into the graft in a complete transection model[1]. A piezoelectric material, consisting of polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE), has been shown to yield a higher level of neuronal differentiation and neurite outgrowth from mouse neuroblastoma cells and rat dorsal root ganglia *in vitro*[2,3]. Piezoelectric conduits for sciatic nerve repair also showed a higher number of myelinated axons than a sciatic nerve graft[4,5]. In this study, PVDF-TrFE was fabricated into an aligned fibrous conduit that released BDNF over time. This conduit was investigated in combination with SCs for the repair of transected spinal cords.

Methods: *Scaffold fabrication:* PVDF-TrFE was dissolved in methyl ethyl ketone and BDNF was dissolved in a PEO solution. The two solutions were mixed and sonicated to create an emulsion. The solution was then electrospun to produce aligned scaffolds using a rotating drum. The scaffolds were then formed into conduits having a diameter about 2.5 mm. Release of active BDNF from the scaffolds was characterized *in vitro* using ELISA. *Transplantation:* Laminectomy was performed from T7 to T9 on female adult Fischer rats followed by a complete transection at T8 (n=6/group). After achieving hemostasis, the conduit was inserted between the stumps and lenti-viral infected Schwann cells expressing green fluorescent protein (GFP-SCs) mixed with Matrigel (BD Sciences) were injected into the conduit via two pre-cut windows on the dorsal side of the conduit. *Behavior test, tissue processing, and analysis:* Incline plane and BBB (open field locomotor test) were performed on animals weekly. Incline plane recorded the maximum angle that the animal can maintain for 5s without sliding and was represented as % recovery. The rats were perfused at 4 weeks post-transplant and cryostat 20µm sagittal sections were stained with antibodies against GFP, GFAP (glial fibrillary acidic protein, astrocyte marker), 5HT (serotonergic axon marker, 5-hydroxytryptamine), and DβH (dopamine β hydroxylase, noradrenergic axon marker). Two-way analysis of variance (ANOVA) and post one-way ANOVA were used to determine the statistical significance between groups ($p<0.05$).

Results: Conduits releasing BDNF showed a significant improvement in % recovery from incline plane test at weeks 3 and 4 ($p<0.05$). BBB scores did not reveal significant improvement between the groups by 4 weeks.

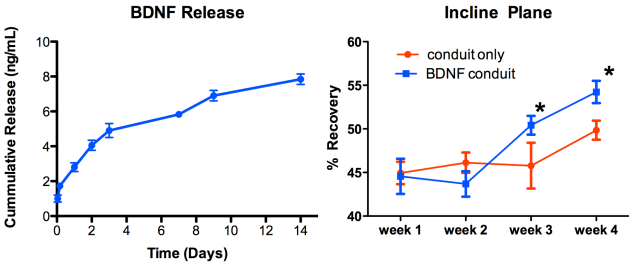

Figure 1 (left). Release profile of BDNF (mean±SD).
Figure 2 (right). Incline plane results (mean±SEM) for aligned conduits with or without BDNF.

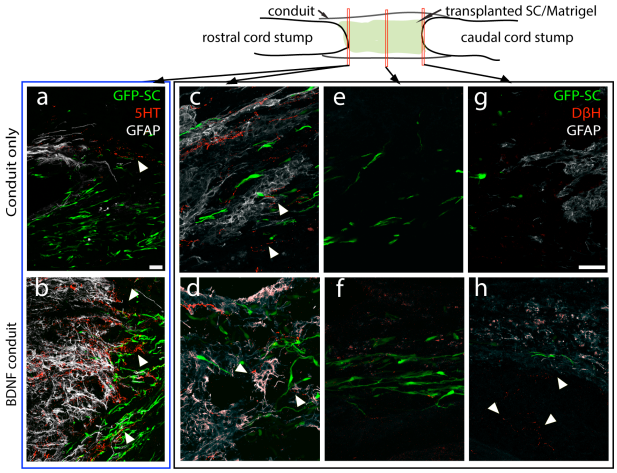

Figure 3: Confocal microscopy images of SC bridges at the rostral (a-d), mid (e&f), and caudal (g&h) locations in conduits with (bottom) or without (top) BDNF at 4 weeks post transplantation (scale bars = 50µm, mag=20x (a-b) and 40x (c-h)). $5HT^+$ axons (arrowheads in a&b) were more numerous in BDNF conduits at the rostral interface. More $D\beta H^+$ axons (arrowheads in c-h) were observed at the rostral end in BDNF conduits (d) and also on the caudal end (h). Irregular $GFAP^+$ borders were observed in both conduits (a-d).

Conclusions: This study demonstrated the release of BDNF from scaffolds *in vitro* and the efficacy of BDNF controlled release from the conduits *in vivo*. These conduits not only increased the number of serotonergic axons at the rostral interface and noradrenergic axons at both interfaces but also improved recovery as shown by the incline plane test at 4 weeks post transplantation.

Reference:

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