Piezoelectric Fibrous Scaffolds for Schwann Cell Induced Spinal Cord Repair

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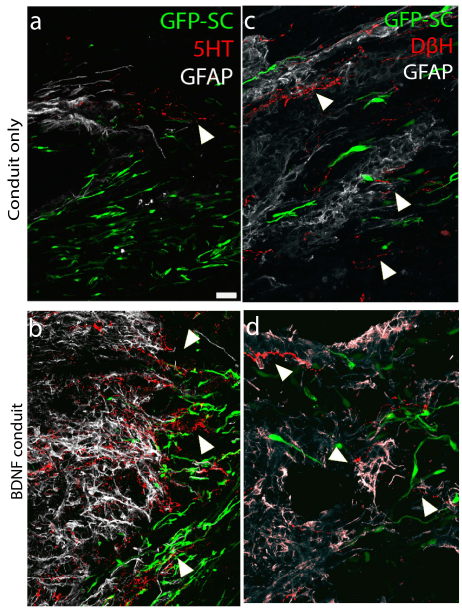
Introduction: Suitable conduits are needed for effective axonal regeneration using Schwann cell (SC) transplant approaches in completely transected spinal cords. Studies have demonstrated SCs introduced into the cord form an irregular cord/SC interface due to the extension of astrocyte processes that enhance brainstem axon regeneration[1]. SCs with additional neurotrophins cause robust axon regeneration into grafts in a complete transection model[2]. Piezoelectric materials have intrinsic electrical properties and piezoelectric conduits lead to a higher number of myelinated axons compared to sciatic nerve grafts for sciatic nerve repair[3,4]. More recently, fibrous scaffolds of polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) have been shown to yield a higher level of neurite outgrowth from rat dorsal root ganglia *in vitro*.[5] Our study investigated the use of SCs and aligned fibrous conduits made from a piezoelectric material, PVDF-TrFE, that provided controlled release of brain-derived neurotrophic factor (BDNF) to enhance spinal cord repair.

Materials and Methods: *Scaffold fabrication and characterization:* PVDF-TrFE in methyl ethyl ketone and BDNF in a PEO solution were mixed and sonicated to create an emulsion. The solution was then electrospun to produce aligned scaffolds using a rotating drum then formed into conduits (diameter~2.5mm). Average fiber size and degree of alignment was characterized using scanning electron microscopy (SEM) and image analysis. The electrical activity was also measured in vitro. The release rate 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 SCs expressing green fluorescent protein (GFP-SCs) mixed with Matrigel (BD Sciences) were injected into the conduit. *Behavior test, tissue processing, and analysis:* Incline plane tests 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 wk post-transplant; cryostat 20µm sagittal sections were prepared. The 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 and Discussion: PVDF-TrFE scaffolds had an average fiber diameter of 1.11±0.06µm and degree of alignment of 82%. Controlled release of BDNF from the scaffolds *in vitro*, was approximately 2 ng/mL within the first 24 h and a total of 8 ng/mL released over 2 wks. *In vivo*, PVDF-TrFE conduits releasing BDNF (BDNF conduit) showed a significant improvement in incline plane at wks 3 and 4 compared to PVDF-TrFE conduit without release (Conduit only) ($p<0.05$). By immunohistochemistry, more 5HT and DβH axons were observed at the rostral end in BDNF conduits (Figure 1). More DβH axons were also observed on the caudal end in BDNF conduits. Irregular $GFAP^+$ borders were observed in both BDNF conduit and Conduit only groups.

Conclusions: This study demonstrated the efficacy of BDNF controlled release from the scaffold *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 in the incline plane test at 4 wk post transplantation.

Figure 1: Confocal microscopy images of SC bridges at the rostral interfaces in conduits with (bottom) or without (top) BDNF at 4 wk post transplantation (scale bars = 50µm, mag=20x (a-b) and 40x (c-d)). 5HT (arrowheads in a&b) and DβH axons (arrowheads in c-d) were more numerous in BDNF conduits.

References:

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