Schwann Cells and Electrical Stimulation: Enhanced Migration and Neurotrophic Factors to Aid PNS Repair

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Introduction: Endogenous electrical fields regulate wound healing, embryonic development, cell migration and axonal outgrowth. Exogenous electrical stimulation (e.g. deep brain stimulation (DBS)) is used to treat a number of diseases and disorders, such as Parkinson's disease and epilepsy in humans[1] and is under investigation for spinal cord[2] and peripheral nerve injury (PNS). Despite its broad use, mechanistic understanding of electrical stimulation to local neurons and glia is not well understood. While peripheral nerves have remarkable capacity for spontaneous regeneration; functional recovery after severe injury is poor. Exogenous stimulation improves axonal growth in rat models of PNS injury and in humans following nerve crush and resident glia, Schwann cells (SC) are believed to play a role, however, a clear understanding of electrically-induced changes to SC have not been widely examined. In this work, changes to the glial phenotype post-stimulation were examined by soluble factor release, migration and evaluation of neurite outgrowth in a 3D biomaterial.

Materials and Methods: Primary neonatal rat SC and dorsal root ganglia neurons were isolated and cultured as described previously. SC were encapsulated within the center of an acellular 3D collagen-Matrigel^{TM} constructs, allowed to incubate overnight and exposed to 50 mV/mm (1 mA, DC) in a custom-designed chamber for 8 hrs. Following ES, growth medium was replaced and the non-neural cells (SC and EC) were incubated for an additional 24 hrs. Samples were fixed, immunostained, and imaged prior to analysis of neurite outgrowth/cell migration and directionality with NIH ImageJ software. Cell-conditioned medium was collected for the cytokine array (RayBiotech) and ELISA. Statistical significance was determined by conducting a two-tailed ANOVA in Excel with $p<0.05$; n=3 for all experiments.

Results and Discussion: SC were electrically stimulated for 8 hr at 50 mV/mm, post-stimulation, the hydrogels were transferred to fresh medium and 24hr post-stimulation, the medium was collected from both the EF-stimulated and unstimulated paired control. Significant increases in SC migration due to ES were observed. Similarly, neurite outgrowth increases significantly in the presence of exogenously-stimulated SC (ES-SC) relative to unstimulated control. Changes in soluble factor release from ES-SC were probed using a cytokine array. Significant increases in NGF, MMP-2 and MMP-13 were observed for ES-SC. Increased NGF was confirmed by ELISA and remains higher than unstimulated control up to 4 days post-stimulation. NGF can be triggered by either AC or DC stimulation and can be re-triggered by a subsequent stimulation. No directional bias was observed for SC migration. This suggests that local SC phenotype can be enhanced by ES to support axonal regrowth (re-cellularization and enhanced neurotrophic support). Similarly, efforts are underway to confirm the increases in MMP-2 and -13 as well as explore additional MMP/MMP inhibitors known to participate post-injury. Initial studies suggest both increased MMP gene expression and down-regulation of MMP inhibitors (TIMP) are observed following ES treatment. MMPs are known to be involved in tissue remodeling, which many explain the increased in Schwann cell migration. Similar increases in migration were observed by ES-endothelial cells suggesting that ES-induced migration may be more universal phenomena. Application of MMP inhibitors prevented migration in the biomaterial despite ES, but ES-induced SC exhibit increased NGF production, suggesting that EF-induced migration and EF-induced NGF production are mediated through different pathways.

Conclusions: Exogenous electrical stimulation is a powerful tool that is being used in the clinic and impacts both neuronal and supports cells locally. While, ES directly supports greater and longer neurites, there are significant changes to the SC both by enhanced migration and release of NGF that indirectly contribute to enhanced neurite outgrowth. Sensitivity to the ES parameters is needed to develop therapeutically effective stimulation regime that directly stimulate axonal growth but indirectly support axons via Schwann cell migration/neurotrophic support to promote greater re-growth and functional recovery

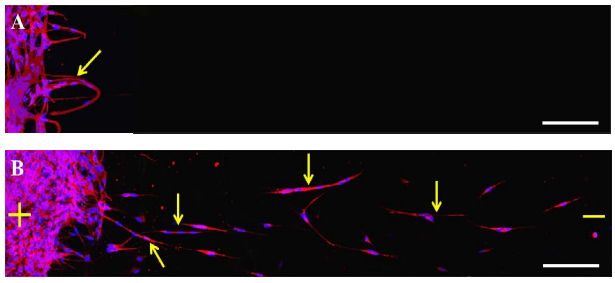

Fig. 1. Representative images of Schwann cell migration within composite 3D hydrogels following electrical stimulation showing increased migratory behavior (left). bar = 100um. Blue = DAPI Nuclei, Red = Actin.

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