Human Schwann Cell Stimulation Through HA-CNT Nanofibers

Judy Senanayake, Harini Sundararaghavan
Department of Biomedical Engineering, Wayne State University

Statement of purpose: At present roughly 800,000 cases of peripheral nerve injury are reported with an economic impact of $1.32 to $1.93 billion dollars in the United States per year (Tajdaran, Chan, Gordon, & Borschel, 2019). Peripheral nerve injuries can cause partial or complete severance of the nerve leading to neuronal death and damages to the supporting Schwann cells and the myelin sheaths at the cellular levels (Rajaram, Chen, & Schreyer, 2012). Neural autografts and allografts have been considered the gold standard for nerve regeneration, though each has its own drawbacks including lack of donor grafts and immunogenicity, respectively. Bioengineered scaffolds integrated with multiple guidance cues such as the cellular, chemical, surface alignment and mechanical/electrical cues are being widely investigated. Guided tissue regeneration combined with electrical stimulation is considered as an effective approach in reconstructive surgery of peripheral nerves.

We developed a conductive nanofibrous polymer with FDA approved Hyaluronic acid (HA) with Multiwalled Carbon Nanotubes (MWCNT) to incorporate electrical stimulation with multiple guidance cues (Steel, Azar, & Sundararaghavan, 2020). Our previous experiment on DRGs cultured on 0.01% HA-CNT align fibers indicated significant neurite extensions upon delivery of exogenous electrical stimuli through the custom stimulation well plates. We hypothesized that electrospun conductive nanofibrous scaffold would enhance the migration of Schwann cell populations and increase growth factor release by utilizing biphasic stimulation.

Methods: Aligned HA and 0.01% HA-CNT fibers were prepared by electrospinning on a rotating mandrel with a flowrate of 1.6-1.7mL/h and a voltage of 16-17kV. Immortalized Schwann cells from NF1 patient peripheral nerves (ipn 02.8) were utilized for experiments (Kraniak, Chalasani, Wallace, & Mattingly, 2018) (a) *Attachment experiment:* The fibers were rinsed with 1XPBS and stored for 48 hours before the experiment. The fibers were coated with rat tail collagen in acetic acid before all experiments. Ipn 02.8 cells were cultured on the coated and control fibers for 24 hours before immunostaining with S100b. Fluorescence microscopy of collagen coated, and control fibers were analyzed to determine the role of incorporating conductive polymers (Fig. 1) (b) *Electrical stimulation:* ipn 02.8 cells were cultured on custom stimulation well plates for 24 hours prior to stimulation. The wells were stimulated at 20Hz biphasic square wave of 200 and 100mV/mm for 30mins. The stimulated plates were incubated for 48 hours and the supernatant was collected to test for expression of growth factors (NGF) through an ELISA assay.

Results: The attachment experiments led to confirmation of cellular preference for collagen coated fibers compared to plain fibers. The aspect ratio data confirms that ipn 02.8 cell elongation and spread across fibers is highest in HA-CNT fibers as seen in fig 1(c). Fluorescence microscopic images on fig 1. indicates that cellular elongation occurs on collagen coated fibers and has the highest spread area.

Electrical stimulation of ipn 02.8 cells caused significant morphological changes including the increased cellular proliferation and elongation. Comparison of two different voltages applied across the HA-CNT fibers demonstrated that 200mV/mm resulted in spheroid formation possibly due to rapid cell proliferation whereas 100mV/mm resulted in an elongated cells across the fibers (fig. 2)

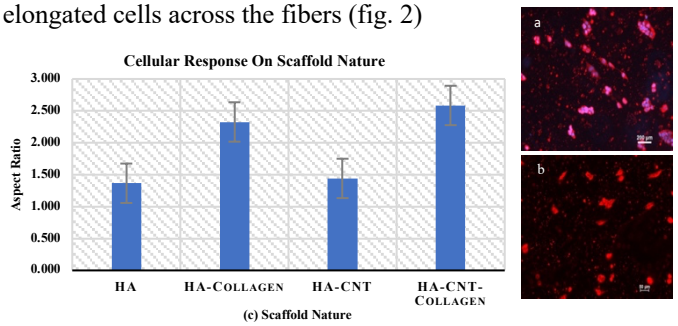

Fig 1: ipn 02.8 cells cultured on HA-CNT and HA fibers in the presence and absence of collagen: HA-CNT collagen coated (a), HA- collagen coated (b)
Aspect ratio comparison between different scaffold surfaces of ipn 02.8 cells cultured for 24 hours (c)

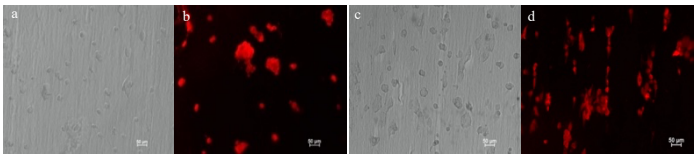

Fig 2:ipn 02.8 cells stimulated at 200mV/mm (a,b) and 100mV/mm (c,d) before (a,c) and after (b,d) stimulation

Conclusion & Future Direction:

We have shown that we can stimulate human Schwann cells in our HA-CNT nanofiber system and Schwann cells respond to stimulation. ELISA studies of NGF release are ongoing.

Future work will include quantification of the cellular proliferation rates and evaluation of the impact on neuronal cells co-cultured with neuronal cells.

Reference:

Kraniak, J. M., Chalasani, A., Wallace, M. R., & Mattingly, R. R. (2018). Development of 3D culture models of plexiform neurofibroma and initial application for phenotypic characterization and drug screening. *Experimental Neurology, 299*(Pt B), 289-298. doi:10.1016/j.expneurol.2017.10.012

Rajaram, A., Chen, X. B., & Schreyer, D. J. (2012). Strategic design and recent fabrication techniques for bioengineered tissue scaffolds to improve peripheral nerve regeneration. *Tissue Eng Part B Rev, 18*(6), 454-467. doi:10.1089/ten.TEB.2012.0006

Steel, E. M., Azar, J.-Y., & Sundararaghavan, H. G. (2020). Electrospun hyaluronic acid-carbon nanotube nanofibers for neural engineering. *Materialia, 9*, 100581. doi:10.1016/j.mtla.2019.100581

Tajdaran, K., Chan, K., Gordon, T., & Borschel, G. H. (2019). Matrices, scaffolds, and carriers for protein and molecule delivery in peripheral nerve regeneration. *Exp Neurol, 319*, 112817. doi:10.1016/j.expneurol.2018.08.014