Dynamic Nerve Growth Factor Delivery for Directed Neurite Outgrowth

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Statement of Purpose: Neural tissue engineering strategies aim at regenerative therapies for both peripheral and central nervous system injuries. There has been considerably more success in the peripheral nervous system, where for smaller injuries, nerve stumps can be surgically reattached. For critical size nerve defects, the gold standard is the use of an autologous nerve graft; however this requires two surgeries, potential donor site morbidity and only partial recovery.[1] There is currently no therapy for spinal cord injuries, which are further complicated by the formation of the glial scar, a physical and chemical barrier to nerve regrowth. To address these limitations, research targets fabricating synthetic nerve growth conduits (NGC) that actively direct neurons across the injury gap. In this study, we combine PLGA microspheres with electrospun fibers to develop patterned scaffolds for directed nerve repair. Future work will investigate these scaffolds in a rat sciatic nerve injury model and ultimately in a rat spinal cord injury model.

Methods: Methacrylated Hyaluronic Acid (MeHA) was synthesized as previously described and conjugated with RGD through a Michael's addition reaction.[2] PLGA microspheres were fabricated through a double emulsion, sonication method. 65:35 PLGA (80 mg/ml) was dissolved in dichloromethane (DCM) and then combined with nerve growth factor (NGF, 50 mg/ml) and polyvinyl alcohol (PVA) dissolved in water through sonication. The second emulsion was created by transferring into a solution of PVA dissolved in water. Prior to electrospinning, microspheres were dispersed in the electrospinning solution (DI water, MeHA, PEO, I2959) at a concentration of 200 mg microspheres/ml (~500 ng NGF/ml) and electrospun. For cell studies, recombinant mouse β-NGF (R and D systems) was encapsulated (empty spheres were used as a control). Patterns were fabricated by offsetting spinnerets by 4 cm in order to create a gradient across the scaffold (Figure 1). By changing the offset distance (x), we can alter the gradient properties. A single Dorsal Root Ganglia (DRG) harvested from 7 day old chick embryos was placed on each scaffold and cells were cultured for 5 days. After 5 days, cells are fixed and stained using anti-neurofilament 200 followed by a FITC secondary antibody.

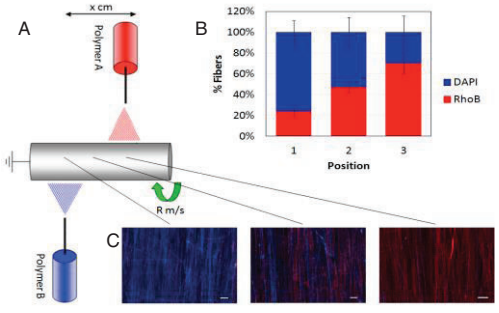

Figure 1: Electrospun gradient scaffold fabrication. Rhodamine and DAPI were included for visualization.

Results: PLGA microspheres have been successfully fabricated, loaded with NGF, and electrospun into MeHA scaffolds as visualized through SEM (Figure 2). Using Image J to quantify microsphere size, we found them to be 40 μm ± 17 μm. The electrospinning procedure does not appear to have an effect on microsphere morphology or release, however, microsphere size does affect scaffold porosity. Therefore, all controls are prepared with scaffolds containing empty microspheres to ensure this change in porosity does not influence nerve growth. NGF loaded microspheres significantly increase neurite outgrowth compared to empty spheres (Figure 2). Protein release from microspheres was assessed by using bovine serum albumin (BSA) as a model protein and tested through a BCA assay (Thermo). 65:35 PLGA microspheres release protein for up to 80 days (data not shown). By altering the L:G ratio in the PLGA layer of the microspheres, we can control the dynamics of growth factor release from days-months and have the potential to include multiple growth factors with release over varying time points.[3]

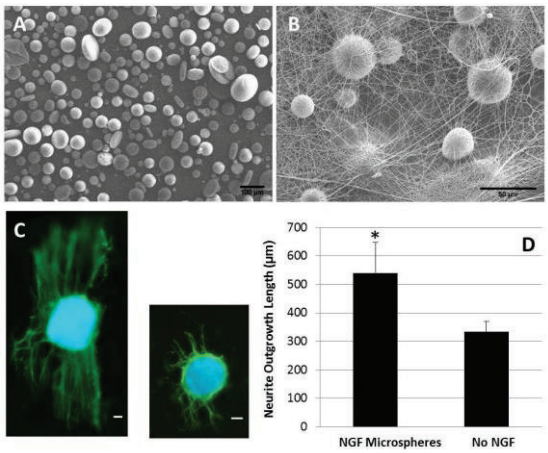

Figure 2: PLGA microspheres electrospun into HA scaffolds. We have shown significantly increased DRG neurite outgrowth on scaffolds containing NGF loaded microspheres compared to empty microspheres ($p<0.05$).

Conclusions: We have shown that NGF loaded microspheres are capable of directing nerve growth after electrospinning into a hyaluronic acid scaffold. Future work will include these scaffolds into a customized NGC. Future work will include layering electrospun scaffolds into a hyaluronic acid based hydrogel and implanting into a rat sciatic nerve injury model.

References:

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3. Shive, M.S. and J.M. Anderson, Adv Drug Deliv Rev, 1997. **28**(1): p. 5-24.