An Electrospun Scaffold Composed of Basal Lamina Proteins for Use in Neural Tissue Repair

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Statement of Purpose: Although peripheral nerve injury is common, successfully repairing transected nerves has proven difficult. Due to collagenous and glial scar tissue formation, chances of functional recovery are diminished with increasing size of the nerve gap. Currently, the preferred method of nerve repair is autografting, harvesting a portion of nerve tissue from another part of the body in order to replace the injured tissue. Clearly this method is limited by the availability of donor tissue, the risk of losing function at the donor site and the risk of undergoing multiple surgeries. Allografts, from different individuals of the same species, and xenografts, from a different species, are also options for nerve repair however there is a high incidence of immune response with these materials. Other tissue types may also be used to bridge the nerve gap including muscle tissue, veins, arteries and tendons. These materials require multiple surgeries which increase the chance of complications and they do not offer significantly better recovery of the damaged nerve [1].

In order to overcome these issues, various biomaterials have been investigated to bridge nerve gaps. The ideal material should be biodegradable and should promote cell adhesion, outgrowth and proliferation without eliciting an immune response. A suitable scaffold should closely resemble the native extracellular matrix in composition and topography[2]. In order to meet these requirements, we have chosen to electrospin a mixture of proteins and proteoglycans extracted from BD Matrigel (BD Biosciences, Bedford, MA). Alignment of these fibers is suspected to expedite cell migration across the nerve gap.

Methods: Protein was extracted from growth factor reduced BD Matrigel by acid precipitation. The material was then dissolved in 1,1,1,3,3,3-hexafluoroisopropanol. The solution was electrospun using a custom apparatus (Figure 1), first yielding randomly oriented nanofibers. Later two charged aluminum plates designed to introduce

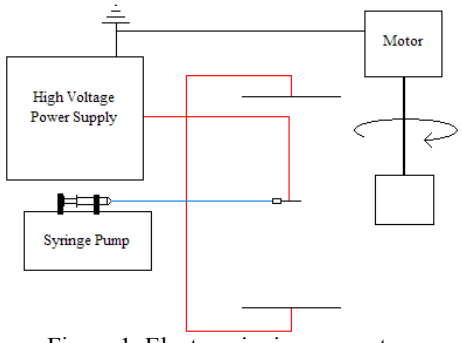

Figure 1. Electrospinning apparatus

a secondary electrical field were added. This field, along with the rotational velocity of the grounded collector resulted in aligned nanofibers. Scanning electron microscopy (SEM) was used to visualize fibers. ImageJ software (NIH, Bethesda, MD) was used to determine fiber orientation and diameter. Schwann cells were grown on random and aligned nanofibers for 24 hours and then dual stained with antibody for s-100 and DAPI.

Results: SEM revealed that rotational velocity and adding a secondary electrical field were sufficient to create aligned nanofibers out of BD Matrigel extract (Figures 2 and 3).

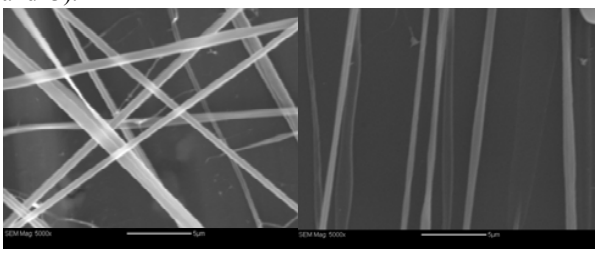

Figure 2. SEM images of random (left) and aligned nanofibers (right)

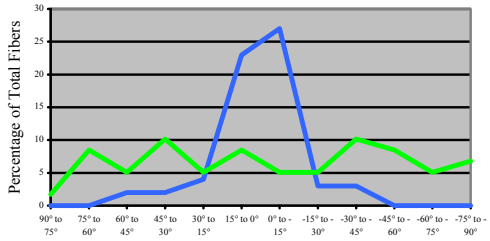

Figure 3. Graph of fiber alignment of random fibers (green) and aligned fibers (blue)

Dual staining with anti-s-100 and DAPI revealed that Schwann cells orient themselves in the same direction as the nanofibers (Figure 4).

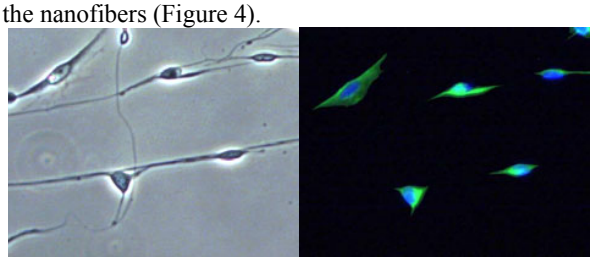

Figure 4. Schwann cells attached to fibers (left) and dual staining of Schwann cells with anti- s-100 (green) and DAPI (blue) (right)

Conclusions: Schwann cell orientation directly correlates with fiber orientation. Scaffolds of aligned nanofibers may be used to reduce healing time, resulting in increased functional recovery. Further studies involving the construction of three dimensional nerve conduits for in vitro studies are being carried out. Future studies are planned that will utilized these nanofibers as fillers within nerve conduits for treatment of sciatic nerve injury.

References:

[1]Siemionow M. Microsurg. 2010:30:7:574-588.
[2]Subramanian A. J Biomed Sci. 2009:16:108.