Magnetic Alignment of Electrospun Poly-l lactic acid Fibers for Directed Cell Guidance after Spinal Cord Injury

Christopher Johnson[1], Debmalya Gangulay[1], Simon McCarthy[1], Silvio Dutz[2], Ryan Gilbert[1]
[1]Rensselaer Polytechnic Institute Troy, New York, [2]Technische Universität Ilmenau, Ilmenau, Germany

Statement of Purpose: Contusive spinal cord injury interrupts white matter tracks and leaves an irregular cyst in the soft tissue of the spinal cord. Two of the major barriers to regeneration after spinal cord injury (SCI) are the glial scar and the cyst filled with inhibitory cellular debris. Aligned electrospun fibers are remarkably capable of reversing the scar-like formation at the boundary of the cyst [1] and of guiding regenerating tissue across a spinal cord injury lesion site [2]. The major drawback of electrospun fibrous scaffolds is their implantation requires placement within hemisection or complete transection injury models; models that do not represent contusive injury found in most patients with SCI. Therefore, our aim for this study was to prepare magnetic electrospun fibers that could be injected, then self-align to bridge the gap in situ. The success of this aim would combine the minimally invasive benefits of an injectable hydrogel with the benefits of a guidance conduit, to guide regenerating neurons and glia across a lesion site.

Methods: Electrospun fibers were prepared using the base solution 8% (wt/wt) PLLA in chloroform. Oleic acid coated superparamagnetic iron oxide nanoparticles (SPIONs) (20nm in diameter, obtained from collaborator Silvio Dutz) were incorporated at 2%, 4%, 6%, and 8% of the weight of PLLA. Electrospinning was performed using previously described methods [2]. The specific controlled parameters during electrospinning were: 2.0 ml/hr pump rate, 1000 rpm rotation speed, 15 kV voltage, 32-33% humidity, and a 5cm gap between the collection wheel and needle tip. Fibers were analyzed using scanning electron microscopy (SEM) to examine the influence of incorporated SPIONs on fiber diameter, Transmission electron microscopy (TEM) to verify SPION incorporation, and a vibrating sample magnetometer (VSM) to determine the magnetic capability of each composite material. To assess the ability of fibers to align within a magnetic field, samples were placed in a non-linear magnetic field established by two 0.5 inch neodymium magnets (BH_{max} = 48MGOe) separated by 35mm. Disorganized fibers were loaded into an 18G syringe, injected into a rudimentary centrifuge tube cavity model, and observed to align. Next, fibers were incorporated into a 10 mg/ml fibrinogen solution (Sigma) containing P2 Sprague-Dawley rat dorsal root ganglia (DRG). The fibers were magnetically dragged towards the DRG to assess the ability of these fibers to direct neurite extension. Thrombin (Sigma) was added to entrap the DRG and aligned fibers.

Results: The results show that 2%, 4%, and 6% wt SPION/wt PLLA can be incorporated without affecting the electrospinning process or fiber outcome. All fibers had a similar physical appearance to control fibers (not shown) by SEM. Fibers containing 8% SPIONs only formed when the electrospinning gap distance was increased to 8 cm. Based on previous findings with drug incorporation into fibers, we hypothesized that the fiber diameter would decrease with increasing SPION content. Our results showed no obvious trend in diameter. All fibers possessed diameters around 1.5-2.0µm (Fig 1 G). TEM showed the SPION particles were incorporated into the fibers and showed that the nanoparticles appear to clump up in the fibers rather than disperse evenly (Fig 1 B,E). There was a noticeable difference in the fibers on the macro scale. Increased SPION % led to fibers that were increasingly brown in color (Fig 1 C,F). The VSM showed that the nanofiber composites retain the superparamagnetic nature of the nanoparticles and showed significant differences in magnetization that was directly related to SPION loading content (Fig 1 H). In practical terms, this meant that the 6% SPION fibers aligned significantly faster (1.2 sec) in a field compared to 2% SPION fibers (3.4 sec) (Fig 1 I, J). As a result 6% SPION fibers were used for alignment within culture experiments. After successful magnetic manipulation of the fibers, the DRG were cultured for 2 days, using a fibrin hydrogel alone as a control.

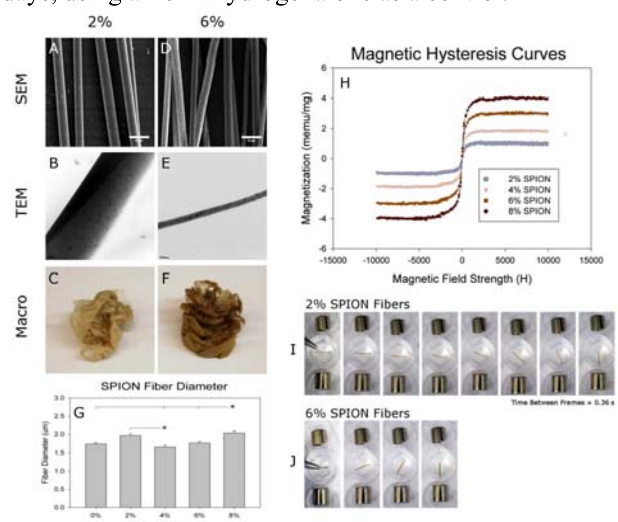

Figure 1. Shows the SEM, TEM, and Macro images of 2% (A-C) and 4% (D-F) SPION fibers. The diameter analysis for each group is G, with * denoting significance (n=3). H shows the magnetic hysteresis curves for each sample group. I shows the alignment of one 2% group of fibers, and J shows the alignment of 6% fibers. The interval between images was 0.36 sec.

Conclusions: To our knowledge, this is the first time that an injectable hydrogel-electrospun fiber composite scaffold has been proposed for treatment of SCI. This preliminary work optimizes magnetic fibers for alignment *in situ*. The 6% SPION fibers display the greatest magnetic movement without requiring significant electrospinning modification. . This innovative approach will ultimately enable the incorporation of topographical guidance cues into an injectable scaffold. Future work focuses on applying this approach to a SCI animal model.

References: [1] Gelain *F. ACS Nano*. 2011:5:227–236.
[2] Hurtado *A*, *Biomaterials. 2011:*32:6068–6079.