PERSPECTIVE

Electrospun fibers: a guiding scaffold for research and regeneration of the spinal cord

The challenge to regenerating the nervous system compared to other tissues in the body is the complexity of the tissue. For example, a small amount of scar tissue formation after an injury to the skin may have few negative side effects, but any scar tissue in the central nervous system is a major physical and chemical barrier to nerve regeneration (Sofroniew, 2009). An ideal treatment for spinal cord injury (SCI) would overcome three major barriers to regeneration: the initial and persistent inflammatory response, physical and chemical barriers in the glial and fibrous scar tissues, and guidance of nerves across the injury site to re-innervation. A significant economic and scientific investment has been placed in mitigating neuroinflammation, but this has not resulted in recovery of lost function. Based on the current literature, any treatment that focuses on one of the three barriers to regeneration is insufficient for functional regeneration after SCI. To date, the only successful attempt to restore lost function has combined digestion of the glial scar with chondroitinase ABC (ChABC) and application of a nerve graft. The combined treatment of ChABC and the peripheral nerve graft to guide and support nerves after a complete transection of a rat spinal cord was able to completely restore lost bladder function (Lee et al., 2013b).

Electrospun fibrous scaffolds are a material approach to overcoming the barrier of nerve guidance (reviewed by Schaub et al., 2015). Electrospinning is a method of generating fibers with diameters on the order of nanometers to micrometers. The geometric properties of electrospun fibers are easily tuned, and there exists a significant body of literature focusing on the optimal properties of a fibrous scaffold for robust neurite growth. Over a decade ago it was discovered that aligned, electrospun fibers led to neurite extension along the length of the aligned fibers (Yang et al., 2005). This result has been repeated in the majority of the literature, including *in vivo* where rostral and caudal axons of a completely transected rat spinal cord grew into the injured region on aligned fibrous scaffolds, and to a significantly lesser extent on randomly aligned fibrous scaffolds (Hurtado et al., 2011). In addition to fiber alignment, fiber diameter has been found to play a role in axon guidance, where axonal guidance is better on micrometer diameter fibers compared to nanometer diameter fibers (Wang et al., 2010). The current state of the literature indicates good agreement across multiple studies regarding how neurons respond to electrospun fiber alignment and diameter. In general, neurite extension is greater on aligned electrospun fibers than on randomly aligned fibers, and neurite extension is greater on fibers with micrometer diameters compared to neurite extension on fibers with a nanoscale fiber diameter.

One attractive aspect of the electrospinning process is the variety of polymers that may be used in the fabrication process, and material selection will play a critical role in both basic and applied neuroscience research. Unlike many other tissues where it is desirable to have a polymer that degrades in a matter of weeks to permit cells to replace the material with native tissue, nerve regeneration in spinal cord injury will likely take months. This is made evident in the study by Lee et al. (2013b) where recovery of bladder function required multiple months of nerve regeneration (Lee et al., 2013b). Therefore, slowly degrading polymers will be critically important in the application of electrospun fibers for spinal cord repair so that fibers that guide neurons do not degrade prior to synaptogenesis. The slow degradation requirement has made polylactic acid (PLA) and polycaprolactone (PCL) attractive polymers for nerve regeneration, and these two polymers are used routinely throughout the electrospun fiber literature (Schaub et al., 2016). One disadvantage to these polymers is that they are hydrophobic and tend to pose problems for cell adhesion unless the fibers are coated with a protein (*e.g.*, laminin) or a charged molecule (*e.g.*, polylysine). Counterintuitively, improving the hydrophilicity of electrospun scaffolds made of PLA by chemically modifying the surface of electrospun fibers with different charged molecules appears to provide no improvement in neurite extension (Schaub et al., 2015b). The work by Schaub et al. (2015b) demonstrates the need for additional work to understand how materials influence neurite extension, and this need will become increasingly evident as the field moves forward and these materials are placed into animal models of SCI more frequently. Hurtado et al. (2011) demonstrated significant nerve growth into electrospun scaffolds made from PLA placed in the excised region of a rat spinal cord, but there was little to no nerve growth when electrospun scaffolds composed of collagen were placed into the excised region of a rat spinal cord. Liu et al. (2012a) proposed that the difference in results between the two studies could be the differences in diameter between the two studies, since the collagen fibers were ~200 nm while the PLA fibers were 1.6 μm. Therefore, as research into electrospun fibers progresses, there is a need to study both neurite extension and non-neuronal cell types to electrospun fibers composed of different materials while controlling the geometric properties known to affect neurite extension.

Another attractive aspect of electrospun fibers is the ability to load the fibers with drugs or protein to improve neurite extension or mitigate the effects of the glial scar. The general approach to encapsulating a drug or protein in the fibers is to add the molecule to the electrospinning solution prior to fiber fabrication. Proteins such as nerve growth factor that promote nerve growth (Chew et al., 2005) have been successfully released from electrospun fibers and maintain their bioactivity. The majority of the drug delivery data to date has focused on including bioactive agents to promote nerve growth, but as the field advances there will be a need for studies that show electrospun fibers are capable of releasing agents that mitigate the other two barriers to nerve regeneration: the glial scar and inflammation. A study by Schaub and Gilbert (2011) that involved the release of 6-aminonicotinamide from electrospun polylactic acid was an early attempt at targeting astrocytes,

with the hope of mitigating the effects of reactive astrocytes. A more recent attempt to address the glial scar was a unique approach to releasing proteins from the fibers, which involved enzymatic linkage of ChABC to the surface of electrospun collagen fibers (Liu et al., 2012b). It is well known that ChABC is highly unstable, but crosslinking ChABC to the surface of electrospun fibers caused sustained release of bioactive ChABC for one month. From these early studies, there is significant room for novel work to be done involving drug and protein release from electrospun fibers, and permits electrospun fibers to help address the issues of inflammation and the inhibitory glial scar.

Until recently, the primary focus of applying electrospun fibers to the spinal cord has been on guiding neurons. There has been significantly less attention on how other cell types interact with the fibrous scaffolds. One of the arguments for the use of the peripheral nerve graft for SCI repair is the trophic support provided to growing axons (Lee et al., 2013b). Therefore, interaction of glia with electrospun fibers is likely a critical factor in the successful application of these materials. There is a limited amount of literature on the topic of glia and electrospun fibers, but a small body of research suggests glia interact favorably with electrospun fibers. For example, reactive astrocytes in an injured spinal cord have reduced expression of the glutamate transporter GLT-1, and reduced GLT-1 expression is thought to create a persistent and excitotoxic environment at the injury site (Lepore et al., 2011). Astrocytes that are cultured on flat surfaces do not express GLT-1, but astrocytes cultured on random or aligned electrospun fibers express GLT-1 (Zuidema et al., 2014). Further, the study by Zuidema et al. (2014) also demonstrated that astrocyte migration is predominantly along the length of electrospun fibers, confirming the observations of Hurtado et al. (2011) that astrocytes migrate into electrospun scaffolds implanted into rat spinal cords. In addition to astrocytes, oligodendrocytes are known to myelinate electrospun fibers made of a synthetic material (Lee et al., 2013a). This finding suggests the process of myelination may be controlled by the physical size of an axon rather than surface proteins or extracellular matrix. The preliminary findings of glial interactions with electrospun fibers suggest the importance of electrospun fibers for basic neuroscience research, and may prove critically necessary to performing basic neuroscience research *in vitro*.

Electrospun fibers have received a lot of attention for nerve regeneration applications due to their ability to guide axons, but there is growing evidence of their utility for fundamental neuroscience research. While the application of electrospun fibers to nerve regeneration is only a little over a decade old, it is a field of increased interest due to the versatility and utility of the process used to generate the material. Use of this material in nerve regeneration applications and basic neuroscience research will help drive a clinical approach to functional recovery after SCI.

This work was funded by the National Research Council Research Associate Program fellowship awarded to NJS.

Nicholas J. Schaub*

National Institute of Standards & Technology, Biosystems and Biomaterials Division, Gaithersburg, MD, USA

***Correspondence to:** Nicholas J. Schaub, Ph.D., nicholas.schaub@nist.gov.*
***Accepted:** 2016-11-01*
***orcid:** 0000-0002-9197-7233 (Nicholas J. Schaub)*
***doi:** 10.4103/1673-5374.194719*
***How to cite this article:** Schaub NJ (2016) Electrospun fibers: a guiding scaffold for research and regeneration of the spinal cord. Neural Regen Res 11(11):1764-1765.*

References

Chew SY, Wen J, Yim EK, Leong KW (2005) Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules 6:2017-2024.

Hurtado A, Cregg JM, Wang HB, Wendell DF, Oudega M, Gilbert RJ, McDonald JW (2011) Robust CNS regeneration after complete spinal cord transection using aligned poly-l-lactic acid microfibers. Biomaterials 32:6068-6079.

Lee S, Chong SY, Tuck SJ, Corey JM, Chan JR (2013a) A rapid and reproducible assay for modeling myelination by oligodendrocytes using engineered nanofibers. Nat Protoc 8:771-782.

Lee YS, Lin CY, Jiang HH, Depaul M, Lin VW, Silver J (2013b) Nerve regeneration restores supraspinal control of bladder function after complete spinal cord injury. J Neurosci 33:10591-10606.

Lepore AC, O'donnell J, Kim AS, Yang EJ, Tuteja A, Haidet-Phillips A, O'Banion CP, Maragakis NJ (2011) Reduction in expression of the astrocyte glutamate transporter, GLT1, worsens functional and histological outcomes following traumatic spinal cord injury. Glia 59:1996-2005.

Liu T, Houle JD, Xu J, Chan BP, Chew SY (2012a) Nanofibrous collagen nerve conduits for spinal cord repair. Tissue Eng Part A 18:1057-1066.

Liu T, Xu J, Chan BP, Chew SY (2012b) Sustained release of neurotrophin-3 and chondroitinase ABC from electrospun collagen nanofiber scaffold for spinal cord injury repair. J Biomed Mater Res A 100A:236-242.

Schaub NJ, Gilbert RJ (2011) Controlled release of 6-aminonicotinamide from aligned, electrospun fibers alters astrocyte metabolism and dorsal root ganglia neurite outgrowth. J Neural Eng 8:46026.

Schaub NJ, Johnson CD, Cooper B, Gilbert RJ (2016) Electrospun fibers for spinal cord injury research and regeneration. J Neurotrauma 33:1405-1415.

Schaub NJ, Le Beux C, Miao J, Linhardt RJ, Alauzun JG, Laurencin D, Gilbert RJ (2015b) The effect of surface modification of aligned poly-L-lactic acid electrospun fibers on fiber degradation and neurite extension. PLoS One 10:e0136780.

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638-647.

Wang HB, Mullins ME, Cregg JM, McCarthy CW, Gilbert RJ (2010) Varying the diameter of aligned electrospun fibers alters neurite outgrowth and Schwann cell migration. Acta Biomater 6:2970-2978.

Yang F, Murugan R, Wang S, Ramakrishna S (2005) Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials 26:2603-2610.

Zuidema JM, Hyzinski-García MC, Van Vlasselaer K, Zaccor NW, Plopper GE, Mongin AA, Gilbert RJ (2014) Enhanced GLT-1 mediated glutamate uptake and migration of primary astrocytes directed by fibronectin-coated electrospun poly-L-lactic acid fibers. Biomaterials 35:1439-1449.