

## Differentiation of Human Mesenchymal Stem Cells to Schwann Cells on Electrospun Nanofibers

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### Abstract:

Injuries to the peripheral nervous system (PNS) are irreparable even with surgical intervention. Tissue engineering strategies use a combination of cellular therapies and biocompatible scaffolds in an effort to restore full function to damaged nerves. In this study, electrospun nanofibers were used as the substrate for investigating the differentiation of the human mesenchymal stem cells (hMSCs) into Schwann cell lineage for nerve repair. We investigated the effects of fiber diameter and cell seeding density on the differentiation process. Poly( $\epsilon$ -caprolactone) nanofibers of various diameters were fabricated using the electrospinning technique and their average diameters were assessed via scanning electron microscopy. The hMSC-derived Schwann cells were visualized through immunofluorescence microscopy for S100, a widely recognized Schwann cell marker.

### Introduction:

The human body possesses very limited regenerative capabilities. For example, severe damage to the peripheral nervous system is irreparable even with surgical intervention due to the limited availability of Schwann cells (SCs), a type of glial cell that supports the extension of axons and communication between synapses. Therefore, this project aims at supplementing hMSC-derived SCs to facilitate the repair of damaged nerves to their full function in combination with biocompatible scaffolds, constructs that provide an encouraging environment for the regeneration.

The electrospinning technique is a simple technique requiring little equipment, but has the ability to produce fibers from biocompatible materials with diameters in the nanometer range. These nanofibers resemble the structure of the extracellular matrix (ECM) making them an effective scaffold. Electrospun nanofibers are particularly attractive for use with nerves and other anisotropic tissues because the electrospinning technique readily allows for uniaxial alignment of nanofibers, mimicking their anisotropic anatomy. In this study, poly( $\epsilon$ -caprolactone) (PCL) electrospun nanofibers were used as the substrate for

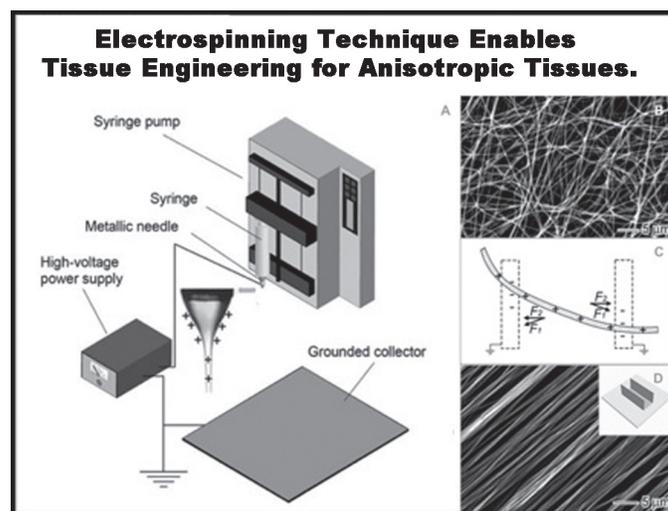


Figure 1: In the (A) electrospinning technique, a high voltage was applied to a syringe containing a polymer dissolved in solution — causing the droplet at the tip to form a Taylor cone. A thin jet stream emerged at the apex of that cone and traveled toward a grounded collector. (B) Fibers randomly oriented, but could be (D) readily aligned using a staple collector, which applied an (C) electrostatic force to the incoming fiber.

investigating the differentiation of hMSCs into Schwann cell lineage for nerve repair. Bone marrow derived hMSCs were chosen particularly due to their clinical relevancy. We investigate the effects of fiber diameter and cell seeding density on the differentiation process, two parameters chosen due to their correlation to conditions governing the initial development of the PNS.

### Methods:

Uniaxially aligned electrospun nanofibers were constructed using the electrospinning technique outlined in Figure 1 from PCL, a biocompatible polymer, dissolved in various solvent systems and spun at various parameters to achieve three distinct diameter sizes. Samples of each fiber type were assessed for size

and morphology using scanning electron microscopy, which is shown in Figure 2. PCL nanofibers were sterilized with ethanol and coated with collagen for cell seeding.

The hMSCs were cultured up to P5 in Dulbecco's modified eagle medium (DMEM). Once confluent, cells were detached by trypsin and seeded on the PCL nanofibers at  $1 \times 10^5$  cells/mL for the fiber diameter study, and both  $1 \times 10^5$  and  $1 \times 10^4$  cells/mL on the intermediate fiber for the cell density study.

The differentiation of hMSC was carried out over a 2.5 week period using an established technique prescribing neuronal inducing factors including  $\beta$ -mercaptoethanol, retinoic acid, platelet derived growth factor AA, nerve growth factor, and forskolin as supplements in DMEM media. The hMSC-derived Schwann cells were visualized through immunofluorescence microscopy for S100, a cytoplasmic protein widely used as a Schwann cell marker.

### Results and Discussion:

Differentiation to Schwann cell lineage was observed on all fiber types (Figure 3), but clear advantage could be seen in the thick fibers, which provided a larger surface area. We predict this larger surface area would promote myelination in co-culture with neurons. In the case of cell density (Figure 4), advantage was obvious in  $1 \times 10^5$  cells/mL, but the upper limit of cell density should be tested. Further optimization of the culture system should be investigated, but, even in its current state, this system has great promise. The ability to culture healthy Schwann cells for cellular therapies without introducing donor site morbidity could be highly impactful in achieving full PNS nerve repair.

### Future Work:

In the future, we seek to apply this approach to nerve repair in model animals for *in vivo* studies in rats with the eventual goal of successful nerve regeneration in human subjects.

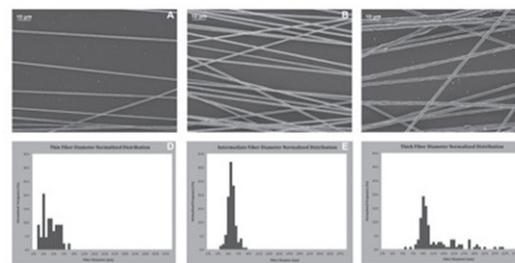
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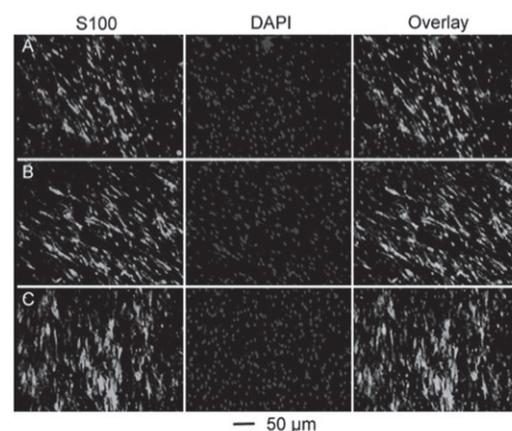
### References:

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## 2 Electrospun Nanofiber Scaffolds for hMSC Differentiation to Schwann Cells.



## 3 Visualization of Differentiation Results in Fiber Diameter Study.



## 4 Visualization of Differentiation Results in Cell Density Study.

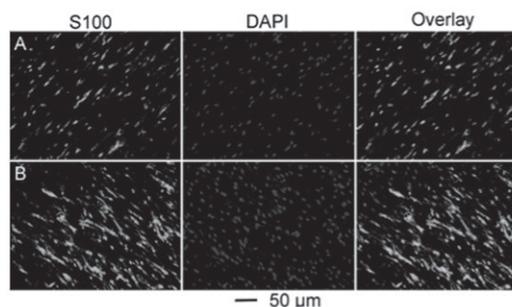


Figure 2, top: (A-C) SEM images taken at 1,000x magnification and (D-F) fiber diameter distribution data for each fiber type. The average diameters of the (L-R) thin, intermediate, and thick fibers were  $484.5 \pm 31\%$  nm,  $617.5 \pm 13\%$  nm, and  $1.198 \pm 35\%$   $\mu$ m, respectively.

Figure 3, middle: Differentiated hMSCs were imaged using fluorescent microscopy for (L-R) S100 and DAPI stains, and the overlay depicts success of differentiation to Schwann cell lineage on (A-C) thin, intermediate, and thick PCL nanofibers.

Figure 4, bottom: Differentiated hMSCs were imaged using fluorescent microscopy for (L-R) S100 and DAPI stains, and the overlay depicts success of differentiation to Schwann cell lineage for hMSC seeding densities of (A)  $1 \times 10^4$  and (B)  $1 \times 10^5$  cells/mL on PCL nanofibers.