Aligning poly(L-lactic acid) nanofibers with magnetic Fe_3O_4 nanoparticles using modified electrospinning methods

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Abstract: *Electrospinning is a simple and adaptable method for generating nanofibers. Due to the instability of the electrified polymer jet, polymer nanofibers are usually collected in random, tangled orientations. Alignment of nanofibers would have practical applications for use as cardiac tissue which have cells that grow in an aligned manner, as well as for neural tissue engineering as guidance channels. Three wt% poly(L-lactic acid) in hexafluoro-2- proponol was mixed in a 95/5 ratio with* Fe_3O_4 *nanoparticles in ethanol. Solutions were sonicated, and then vortexed for one min and electruspun, with a grounded plane being cable wire with 60 amps of current running through the wires to create a magnetic field. In vitro cellular uptake was analyzed with PC12 cells and Schwann cells. Scanning Electron Microscopy (SEM) photos show the cells moving, attaching and growing in the direction of the general fiber orientation. Fiber morphology was analyzed using SEM and mechanical strength was tested in the longitudinal and transverse directions using a nanotensile tester.*

1. INTRODUCTION

Polymer nanofibers can be used for many biological and medical purposes. For tissue engineering, the nanofibers can be made to mimic the extracellular matrix's structure, chemical composition and mechanical properties [1]. Polymer nanofibers are beneficial because they have a large surface area to volume ratio, allowing for better cell adhesion [2]. Acting as scaffolds, the nanofibers direct cellular behavior until the cells can repopulate and create their own matrix, at which point the nanofiber scaffold degrades [2].

One method for creating nanofibers is through electrospinning. The basic setup for electrospinning consists of a high voltage power supply, a spinneret (a metallic needle) and a grounded collector [3]. Polymer solution is put in a syringe, and the spinneret is placed in the syringe. A high voltage is applied, and the polymer at the tip becomes highly electrified [3]. The polymer drop then experiences the electrostatic repulsion between the surface charges and the coulombic force exerted by the electric field [2]. The electrostatic forces overcome the surface tension of the polymer, and a liquid jet is emitted from the syringe, and polymer is collected on the drum [2].

Although the setup for electrospinning is very simple, the spinning mechanism can be complicated. Due to the instability and thinning of the polymer jet, the charged fiber is usually deposited as randomly oriented mats [2]. For many applications it is ideal to control the orientation of the fibers. Aligned nanofibers would be better at mimicking both cardiac tissue and neural tissue to act as guidance channels, allowing cells to grow uniaxially. This research is focused on finding ways of aligning nanofiber polymers- specifically poly(L-lactic acid) by modifying the electrospinning set–up.

The following report describes the first alignment of PLLA fiber mixed with magnetic nanoparticles by using the electrospinning method. The magnetic iron oxide nanoparticles are manipulated with a current and magnetic field. The material used, PLLA, is available from renewable sources, including corn, sugar and dairy products, and is easily biodegraded back to lactic acid or recycled to lactide monomer which renders it harmless in vivo. It is a polyester with good biodegradability, biocompatibility, reasonably good mechanical properties and processibility in forming fibers [5]. It has been studied extensively as a material for biomedical applications and drug delivery [5].

The nanoparticles were added in hopes that they would help the polymer align with the magnetic field. The magnetized magnetic nanoparticles are expected to be influenced by the magnetic field. As shown in Figure 1, the particles will tend to straighten the nanofiber by forcing it into the low energy position in line with the magnetic field [4].

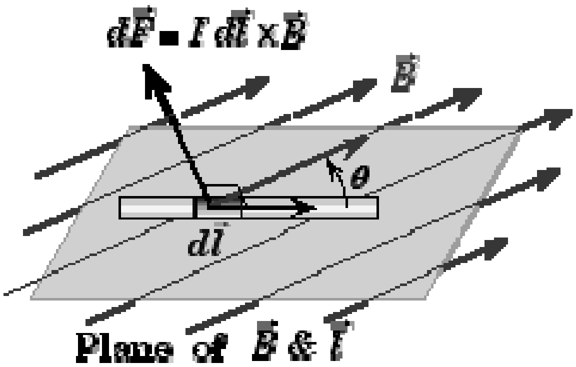

Figure 1 *Representation of fiber length immersed in a magnetic field. I, current; B, magnetic field;* θ*, initial angle between fiber and B; dl, represents a magnetite nanoparticle.*

The magnetic moments of the particles are mostly in a random orientation, but enough are aligned with the nanoparticle so that their sum forms a nanomagnet similar in concept to Figure 2.

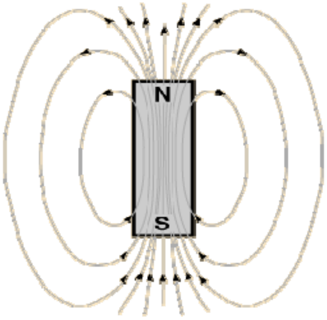

Figure 2 *Field representation around a nanoparticle that is made up of many magnetic moments [2].*

With enough of these nanoparticle magnetic fields and moments, it is expected that the fibers would align with a given magnetic field. The PLLA solution itself has a slight negative charge which would also contribute to orienting the fibers.

2. MATERIALS AND METHODS

2.1 Nanoparticle and PLLA synthesis

Three wt% poly(L-lactide) solution was created by dissolving PLLA in hexafluoro-2-proponol and mixing the solution for 24 h. Ten wt% Fe_3O_4 nanoparticles were obtained and dissolved in ethanol. The PLLA and nanoparticle solutions were then mixed in a 95/5 ratio of PLLA to nanoparticles. The resulting mixture was then sonicated to ensure homogeneity, and vortexed for 1 min before each use.

2.2 Creating the Magnetic Field

Cable ribbon was soldered together to create a ribbon where current could pass through. The ends were soldered to wires and 60 Amps (Electronic Measurements Inc, SCR Power Supply) was run through the cable ribbon, shown in Figure 3, during scaffold fabrication.

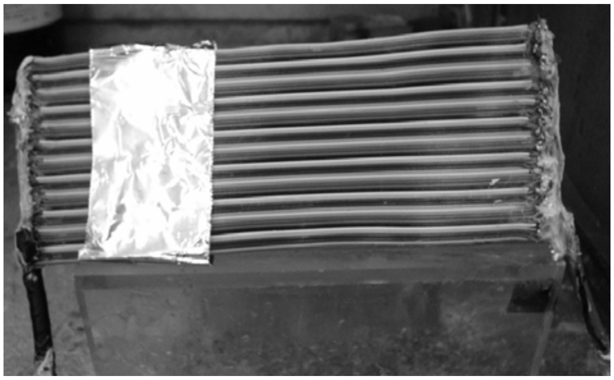

Figure 3 *Cable ribbon was used to run 60 Amps of current and create a magnetic field.*

2.3 Scaffold Creation

A DC voltage of 20 kV (High DC power supply, Del Electronics Corp.) was applied between the syringe tip and the cable ribbon collector. The tip of the syringe to the cable ribbon was set 20 in apart. PLLA solution was spun with and without Fe_3O_4 nanoparticles. The resulting scaffold is shown in Figure 4.

2.4 Cell Attachment

Scaffolds were cultured with Schwann and PC12 cells.

2.5 Characterization

SEM photos of the fiber with Schwann and PC-12 cells were taken using a JSM 7000. Mechanical strength was tested in the transverse and longitudinal direction using a nanotensile tester.

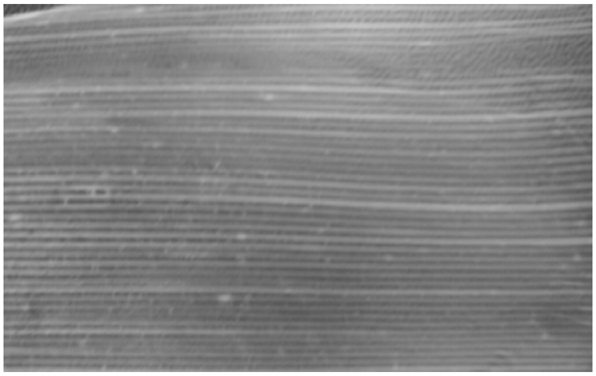

Figure 4. *Scaffold created using cable ribbon.*

3. RESULTS AND DISCUSSION

3.1 Mechanical Strength

In the longitudinal direction, the stress reached a tensile strength of 0.09 Mpa, with a modulus of 24.8 MPa (Figure 5). In the transverse direction, Figure 5 shows a fluctuating stress due to a lower mechanical strength and therefore more elasticity of the nanofibers. With a greater displacement, the polymer strands untangled in the transverse direction, stretching elastically until broken. Figure 5 shows the difference in the stress-strain graphs between longitudinal and transverse directions.

These data suggest that there is more mechanical strength in the longitudinal direction, showing that there are more fibers aligned in one direction than the other.

3.2 Cellular Uptake

The adherence of Schwann cells was tested on the fiber with nanoparticles, and it was seen that the cells seemed to grow in oriented directions, following the general fiber path. As seen in Figure 6, the more aligned fibers are in the center, while the outer areas of the SEM photo show the tangled regions between the linear ridges of aligned fibers.

PC12 cells were also tested on the fibers. Although there seems to be the same amount of cells on both the aligned and tangled regions, on the aligned regions, the cells seem to stretch in the direction of the fiber. Figure 7 shows an image of the PC12 cells on the fiber. A close-up image of the same cells is also shown to be able to distinguish fiber orientation better.

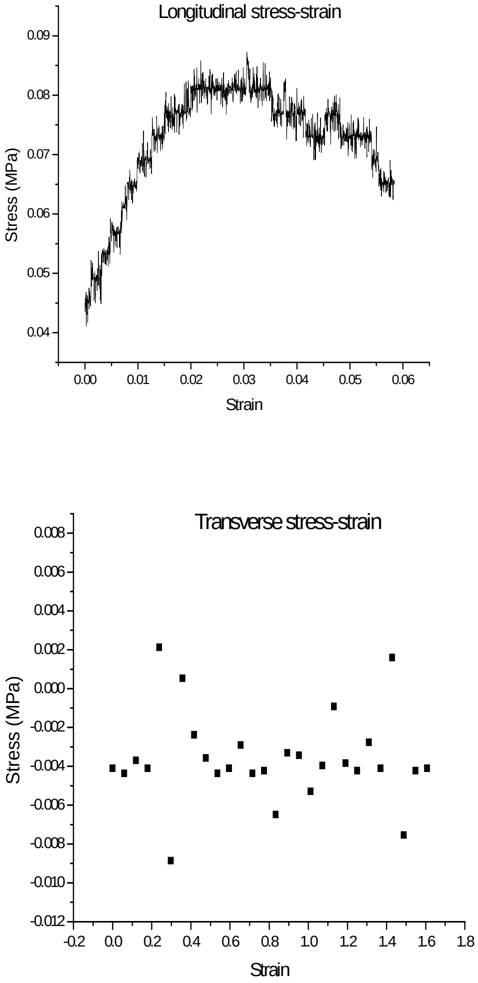

Figure 5 *Longitudinal and transverse stress-strain.*

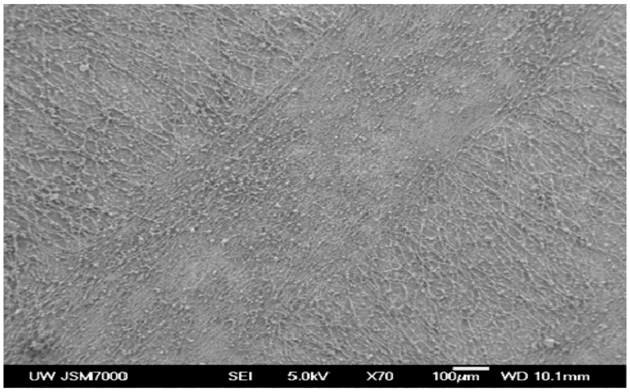

Figure 6 *SEM photo of cell growth of Schwann cells on scaffold.*

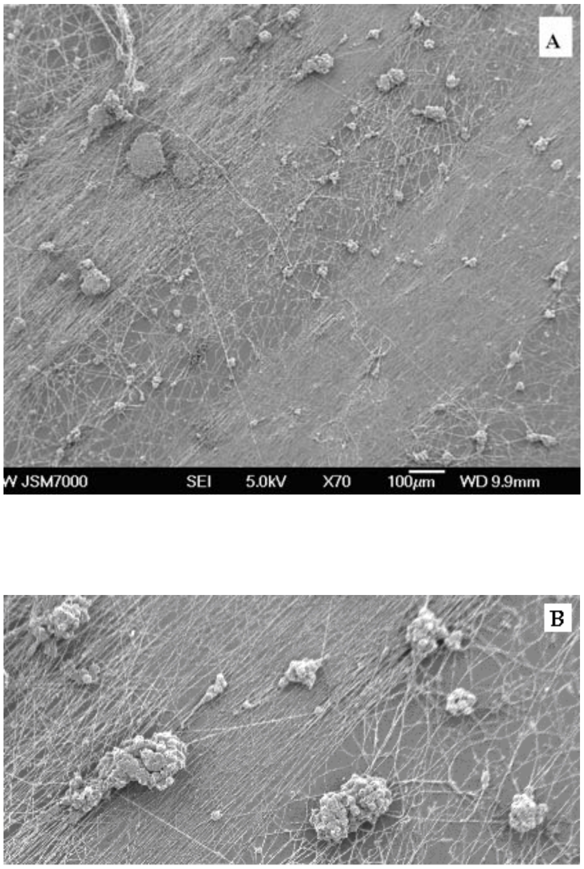

Figure 7 *(A) PC12 cells on scaffold. (B) Close-up to distinguish fiber orientation.*

CONCLUSIONS

Scaffolds showing regions of alignment were created using a solution of PLLA and magnetic nanoparticles in the presence of a magnetic field. The mechanical strength is greater in the longitudinal direction compared to the transverse direction suggesting that more fibers are oriented in the longitudinal direction. Schwann and PC12 cells cultured on the fibers showed directional growth. Future work would include attempting this method with different biocompatible polymers and nanoparticles, as well as investigating cell adherence with stacked layers of aligned electrospun fiber.

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REFERENCES

1. Bhattarai N, Edmondson D, Veiseh O, Matsen FA, Zhang MQ. Electrospun Chitosan-based nonofibers and their cellular compatibility. Biomaterials 2005;26:6176-6184.
2. Boudriot U, Dersch R, Greiner A, Wendorff J. Electrospinning approaches toward scaffold engineering-A brief overview. Artificial Organs. 2006;30:785-792.
3. Pana, H, Li Luming, Hua L, and Cuia X. Continuous aligned polymer fibers produced by a modified electrospinning method. Polymer 2006;47:14.
4. Purcell, Edward, M., Electricity and Magnetism: Berkeley Physics Course, Vol. 2, 2nd ed., McGraw-Hill, Inc., 1985.
5. Zhang X, Hua H, Shen X, Yang Q. In vitro degradation and biocompatibility of poly(l-lactic acid)/chitosan fiber composites. Polymer 2006; 1005-1011.