Three Dimensional Aligned Individual Nano-fibers For Neural Tissue Engineering

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Statement of Purpose: It has been shown that nanofibers have many favorable characteristics as tissue engineering scaffolds. Alignment of fibers in scaffolds allows directional guidance, which is an important characteristic when repairing tissues such as nerves, tendons, and muscles. Aligned nano-fibrous scaffolds can be fabricated by electrospinning onto a fast rotating mandrel. However, the as-fabricated scaffold is in a very dense mat form due to the adhesion between each individual fiber during the fabrication process. The dense mat form of scaffold can only allow cells to grow on the surface of the dense mat and greatly prevents cell penetration inside the scaffold. If used for neural repair, for example, regenerating neurites can only grow on the surface, which greatly limits the possible number of regenerating neurites in a confined volume. Therefore, researchers are facing great difficulties in applying electrospun nanofibers for neural repair in vivo. To solve this problem, a novel method of fabricating loose 3-dimensional bundles of aligned nanofibers was developed in our lab and evaluated in vitro as a scaffold for guided axonal regeneration.

Methods: Nanofibers can be induced to align by manipulation of the electric field.[1] When nanofibers are spun onto a collecting device consisting of two grounded parallel plates, fibers are deposited across the gap between the plates in a highly aligned configuration. Utilizing this principal, a dynamic collecting device was developed that could collect large 3-D-dimensional arrays of individual aligned nanofibers. Fibers were electrospun through a 23g needle from solutions of 8-18% wt PCL dissolved in 75/25 dichloromethane/dimethylformamide. Applied voltage was 5-15 kV, drop heights were 7-15cm, and flow rates were between 0.008- 0.032 ml/min. Fibers were vacuum dried to evaporate any residual solvent and bundled for in vitro neurite outgrowth assay. Aligned fibers in dense mats were used as control. In addition, the aligned 3D individual fibers were functionalized by direct loading brain-derived neurotrophic factor (BDNF) into the nanofibers during the electrospinning process. To slowdown the BDNF release, heparin was loaded into the nanofibers during the spinning process. BDNF release was measured over 4 weeks using ELISA. DRGs were cultured on 3-D bundles of varying fiber diameters and BDNF concentrations. Neurite outgrowth was evaluated and compared for different scaffold compositions.

Results/Discussion: Special devices with grounded mobile metal collecting tracks were used to collect 3-D fiber arrays with fiber lengths of 2.5, 4.5, and 9 cm and areas of 15, 60, and 135 cm^2 respectively. The mobile collecting track was moved at approximately 0.5 cm/min. Decreasing the speed of the track movement increases the fiber density, but it was observed that a maximum fiber density was reached at around 0.5 cm/min. A uniform aligned fiber distribution was present throughout the 3-D arrays as shown in figure 1. Fiber bundles of average diameters of 250nm, 500nm, and 1 μm were fabricated and used as scaffolds for in vitro culture of DRGs. It was observed that the penetration of regenerating neurites was much greater when grown on the novel individual nanofiber arrays as compared to aligned nanofiber dense mats fabricated by the conventional rotating mandrel technique. Heparin and BDNF were incorporated into nanofibers at different concentrations. Steady release of BDNF was observed for both heparin containing fibers and heparin null fibers. The release of BDNF from heparin null fibers was significantly faster than heparin containing fibers. By varying heparin and BDNF loading, BDNF release can be very well tuned for optimal neurite outgrowth. BDNF released from nanofibers remained bioactive after electrospinning. DRGs extended neurites on the fiber scaffolds over 7 days with faster growth observed on nanofibers loaded with BDNF.

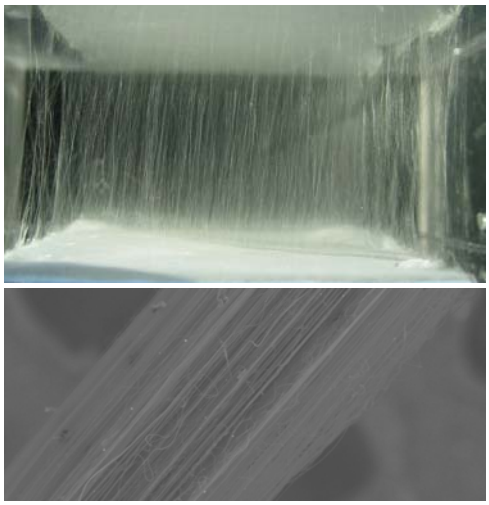

Figure 1. 3-D arrays of aligned individual nanofibers.

Conclusions: It has been demonstrated that 3-D aligned individual nanofiber arrays can be obtained using a parallel plate/dynamic track device. Fiber bundles can be collected from these arrays after complete evaporation of residual solvent from the individual nanofibers to prevent adhesion between nanofibers. Nanofiber arrays can be made bioactive for neural repair by incorporating BNDF during the electrospinning process. Studies in progress are testing nanofiber entubulated bridging devices for sciatic nerve and spinal cord repair.

References: 1. Li et al. Nanoletters. 2003;3:1167-1171.

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