Generating Protein Gradients on Polymeric Nanofibrous Scaffolds for Neural Tissue Engineering

Chandra M. Valmikinathan, Neha.G.Golwala, Xiaojun Yu
Stevens Institute of Technology, Hoboken, NJ

Introduction: Nerve injuries affect hundreds of thousands of patients, and a considerable amount of research has focused on regeneration of the damaged nerves (1,2). Promising evidence has been found for protein gradient-guided neurite growth (3,4). Electrospinning is a facile technique that has opened gates to manipulate tissues at the nanoscale by providing an analog to mimic the ECM structure. Electrospun fibers are easy to fabricate and have a large surface area to volume ratio due to their nanofibrous structure. In this study, we have created a gradient of an extracellular matrix protein, Fibronectin, on nanofibrous scaffolds through crosslinking it to ferritin, a magnetically inducible and biocompatible protein with functional amine and carboxylic groups on the surface (5-7).

Materials and Methods: A 10% solution of Polycaprolactone (PCL) in hexa fluoro isopropanol (HFIP), and Poly (ethylene glycol) (PEG)-diamine was added to the PCL solution, in the ratio of 1:10 to form a blend. Poly (ethylene glycol) (PEG)-diamine provides amine groups for protein crosslinking. The solution was electrospun onto glass substrates to form nanofibrous scaffolds.

Different concentrations of fibronectin (0, 0.05, 0.5, 5 mg/mL) were crosslinked to ferritin (0.5 mg/ml) for 2 hours in MES solutions containing 10mM EDC. The scaffolds were then placed on magnets and the ferritin-fibronectin solutions were added onto the scaffolds to form a gradient of increasing fibronectin concentrations and were left to crosslink at room temperature for 2 hours.

The morphology of the scaffolds was characterized with optical and scanning electron microscope (SEM). The chemical groups on the scaffolds were characterized using Fourier transform infrared spectroscopy (FTIR). To distinguish the crosslinking from the adsorption of proteins on the surface, fluorescent antibody binding experiments were performed on the fibronectin crosslinked or adsorbed after continuous washing for 2 hours. Mouse anti human fibronectin was tagged to the fibronectin molecule which in turn was bound to Fluorescein isothiocyanate (FITC) containing anti-mouse antibodies.

Schwann cells were cultured on the scaffolds in complete DMEM containing 10% serum and 1% penicillin-streptomycin, for 24 hours and the cell density was analyzed visually using methylene blue staining.

Results and Discussions: The scaffolds made from electrospinning are shown in Fig 1.

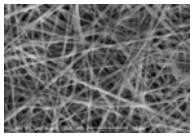

Figure 1. Scanning electron micrograph of PCL fibers produced by electrospinning.

As shown in the FTIR spectra, the CO-NH linkage bond (1600 cm), which is characteristic to the protein linkage, was observed on nanofibrous scaffolds modified with fibronectin (Fig. 2).

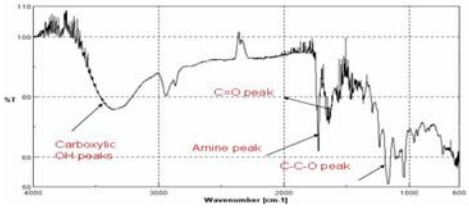

Figure 2: FTIR spectra showing the PEG groups in the fibers and the CO-NH linkage in the protein.

As probed by the FITC tagged antibody, strong fluorescence was observed on the fibrous scaffolds that were crosslinked with proteins (Fig 3A); while no fluorescence was observed on the control fibrous scaffolds that had only protein adsorption followed by washing with PBS (Fig. 3B).

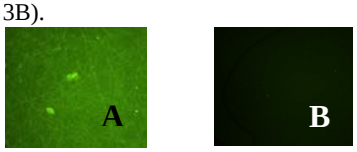

Figure 3: A) Fibrous PCL amine showing crosslinked fibronectin B)Adsorbed fibronectin was washed off and was not seen on the fibers.

The fluorescent intensities on the scaffolds increased with the increase in fibronectin concentrations (Fig.4A). The cell density on the scaffolds also increased with the increase with the increase in fibronectin concentrations (Fig. 4B). These results indicated the formation of fibronectin gradient on the fibrous scaffolds and further guided preferential cell attachment.

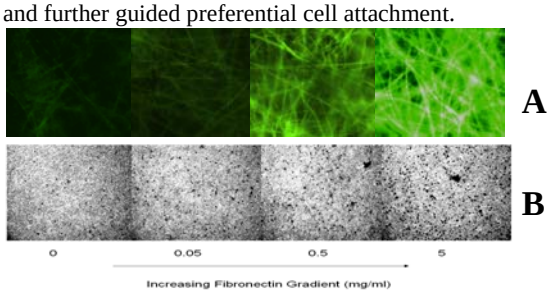

Figure 4: Ferritin- fibronectin gradients on PCL-PEG amine (A) showing the gradient of fluorescent antibody bound to fibronectin on scaffolds (B) Shows increased cell numbers with increasing protein amounts along the length of the scaffolds.

Conclusions: A fibronectin gradient can be successfully established on polymeric nanofibrous scaffolds by chemical crosslinking under magnetic field, and the fibronectin gradient on the nanofibrous scaffolds can guide preferential attachment of Schwann cells. The scaffolds could be potentially used in neural tissue engineering for guiding nerve regeneration.

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