Excimer Laser Channel Creation in Polyethersulfone Hollow Fibers For Compartmentalized *In Vitro* Neuronal Culture Models

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Introduction

We report here using KrF excimer laser ablation to generate specifically designed channels within the walls of hollow polymer fibers to force compartmentalization of the neuronal cell bodies from their axons to create a novel scaffold for *in vitro* neural tissue engineering. Previously, Nakayama and Matsuda in 1994 created pores in polymer scaffolds for cardiovascular tissue engineering using an excimer laser.[1] This trend continued with Tiaw et. al. by producing a porous polymer with excimer laser ablation for a possible skin substitute.[2] The channels we create with an excimer laser can allow neuronal axons to grow within a three dimensional space inside the fibers where we can apply gradients of clinically relevant chemotropic or pharmacological reagents. These scaffolds can be used to provide more appropriate *in vitro* growth and differentiation of neuronal cells, enabling a system for superior testing of directed neurite outgrowth and behavior.

Materials and Methods

Scaffold Preparation. 500µm outer diameter microporous polyethersulfone (PES) hollow fibers (Membrana, Wuppertal, Germany) were modified with a KrF 248 nm nanosecond excimer laser (Lambda-Physik EMG-202). Single rows of 5µm channels were created in fiber walls by shaping beam with a copper mask and using 35-50 pulses at a peak surface fluence of 1000-1200 mJ/cm^2.

Flat sheet membrane studies. Flat sheet membrane counterparts to the PES hollow fibers (Membrana, Wuppertal, Germany) were used to analyze surface property changes due to excimer laser ablation. Laser fluence was adjusted to account for exclusion of mask to modify entire membrane surface areas with similar peak surface fluences as used in channel creation. The membrane surfaces were modified with peak fluences ranging from 300-1200 mJ/cm^2. Unmodified membrane properties were compared to laser modified membrane surfaces using contact angle. Neuron-like PC12 adhesion to these different surfaces was quantified by using CyQuant®.

Single fiber culture studies. To test neuronal cell activity on modified scaffolds we incorporate a single fiber into a 35mm diameter Petri dish with a divider sealed perpendicularly with polyurethane across top of fiber. Bioactivity of growth factor gradients through fibers were quantified by PC12 cell neurite outgrowth after application of NGF to the opposite dish compartment. Experiments to test axonal growth into laser modified channels were performed by differentiating adult rat neural stem cells isolated from the subventricular zone (NSCs) in single fiber culture models with 20 ng/ml bFGF gradients.

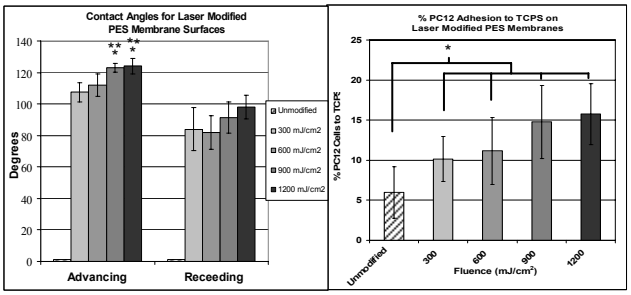

Figure 1. Laser modified membrane properties a) contact angle data n=10, * p< 0.05 compared to F=300 mJ/cm2 and ** p< 0.05 compared to F=600 mJ/cm2; and b) PC12 adhesion normalized to tissue culture polystyrene (TCPS) n=5, * p < 0.05.

Results and Discussion

Excimer laser modification of PES hollow fibers results in a reproducible method in generating 5µm channels for neuronal axon ingrowth. Laser modification of membrane surfaces results in increased hydrophobicity with increasing fluence (Figure 1a). In correlation with this is enhanced PC12 adhesion on laser modified membrane surfaces when compared to unmodified surfaces (Figure 1b). Therefore, channel surfaces themselves can attract neuronal axons to grow into scaffold walls. Also, bioactivity assay using NGF-PC12 activation revealed continuous growth factor gradient in single fiber culture model for 48 hours (data not shown).

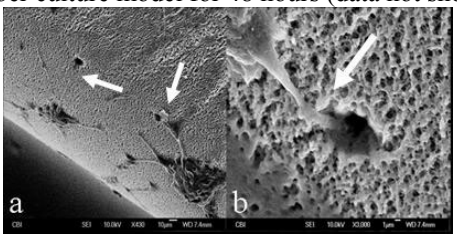

Figure 2. SEMs of a) differentiated NSCs on PES fiber with laser modified channels and b) axon penetrating fiber wall through channel (arrows).

Differentiation of NSCs in single fiber culture models showed increased number of neurons on fibers including laser modified channels versus fibers with no channels. β-III-tubulin staining was used to confirm axon ingrowth into channels (data not shown).

Conclusions

With excimer laser modification of PES hollow fibers we are able to produce scaffolds that compartmentalize neuronal cell bodies from their axons. Using these scaffolds we can further manipulate and control axonal growth within *in vitro* neuronal cultures.

References

1. Nakayama Y, Matsuda T. ASAIO J. 1994 Jul-Sep; 40(3): M590-593.
2. Tiaw KS, Goh SW, Hong M, Wang Z, Lan B, Teoh SH. Biomaterials. 2005 Mar; 26(7): 763-769.