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ScienceDirect
Acta Biomaterialia 4 (2008) 244–255
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Excimer laser channel creation in polyethersulfone hollow fibers for compartmentalized in vitro neuronal cell culture scaffolds

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Received 18 March 2007; received in revised form 28 September 2007; accepted 3 October 2007
Available online 24 October 2007

Abstract

Hollow fiber scaffolds that compartmentalize axonal processes from their cell bodies can enable neuronal cultures with directed neurite outgrowth within a three-dimensional (3-D) space for controlling neuronal cell networking in vitro. Controllable 3-D neuronal networks in vitro could provide tools for studying neurobiological events. In order to create such a scaffold, polyethersulfone (PES) microporous hollow fibers were ablated with a KrF excimer laser to generate specifically designed channels for directing neurite outgrowth into the luminal compartments of the fibers. Excimer laser modification is demonstrated as a reproducible method to generate 5 μm diameter channels within PES hollow fiber walls that allow compartmentalization of neuronal cell bodies from their axons. Laser modification of counterpart flat sheet PES membranes with peak surface fluences of 1.2 J cm^{-2} results in increased hydrophobicity and laminin adsorption on the surface compared with the unmodified PES surface. This is correlated to enhanced PC12 cell adhesion with increasing fluence onto laser-modified PES membrane surfaces coated with laminin when compared with unmodified surfaces. Adult rat neural progenitor cells differentiated on PES fibers with laser-created channels resulted in spontaneous cell process growth into the channels of the scaffold wall while preventing entrance of cell bodies. Therefore, laser-modified PES fibers serve as scaffolds with channels conducive to directing neuronal cell process growth. These hollow fiber scaffolds can potentially be used in combination with perfusion and oxygenation hollow fiber membrane sets to construct a hollow fiber-based 3-D bioreactor for controlling and studying in vitro neuronal networking in three dimensions between compartmentalized cultures.

Keywords: Laser ablation; PES fibers; Neural progenitor cells; PC12

1. Introduction

Recent trends for studying neurobiological mechanisms in vitro have been to develop substrates that can control specific arrangements of neuronal axon growth and synaptic connectivity in neuronal networks. Advantages of these neuronal cell cultures lie in the ability to separate multiple interactions in order to study single events. Information gathered from studying these cultures could permit synthetic manipulation of events such as directed axonal regeneration. For example, Aguayo et al. demonstrated that central nervous system neurons retain the ability to regenerate after damage given the proper environment [1,2]. An in vitro system for neuronal cell cultures allowing controlled growth and signaling in a three-dimensional (3-D) space could be used as a diagnostic tool for unveiling

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doi:10.1016/j.actbio.2007.10.004

the permissive environments for nerve regeneration. The system could specifically be used to test diffusible molecules such as drugs and therapeutic proteins on their effects on directed neurite outgrowth for nerve regeneration. The responses of these 3-D neuronal cell cultures could translate better from the in vitro models to animal models and clinical applications for deriving therapeutic strategies enhancing nerve regeneration.

Hirono et al. in 1988 was one of the first groups to realize patterned substrates could control axonal outgrowth in neuronal cell cultures in vitro [3]. Many groups since then have studied different methods for creating structured biomaterial surfaces for controlling axonal guidance. Most of the knowledge within this field has come from creation of nerve guide conduits used for in vivo nerve gap repair. These nerve guides are typically a hollow tube that the cut nerve stumps are placed in to be guided to regenerate. The lumens of the nerve guides provide the contact guidance cue for the proximal nerve end to grow directly towards its distal end. Other aspects found in these studies to be important for guiding axonal regeneration in vivo have been the nerve guide conduit material properties, dimensions, and configuration [4–12]. Methods to further improve functional nerve regeneration within these conduits have ranged from surface modifications or natural matrix incorporation (collagen or fibrin gels) to enhance cell adhesion, growth factor immobilization to conduit walls or microcarrier incorporation, and use of novel biomaterial surfaces, i.e. electrically conducting polymers [13–28].

The topography of biomaterial surfaces used for directing neurite outgrowth is currently a useful topic in nerve tissue engineering in order to devise methods for improving nerve regeneration in vivo. One extensively studied method in generating these structured surfaces has been the microfabrication technology of photolithography. This method can be used to create different topographical structures on a surface to construct grooved pathways for specific axonal growth [29–33]. Another attractive method for surface patterning has been the technique of microcontact printing. Wheeler et al. used microcontact printing to direct hippocampal neuronal growth on lysine and laminin patterned surfaces [34]. Others have created microcontact printed surfaces with even more complex patterns using adhesive proteins and molecules [35–39]. Thiebaud et al. have combined photolithography and microcontact printing in order to create a microfluidic device for continued in vitro growth of specific neuronal cell arrangements [40]. Using these two methods to pattern substrates in more than two dimensions can be very complex. It is possible to stack layers of the modified surfaces to create 3-D scaffolds, but this has not yet been achieved for neuronal cell culture scaffolds with the exception of creating a tube out of the layer for use as in vivo peripheral nerve guide conduits [29,41,42]. A technique that could also be scaled up to achieve 3-D neuronal in vitro scaffolds is that of polymer/extracellular matrix protein inkjet printing [43]. These studies will all help lead to the establishment of scaffolds used for generating in vitro systems for controlled neuronal cell networks

We report here the development of a scaffold for controlling specific neuronal cell body and axonal process outgrowth in vitro by using excimer laser ablation modifications in microporous polyethersulfone (PES) hollow fibers. Excimer laser ablation for tissue engineering applications was performed in 1994 by Nakayama and Matsuda to create pores in polymer scaffolds for cardiovascular tissue engineering [44]. Further work was performed by Tiaw et al. to produce porous poly(caprolactone) sheets with excimer laser ablation for a possible skin substitute [45]. There have been other reports on the use of excimer laser ablation on polymer scaffolds to manipulate cell growth, but none for controlling neuronal cell growth [46–49]. In this study, excimer laser ablation was utilized to generate specifically sized channels within the walls of PES hollow fibers in order to compartmentalize growth of neuronal cell bodies from their axonal processes and further facilitate directed process growth into the 3-D space of the fiber lumens. Compartmentalization of neuronal cell bodies from their axons may lead to controlled synaptic networking between cell bodies within a 3-D space. We plan to further incorporate these scaffolds into a hollow fiber-based bioreactor to establish high-density 3-D in vitro neuronal cell cultures with defined axonal pathways in the directions of the lumens of the scaffolds to create a tool for studying 3-D neuronal networking in vitro. The growth of only the neuronal axons into the fiber scaffolds allows a direct method of analyzing directed neurite outgrowth within a 3-D space at high densities that more accurately mimics the in vivo environment.

2. Materials and methods

2.1. Laser modification of PES flat sheet membranes and hollow fibers

Ultrafiltration microporous polyethersulfone hollow fibers (MicroPES TF10, Membrana, Wuppertal, Germany) with inner diameter of 300 ± 40 μm, wall thickness of 100 ± 25 μm and maximum pore size of 0.5 μm were used. PES flat sheet membrane counterparts (Membrana, Wuppertal, Germany) to these fibers with similar pore structure were also used to enable characterization of laser modifications on 2-D surfaces. All laser modifications were performed with a KrF 248 nm nanosecond excimer laser (Lambda-Physik EMG-202) with a full-width at half-maximum pulse duration of 25 ns at a pulse frequency of 2 Hz, at room temperature in an open air atmosphere. The laser beam path through the mask projection system to the sample stage is shown in Fig. 1. Laser pulse energy was controlled at position Fig. 1a by rotation of a variable attenuator plate (JPSA Laser UM-VA) and measured with a Molectron JD-100 pyroelectric joulemeter.

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