Acta Biomaterialia 8 (2012) 2628–2638

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

# Preparation of uniaxial multichannel silk fibroin scaffolds for guiding primary neurons

Qiang Zhang [a], Yahong Zhao [b], Shuqin Yan [a], Yumin Yang [b], Huijing Zhao [a], Mingzhong Li [a,*], Shenzhou Lu [a], David L. Kaplan [c]

[a] National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, No. 199 Ren'ai Road, Industrial Park, Suzhou 215123, PR China
[b] Jiangsu Key Laboratory of Neuroregeneration, Nantong University, No. 19 Qixiu Road, Nantong 226001, PR China
[c] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

ARTICLE INFO

*Article history:*
Received 28 December 2011
Received in revised form 29 February 2012
Accepted 20 March 2012
Available online 28 March 2012

*Keywords:*
Silk
Scaffold
Neural cell
Nerve guide
In vitro test

ABSTRACT

Physical guidance cues have been exploited to stimulate neuron adhesion and neurite outgrowth. In the present study, three-dimensional (3-D) silk fibroin scaffolds with uniaxial multichannels (42–142 μm in diameter) were prepared by a directional temperature field freezing technique, followed by lyophilization. By varying the initial silk fibroin concentration, the chemical potential and quantity of free water around cylindrical ice crystals could be controlled to control the cross-section morphology of the scaffold channels. Aligned ridges also formed on the inner surface of the multichannels in parallel to the direction of the channels. In vitro, primary hippocampal neurons were seeded in these 3-D silk fibroin scaffolds with uniaxial multichannels of ~120 μm in diameter. The morphology of the neurons was multipolar and alignment along the scaffold channels was observed. Cell–cell networks and cell–matrix interactions established by newly formed axons were observed after 7 days in culture. These neurons expressed β-III-tubulin, nerve filament and microtubule-associated protein, while glial fibrillary acidic protein immunofluorescence was barely above background. The ridges on the inner surface of the channels played a critical role in the adhesion and extension of neurons by providing continuous contact guidance. These new 3-D silk scaffolds with uniaxial multichannels provided a favorable microenvironment for the development of hippocampal neurons by guiding axonal elongation and cell migration.



## 1. Introduction

In regeneration of the central nerve system (CNS), the microenvironment surrounding an injury site in the CNS is usually incompatible with axonal regeneration, as a result of the deficiency of extracellular matrix (ECM) and neurotrophic factors, the existence of inhibitive factors and glial scar, etc. [1,2]. This challenge motivates the continued development of novel materials and fabrication processes to produce scaffolds for the targeted guidance of regenerating axons. Oriented pores and topographical cues or micropatterns in the micron and nanoscale regime are effective for guiding adhesion, extension and migration of neurons and glial cells [3–5].

Numerous studies have indicated that artificial nerve grafts with special oriented channels provide physical cues to guide the linear growth of axons across a site of injury [6,7]. For example, the effect of chitosan biomaterials with different topologies (film, porous scaffold and multichannel conduits) on the differentiation and proliferation of neural stem cells was investigated [8]. After 5 days in culture, chitosan conduits prepared by lyophilization with multichannels in the inner part of the conduit assisted more neural stem cells than films or porous scaffolds, due to elongation along a preferred axis in the microchannels [8]. Fibronectin mats with oriented pores were prepared by lyophilization and implanted in the damaged spinal cord of adult rats, and robust, correctly oriented axonal growth was observed [7]. Freeze-dried agarose scaffolds with uniaxial channels also supported linear axonal growth in adult rats with spinal cord injuries [4]. Aligned polycaprolactone/gelatin (PCL/gelatin) nanofibrous scaffolds were fabricated by electrospinning. Schwann cells seeded on the PCL/gelatin nanofibrous scaffolds possess bipolar extensions with spindle-shaped morphology along with the aligned nanofibers [9]. Poly (ethylene glycol) hydrogel patterned surfaces formed by cross-linking permitted the adhesion and directed growth of neuronal cell, enabling regrowth of axons to bridge a spinal cord injury without interference from glial scars [10]. Many studies generally confirm that scaffolds with axially aligned pores support targeted axonal elongation and may also be an effective means for the local delivery of exogenous support cells and growth factors to the site of injury [11,12]. These studies suggested that

* Corresponding author. Tel.: +86 512 6706 1150; fax: +86 512 6724 6786.
*E-mail address:* mzli@suda.edu.cn (M. Li).

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http://dx.doi.org/10.1016/j.actbio.2012.03.033