Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 419–429
Biomaterials
www.elsevier.com/locate/biomaterials

Multiple-channel scaffolds to promote spinal cord axon regeneration

Michael J. Moore[a], Jonathan A. Friedman[b], Eric B. Lewellyn[c], Sara M. Mantila[a], Aaron J. Krych[d], Syed Ameenuddin[c], Andrew M. Knight[c], Lichun Lu[a,e], Bradford L. Currier[d,e], Robert J. Spinner[d,f], Richard W. Marsh[d,f], Anthony J. Windebank[c,d], Michael J. Yaszemski[a,d,e,*]

[a]*Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[b]*Section of Neurosurgery, Dartmouth Hitchcock Medical Center, Lebanon, NH 03756, USA*
[c]*Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[d]*Mayo Medical School, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[e]*Department of Orthopedic Surgery, Mayo Clinic, MS 3-75, 200 First Street, SW, Rochester, MN 55905, USA*
[f]*Department of Neurologic Surgery, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, USA*

Received 30 June 2005; accepted 27 July 2005
Available online 31 August 2005

Abstract

As molecular, cellular, and tissue-level treatments for spinal cord injury are discovered, it is likely that combinations of such treatments will be necessary to elicit functional recovery in animal models or patients. We describe multiple-channel, biodegradable scaffolds that serve as the basis for a model to investigate simultaneously the effects on axon regeneration of scaffold architecture, transplanted cells, and locally delivered molecular agents. Poly(lactic-*co*-glycolic acid) (PLGA) with copolymer ratio 85:15 was used for these initial experiments. Injection molding with rapid solvent evaporation resulted in scaffolds with a plurality of distinct channels running parallel along the length of the scaffolds. The feasibility of creating scaffolds with various channel sizes and geometries was demonstrated. Walls separating open channels were found to possess void fractions as high as 89%, with accessible void fractions as high as 90% through connections 220 μm or larger. Scaffolds degraded in vitro over a period of 30 weeks, over which time-sustained delivery of a surrogate drug was observed for 12 weeks. Primary neonatal Schwann cells were distributed in the channels of the scaffold and remained viable in tissue culture for at least 48 h. Schwann-cell containing scaffolds implanted into transected adult rat spinal cords contained regenerating axons at one month post-operation. Axon regeneration was demonstrated by three-dimensional reconstruction of serial histological sections.

Keywords: Nerve tissue engineering; Scaffold; Microstructure; Schwann cell; Drug release; Image analysis

1. Introduction

Advances in neuroscience over the past 2 decades begin to offer hope for spinal cord injury (SCI) victims. Since the demonstration in 1980 that central nervous system (CNS) axons have the capacity to regenerate within peripheral nervous system (PNS) grafts [1], much has been accomplished toward understanding factors that contribute to a physiologically permissive environment. Mechanisms of injury, of regeneration, and of inhibition to regeneration are being delineated, and several promising treatment strategies have arisen. Transplantation of a variety of cell types, including Schwann cells [2,3], olfactory ensheathing glial cells [4,5], or neural stem cells [6,7], has resulted in axon regeneration and limited functional improvement after spinal cord injury in rats. Molecular therapies that work

*Corresponding author. Department of Orthopedic Surgery, Mayo Clinic, MS 3-75, 200 First Street SW, Rochester, MN 55905, USA. Tel.: +1 507 284 2267; fax: +1 507 284-5075.
E-mail address: yaszemski.michael@mayo.edu (M.J. Yaszemski).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.07.045

to promote regeneration, such as administration of neurotrophins [8–10], and those that target deleterious inhibition of regeneration, such as chondroitinase ABC [11–15], have also yielded favorable results. Synthetic biomaterials have been investigated for their ability to reconstruct spinal cord tissue architecture, to provide guidance for regenerating axons, and to prevent the infiltration of scar tissue [16–19]. A comprehensive review of neural regeneration strategies was recently provided by Schmidt and Baier Leach [20].

Despite recent advances, the limited demonstration of functional improvement in animal models has prevented advancement of any regenerative therapy to clinical use. This may be due in large part to the multifaceted nature of spinal cord injuries, which presents a major challenge to therapeutic development. Primary mechanical trauma to the cord induces secondary injury consisting of a complex cascade of molecular events that lead to the loss of myelin and the formation of a glial scar [21,22]. Therefore, in order for viable treatment strategies to be realized clinically, it is likely that combinations of current therapeutic approaches must be used. Indeed, combinatorial approaches have already shown promise in animal models. For example, administration of neurotrophins enhanced axon growth into Schwann-cell seeded guidance channels and increased integration into the graft–host interface [23]. Synergistic effects on CNS axon regeneration have been demonstrated when strategies promoting regeneration and antagonizing inhibition were used simultaneously [24,25].

An elegant solution may lie in the design of a bio-artificial graft that targets injury mechanisms at the molecular, cellular, and tissue levels. Biodegradable polymers can simultaneously provide a tissue scaffold, a cell delivery vehicle, and a reservoir for sustained drug delivery [16]. This integrative approach suggests a possible treatment strategy and may serve as an in vivo model for studying optimization of various combinations of treatments. We describe techniques for producing biodegradable polymer scaffolds with parallel-channel architecture that can be systematically modified. They may be seeded with multiple cell types arranged spatially in anatomically relevant locations and may serve as a vehicle for sustained drug delivery. We quantitatively describe the scaffolds' architecture, their in vitro degradation profile, their drug delivery characteristics, their biocompatibility with Schwann cells in culture, and their promotion of in vivo axon regeneration.

2. Materials and methods

2.1. Scaffold fabrication

Biodegradable scaffolds with controlled, parallel-channel architecture were fabricated by an injection molding, solvent evaporation technique. Cylindrical, Teflon molds, with diameter 3.0 mm were fitted with Delrin spacers containing an array of seven, uniformly spaced, 508- or 660-μm stainless-steel wires (Malin, Cleveland, OH), as shown in Fig. 1A. The wire arrays were spray-coated with a minimal amount of Ease Release 200 (Mann Formulated Products, Easton, PA) mold lubricant to facilitate removal. A concentrated solution of poly(D,L-lactic-*co*-glycolic acid) (PLGA, Alkermes, Cambridge, MA), with copolymer ratio 85:15 lactide:glycolide and number average molecular weight (M_n) 75,000, was made by adding 1.0 g PLGA to 2.0 ml dichloromethane (DCM) in a glass vial and shaking vigorously for 3 h. The viscous PLGA solution was injected with a syringe through a 16-gauge hypodermic needle from the bottom of each mold until solution was seen escaping through the top.

Polymer-filled molds were vacuum-dried for at least 24 h with a high-vacuum pump (VP 190, Savant Instruments, Holbrook, NY) connected to a condensation trap (RT 4104, Savant Instruments). Vacuum drying removed the solvent and created pores in the walls of the scaffolds. Scaffolds to be used in cell culture or in vivo experiments were washed in ethanol for 30 min with gentle shaking both to disinfect the scaffolds and to remove any residual mold lubricant. Scaffolds were again vacuum-dried for 24 h to remove the ethanol, then sealed in sterilized glass vials and stored desiccated at 4 °C until further use.

Creation of scaffolds with more complex, biomimetic architecture was demonstrated by fabrication of molds using computer-aided design (CAD) and solid freeform fabrication (SFF). A PatternMaster three-dimensional (3-D), piezo-inkjet printer (Solidscape, Merrimack, NH) was used to create wax molds from models designed manually with ProEngineer software (Parametric Technology Corp., Needham, MA). Fig. 1B shows one wax mold manufactured by the PatternMaster. Injection of PLGA polymer solution followed by vacuum-evaporation of solvent resulted in PLGA scaffolds 1 cm in diameter. These were used to test the principle that complex architecture mimicking the structure of the human spinal cord can be fabricated.

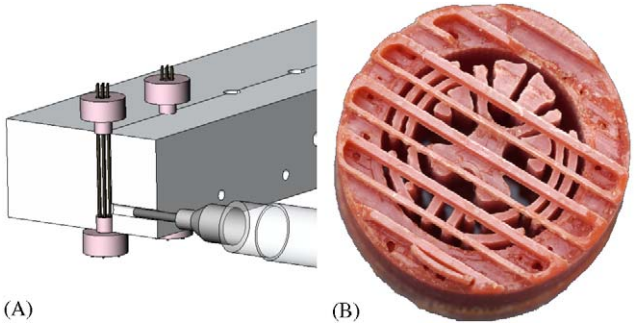

Fig. 1. Injection molds for fabrication of multiple-channel scaffolds. (A) Cut-away view of parallel-wire injection molding apparatus. Mold inner diameter = 3.0 mm. Polymer solution was injected with a syringe through an injection canal, as shown in the model. (B) Photograph of wax mold fabricated with PatternMaster for creation of scaffold by injection of PLGA. Parallel cross bars are for support of inner mold parts. Mold inner diameter = 1 cm.