Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 430–442
Biomaterials
www.elsevier.com/locate/biomaterials

Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modified to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord

Andres Hurtado[a], Lawrence D.F. Moon[a], Véronique Maquet[c,d], Bas Blits[a], Robert Jérôme[c,d], Martin Oudega[a,b,*]

[a]*The Miami Project to Cure Paralysis, University of Miami School of Medicine, P.O. Box 016960, R-48, Miami, FL 33136, USA*
[b]*Department of Neurological Surgery, University of Miami School of Medicine, Miami, USA*
[c]*Center for Education and Research on Macromolecules (CERM), University of Liège, Sart-Tilman, 4000 Liège, Belgium*
[d]*Interfacultary Center for Biomaterials, University of Liège, Sart-Tilman, 4000 Liège, Belgium*

Received 25 June 2005; accepted 1 July 2005
Available online 18 August 2005

Abstract

Freeze-dried poly(D,L-lactic acid) macroporous scaffold filled with a fibrin solution containing Schwann cells (SCs) lentivirally transduced to produce and secrete D15A, a bi-functional neurotrophin with brain-derived neurotrophic factor and neurotrophin-3 activity, and to express green fluorescent protein (GFP) were implanted in the completely transected adult rat thoracic spinal cord. Control rats were similarly injured and then implanted with scaffolds containing the fibrin solution with SCs lentivirally transduced to produce express GFP only or with the fibrin solution only. Transgene production and biological activity in vitro, SC survival within the scaffold in vitro and in vivo, scaffold integration, axonal regeneration and myelination, and hind limb motor function were analyzed at 1, 2, and 6 weeks after implantation. In vitro, lentivirally transduced SCs produced 87.5 ng/24 h/10^6 cells of D15A as measured by neurotrophin-3 activity in ELISA. The secreted D15A was biologically active as evidenced by its promotion of neurite outgrowth of dorsal root ganglion neurons in culture. In vitro, SCs expressing GFP were present in the scaffolds for up to 6 h, the end of a typical surgery session. Implantation of SC-seeded scaffolds caused modest loss of spinal nervous tissue. Reactive astrocytes and chondroitin sulfate glycosaminoglycans were present in spinal tissue adjacent to the scaffold. Vascularization of the scaffold was ongoing at 1 week post-implantation. There were no apparent differences in scaffold integration and blood vessel formation between groups. A decreasing number of implanted (GFP-positive) SCs were found within the scaffold during the first 3 days after implantation. Apoptosis was identified as one of the mechanisms of cell death. At 1 week and later time points after implantation, few of the implanted SCs were present in the scaffold. Neurofilament-positive axons were found in the scaffold. At 6 weeks post-grafting, myelinated axons were observed within and at the external surface of the scaffold. Axons did not grow from the scaffold into the caudal cord. All groups demonstrated a similar improvement of hind limb motor function. Our findings demonstrated that few seeded SCs survived in vivo, which could account for the modest axonal regeneration response into and across the scaffold. For the development of SC-seeded macroporous scaffolds that effectively promote axonal regeneration in the injured spinal cord, the survival and/or total number of SCs in the scaffold needs to be improved.

Keywords: CNS regeneration; Schwann cells; BDNF; NT-3; Poly (α-hydroxyacid); Biodegradable; Polymer; Spinal cord injury; Apoptosis; Caspase-3

*Corresponding author. The Miami Project to Cure Paralysis, University of Miami School of Medicine, P.O. Box 016960, R-48, Miami, FL 33136, USA. Tel.: +1 305 243 7161; fax: +1 305 243 9324.
E-mail address: moudega@miami.edu (M. Oudega).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.07.014

1. Introduction

Injury to the adult mammalian spinal cord elicits a cascade of pathophysiological events that results in loss of nervous tissue, and, consequently, in partial or complete loss of neurological functions. For restoration of locomotor function following an injury, damaged axons need to regenerate across and beyond the injury site and form synaptic connections with neurons involved in motor function. Implantation of cells within the injured area may provide the regenerating axons with a substrate that will promote and support axonal growth towards the denervated neurons.

The efficacy of Schwann cells (SCs) to promote axonal regeneration and myelination in the injured adult rat spinal cord has been demonstrated repeatedly [1–4]. Following a complete transection and removal of several millimeters of the spinal cord, grafting a non-degradable polyacrylnitril/polyvinylchloride (PAN/PVC) tubular scaffold filled with SCs that bridged both cord stumps, which were placed within the scaffold, resulted in axonal regeneration and myelination [1,3–5]. The use of a tubular scaffold for cell implantation in the injured cord may be beneficial to the overall axonal growth process by preventing scar formation, allowing accumulation of growth-promoting molecules, and serving as a protective casing for the implant. On the other hand, it can be anticipated that a non-degradable tubular scaffold becomes harmful during the later stages of the growth/recovery response by constricting the spinal cord and/or eliciting a foreign body response. The use of biodegradable scaffolds to implant axonal growth-promoting substrates may circumvent these potential problems.

A widely used biodegradable material in tissue engineering is poly(D,L-lactic acid) (PLA). In vivo and in vitro, PLA and its breakdown products were demonstrated to be biocompatible with spinal cord tissue and with SCs [6]. Implantation of a PLA single-channel tubular scaffold containing SC into the completely transected adult rat spinal cord resulted at first in an axonal regeneration response comparable to that seen with a SC-filled non-degradable PAN/PVC channel [7]. However, in time axonal growth was obstructed due to the collapse of the PLA scaffold. Recently, Patist et al. [8] demonstrated that the implantation of a macroporous PLA tubular scaffold in the transected rat spinal cord elicited a modest axonal regeneration response. These particular scaffolds were prepared by a thermally induced polymer-solvent phase separation process and contained longitudinally oriented macropores connected to each other by a network of micropores [8]. It was proposed that seeding these scaffolds with SCs prior to implantation would be beneficial for the overall axonal growth response [8].

Here, we have filled a freeze-dried PLA macroporous guidance scaffold with genetically modified rat SCs and analyzed cell survival, scaffold integration, and the axonal regeneration response following implantation into the completely transected adult rat thoracic spinal cord. Control rats were implanted with scaffolds filled with a fibrin solution with or without SCs genetically modified to express green fluorescent protein (GFP) for easy and reliable identification of the SCs. Experimental rats were grafted with scaffolds with the fibrin solution with SCs genetically modified to express GFP and, in addition, to produce and secrete a bi-functional neurotrophin (D15A), a molecule with brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) activity [9], to evaluate the effects of elevated levels of neurotrophins within the graft environment [3,10].

2. Materials and methods

2.1. Animals

Female Fischer rats ($n = 37$, 160–180 g; Charles River Laboratories, Wilmington, NC) were housed according to NIH and USDA guidelines. The Institutional Animal Care and Use Committee of the University of Miami approved all animal procedures. Rats were anesthetized using a mixture of ketamine (42.8 mg/ml), xylazine (98.6 mg/ml) and acepromazine (1.4 mg/ml) at 0.06 ml/100 g body weight, intramuscularly. The backs were shaved and aseptically prepared. Lacrilube ophthalmic ointment (Allergen Pharmaceuticals, Irvine, CA) was applied to the eyes to prevent drying and gentamicin (0.03 ml, Buck, Inc., Owings Mills, MO) was given intramuscularly. During surgery the rats were kept on a heating pad to maintain the body temperature at $37 \pm 0.5\,^{\circ}C$.

2.2. Purification of Schwann cells

Highly purified SC cultures were obtained from sciatic nerves of adult female Fischer rats (Charles River Laboratories) as described previously [11]. Dissociated SCs were cultured on poly-L-lysine-coated tissue culture dishes in DF-10S medium supplemented with the mitogens, bovine pituitary extract (2 mg/ml), forskolin (0.8 g/ml), and heregulin (2.5 nM). The addition of heregulin is a modification of the original protocol. To determine the purity of the SCs used for seeding the polymer scaffolds, samples of the harvested cells were plated onto culture dishes, cultured for 3 h, stained for S100, and then coverslipped with Citifluor (UKC Chemical Laboratory, Canterbury, England) with 100 μM Hoechst nuclear dye (Sigma, St. Louis, MO) to compare numbers of S100-positive cells with Hoechst-labeled cells. The purity of the SCs used for seeding the scaffolds was 95–98%.

2.3. Preparation of lentiviral vectors and transduction of SCs

The ViraPower Lentiviral Expression System (Invitrogen) was used to generate lentiviral vectors (LVs) encoding only