Journal of Neuroscience Methods 232 (2014) 84–92

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

A two-compartment organotypic model of mammalian peripheral nerve repair

Rezina Siddique[a,1], Alka Vyas[b,1], Nitish Thakor[a,c], Thomas M. Brushart[b,d,*]

[a] Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Department of Orthopaedic Surgery, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA
[c] SINAPSE Institute, National University of Singapore, Singapore
[d] Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, MD 21287, USA

HIGHLIGHTS

- We describe the first two-compartment organotypic model of peripheral nerve repair.
- Motor axons regenerate through three-dimensional peripheral nerve segments that can be transected and repaired.
- The environment of regenerating axons can be modified without altering that of parent motoneurons.
- This model is ideal for studying the effects of pathway-derived growth factors on regenerating motor axons.

ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 5 May 2014
Accepted 6 May 2014

Keywords:
Regeneration
Organotypic
Spinal cord
Peripheral nerve
YFP

ABSTRACT

Background: Schwann cells in the distal stump of transected nerve upregulate growth factors that support regeneration on a modality-specific basis. It is unclear, however, which of these preferentially support motor axon regeneration. Identification of these factors will require a model that can isolate growth factor effects to growing axons while reproducing the complex three-dimensional structure of peripheral nerve.
New method: A two-compartment PDMS base is topped by a collagen-coated membrane that supports a spinal cord cross-section above one compartment. Fluorescent motoneurons in this section reinnervate a segment of peripheral nerve that directs axons through a water-tight barrier to the second compartment, where nerve repair is performed.
Results: Motoneurons remain healthy for several weeks. The axons they project through the water-tight barrier survive transection and cross a nerve repair in substantial numbers to reinnervate an additional nerve segment. Fluidic isolation of the two compartments was confirmed with a dye leakage test, and the physiologic integrity of the system was tested by retrograde labeling of only those motor neurons to which tracer was exposed and by limitation of toxin effects to a single compartment.
Comparison with existing methods: Nerve repair cannot be modeled in monolayer cell culture. Our previous organotypic model accurately modeled nerve repair, but did not allow individual control of motoneuron and growth cone environments.
Conclusions: This model isolates treatment effects to growing axons while reproducing the complex three-dimensional structure of peripheral nerve. Additionally, it facilitates surgical manipulation of tissues and high-resolution imaging.

1. Introduction

The role of pathway-derived growth factors in promoting motoneuron regeneration is poorly understood. Several growth factors are upregulated by denervated Schwann cells in the distal nerve stump soon after injury (Liu and Snider, 2001). Recently, anatomical and functional subsets of these Schwann cells have

Abbreviations: GDNF, glial cell-line derived neurotrophic factor; DMSO, dimethylsulfoxide; DRG, dorsal root ganglion; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NGF, nerve growth factor; PDMS, polydimethylsiloxane; YFP, yellow fluorescent protein.

* Corresponding author at: Johns Hopkins Orthopaedics, 601 N. Caroline Street, Baltimore, MD 21287, USA. Tel.: +1 410 955 9663; fax: +1 410 502 6816.
E-mail address: tbrusha@jhmi.edu (T.M. Brushart).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jneumeth.2014.05.005
0165-0270/

been characterized by unique growth factor profiles that have been found to support regeneration of sensory and motor axons on a modality-specific basis (Höke et al., 2006; Brushart et al., 2013). Although upregulation of several growth factors differs between sensory and motor nerve, it is not clear which of these factors could be responsible for the modality-specific support of motor axon regeneration. Identification of these factors will require a model that can both localize growth factor effects to growing axons and duplicate the complex three-dimensional architecture of peripheral nerve.

Growth factor effects on regeneration are isolated most easily *in vitro*. Cell culture devices such as the Campenot chamber and its microfluidic counterparts are able to isolate growth factor effects to the growing axon. However, the three-dimensional configuration of extracellular matrix components is especially difficult to model *in vitro* (Tucker et al., 2006). As a result, currently available techniques cannot reproduce the three dimensional structure of nerve, and thus cannot model nerve repair accurately (Campenot, 1977; Park et al., 2006; Yang et al., 2009).

Attempts to determine the role of pathway-derived growth factors *in vivo* are hampered by the complexity of the peri-axonal environment and by the paucity of relevant conditional knockout mice. Growth factors are produced not only by Schwann cells, but also by infiltrating macrophages, central glia, neurons that synapse on the regenerating motoneuron, and by the neuron itself. These growth factors can also have multiple effects that influence regeneration indirectly, such as promoting neuronal survival, signaling axonal injury to the neuron, and modulating Schwann cell behavior during Wallerian degeneration (Makwana and Raivich, 2005). Clearly, there is a need for a platform that selectively controls the growth factor environment within the three-dimensional structure of peripheral nerve. To address this need, our lab developed the first *in vitro* model of adult mammalian nerve repair in an organotypic co-culture system (Vyas et al., 2010). Organotypic cultures are prepared from nervous tissue without dissociation, and thus preserve the three dimensional cytoarchitecture within both spinal cord and peripheral nerve (Rothstein et al., 1993; Gähwiler et al., 1997). Additionally, organotypic culture of motoneurons overcomes the difficulties encountered when maintaining these cells in a monolayer environment (Kaal et al., 1997).

In our previously described *in vitro* model of nerve repair spinal cord sections from mice expressing yellow fluorescent protein (YFP) in their motoneurons were co-cultured with freshly-harvested segments of peripheral nerve (Vyas et al., 2010). To reconstruct ventral roots, these nerve segments were opposed to the ventral portion of the spinal cord section adjacent to the motor neuron pool to promote the ingrowth of YFP-expressing motor axons. After a week in culture, once the new ventral roots had been reinnervated, they were transected and nerve repair was performed by opposing their cut ends to freshly-harvested nerve grafts. As initially described, organotypic cultures were grown on a Transwell© collagen-coated insert within a 6-well plate. The height of the Transwell© enclosure compromised our ability to perform microsurgery on the cultured tissue and to achieve the working distances required for high resolution imaging. The Transwell© construct is designed to be imaged from below; image quality is degraded by the fluid and plastic beneath the membrane, and magnification is limited by the distance between lens and fluorescent tissue. Additionally, this construct did not permit selective manipulation of the nerve repair environment without simultaneously altering that of the parent neuron.

To overcome the physical limitations of the Transwell© construct, the walls of the membrane insert were shortened to increase mechanical access to the membrane for microsurgery and imaging. Fluidic isolation of motoneuron and regeneration compartments was obtained by replacing the 6-well plate with a low-profile two-compartment poly(dimethylsiloxane) (PDMS) base. Motor axons were conveyed from the motoneuron compartment into the nerve repair compartment through reconstructed ventral root that passed through a water-tight barrier. The result of these modifications is a biocompatible organotypic system that facilitates tissue manipulation and photography while permitting individual control of motoneuron and nerve repair environments. Growth factor effects can be studied in each compartment by adding growth factors or by blocking growth factor function with antibodies or siRNA. This model also has the potential to facilitate studies of Wallerian degeneration, myelination, and axonal pathfinding.

2. Materials and methods

2.1. Fabrication of compartmentalized culture system

The completed device consists of two principle components: a PDMS reservoir for culture medium that is divided into two compartments by a central partition, and a superimposed Transwell© membrane (Corning, Acton, MA) that provides a surface for the growth of spinal cord and peripheral nerve co-cultures. The dimensions of these components are illustrated in Fig. 1.

PDMS reservoirs are replicas from a master mold created by micromachining a negative relief of the device design into aluminum. Aluminum provides a robust surface for molding that is both reusable and economical. The dimensions of the PDMS reservoir are determined by the configuration of the Transwell© insert, the space required for media supply and exchange around the periphery of the insert, and the PDMS surface area required for adhesion to a glass substrate (Fig. 1). The outer wall of the reservoir is circular with a 38 mm outer diameter and 33 mm inner diameter. The Transwell© is suspended over the media by the central partition, and by two steps placed at 90 degrees to the axis of the partition. The partition is 28 mm long and 1 mm tall, and bisects the device into two separate compartments. In order to provide adequate support for the membrane and to ensure bonding to the glass substrate, the partition is 5 mm wide along most of its length. At the center of the device, a 3 mm-long segment of the partition narrows to 2 mm wide to maximize media circulation to the nerve as it passes through the grease barrier between compartments (Fig. 1B). To minimize sagging of the membrane beneath the spinal cord section, the central narrow segment of the partition is flanked by two 1 mm-wide buttresses that extend perpendicular to its axis. A 0.25 mm thick film of PDMS connects the base of the buttresses to enhance their bonding to the glass beneath, and to promote the flow of media beneath the membrane so that bubbles do not form under the spinal cord explant.

The Transwell© membrane receives additional support from two 5 mm-wide peripheral steps that are aligned on an axis at 90 degrees to that of the partition (Fig. 1A). The lower stair step, which supports the Transwell© insert, is 1 mm above the floor of the reservoir, and thus in the same plain as the surface of the partition; the upper step is the full height of the device, and limits horizontal movement of the Transwell©. The inner edge of the upper step extends 2.5 mm horizontally from the inner wall, creating a space 28 mm in diameter for the Transwell©. Once a Transwell© is placed into the device, the spaces around the Transwell© between the steps and the partition serve as access points for media exchange.

Cross-linked PDMS (sold as Sylgard 184, Dow Corning, Midland, MI) is used to make replicas of the reservoir using standard soft lithography techniques as described in detail elsewhere (Xia and Whitesides, 1998). Fig. 2 summarizes the device process flow. Briefly, the base and crosslinker are mixed thoroughly in a ratio of 10:1, and placed under vacuum to remove bubbles. The PDMS is