ABSTRACT

INTRODUCTION

Conventional histologic assessment techniques of neural regeneration such as toluidine blue staining or immunohistochemistry for neural markers are expensive, laborious, time-intensive, and can involve hazardous chemicals. Additionally, subjectivity is a concern as distinguishing nerve from surrounding tissue can be problematic in the distal segment of regenerating nerve.

Transgenic murine models exist that enable stain-free axon counts, and these reporter models can be combined with the use of modern microscopy techniques to accelerate and streamline the testing of peripheral nerve conduits.

METHODS

A rapid, non-toxic, and stain-free frozen section protocol suitable for assessment of neural regeneration by brightfield and confocal laser scanning microscope was developed. Interposition repair of a sciatic nerve defect in Thy1:2YFP-16 mice was performed using various types of biocompatible neural conduits, and regeneration assessed at six weeks.

RESULTS

Processing time for axon counting was shortened from two weeks to three days, and stain costs eliminated. Confocal fluorescent microscopy images revealed excellent morphology of regenerating axons, with clear elucidation of permissive vs. restrictive conduit environments.

CONCLUSIONS

A rapid and cost-efficient platform for assessment of neural regeneration suitable for testing of novel neural conduit designs has been described.

INTRODUCTION

Histologic assessment is fundamental to the study of nerve regeneration. Hematoxylin and eosin (H&E) and Masson's trichrome staining (TB) are used to visualize extracellular matrix staining. Conventional techniques for nerve histomorphometry include osmium tetroxide (OsO4) of paraffin or frozen sections.

Conventional methods are labor-intensive, involve hazardous agents, and provide images that may be difficult to interpret and quantify. Transgenic fluorescent reporter models exist to enable stain-free imaging of axons (e.g. Thy1:YFP mouse, Thy1-GFP rat). Frozen section techniques may be used for rapid processing of transgenic nerve, with visualization using wide-field or scanning fluorescent microscopy.

METHODS

Subjects and Surgery

• Five adult female B6.Cg-Tg[Thy1:YFP(m31m)] mice used

• Proprietary conduits designed with a chitosan-based nerve and collagen core obtained from the Dartmouth School of Engineering.

• Animals underwent 15 minute surgery under isoflurane with appropriate peri-operative anesthetics.

• Left hind limb shaved, inoculated, and dissected to find the sciatic nerve (Fig. 1A-B).

• Sciatic n. transected, and 7mm conduit placed (Fig. 1C).

• Single suture repair performed at both ends with 10-0 nylon.

• Conduit nerve complexes harvested at 6 weeks after perfusion.

Histology

• Post fixation in 2% PFA, 30% Sucrose dehydration

• Snap frozen in OCT with liquid nitrogen

• Samples transected in half lengthwise, followed by longitudinal cryosectioning of the proximal end and axonal sectioning of the distal end into 20 µm sections.

• Fluoromyelin staining (1:300) per protocol.

• Imaging

• Leica SP8 Confocal Microscopy at 40x magnification

• Z-stacks displayed as maximum projections

• Contrast enhancements in Fiji Image software.

REFERENCES


RESULTS AND DISCUSSION

• Processing time for axonal assessment was shortened from 2 weeks to three days, and stain costs eliminated.

• Excellent qualitative morphological detail of axonal regeneration patterns is observed.

• Due to their tendency towards robust neural regeneration, use of rodents to study nerve conduit performance has been called into question.

• Herein we demonstrate that discrimination between permissive and repulsive conduit environments may be clearly visualized in a transgenic murine model.

• Histological assessment is a critical adjunct to functional assessment of conduit performance in rodent models as axons may grow around a conduit and evade the core.

• The present model is more efficient than conventional techniques using rats in that:

• Multiple conduit designs can be feasibly assessed in a short time frame and with limited resources

• Conduct performance can be assessed as early as 3-4 weeks after implant

• Smaller footprint (5 mice per cage)

• Less labor-intensive tissue processing

• Leg autotomy rate in mice is low, minimizing subject loss

• Quantitative analysis of axon regeneration is facilitated by label expression only within viable axons

CONCLUSIONS

A rapid and cost-efficient platform for assessment of neural regeneration suitable for testing of novel neural conduit designs has been described.

A High-Throughput Mouse Platform for Nerve Conduit Assessment

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Figure 1. (A) Schematic of Thy1:YFP mice used for sciatic nerve transection. Intraoperative photos of (B) Left sciatic nerve exposed, and (C) Post-transaction and conduit placement.

Figure 2. (A), (C), (E) Proximal longitudinal sections of conduits. (B), (D), (F) Distal axial sections. Permissive nature of chitosan shell-only conduit seen as axons traverse through conduit in longitudinal section (A2), with evidence of axonal penetration to distal segment (B2). Conversely, a growth-restrictive environment is seen in shell + core conduits (C2-F2). One model of growth restriction shows a 180 degree reversal of growth out of the conduit (C2), reflected by sparse axonal penetration into the distal conduit (D2). An alternate restrictive pattern shows evasion of the core with splaying of axons around the periphery (E2), corroborated by nearly no axons seen at the distal conduit (F2). Fluoromyelin Red was seen to only stain not myelin (not shown), but act as an effective counterstain for labeling of the conduit shell and core. YFP (Axons) – yellow, Fluoromyelin Red (Background) – Red. Scale bar, 500 µm.