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A Novel Conduit-Based Coaptation Device for Primary Nerve Repair

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Abstract

Background: Conduit-based nerve repairs are commonly used for small nerve gaps, whereas primary repair may be performed if there is no tension on nerve endings. We hypothesize that a conduit-based nerve coaptation device will improve nerve repair outcomes by avoiding sutures at the nerve repair site and utilizing the advantages of a conduit-based repair.

Methods: The left sciatic nerves of female Sprague-Dawley rats were transected and repaired using a novel conduit-based device. The conduit-based device group was compared to a control group of rats that underwent a standard end-to-end microsurgical repair of the sciatic nerve. Animals underwent behavioral assessments at weekly intervals post-operatively using the sciatic functional index (SFI) test. Animals were sacrificed at four weeks to obtain motor axon counts from immunohistochemistry. A sub-group of animals were sacrificed immediately post repair to obtain MRI images.

Results: SFI scores were superior in rats which received conduit-based repairs compared to the control group. Motor axon counts distal to the injury in the device group at four weeks were statistically superior to the control group. MRI tractography was used to demonstrate repair of two nerves using the novel conduit device.

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Conflict of Interest Statement:

The authors declare that there is no conflict of interest.

Statement of Animals Rights:

All institutional and national guidelines for the care and use of laboratory animals were followed.

Conclusions: A conduit-based nerve coaptation device avoids sutures at the nerve repair site and leads to improved outcomes in a rat model. Conduit-based nerve repair devices have the potential to standardize nerve repairs while improving outcomes.

Keywords

Microsurgery; Nerve injury; Nerve repair; Nerve conduit; Diffusion tensor imaging

Introduction

Nerve repair technique is dependent upon the mechanism of injury and the length of the gap between severed nerve endings. The most optimal method is a primary end-to-end anastomosis with no tension¹. However, a primary nerve repair not only requires an experienced microsurgeon but also multiple sutures at the repair site. The use of sutures at nerve repair sites has been correlated with increased scar tissue and decreased conductance^{2,3}. Avoidance of sutures at the repair site could potentially improve outcomes after peripheral nerve injury.

Conduit-based nerve repair has become a novel method for short gap repairs. The concept of nerve conduits is to create a microenvironment that promotes nerve growth in a specific and directed manner⁴. However, nerve conduits have not been investigated in their utility in primary repair. Nerve conduits have been extensively investigated and found to be clinically effective, easy to use, and non-inflammatory⁵⁻⁷. A method incorporating the gold standard of primary nerve repair and a nerve conduit could represent a novel technique in nerve repair.

The purpose of this study was to investigate a nerve repair device with a nerve conduit that avoids sutures at the coaptation site. The nerve conduit provides an environment that promotes nerve growth, and avoiding sutures at the coaptation site helps reduce inflammation of the repair site. In addition, developing a nerve coaptation device could help standardize nerve repair and improve outcomes.

Materials and Methods

Experimental Animals

Adult female Sprague-Dawley rats (250g; Charles River Laboratories, Wilmington, MA) were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at our institution. Animals were supplied with water and food (Purina Rodent Diet) at all times. Animal cages were inspected daily to ensure animal health and cleanliness of housing by the veterinary staff and surgical research personnel.

Experimental Design

Eighteen female Sprague-Dawley rats were divided into two groups (Figure 1). A sciatic nerve injury model was used, and all rats underwent a complete nerve transection with an immediate nerve repair. The control group received a standard microsurgical repair. The experimental group received a repair with a nerve coaptation device.

Nerve coaptation device

The nerve coaptation device was hand assembled and consisted of a porcine sub-mucosal extracellular matrix nerve tube (Axogen, Alachua, FL, USA). A novel pulley system was constructed within the nerve tube (Figure 2). The device required two sutures placed 180° apart on both nerve endings. After sutures were placed and tied down to the nerve endings, the nerve could be manipulated into the nerve conduit. The nerve endings were drawn into the conduit until they were aligned with each other. The nerve conduit was designed to be larger than the nerve itself. The nerve conduit was then cinched down on the nerve to ensure an adequate end-to-end anastomosis with good alignment.

Surgical Procedures

After anesthesia with inhaled 2% isoflurane, a 2 cm incision was made parallel and just posterior to the left femur. The cephalad border of the biceps femoris was freed to allow for optimal exposure to the left sciatic nerve. The nerve was irrigated with Plasmalyte-A (Baxter: Deerfield, IL). A complete transection of the left sciatic nerve was made to imitate a Sunderland type V nerve injury, and the wound was irrigated with PlasmaLyte-A.

Using microsurgical technique, a standard end-to-end nerve repair was performed using 9–0 ethilon (Ethicon, Somerville, NJ, USA) for control animals. For experimental animals, the repair was performed using our novel nerve coaptation device. Using the coaptation device, the nerve endings were aligned together, similar to a standard repair.

After the nerve repair was completed, the wound was irrigated with Lactated Ringer's (Hospira, Lake Forest, IL, USA). The biceps femoris and skin were re-approximated using a 5–0 monocryl suture (Ethicon, Somerville, NJ, USA). All survival animals received a subcutaneous injection of ketoprofen (5mg/kg) and were allowed to recover from anesthesia.

After recovery, the animals were returned to the housing facility and monitored for weight loss, infection, and other morbidities. At four weeks, animals were again anesthetized, and nerve harvests were performed. After nerve harvest, the animals were euthanized via intracardiac injection of euthasol (euthanasia solution).

Sciatic Functional Index

Behavioral assessments using sciatic functional index (SFI) were performed at three days and weekly intervals post-operatively. SFI is a measure of sciatic function that includes both sensory feedback and motor control. The animals traversed a walking beam after their hind legs were inked, and six consecutive footprints were recorded. Print length (NPL/EPL), toe spread (NTS/ETS), and intermediary toe spread (NIT/EIT) were measured for both the normal and experimental limbs. SFI scores were calculated using the following formula: $SFI = -38.3([EPL-NPL]/NPL) + 109.5([ETS-NTS]/NTS) + 13.3([EIT-NIT]/NIT) - 8.8$.

Histology

At four weeks post-operatively, the sciatic nerve was harvested for histological analysis of motor axon counts. A 2 cm segment was harvested, and proximal and distal sections were fixed for immunohistochemistry.

Immunohistochemical staining was performed using commercial antibodies specifically directed against choline acetyltransferase (Millipore, Temecula, CA, USA). Choline acetyltransferase staining is positive in motor neurons. Formalin-fixed paraffin embedded tissues were sectioned at 5 μ m, placed on slides and warmed overnight at 60° C. Slides were deparaffinized and rehydrated with graded alcohols ending in Tris buffered saline (TBS-T Wash Buffer, LabVision, Freemont, CA, USA). For choline acetyltransferase staining, heat mediated target retrieval was performed in 1X Target Retrieval Buffer (DAKO, Carpinteria, CA, USA). Endogenous peroxidases were blocked as before. Non-specific background, secondary, and tertiary labeling of target was accomplished by use of Vector's ABC Elite Goat IgG kit (Vector Laboratories, Burlingame, CA, USA). Primary antibody to choline acetyltransferase was used at 1:200 for 1 h. Slides were rinsed with TBS-T between each reagent treatment and all steps were carried out at room temperature unless otherwise noted. Visualization was achieved with DAB+ chromogen (DAKO). Slides were counterstained with Mayer's hematoxylin, dehydrated through a series of alcohols and xylenes, and then coverslipped with Acrytol Mounting Media (Surgipath, Richmond, IL, USA).

We examined all stained slides using an Olympus Vanox-TAH-2 light microscope (Olympus, Center Valley, PA, USA) interfaced to a Pixera Pro 600 HS digital camera (Pixera Corporation, Santa Clara, CA, USA). We captured multiple digital photomicrographs at 10x using Viewfinder V3.0.1 (Pixera Corporation). For each nerve processed, we photographed representative cross-sections proximal to the site of injury and distally in the sciatic nerve. To count axons, the number of stained axons on each photomicrograph, we used Fiji, an open source image processing software with a method that has been previously reported⁸. We determined the total number of axons per cross-section by adding the axon totals from all representative photomicrographs or a given cross-section; we took care to avoid double-counting axons.

Magnetic Resonance Imaging

A subset of animals had nerves imaged with magnetic resonance imaging (MRI) post-operatively (n=3 in each group). Nerves were harvested immediately after repair for imaging. After 24 h of post-fixation, excised nerves were placed in PBS + 2 mM gadopentetic acid (Gd-DTPA) (Magnevist, Bayer HealthCare, Wayne, NJ, USA) at 4 °C for at least 24 h before imaging and trimmed to ~1 cm in length with the injury site centered. MRI was performed on a 9.4T 21-cm horizontal bore Agilent DirectDrive scanner (Agilent Technologies, Santa Clara, CA, USA) using a 38-mm Litz quadrature coil (Doty Scientific, Columbia, SC, USA) for radio frequency (RF) transmission and reception. Diffusion tensor imaging (DTI) data were acquired using a three-dimensional diffusion-weighted spin-echo sequence. Image data reconstruction was performed using in-house written code in MATLAB (Mathworks, Natick, MA, USA). Diffusion tensor estimation and tractography were performed using ExploreDTI (exploredti.com).

Statistical Analysis

Appropriate statistical analysis including student's t-test and two-way analysis of variance with the Bonferroni multiple comparison method were employed to specifically compare treatment and control groups. All *p* values were two-tailed where appropriate, and

significance was determined at $p < 0.05$. The sample size ($n=5$) was calculated for a power of 80% to detect a 200 axon difference between each group.

Results

Nerve coaptation

Figure 3 demonstrates the steps of the nerve coaptation device. The device was easily sutured to the cut nerve endings. Only two sutures were required for coaptation, and the cinching mechanism of the device ensured an adequate end-to-end anastomosis.

Sciatic Functional Index

Behavioral assessments were evaluated at three days and at weekly intervals post-operatively via the SFI. Animals that underwent nerve repair with our novel device demonstrated superior SFI scores compared to standard repair. The difference reached statistical significance at week 3 and remained superior at week 4. Figure 4 demonstrates SFI scores of both groups through four weeks.

Immunohistochemistry

Motor axon counts were increased distally in nerves repaired with our novel repair device ($p = 0.03$). As expected, there was no difference in motor axon counts proximally in the nerve between the two groups. (Figure 5). Figure 6 shows representative photomicrographs of these nerve segments.

MRI

Figure 7 demonstrates MRI tractography of two nerves using the novel conduit device. The repaired tracts appear continuous, and this finding indicates good alignment with our device.

Discussion

Our study demonstrates a potential new strategy for nerve repair. Our novel conduit-based nerve repair device avoids sutures at the anastomosis and introduces a method for using a nerve conduit in a primary repair of a nerve. In this study, rat behavioral studies and histology were superior in the group using our nerve repair device compared to control repairs.

Even though the most common method for nerve repair is a primary anastomosis, surgeons are becoming increasingly interested in alternative methods for repair⁹. A popular method for nerve repair is fibrin glue, which is touted as a quicker and easier alternative to microsutures. A recent systematic review of fibrin glue nerve repair found most studies favoring fibrin glue over microsutures¹⁰. However, fibrin glue repairs have been cited for lacking tensile strength and dehiscence of repairs^{11,12}. Thus, at this time there is no method that has replaced a microsurgical repair.

Despite being the gold standard, microsurgical repair may have many disadvantages. Histopathologic examination of nerves that have been primarily repaired show degenerative nerve bundles and prominent granulomas². Primary microsurgical repairs even when

performed well may be under tension secondary to the traumatic nature of nerve injury¹³. Additionally, microsurgical repairs are time-consuming and require certain expertise, which is often not available. With these considerations, it would be beneficial to develop new strategies for nerve repair.

A conduit-based nerve repair device offers a few advantages compared to other current nerve repair strategies. One is that it avoids microsutures at the nerve repair site. This should help avoid scarring and inflammatory changes that may impede nerve growth. Secondly, it utilizes a proven nerve conduit which promotes axonal growth and protects the repair site. Finally, the device does not require an experienced microsurgeon to use.

Utilizing nerve conduits is an ideal method for nerve repair because nerve conduits have the potential to offer an ideal environment for nerve growth¹⁴. Nerve conduits are most often used for nerve gap repairs⁴, but extending the use of nerve conduits may be beneficial for nerve repair. Nerve conduits provide a better environment for nerve outgrowth and offer protection from the inflammation of the surrounding injury. Our study did not address a potential gap in nerve repair, but this device may be useful for repairing nerves and leaving a gap in order to avoid tension at the repair site. However, this will need to be investigated further.

There are some limitations to our current study, and this warrants further inquiry. Our current investigation was in a rat sciatic nerve injury model, and even though there were statistically superior outcomes with our device, this will need to be confirmed in human and larger nerve studies. Rodent models offer similar nerve physiology and are accepted translational models, but clinical studies are required. Our trial had a relatively small sample size, and yet statistically significant outcomes were discovered. A large tier study may uncover further conclusions about a conduit based repair device. Finally, in order to make conclusions about the immune response of a device that avoids sutures at the repair site, a study that investigates the immune response at the nerve repair site is required. Unfortunately, study of the pathology of the repair site was outside the scope of this small study, but it will serve as the focus of future studies.

Despite our limitations, this study provides insight into a new method for nerve repair. Use of a nerve repair device has many advantages, and given current dismal outcomes of peripheral nerve injury, new approaches to nerve repair may provide better outcomes. Our current study presented a conduit-based nerve coaptation device that avoids sutures at the nerve repair site and leads to improved outcomes in a rat model. Development of a nerve repair device such as the one presented here, may help standardize nerve repairs and outcomes.

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Figure 1:
Study Design

Figure 2:
Schematic drawing of novel nerve repair device

Figure 3:
Steps for nerve coaptation (a) placement of device prior to repair (b) nerve device after placement of sutures (c) nerves being pulled into the conduit (d) final coaptation of nerve

Figure 4:
Sciatic Functional Index

Figure 5:
Motor Axon Counts

Figure 6:
Representative photomicrographs of motor axons of distal nerves (a) control (b) device

Figure 7:
MRI tractography of nerves repaired with novel device.