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Side-to-side nerve bridges reduce muscle atrophy after peripheral nerve injury in a rodent model

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ABSTRACT

Background: Peripheral nerve injury can result in muscle atrophy and long-term disability. We hypothesize that creating a side-to-side bridge to link an injured nerve with a healthy nerve will reduce muscle atrophy and improve muscle function.

Methods: Sprague-Dawley rats were divided into four groups ($n = 7$ per group). Group 1: transection only—a 10-mm gap was created in the proximal tibial nerve; group 2: transected plus repaired—the transected tibial nerve was repaired; group 3: transected plus repaired plus nerve bridge—transected nerve repaired with a distal nerve bridge between the tibial and peroneal nerves *via* epineurial windows; and group 4: transected plus nerve bridge—transected tibial nerve left unrepaired and distal bridge added. Gait was assessed every 2 wk. At 90 d the following measures were determined: gastrocnemius mass, muscle and nerve nuclear density, and axonal infiltration into the nerve bridge.

Results: Groups 3 and 4 had greater improvements in walking track recovery than groups 1 and 2. Group 3's gastrocnemius muscles exhibited the least amount of atrophy. Groups 1, 2, and 4 exhibited greater histologic appearance of muscle breakdown compared with group 3 and control muscle. Finally, most bridges in groups 3 and 4 had neuronal sprouting *via* the epineurial windows.

Conclusions: Our study demonstrated reduced muscle atrophy with a side-to-side nerve bridge in the setting of peripheral nerve injury. These results support the application of novel side-to-side bridges in combination with traditional end-to-end neurorrhaphy to preserve muscle viability after peripheral nerve injuries.

1. Introduction

There is an enormous clinical need for improved outcomes after nerve repair in the setting of trauma, fractures, and nerve plexus injuries [1,2]. Axonal regeneration is a slow process occurring at approximately 1 mm/d [3]. In the case of proximal peripheral nerve injury, the process of regeneration and reinnervation may take years because of the large distance from

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the point of injury to the site of distal muscle innervation. During this time, the denervated end organs undergo histologic changes that are consistent with muscle atrophy and infiltration of fibrous tissue [3]. These changes lead to impaired physiological function, resulting in long-term disabilities.

Currently, the gold standard for repairing nerve gaps are autologous nerve grafts. Unfortunately, this technique is associated with significant donor site morbidity, neuromas, and variable ability to prevent muscle atrophy at the end organ. One strategy to minimize donor site morbidity is to use synthetic conduits, which can bridge short gaps. Present research aims to increase the gap length that can be successfully bridged by using growth factors, stem cells, or a combination of the two along with synthetic conduits [4]. Preliminary results using these enhanced conduits are promising, however, distant muscle atrophy remains a problem.

Another strategy to minimize muscle atrophy is the coaptation of an injured nerve to a healthy donor nerve in an end-to-side fashion. This technique can reinnervate a paralyzed muscle and minimize muscle atrophy. The use of end-to-side neurorrhaphy has resulted in improvements in muscle mass through motor neuron collateral sprouting *via* an epineurial window on the healthy donor nerve to the recipient nerve [5–7]. As an extension of end-to-side nerve coaptation, we propose that connecting a healthy donor nerve to an injured nerve in a side-side fashion *via* a synthetic conduit will result in similar improvements in muscle mass. In the setting of a proximally injured and repaired peripheral nerve, these side-to-side connections may function to babysit the muscle during which time the damaged nerve is regenerating. We have therefore designed the present study to combine the traditional end-to-end neurorrhaphy with a distal side-to-side synthetic nerve bridge. We hypothesize that connecting a healthy nerve to a proximally damaged nerve *via* a distal side-to-side nerve bridge through epineurial windows will reduce muscle atrophy during which time the damaged nerve is regenerating. This combined method is projected to reduce muscle atrophy and improve muscle function.

2. Methods

2.1. Surgical procedures

All procedures were approved by the University of Utah Institutional Animal Care and Use Committee (09-09017). A total of 28 male Sprague-Dawley rats weighing 350–400 g were used for this study. The rats were anesthetized using ketamine 50 mg/kg and xylazine 5 mg/kg by intramuscular injection in the contralateral hind limb [6]. The surgical area was shaved and prepared with betadine. A longitudinal incision was then made in the posterior distal thigh of the hind limb, separating the natural plane between the vertebral head of the biceps femoris and superior gluteal muscles. Under the operative microscope, a 2-cm segment of the sciatic nerve was isolated at its bifurcation into the tibial and peroneal nerves. At this point, the methods differed between the four groups as follows:

Group 1 ($n = 7$): Transection only—a 1-cm segment of the proximal tibial nerve was resected and the free ends were left unrepaired. The incision was then closed (Figs. 1A and 2A).
Group 2 ($n = 7$): Transected plus repaired—the proximal tibial nerve was transected just distal to the bifurcation and repaired with 9-0 nylon epineurial sutures in an end-to-end fashion. The incision was then closed (Figs. 1B and 2B).
Group 3 ($n = 7$): Transected plus repaired plus side-to-side nerve bridge—the proximal tibial nerve was transected and repaired as described in group 2. After this, epineurial windows were created on the tibial and peroneal nerves 1 cm distal to the repair site. A 1.5-mm diameter and 5-mm long collagen nerve guide (NeuraGen; Integra, Plainsboro, NJ) was sutured with 9-0 nylon sutures to the epineurial windows creating a side-to-side nerve bridge. Two sutures were placed at each window and no deliberate axotomy was performed to the donor nerves. The incision was then closed (Figs. 1C and 2C).
Group 4 ($n = 7$): Transected plus no repair plus side-to-side nerve bridge—the proximal tibial nerve was transected and left unrepaired with a 1-cm gap as in group 1. Then, as described in group 3, a side-to-side nerve bridge was created by connecting the peroneal and tibial nerves with a collagen nerve guide (Figs. 1D and 2D).

All animals were given a subcutaneous injection of carprofen (5.0 mg/kg; Rimadyl, Pfizer, New York, NY) after surgery for analgesia and every 12 h after surgery if the signs of pain still persisted (up to three doses total). At 90 d postoperatively, the animals were sacrificed.

2.2. Walking track analysis

Preoperatively and every 2 wk after surgery, a functional assessment of the animals' gait was performed as previously

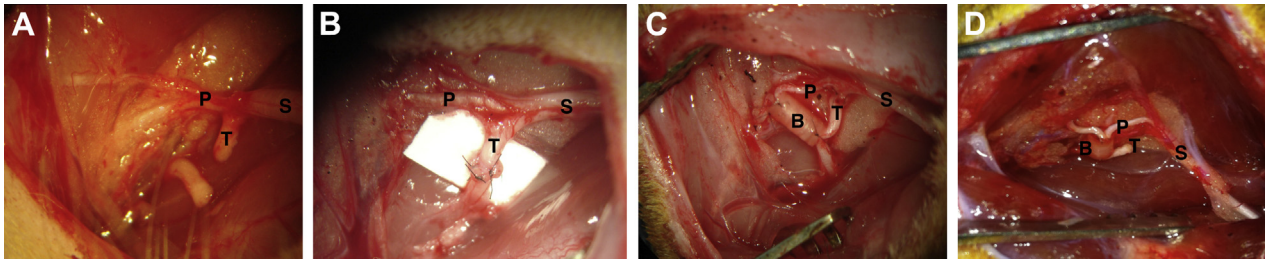

Fig. 1 – Photographs of the three different experimental groups. (A) Group 1: transected tibial nerve (T). (B) Group 2: tibial nerve (T) transected and then repaired. (C) Group 3: tibial nerve (T) transected and repaired combined with a side-to-side bridge between the tibial (T) and peroneal (P) nerves. (D) Group 4: tibial nerve transected and side-to-side nerve bridge. S = sciatic nerve; T = tibial nerve; P = peroneal nerve; B = side-to-side bridge. (Color version of figure is available online.)