Motor Nerve Architecture and Peripheral Nerve Regeneration

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Abstract

Purpose: Sensory nerve autografting is the standard of care for injuries resulting in a nerve gap. Recent work demonstrates inferior regeneration with sensory grafts compared to motor nerve graft repairs. Improved regeneration with motor grafting may be a result of either the nerve's cellular content, or architecture. To define the role of nerve architecture, our study evaluates regeneration through acellular motor and sensory nerve grafts. ***Methods:*** Twenty-four Lewis rats underwent tibial nerve repairs with 5mm motor or sensory nerve cable isografts. Grafts were harvested and acellularized with 7 weeks of cold preservation in University of Wisconsin solution, leaving only the laminin ultrastructure of the Schwann cell (SC) basal lamina tubes. Control animals received fresh motor or sensory isografts. Nerves were harvested after 4 weeks and histomorphometric analysis of the regenerating nerves was conducted for comparison. ***Results:*** Histomorphometric analysis distal to the repair revealed more robust nerve regeneration in both acellular and cellular motor grafts. In contrast, sensory graft groups showed poor regeneration with significantly decreased percent nerve, nerve fiber count, and density when compared to the motor graft groups ($P < 0.05$). ***Conclusions:*** Nerve architecture plays an important role in nerve regeneration through grafts of differing modalities. Motor nerves have larger SC basal lamina tubes allowing more nerve fibers to cross a nerve graft. The importance of nerve architecture may partly explain the suboptimal clinical results seen with sensory nerve grafting techniques.

Introduction

Sensory nerve autografting is currently the accepted technique for reconstruction of peripheral nerve defects despite suboptimal functional recovery[1]. In the rodent model, motor nerve grafts result in improved nerve regeneration as compared to sensory nerve grafts[2,3]. The governing mechanisms for this phenomenon are not well understood. To evaluate the role of nerve cellularity alone, we used silicone conduits filled with minced motor, sensory, or mixed nerves to repair a sciatic nerve defect in a rat model. With disrupted graft architecture but intact cellularity (i.e., SCs) the benefit seen with motor graft material was lost[4] implying that nerve architecture (SC basal lamina tube size) may play an important role in nerve regeneration. Schwann cell basal lamina tubes are smaller in sensory nerves than in motor nerves (Fig 2A). Cold preservation in University of Wisconsin solution (UW) for 7 weeks completely decellularizes nerve grafts, while leaving intact the basal lamina tube architecture and laminin in the extracellular membrane[5]. Thus, this study evaluates the impact of nerve architecture (size of Schwann cell basal lamina tubes) by studying nerve regeneration across acellular motor and sensory grafts.

Methods and Materials

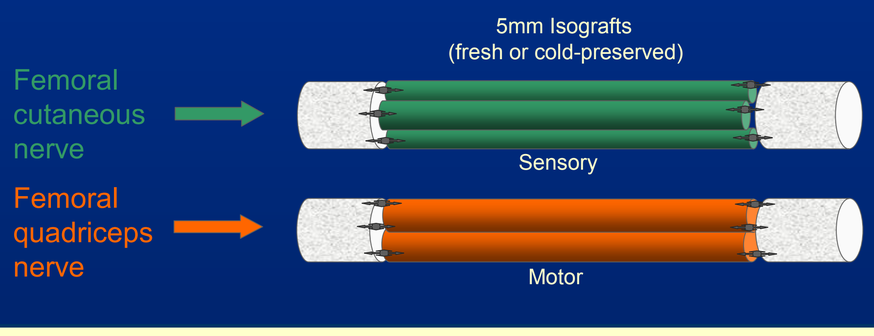
Experimental Design: Twenty-four rats were randomized to each of 2 fresh graft recipient groups and 6 animals each were randomized to 2 acellular graft recipient groups. An additional 18 animals served as nerve graft donors. In all experimental animals, a 5 mm tibial nerve gap was created and immediately reconstructed with a nerve cable graft of equivalent length. Experimental groups were defined based upon the cellularity and composition (motor or sensory) of cable grafts used for reconstruction. Motor nerve grafts were derived from the femoral motor branch to the quadriceps and sensory nerve grafts were derived from the femoral saphenous branch.

Six animals per group were sacrificed at 4 weeks, and the nerve tissue was harvested for histomorphometric analysis. An additional 6 animals in the fresh nerve graft groups were used for weekly walking track analysis and at a 6 week endpoint, bilateral gastrocnemii were harvested for wet weight ratios as a measure of functional recovery (Table I).

Table I: Experimental Design

Group	N	Description	Outcome Measure(s)	Endpoint
I	12	Fresh Sensory Isograft	6: Histomorphometry	4 weeks
			6: Weekly walking tracks, wet muscle mass ratios	6 weeks
II	12	Fresh Motor Isograft	6: Histomorphometry	4 weeks
			6: Weekly walking tracks, wet muscle mass ratios	6 weeks
III	6	Cold Preserved Sensory Isograft	All: Histomorphometry	4 weeks
IV	6	Cold Preserved Motor Isograft	All: Histomorphometry	4 weeks

Figure 1. Sensory and motor cable graft repair of the tibial nerve



Operative Procedures: Surgical procedures were performed under sterile conditions with the aid of a Wild M651 operating microscope. Animals were anesthetized by subcutaneous injection of a mixture of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg). All procedures were approved by the institutional animal studies committee.

For donor nerve harvests, an oblique cutaneous groin incision was made and the saphenous nerve (sensory) and the motor branch to the quadriceps were identified at the branch point from the femoral nerve. The nerves were then neurolysed, resected, and divided into 5mm segments for later use as cable grafts. In the cold preserved nerve groups, nerves were placed into 15 ml of University of Wisconsin (UW) solution supplemented with penicillin G (200,000 U/L), regular insulin (40 U/L), and dexamethasone (16 mg/L) at the time of harvest and maintained at 4°C for 7 weeks. Cold preservation solution was changed weekly until the time of implantation.

In the recipient animals, a gluteal muscle-splitting incision was used to expose the sciatic nerve which was then neurolysed to the area of the sciatic trifurcation where the tibial branch was identified and isolated from the neighboring sural and peroneal nerves. A 5 mm segment of the tibial nerve beginning approximately 4mm distal to the trifurcation was resected. Cable grafts were stacked using a single 11-0 nylon rosette stitch to bind the grafts at each end before implantation. Double cable motor grafts and triple cable sensory grafts were used to maintain a similar cross-sectional area. Grafts were then inset using a several 11-0 nylon epineurial stitches under 40X magnification.

At the endpoint, the tibial nerve was approached and the graft was neurolysed and resected to include the tibial nerve 3mm proximal and at least 5mm distal to the graft region. The tissue was fixed in 3% glutaraldehyde and stored at 4° C prior to histomorphometric analysis.

Functional Measures: At the 6 week endpoint, bilateral gastrocnemii were surgically approached using a posterior tibial incision. These muscles were divided from surrounding tissue, resected, and weighed. The wet weight ratio between experimental (right) and control (left) sides was measured. Additionally, the hind feet of the rat was dipped in X-ray film developer and the rat was then allowed to walk down a track composed of exposed X-ray film; the hind footprints appeared as the developer reacted with the film. Weekly measurements of the footprints from the walking tracks were used to calculate the print length factor as a measure of the tibial function index.

Results

Histology: Qualitative analysis of nerve sections distal to the cable graft site showed robust regeneration in motor graft groups compared to sensory graft groups (Fig 2B). Electron micrographs of the acellular groups showed robust regeneration in the motor group, while in the sensory group, multiple empty SC basal lamina tubes suggest a possible size barrier to reinnervation.

Nerve Histomorphometry: Analysis of the distal tibial nerve tissue harvested from each group revealed significant differences in multiple histomorphometric parameters (Fig 4). The total number of fibers, nerve density, and percent nerve all showed significantly greater regeneration in the motor graft groups ($p < 0.05$).

Walking track analysis: Weekly analysis of the print length factor as an indicator of tibial functional recovery demonstrated a trend toward improved recovery with motor compared to sensory grafting.

Wet muscle mass ratios: Gastrocnemius wet muscle masses demonstrated a significantly greater return of muscle mass in the motor graft group ($p < 0.05$), (Fig 3).

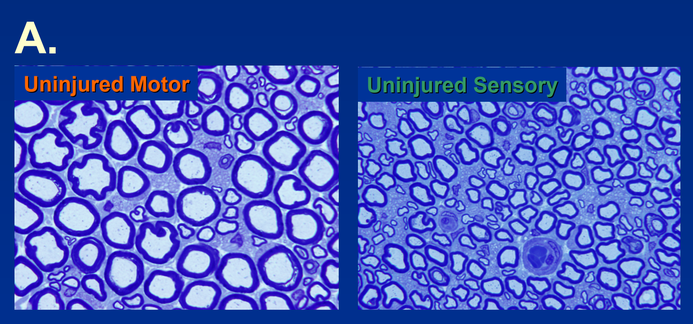


Figure 2. Nerve Histology

A) Normal motor and sensory nerve architecture demonstrates the larger motor SC basal lamina tube size

B) Sections distal to the various graft repairs demonstrates significant regeneration in the motor graft groups compared to the sensory groups irrespective of cellularity *(all images taken at 1000X magnification).*

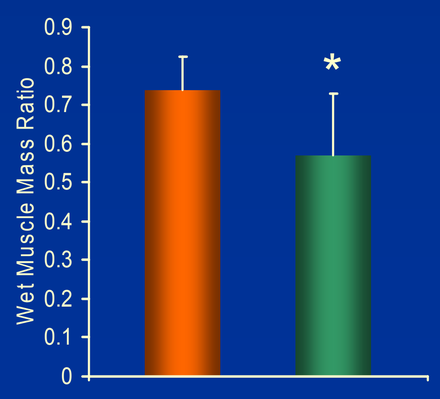


Figure 3. Wet muscle mass ratios (*significant difference marked with an asterisk)*

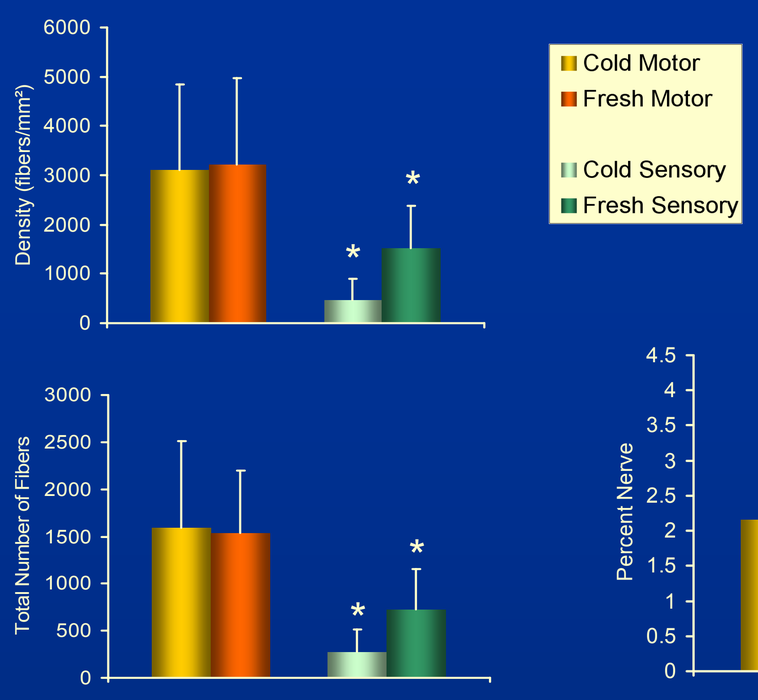


Figure 4. Histomorphometric comparisons of nerve regeneration. The sensory nerve graft group's exhibit significantly reduced nerve fiber counts, nerve density, and percent nerve when compared to motor groups ($p < 0.05$), *marked with an asterisk*.

Conclusions

This study demonstrates that motor nerve grafting has a clear functional advantage over sensory grafts, and that this can be attributed to motor nerve architecture. Regardless of cellularity, more fibers cross a motor nerve graft than a sensory graft. Motor nerve grafts have larger diameter endoneurial tubes which allow for more nerve fibers to cross a nerve defect.

While the reason for improved axonal regeneration in motor compared with sensory grafts is likely multifactorial, this study suggests that motor nerve architecture, regardless of neurotrophic support or biochemical factors, has an independent beneficial effect on nerve grafting. Sensory nerves have a higher fiber count, indicating that more Schwann cell basal lamina tubes are available, and there is evidence that supports that regenerating axons prefer to grow along SC basal lamina tubes[6]. However, the size of Schwann cell basal lamina tubes appears to be a crucial factor when nerve fibers are selecting an endoneurial tube.

The use of musculocutaneous flaps requiring sacrifice of the transferred motor nerve, illustrates that these accompanying motor nerves are functionally expendable. Multiple expendable motor nerves exist and can be harvested and used with limited morbidity to bridge short defects in nerves with critical function. This finding has implications for our immediate clinical management of nerve gaps, and can guide the future development of tissue engineered nerve conduits constructed with SC basal lamina tubes at an "optimal diameter" for motor nerve regeneration.

References

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