New Pure Motor Nerve Experimental Model for the Comparative Study between End-to-End and End-to-Side Neurorrhaphy in Free Muscle Flap Neurotization

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ABSTRACT

The present study demonstrates a new experimental model to compare the efficacy of end-to-end and end-to-end neurorrhaphy in free muscle flap neurotization. Forty animals were used, divided into four equal groups named A, B, C and D. The peripheral stump of the thoracodorsal nerve was stitched end-to-end in groups A and C and end-to-side in groups B and D to the long thoracic nerve. Free functional muscle transfer was simulated by putting vascular clamps to the thoracodorsal artery (FD SS8R, F: 15 to 20 g) and vein (FD SS6R, F: 10 to 15 g) for 60 minutes and transecting and then restitching the origin and insertion of the latissimus dorsi muscle. Electromyographic and histological studies were performed 150 days following completion of the experiment. The results could indicate the possibility that end-to-side neurorrhaphy might be used in free functional muscle transfer as an alternative to end-to-end neurorrhaphy. We believe that the proposed experimental model is useful for the comparative study between end-to-end and end-to-side neurorrhaphy in free muscle flap neurotization, as these are pure motor nerves and innervate synergistic muscles, are in close approximation, and have similar diameters.

KEYWORDS: End-to-side neurorrhaphy, functional muscle, free muscle transfer

The terminolateral *neurorrhaphy* was first introduced in the early 1900s by Balance et al[1] and Kennedy[2] as a method of nerve repair in patients with facial palsy and by Harris and Low in 1903[3] in cases of brachial plexus palsy. These early studies confirmed regeneration of nerve axons through the end-to-side neurorrhaphy site, but the functional results were poor, so the method was quickly abandoned. The end-to-side technique was revived in the 1990s by Viterbo et al, who demonstrated satisfactory results in experimental[4] and clinical[5] studies. After Viterbo et al, several other authors demonstrated the efficacy of the end-to-side neurorrhaphy

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both experimentally[6–8] and clinically.[9,10] Despite the encouraging clinical results, failures have also been reported,[11] and recovery of motor function was rarely obtained.[8]

Based on the observations of Sundine et al,[12] we chose to use two pure motor nerves, the thoracodorsal and long thoracic, to control the efficacy of end-to-end and end-to-side neurorrhaphy in free functional muscle transfer. This model was selected to increase the number of motor nerve axons that reach the motor plates and consequently improve the functional result. Furthermore, these two nerves supply synergistic muscles, an essential fact if adequate muscle power with well-coordinated muscle function is to be achieved.[10,13]

MATERIALS AND METHODS

Forty adult male New Zealand White rabbits weighing ~2.8 kg were divided into four equal groups (A, B, C, and D). The animals were individually housed, given food and water ad libitum, and exposed to a 12-hour light-dark cycle. For each operation, the rabbits were anesthetized with an intramuscular injection of a 3:2 mixture of ketamine hydrochloride 100 mg/dL and xylazine hydrochloride 20 mg/dL. The animals that presented acute anesthesiological problem and those that had surgical site infection were excluded from the study.

Operative Technique

An incision from the axillary fold up to the lower third of the midaxillary line was performed at the right side of every animal. The skin and panniculus carnosus were reflected upward, and the latissimus dorsi muscle was identified. The latissimus dorsi muscle was separated from its interdigitations with serratus anterior and the thoracodorsal neurovascular bundle, and long thoracic nerves were then identified and meticulously dissected (Fig. 1). The thoracodorsal nerve was severed ~1 cm central to its entrance point into the latissimus dorsi muscle, and the proximal stump was reflected cephalad and buried in the upper limb musculature. In the groups where an end-to-side neurorrhaphy was performed (Fig. 2), the peripheral stump of the thoracodorsal nerve was stitched to a perineural window ~1 mm in diameter at the long thoracic nerve. The perineural window was created with the use of two pairs of microvascular forceps pulling into opposite directions, and its formation was confirmed by the "mushrooming" of the protruding endoneurium.

All surgical procedures were performed by the same surgeon (G.-A.S.), and neurorrhaphies were performed under microscope magnification using 11–0 nylon interrupted sutures.

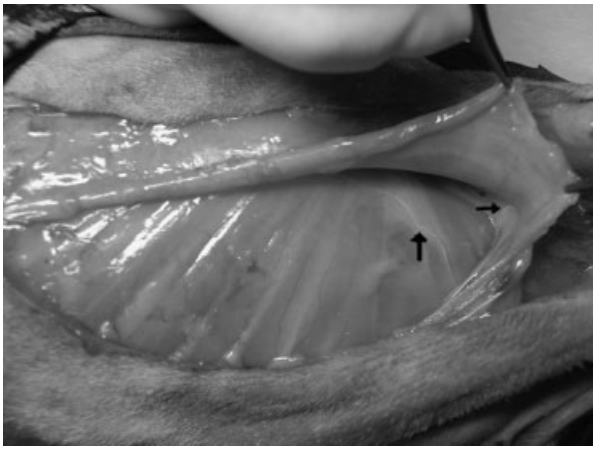

Figure 1 Exposure of the long thoracic and thoracodorsal nerves. The vertical arrow points to the long thoracic nerve, and the horizontal arrow points to the thoracodorsal nerve.

In group A, the long thoracic nerve was transected ~1 cm proximal to its entrance point into the serratus anterior muscle, and its proximal stump was stitched end-to-end with the peripheral stump of the thoracodorsal nerve. In group B, a perineural window was created in the long thoracic nerve at a location to facilitate tension-free neurorrhaphy with the distal end of the thoracodorsal nerve. In group C, the thoracodorsal nerve was stitched end-to-end with the long thoracic nerve as in group A; finally, in group D, an end-to-side neurorrhaphy was performed between the thoracodorsal and long thoracic nerve as in group B. In groups C and

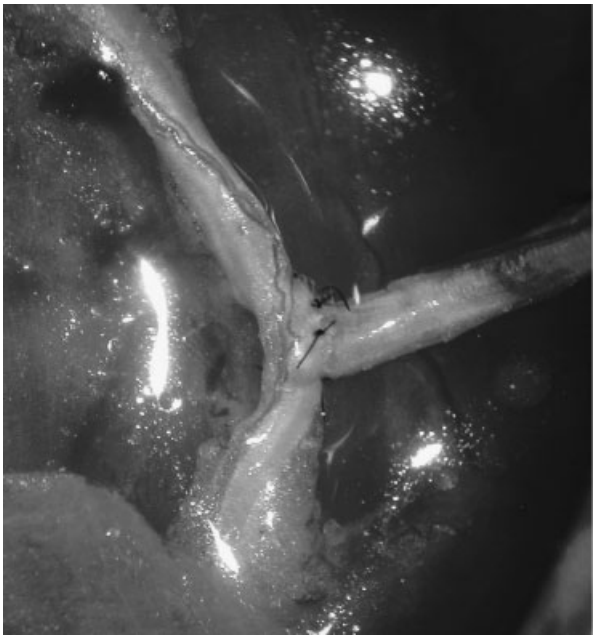

Figure 2 The peripheral stump of the thoracodorsal nerve is stitched end-to-side to a perineural window of the long thoracic nerve.

D, we put clamps to the artery (FD SS8R, F: 15 to 20 g) and the vein (FD SS6R, F: 10 to 15 g) for 60 minutes, then we transected and restitched in place the origin and insertion of latissimus dorsi muscle, imitating free functional muscle transfer.

After 5 months, the site of nerve coaptation was reexposed in the right axilla. In the left axilla, the thoracodorsal and long thoracic nerves were dissected, and electromyography (EMG) was performed. Immediately after the EMG was completed, the neurorrhaphy site, along with 0.5 cm of nerve segment proximal and distal to it, was taken as biopsy specimen. Samples were also taken from the unoperated contralateral thoracodorsal nerve that was used as control and from both latissimus dorsi muscles around the entry point of the thoracodorsal nerve.

Electromyographic Investigation

Electromyography was performed using a clinical MYSTRO 25 + (Medelec Mystro 25 +; Medelec Limited, Manor Way, Old Woking, Surrey, England) system with modified pediatric electrodes. At the operated side, electric stimulators were placed at points proximal to the neurorrhaphy site. A recording electrode was placed in the central area of the latissimus dorsi muscle. The thoracodorsal nerve was periodically stimulated with supramaximal voltage by an electric stimulator, and evoked potentials from the reinnervated latissimus dorsi muscle were recorded. The threshold and the compound muscle action potentials (CMAPs) were measured. The ratio of the CMAPs between the operated and the nonoperated thoracodorsal nerve (P ratio) for each animal was calculated. The P ratio was used as a measure of CMAP recovery.

Histological Examination

The nerve segments were fixed for 12 hours in a solution of 2.5% glutaraldehyde in phosphate-buffered saline at 4%. They were then fixed in 1% osmium tetroxide and dehydrated in series of ethanol solutions, and, finally, they were embedded in Agar 100% resin. The part of the thoracodorsal nerve distal to the neurorrhaphy site at the operated side and the specimen of the contralateral thoracodorsal nerve were cut in serial semithin sections (2 μm thick). The sections were stained with toluidine blue and examined with a light microscope. The muscle biopsies were fixed for 24 hours in 10% buffered formalin, cut in perpendicular sections of 2- to 3-mm thickness, and after dehydration were embedded in paraffin blocks. Subsequently, histological sections 4 μm thick were stained with hematoxylin-eosin. All sections were examined by light microscopy. The morphometric measurements were performed with the use of Image J image-analyzing system (Research services branch, National Institute of Mental Health Bethesda Maryland, USA).

Statistical Analysis

The values of variables were presented using the number of rabbits (n), the mean, the standard deviation (SD), and the median. The comparison of the histological and electromyographic findings among the groups was performed using the one-way analysis of variance model. For the pairwise multiple comparisons among groups, the Scheffe test was used. The assessment of results between the operated and contralateral unoperated side was completed using the independent samples t test. Every test was two-sided with a 0.05 level of significance. The statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS version 10.00).

RESULTS

Two animals from groups A and C and one animal from groups B and D died from *Pasteurella multocida* infection before the second intervention.

Electromyographic Study

The P ratio of the CMAPs between the operated and nonoperated side was used, as mentioned, for the electromyographic assessment. The findings for the rabbits in every group are documented in Table 1 and the descriptive statistics in Table 2. From the evaluation of the average of P ratio (Table 3), there was no statistically significant difference of CMAP ratio between the four groups ($p = 0.430$).

Histological Assessment

The mean diameter for the long thoracic nerve was 1.2 ± 0.1 mm, and mean diameter for the thoracodorsal nerve was 1 ± 0.9 mm. "Operated" refers to the part of

Table 1 Electromyographic Findings: Ratio of the Compound Muscle Action Potentials between the Operated and Contralateral Nonoperated Side

Group A	Group B	Group C	Group D
0.980	0.977	0.150	D 0.298
0.554	0.635	0.544	0.455
0.472	0.203	0.394	0.455
0.543	1.010	0.606	0.320
0.980	0.345	0.659	0.730
0.392	0.534	0.394	0.730
0.980	0.635	0.606	0.496
0.328	0.280	0.659	0.656
	0.534		0.069

Table 2 Descriptive Statistics for the Electromyographic Findings for the Compound Muscle Action Potential in Every Group

Group	Mean	Median	SD	Minimum	Maximum	ν
A	0.65	0.55	0.28	0.33	0.98	8
B	0.57	0.53	0.28	0.20	1.01	9
C	0.50	0.58	0.18	0.15	0.66	8
D	0.47	0.46	0.22	0.07	0.73	9

the thoracodorsal nerve distal to the neurorrhaphy site, and "nonoperated" refers to the contralateral control thoracodorsal nerves at an analogous location. There was no statistically significant difference for the number of nerve axons at the nonoperated side among the four groups ($p = 0.588$), nor was there a statistically significant difference for the number of nerve axons at the operated side ($p = 0.335$) among the four groups (Fig. 3A to E). The comparison of the diameter of nerve axons revealed no statistically significant difference among the four groups for the nonoperated thoracodorsal nerve ($p = 0.242$). The comparison of the diameter of nerve axons at the operated side revealed statistically significantly larger diameter of nerve axons in groups A and C compared with groups B and D ($p < 0.0005$; Table 4).

The latissimus dorsi muscle at the operated side presented fatty degeneration and focal atrophy, which were more extensive in groups B and D (Fig. 4B, D). The muscles in groups A and C did not present significant atrophy (Fig. 4A, C).

DISCUSSION

The study of end-to-side neurorrhaphy is a controversial area. A basic conflict among scientists was whether end-to-side neurorrhaphy should be performed to a "window" created at the donor nerve, and if so, should this window be epineural or perineural? On the subject of window creation, most studies concluded that no significant collateral axonal sprouting exists in the lack of an epineurotomy.[14,15] Concerning the theory of whether the window should also involve the perineurium, the results among scientists were divisive, with some of them presenting similar evidence regardless of the removal of perineurium or not,[16,17] and others supporting the hypothesis that the creation of perineural window enhances the effectiveness of end-to-side neurorrhaphy.[18,19]

Table 3 Parametrical Analysis of the Electromyographic Findings

	RATIO					
				Multiple Comparisons		
Group	*n*	Mean	SD	B	C	D
A	8	0.65	0.28	NS	NS	NS
B	9	0.57	0.28	—	NS	NS
C	8	0.50	0.18	—	—	NS
D	9	0.47	0.22	—	—	—

There is no statistically significant difference between the four groups ($p = 0.430$).

It is known that the perineurium is composed of concentric layers of flattened cells and a dense mesh of collagen fibers.[20] Thus, it creates a diffusion barrier and enhances the maintenance of a stable "internal milieu," protecting the nerve from the entrance of toxic molecules and contributing in the preservation of positive intraneural pressure.[21] It is obvious by its organization that this highly complicated structure is unlikely to allow the passage of nerve axons, except possibly from areas where blood vessels enter or leave the perineurium,[22] and could be used as guides for sprouting nerve axons.[18] Furthermore, the human perineurium is thicker compared with that of laboratory animals, and consequently its penetration by growing nerve axons is more difficult. Additionally, human nerve-regenerating capacity is also poorer.[16] Based on the aforementioned evidence, we chose to create a perineural window.

The present experimental study demonstrates a new experimental model to evaluate the efficacy of end-to-end and end-to-side neurorrhaphy in free functional muscle transfer. Nerve combinations that have been used until now in the literature for the study of end-to-side neurorrhaphy comprise mainly mixed nerves. The most common model is the tibial and peroneal nerve.[4,6,8,18,23,24] Other examples are the musculocutaneous and median nerve,[25] median and ulnar nerve,[26] sciatic and obturator nerve,[27] sciatic nerves bilaterally,[19] and median, ulnar, and radial nerves.[28] In these mixed nerve models, the regenerating aesthetic nerve axons might compete with the regenerating motor nerve axons for the endoneural tubules in the peripheral nerve stump, thus downgrading the number of motor axons that eventually reach the motor plates. Aiming at optimum results, we utilized the thoracodorsal and long thoracic nerves that innervate synergistic muscles.[10,13] These are both pure motor nerves[12]; therefore, in case of a neurorrhaphy, the number of motor nerve axons that reach the motor plates would be maximized and the functional result consequently improved. The nerves are also in close approximation (Fig. 1), which permits effortless coaptation between them in cases of either end-to-end or end-to-side neurorrhaphy (Fig. 2). Finally, the nerves are similar in diameter, allowing an accurate approximation of their stumps in cases of end-to-end neurorrhaphy.

The experimental model we used resulted in collateral sprouting of nerve axons through the end-to-side neurorrhaphy site. This is proved by the existence of nerve fibers in the thoracodorsal nerve distal to the neurorrhaphy location in groups B and D where

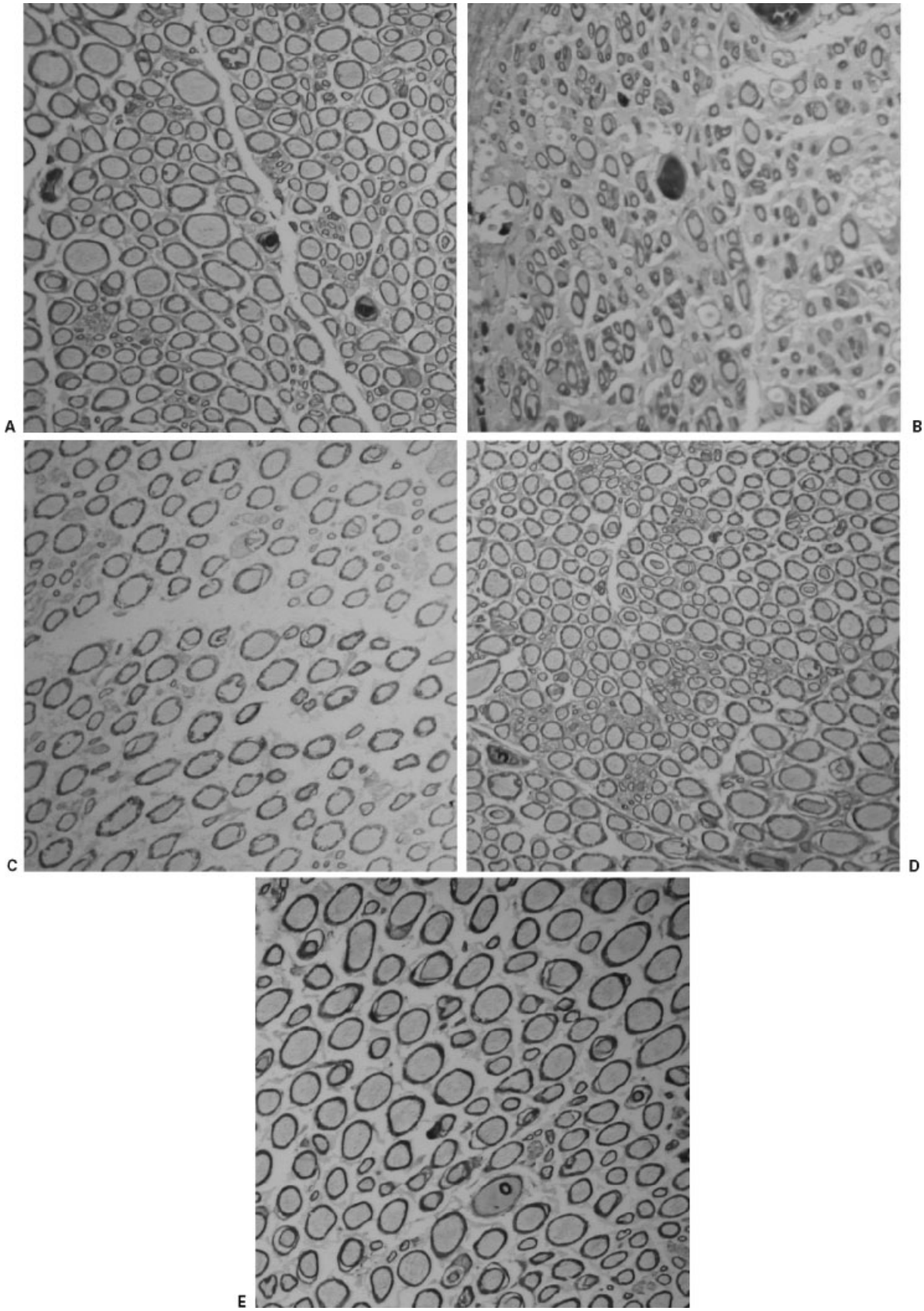

Figure 3 (A–E) Semithin (2 μm thick) sections of the thoracodorsal nerve stained with toluidine blue. (A–D) Sections from the operated thoracodorsal nerve in groups A, B, C, and D, respectively. (E) Sections from the contralateral nonoperated thoracodorsal nerve (initial magnification × 400).

Table 4 Diameter of the Operated Thoracodorsal Nerve among Groups

Operated Thoracodorsal Nerve			
Animal Group	***n***	**Axonal Diameter (μm) (mean ± SD)**	
A	8	5.56	0.55
B	9	4.06	0.51
C	8	5.36	0.43
D	9	4.27	0.42

Groups A and C have statistically significantly larger diameters compared with groups B and D ($p < 0.0005$).

end-to-side neurorrhaphy was performed. The ability of the peripheral nerve stump to "attract" sprouting nevrites from intact nerve axons has been attributed to the fact that it releases neurotrophic and neurotropic factors, such as insulin-like growth factors I and II, fibroblast growth factor, and extracellular stroma molecules such as laminin, fibronectin, and nerve cell adhesion molecules.[29,30]

There was no statistically significant difference for the number of nerve axons for the operated thoracodorsal nerve among the four groups ($p = 0.335$). Groups A and C had statistically significantly larger diameters for the operated thoracodorsal nerve compared with groups B and D ($p < 0.0005$). The latter is an indirect sign of maturity of nerve axons and thus of more efficient nerve regeneration.

Regarding pathology of the muscle specimens, the best results were observed in groups A and C, and groups B and D had graver fatty degeneration and muscle atrophy. It is known that fatty degeneration is one feature that accompanies muscle atrophy. Therefore, the differences in the fatty degeneration could be explained by the difference in the quality of nerve regeneration.

However, it appears that the aforementioned differences, regarding the diameter of regenerating nerve axons and fatty degeneration among groups, do not reflect in the excitability of the muscles among groups, as depicted by the absence of statistically significant difference in CMAP recovery among groups. The information that the CMAP ratio has no statistically

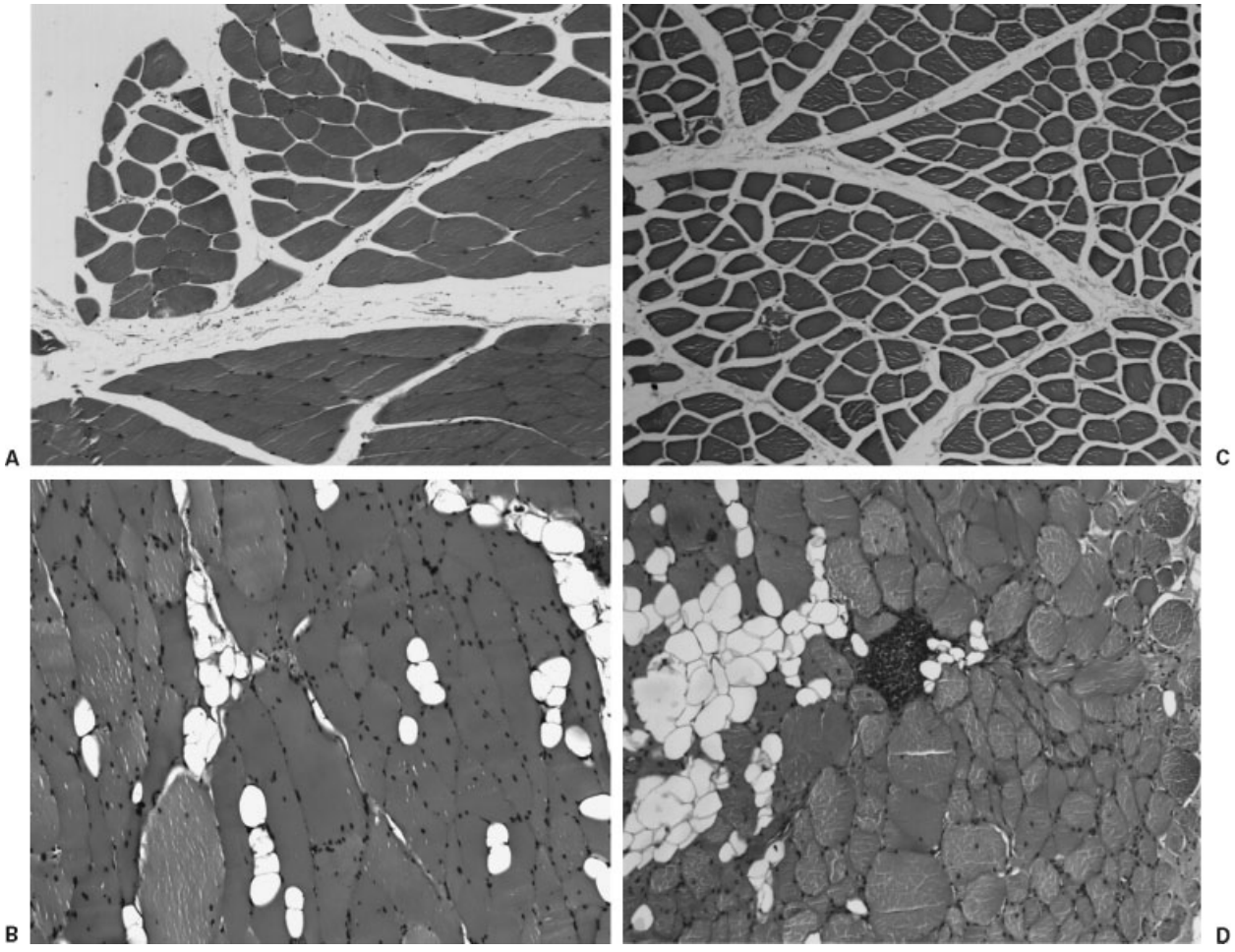

Figure 4 (A–D) Histological sections of the latissimus dorsi muscle at the operated side from groups A, B, C, and D, respectively; 4 μm thick, stained with hematoxylin eosin (initial magnification × 400).

significant difference among the four groups, and especially between groups C and D that simulate free muscle transfer, could indicate the possibility that end-to-side neurorrhaphy might be used in free functional muscle transfer as an alternative to end-to-end neurorrhaphy.

According to our review of the literature, Kalliainen and Kuzon[31] demonstrated comparable results in muscle reinnervation with the use of end-to-end or end-to-side neurorrhaphy of the peroneal nerve to a perineural window of the tibial nerve. There is only one other study, by Giovanoli et al, that used a pure motor nerve model and controlled muscle reinnervation after end-to-side neurorrhaphy in the rabbit.[7] In that study, the authors did not simulate free muscle transfer.

We believe that the proposed experimental model could be useful for the comparative study between end-to-end and end-to side neurorrhaphy in free muscle transfer. The thoracodorsal and long thoracic are purely motor nerves, and therefore the number of motor nerve axons that reach the motor plates is maximized. They innervate synagonistic muscles, a prerequisite if we want adequate muscle power with well-coordinated muscle function. They have similar diameters, and therefore end-to-end neurorrhaphy can be achieved without discrepancy, and they are in close approximation to each other. However, there is no doubt that the usefulness of functional muscle transfers can only be judged by their ability to generate power, and we are currently working on a methodology of assessment of power and force output for the proposed experimental model.

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