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(Degree)
博士（医学）

(Date of Degree)
2012-03-25

(Date of Publication)
2012-10-30

(Resource Type)
doctoral thesis

(Report Number)
甲5604

(URL)
https://hdl.handle.net/20.500.14094/D1005604

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運動および知覚神経再支配を用いた脱神経後の筋萎縮予防効果の検討

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Key words: reinnervation, end-to-side neurorrhaphy, sensory protection, end-to-end neurorrhaphy

Title page

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Comparison of Reinnervation for Preservation of Denervated Muscle Volume with Motor and Sensory Nerve: An Experimental Study

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Summary

Prevention of the atrophy of denervated muscles is essential for a good outcome in facial contouring and oral reconstruction. In this study, we compared the effectiveness of end-to-end and end-to-side neurorrhaphy of the motor nerve, and end-to-end neurorrhaphy of the sensory nerve, all of which are frequently used in such reconstruction for the prevention of muscle atrophy.

Wistar rats were divided into four groups: Group 1, motor nerve division of semimembranosus without repair; Group 2, motor nerve division and end-to-end coaptation to the saphenous nerve; Group 3, motor nerve division and end-to-side coaptation to the sciatic nerve; Group 4, motor nerve division and end-to-end repair.

Measurement of semimembranosus volume, histological evaluation and staining of neuromuscular junctions carried out three months postoperatively revealed that muscle volume preservation was larger in groups 3 and 4 than in the other 2 groups ($p<0.05$), but slightly superior in Group 4 ($p<0.05$). There was no statistical difference between Groups 2 and 1; histologically, muscle architecture was better preserved in Group 2 than in Group 1; reactivation of the neuromuscular junctions was observed in all except Group 1.

End-to-side repair of motor nerves is one of the better options for the preservation of muscle volume when end-to-end nerve repair is not indicated. Sensory protection may also provide some advantages in the preservation of muscle volume.

Key words: reinnervation, end-to-side neurorrhaphy, sensory protection, end-to-end neurorrhaphy

INTRODUCTION

Denervation of the muscle causes loss of contracture and muscle atrophy. In tongue reconstruction, atrophy of the transplanted muscle causes dysfunctional swallowing[1, 2].

Therefore, control of the volume of transplanted muscle is crucial for a good outcome from not only the cosmetic but also the functional aspect.

Various trials have been conducted for the control of muscle atrophy by reinnervation[3-9]. Two known forms of the reinnervation of muscle flap transfers are 1) motor-to-motor reinnervation and 2) sensory-to-motor reinnervation. The first is classified into end-to-end and end-to-side nerve repair, the former of which is considered most effective, although it involves sacrifice of the donor motor nerve; therefore, indications for this method are limited. Recently, collateral sprouting of motor axons in end-to-side nerve repair has been demonstrated and is considered less invasive for motor nerve reinnervation than end-to-end nerve repair[4, 5, 10-14]. Furthermore, during the last decade, experimental studies have proven that sensory nerve reinnervation also preserves muscle volume (sensory protection)[3, 6, 9].

Clinically, the three types of neurorrhaphy are the most practical choices for nerve repair. Studies on each pattern have been carried out; however, no comparison of the three forms has been made. Here, we compared the effectiveness of end-to-end neurorrhaphy of the motor nerve, end-to-side neurorrhaphy of the motor nerve and sensory protection, with the use of a rat model.

MATERIALS AND METHODS

Animals

Wistar rats (adult female rat) used in this study were, in groups, housed in a temperature-controlled colony room with a 12/12-h L/D cycle, in acrylic cages with woodchip bedding and given unlimited access to normal laboratory chow and water. All experiments were carried out with the approval of the Committee on Animal Care and Welfare, Kobe University School of Medicine. The animals were randomly

divided into four groups: 1) division of motor branch of sciatic nerve without repair [denervation (DN)] (n=9); 2) end-to-end coaptation of the motor branch of the sciatic nerve and the saphenous nerve [sensory protection (SP)] (n=10); 3) end-to-side coaptation of the motor branch of the sciatic nerve and the main trunk of the sciatic nerve [end-to-side motor protection (MPes)] (n=11); 4) division of the motor branch of the sciatic nerve and its immediate repair [end-to-end motor protection (MPee)] (n=9). Some animals died during the experimental period; the remaining thirty-nine rats were included in the study. The contralateral limb of each animal was left unoperated on and served as the control (Fig. 1).

Surgical procedure

The animals were anesthetized with intraperitoneally administered sodium pentobarbital (47 mg/kg). Through a transverse skin incision on the medial thigh, the intermuscular septum between the gracilis and the semimembranosus was dissected, and the semimembranosus and motor branches of the sciatic nerve were identified. The nerve was stimulated before its division so as to confirm that it was the motor nerve of the semimembranosus. In group 1[DN], the motor branch of the sciatic nerve was sharply transected under microscopical vision. The distal nerve stump was removed from the muscle, and the proximal nerve stump was removed from the main trunk of the sciatic nerve, leaving a gap of over 10mm to avoid spontaneous reinnervation. In group 2 [SP], the saphenous nerve and the motor branch of the sciatic nerve were sharply transected, and the proximal saphenous nerve and the distal motor branch of the sciatic nerve were coapted with 10-0 nylon under microscopical vision. The proximal motor branch of the sciatic nerve was removed from the main trunk, leaving a gap of over 10mm to avoid spontaneous reinnervation. In group 3 [MPes], the main trunk of the sciatic nerve was exposed and an epineural window was

created. The motor branch of the sciatic nerve was sharply transected and the distal nerve trunk was coapted end-to-side to the main trunk of the sciatic nerve with 10-0 nylon under microscopical vision. The proximal motor branch of the sciatic nerve was removed from the main trunk, leaving a gap of over 10mm to avoid spontaneous reinnervation. In group 4 [MPee], the motor branch of the sciatic nerve was sharply transected and immediately repaired in end-to-end fashion with 10-0 nylon.

Three months postoperatively, the incisions in all the rats were reexplored. The operative site in each animal was examined under a surgical microscope to confirm the continuity of the anastomosed site, and then the operative side semimembranosus and contralateral semimembranosus were harvested, dried by placing in a dry box for 48 hours and weighed with a precision electronic balance. The weight ratio as a percentage of the control was calculated in all the groups.

Histological examination

A sample of each muscle was fixed in 10% formalin, frozen in 1:2 solution of OCT compound (Sakura Tek, Japan) and 20% sucrose PB, cut into 6μm sections in a cryostat at -20℃ and stained with hematoxylin and eosin.

alpha-BTX reaction (Staining of the neuromuscular junctions)

Another sample of each muscle was fixed in 10% formalin and cut into sections, as above. The sections were treated with blocking buffer (PBS containing 1% triton and 4% BSA) for 30min at room temperature, reacted for 1h with Alexa Fluoro 488 conjugated alpha-Bungarotoxin (Invitorogen B13422) (dilution 1:500 in PBS containing 0.1% triton and 1% BSA), counterstained with Evans blue for 5min, washed, coverslipped and examined by fluorescence microscopy (Biozero ; Keyence). Neuromuscular junctions were also analyzed by this method.

Statistical analysis

Statistical analyses were carried out with R version 2.92 (R Foundation for Statistical Computing, Vienna, Austria).

The dry muscle weight ratio of the four methods was compared by the Kruskal-Wallis test and post-hoc Wilcoxon rank sum test with Holm adjustment for multiple comparisons. The trend of the dry muscle weight ratio among the four methods was evaluated by the Jonckheere test.

P values <0.05 were considered statistically significant.

RESULTS

Muscle weight

The dry muscle weight ratios among the four groups are shown in Table 1. There was significant difference among the four methods (Kruskal-Wallis test, $p<0.00001$).

In the post-hoc Wilcoxon rank sum test, both end-to-end and end-to-side motor protection showed higher muscle volume preservation than the other methods ($p<0.05$), with the former being superior to the latter ($p<0.05$). There was no statistical difference between sensory protection and denervation ($p=0.1564$), but the trend of the four methods was significant (Fig. 2).

Histological evaluation

Histological examination of the denervated group showed proliferating connective tissue, general muscle atrophy and destroyed muscle architecture. In the sensory protected group, a change in muscle atrophy was also seen, but muscle architecture was well preserved compared with the denervated group. Both of the motor protected groups showed evidence of better preserved muscle architecture compared with the other two groups. MPee showed much less muscle fiber atrophy than MPes; compared with the control group, however, both groups showed atrophy of muscle fasciculi (Fig. 3).

Alpha-bungarotoxin-binding neuromuscular junction was observed in all groups except the denervation group; in the sensory protected group, however, the number of neuromuscular junctions was small compared with that in the motor protected groups (Fig. 4).

DISCUSSION

Immediately after nerve division, Wallerian degeneration of distal axons and nerve regeneration started simultaneously. Promoting nerve regeneration and minimizing muscle degenerative change after nerve division is conducive to reducing muscle atrophy. Neurorrhaphy of the motor nerve is thought to be the most effective method of maintaining denervated muscles[4, 5, 7, 8], and neurorrhaphy of the sensory nerve is also effective in doing so (the so-called "sensory protection" [3, 6, 9] that reduces muscle atrophy) but is inferior to neurorrhaphy of motor nerves. We prepared two neurorrhaphy models for the motor nerve (end-to-end nerve repair and end-to-side nerve repair) and an end-to-end neurorrhaphy model of the sensory nerve for comparing the effectiveness of retarding muscle atrophy. End-to-end repair of the motor nerve is thought to be the most reliable method. On the other hand, the concept of end-to-side repair of the motor nerve, although known since about one hundred years, was abandoned because of poor results. The concept of the effectiveness of end-to-side nerve repair evaluated by histological and electrophysiological studies has been revived and well discussed[15,16]. The advantages of end-to-side nerve repair are the short distance traveled by regenerating axons in reaching their targets and the tension-free repair. Some investigators have attempted to prove collateral sprouting in noninjurious end-to-side nerve repair models,[17] and others have successfully proved collateral sprouting in two different types of noninjurious end-to-side neurorrhaphy models[18].

In our study, end-to-end repair of the motor nerve showed a significantly high degree of maintaining muscle weight compared with end-to-side nerve repair. Histological examination of muscle specimens showed much less muscle fiber atrophy and fibrosis in end-to-end than in end-to-side nerve repair. The weight of muscles reinnervated by end-to-end nerve repair is significantly greater than that of muscles reinnervated by end-to-side nerve repair, and muscles reinnervated by the former have significantly fewer denervated fibers than muscles reinnervated by the latter[4]. Our results are compatible with theirs, although we did not examine muscle function.

Regenerative sprouting and collateral sprouting are two well-known major forms of nerve regeneration, and both are thought to be intermingled in end-to-side nerve repair because axonal injury during surgery is thought to be inevitable. In our study, reactivation of the neuromuscular junctions was observed in both end-to-end and end-to-side nerve repair. In the former, the neural source was evidently from regenerative sprouting; in the latter, however, the neural source, whether only from regenerative sprouting or, to some extent, also from collateral sprouting, was uncertain.

Nonetheless, some authors have suggested that regenerative axons from collateral sprouting are probably pruned off in the long run[12,18]. Therefore, long-term studies are needed to clarify this issue. Axons from collateral sprouting may obtain neurotrophic factors from surrounding structures that form neuromuscular junctions, and may escape pruning.

The concept of "sensory protection", that sensory reinnervation is capable of preserving the same degree of muscle volume as motor reinnervation, has been known since the 1940s , and some research has been done accordingly. Comparisons of sural nerve-tibial nerve coaptation models with denervation models in rat lower limbs have demonstrated significantly greater muscle volume preservation in sensory

protected models[3]. Other studies have indicated that cutaneous sensory nerves may contain sympathetic fibers related to sweat glands and pilorum muscles, and that these motor fascicles may play a role in the preservation of muscle volume.[8] Others have also described similar results.[6, 9] Our results demonstrated that sensory protected groups tended to preserve more muscle volume compared with denervated groups, but that tendency did not reach a significant difference. Histologically, however, both muscle fiber atrophy and fibrosis were obviously inhibited compared with denervated models.

In our study, one of the possible reasons that a small number of reactivated neuromuscular junctions were observed in the sensory protected model is that motor fascicles, related to sweat glands and pilorum muscles contained in the sensory nerve, might induce the reactivation of the neuromuscular junctions, as previously reported[8]. Another possibility is that, after nerve coaptation, neural signals are transmitted through the regenerated sympathetic axons present on the surface of the somatic nerve system, and subsequently induce the reactivation of the neuromuscular junctions[8]. This reactivated neuromuscular junction may to some extent contribute to the preservation of the denervated muscle. Our results suggest that sensory protection plays a part in maintaining muscle structure but not to a sufficient extent for preserving muscle volume. It is well known that sensory fiber Schwann cells release cytokines[19,20] as well as motor fibers, which may help in inhibiting muscle degeneration until motor reinnervation from surrounding structures occurs. The mechanism of "sensory protection" remains uncertain and warrants further study.

Our results showed a significant difference in muscle volume preservation between MPee and MPes. Although some studies do not support the effectiveness of end-to-side repair of motor nerves[21], it is obviously effective in the preservation of muscle

volume compared with sensory protection groups. Sensory protection may, however, also provide some advantages in muscle volume preservation. In breast reconstruction, wherein abnormal muscle contraction is undesirable, sensory protection may be a good option. On the other hand, facial reanimation, in which muscle function maintenance takes priority over muscle volume preservation, neurorrhaphy of the motor nerve, not of the sensory nerve, should be selected to acquire voluntary muscle movement.

In tongue resection, the hypoglossal nerve sacrificed at tumor resection could be utilized for end-to-end nerve repair; thus the recommendation that end-to-end nerve repair be used for tongue reconstruction. On the other hand, in maxillary tumor resection, the cut end of the motor nerve is seldom exposed in the operating field; therefore, adoption of end-to-side nerve repair seems more reasonable. According to the outcome of our study, we believe end-to-side nerve repair is one of the good options for muscle volume preservation.

Our study involves no transfer of muscles, but since muscles inevitably fall into disuse atrophy when muscle origin and insertion are divided, disuse atrophy may influence the degree of atrophy of the transplanted muscle flap. Therefore, in clinical applications, all this should be taken into consideration. Examination of motor and sensory reinnervation in both muscle transfer and muscle in situ models has revealed differences in muscle mass preservation between the two models, suggesting that muscle transfer with complete muscle attachment may decrease muscle atrophy[8]. Use of a muscle model wherein the origin and insertion are divided might reduce the effect of motor protection. Also, in the muscle transfer model, the inevitable formation of scar tissue around the muscle flap may influence the accuracy of the calculation of muscle volume. Our results are based on a relatively short experimental period;

therefore, a longer period of investigation is needed for evaluating the effectiveness of end-to-side motor and sensory protection. We are presently in the process of investigating the correlation between the number of neuromuscular junctions and the degree of muscle atrophy.

CONCLUSION

An untransferred denervated muscle model was used to compare the effect of end-to-end and end-to-side repair of the motor nerve on the preservation of muscle volume and on sensory protection. End-to-end and end-to-side nerve repair showed the best and good muscle volume preservation, respectively. The sensory protection model also showed muscle volume preservation, but to a lesser extent than the two former methods. We believe that end-to-side repair of motor nerves is one of the better options for muscle volume preservation when end-to-end nerve repair is not indicated. Sensory protection may also provide some advantages in muscle volume preservation.

Funding: None

Conflicts of interest: None declared

Ethical approval: Not required

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Figure legends

Figure 1. Schema of surgical procedure. A. Control B. Group 1, denervation (DN)

C. Group 2, sensory protection (SP) D. Group 3, end-to-side motor protection (MPes) E. Group 4, end-to-end motor protection (MPee)

SciN: sciatic nerve, SN: saphenous nerve (sensory) MBSN: motor branch of sciatic nerve

Figure 2. Dry muscle weight at 3 months expressed as a percentage versus control. The trend of the 4 methods was significant (DN SP MPes MPee, $p<0.001$).

Figure 3. Representative photomicrographs of muscle specimens (3 months postop, HE staining) and macroscopic view of anastomosed nerve (1 month postop). SciN: sciatic nerve, SN: saphenous nerve, MBSN: motor branch of sciatic nerve

A. Control specimen B. End-to-end motor protection (MPee) specimen; muscle structure is almost preserved. C. End-to-side motor protection (MPes) specimen; muscle fibers are almost preserved but slightly atrophic compared with MPee specimens. D. Sensory protection (SP) specimen; muscle fiber structures are well preserved compared with the denervated specimen, but proliferation of connective tissue and muscle fiber atrophy are noticeable. E. Denervated (DN) specimen; muscle fiber structures are destroyed and muscle atrophy is obvious. Scale Bars: 200um.

Figure 4. Neuromuscular staining of each group. Neuromuscular junctions are observed in control specimen (A,B), MPee (C), MPes (D), SP (E), and. No neuromuscular junction is observed in DN (F). Arrows indicate neuromuscular junctions stained by alpha-bungarotoxin. Scale bars:100um (A,C,D,E,F), 20um (B)

Table 1

Dry Muscle Weight 3 months After Operation

Group	Number	Muscle Dry Weight 3 months, % of control(mean±SD)
1 (DN)	9	29.8 ± 7.2
2 (SP)	10	34.7 ± 8.0
3 (MPes)	11	44.8 ± 9.7
4 (MPee)	9	61.7 ± 13.0

DN, denervation; SP, sensory protection; MPes, end-to-side motor protection; MPee, end-to-end motor protection; SD, standard deviation

Figure.1

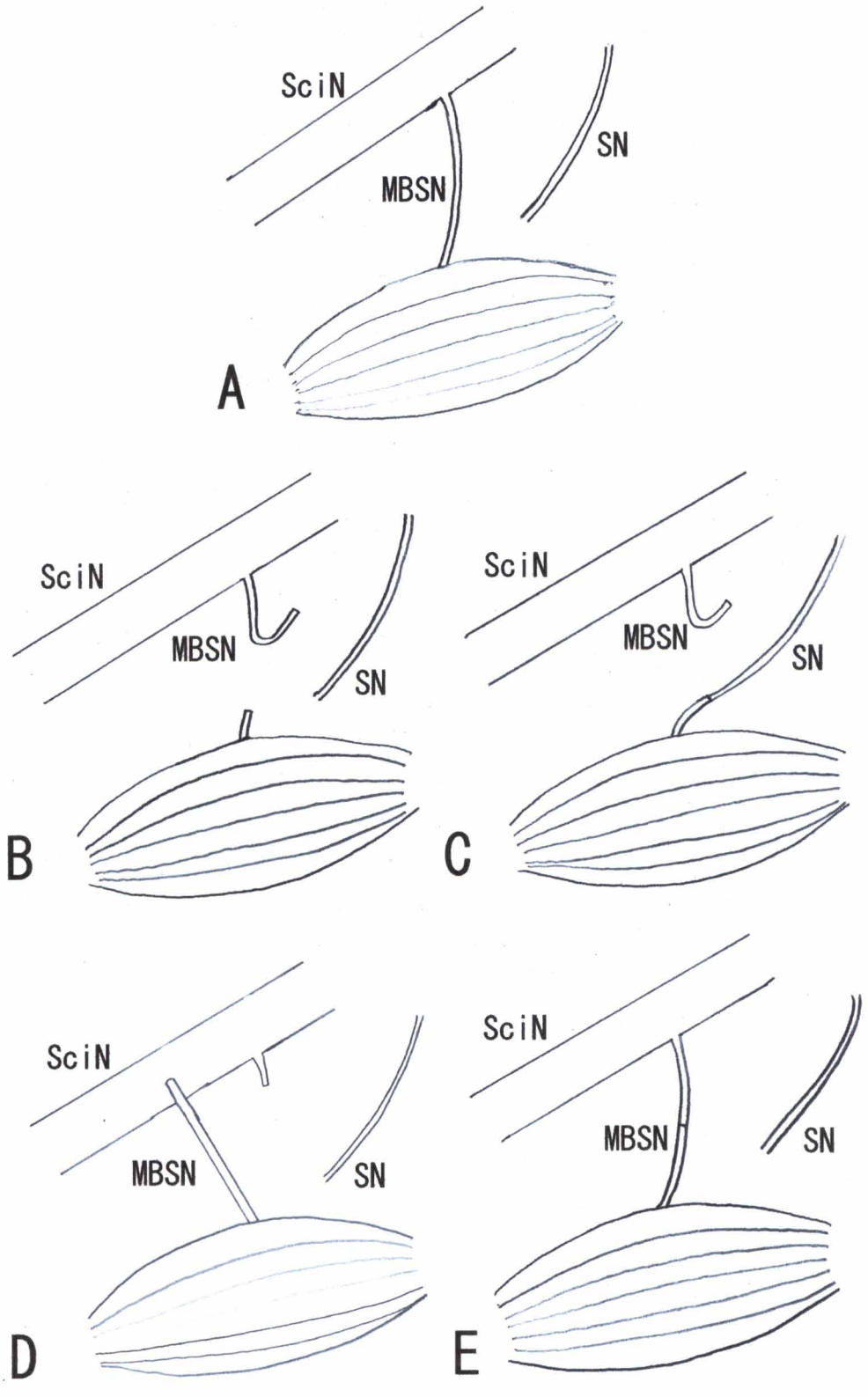
SciN
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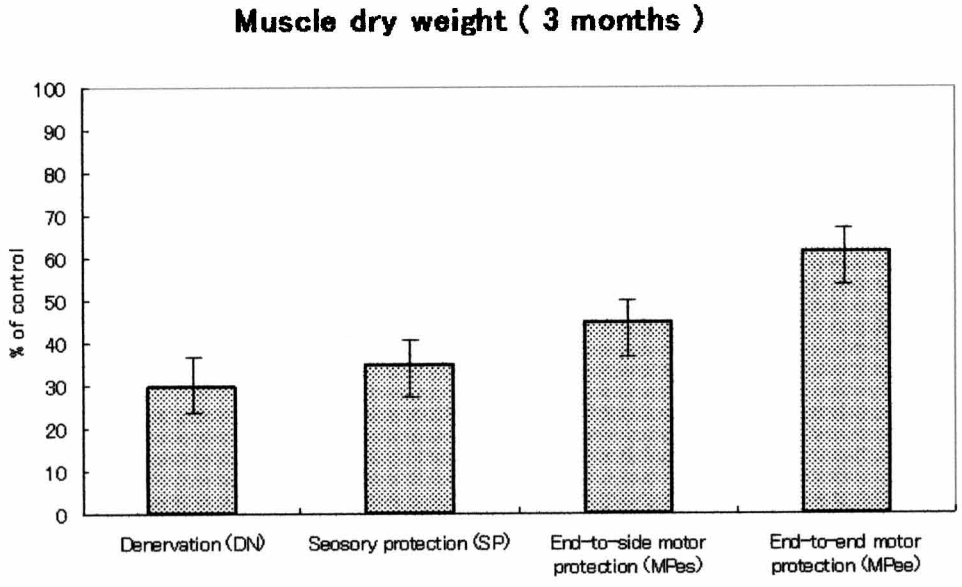
Figure.2



DN ≦ SP ≦ MPes ≦ MPee

(P<0.001, Jonckheere trend test)

Figure.3

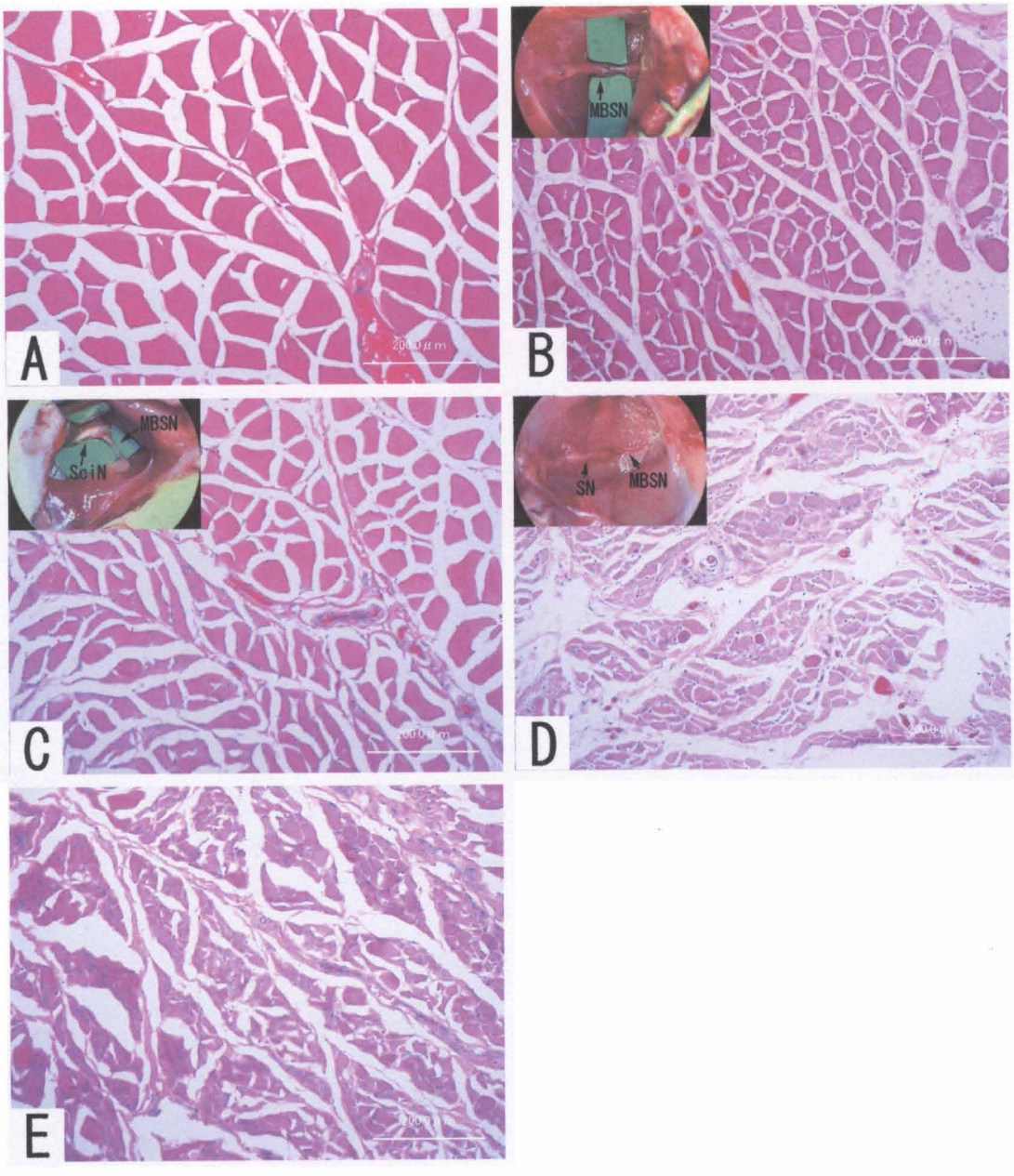


Figure.4