Comparison of surgical techniques used for prevention of neuroma in order to make more clear the role of the epineurium.

An experimental study

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Comparison of surgical techniques used for prevention of neuroma in order to make more clear. The role of the epineurium. An experimental study

AIM: *Several studies have been conducted for the prevention of neuroma and recently published experimental studies include interventions on epineurium. The techniques which include interventions on epinerium were compared to reveal the role of epinurium in neuroma prevention.*
MATERIAL E METHODS: *55 Sprague-Dawley rats were divided into five groups. Two of the groups were negative and positive controls. The proximal nerve stump was left "free" in the negative control group, while the stump was implanted in a muscle pocket in the positive control group following sciatic nerve transection. Experimental groups include epineural ligation, epineural stripping and epineural capping procedures. Follow-up period was six months. After sacrification of the rats, histopathologic and immunohistochemical examinations were conducted as well as real-time PCR studies for the assessment. Statistical analysis was performed.*
RESULTS: *The most prominent neuroma formation was detected in the epineural capping group, while the least neuroma was observed in the epineural ligation group.*
DISCUSSION: *Statistically significant differences were obtained when the three experimental groups were compared with both control groups. Interestingly there was no significant difference in-between the control groups in terms of preventing neuroma formation.*
CONCLUSION: *epineural ligation group were found to be superior to both control groups as well as experimental groups. Use of epineural capping was concluded to increase the formation of neuroma rather than preventing. Intramuscular implantation of nerve stump had no preventive effect on neuroma formation.*

KEY WORDS: Capping, Epineurium, Ligation, Neuroma, Stripping

Introduction

Neuroma is a painful mass located on a nerve stump after injury. It contains irregularly and improperly regenerated axons following injury of a peripheral nerve [1].

Pain makes neuroma an important clinical entity and neuromas that develop after extremity amputations are one of the common causes of stump pain [2]. There are currently 1.7 million individuals with amputated extremities in the United States (USA) and it is predicted that this number will double in 2050 [3].
Approximately 25% of all major amputated individuals are thought to experience chronic pain due to the development of symptomatic neuroma on amputation stump [4-5]. It has been emphasized that the neuroma formation is seen commonly on nerve stumps and should be regard-

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ed as a part of normal nerve healing [6]. When a peripheral nerve is excised and the proximal stump left free, it is predicted that neuroma will occur in a range between an asymptomatic histologic neuroma and a painful symptomatic neuroma.
Experimental studies have been carried out in the literature to prevent unguided axonal regeneration after nerve transection which eventually lead to a painful mass. Techniques such as ligation of proximal nerve stump, implantation into a pocket located on an adjacent muscle, stripping of epineurium to release fascicles or ligation after lengthening of the epineural sheath were used in different experimental studies and their efficacy was shown [7-10]. Despite the success of these methods in different studies, there is no study comparing the results of these methods with each other. For this reason, it is not known exactly which of the mentioned techniques is more effective in preventing neuroma.
In recent years, many studies have been done on the epineurium to prevent neuroma. This study was designed to compare the effects of most common techniques as well as to reveal the role of epinural layer in neuroma formation.

Matherial and Methods

Following the approval of the Institutional Animal Experiment Ethics Committee, the study was carried out in accordance with the Paris Universal Declaration of Animal Rights of 1978. Fifty five adult Sprague-Dawley rats weighing 250 to 300 g each were used.

Surgical Procedure

Before all surgical procedures; rats were anesthetized by intraperitoneal injection of 100 mg/kg ketamine (Ketalar® Flakon, Pfizer) and 15 mg/kg xylazine (Rompun® 2% Flakon, Bayer). The sciatic nerve was exposed after exploration with longitudinally skin incision on the posterior sides of right thighs of the animals.
The operation was continued under operating microscope magnification (OPMI 7, Zeiss, Germany). Fifty-five rats were randomly assigned to 5 groups as 11 rats in each. In all groups, a 2 cm long nerve segment was removed from the proximal part of the sciatic nerve (Fig. 1) and the procedure was performed with appropriate surgical procedure related to each group.
Group 1: (Free nerve stump) (negative control group): No procedure was performed on the proximal nerve stump after nerve segment was removed.
Group 2: (Implantating into the muscle) (positive control group): The proximal stump of sciatic nerve was implanted into a pocket located on the adjacent muscle and was fixed with 8/0 nylon suture.
Group 3: (Epineural ligation): The epineurium of the proximal segment was turned back in the proximal direction to a length of 5 mm, and the axonal structures without the epineurium layer were excised. Five mm-long epineurium layer, which was left intact, was advanced back in the distal direction and brought to its former position, resulting in an epineurium without axonal structures inside. The epineurium ending was tightly closed by attaching to the nail 9/0 nylon suture (Fig. 1A)

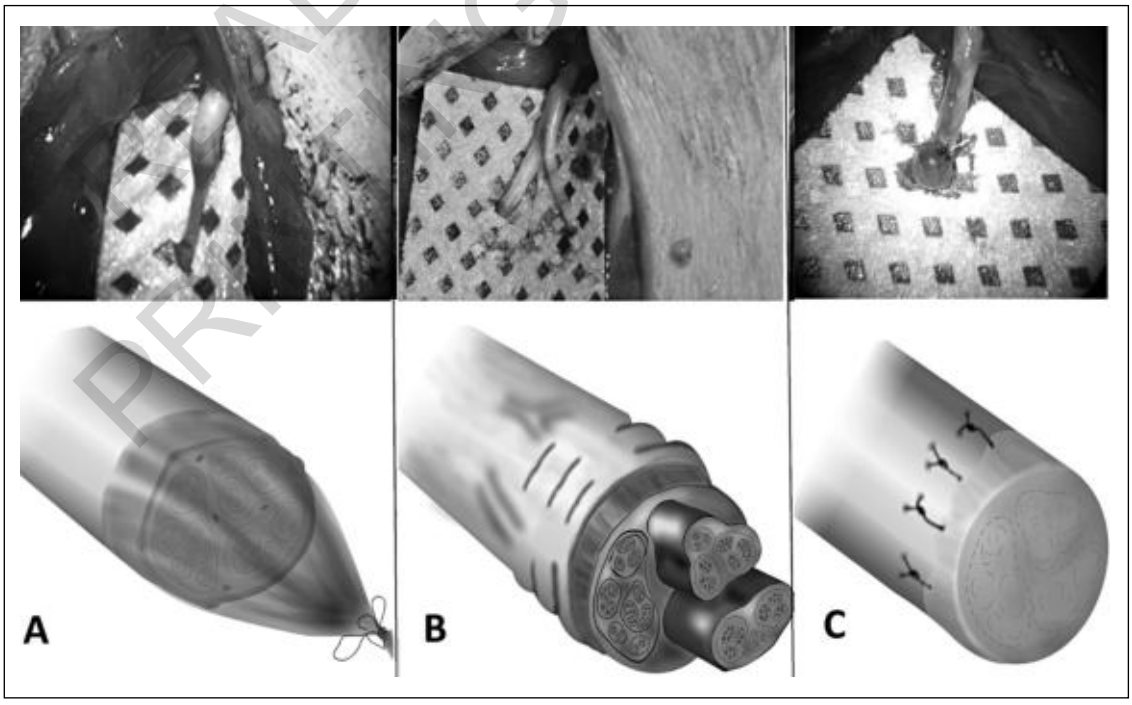

Fig. 1: Experiment groups: A) Epineural ligation group; B) Epineural stripping group; C) Epineural capping group.

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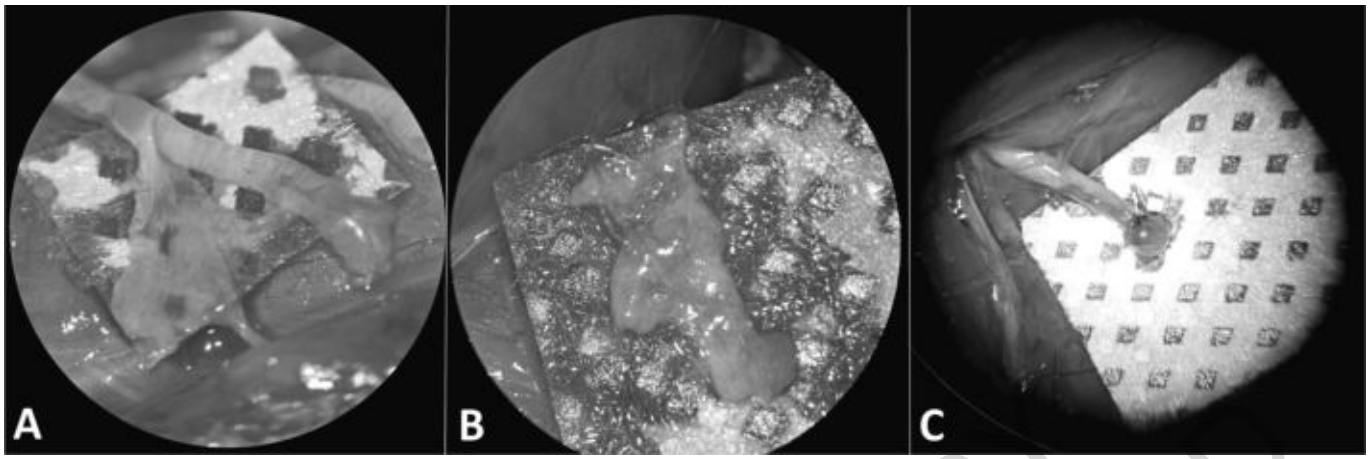

Fig. 2: Capping group: A) and B), Harvest of epineurium layer graft, C) Inset of epineural cap graft.

Group 4: (Epineural stripping): The epineurium at the proximal end of the sciatic nerve was stripped at 5 mm in length and nerve fascicles without the epineurium at the proximal stump were exposed (Fig. 1B).
Group 5: (Epineural capping group): The epineurium of the excised 2-cm-long nerve segment was removed and conserved for use as a graft (Figs. 1C, 2). The epineurium to be used as a graft was epinorally sutured to the proximal stump of sciatic nerve using 9/0 nylon suture to cover all nerve fascicules.
Muscles and skin were sutured after surgical procedures. All animals were hydrated by injecting 5 cc of saline via subcutaneous route. In the postoperative period, the animals were kept in the reanimation section until the anesthesia was completely resolved and then were taken to their cages. The animals were provided with dry food and drinking water that they could access freely in their cages. At a room temperature of 19-22 ºC, a light cycle of 12 hours light - 12 hours darkness was provided in a 40-50% humid environment in accordance with their circadian rhythms. Rats were followed for 6 months and 2 rats were lost from the epineural stripping group within the follow-up period. Two dead rats were removed from study. At the end of follow-up period, the animals were sacrificed after taking 1 cm long specimens for evaluation from the proximal end of the sciatic nerve. Nerve samples from 7 animals from each group were used for morphological and immunohistochemical evaluation, while nerve samples from 4 animals were used for real-time polymerase chain reaction (PCR). Since 2 animals died from the epineural stripping group during the follow-up period, the nerve sample of 2 animals was evaluated by real-time PCR in this group.

Evaluation

Morphological Evaluation

Morphologic evaluations were performed to reveal level of neuroma formation, and ratio of intraneural connective tissue and non-uniformly regenerating axonal structures.
The samples were fixed in 10% neutral buffered formalin solution, embedded into paraffin, and cut into 4 µm sections using ultramicrotome (Leica Ultracut R, Germany). The sections were stained with hematoxylin and eosin (H&E), Toluidine blue and Masson tricrom, and analyzed using an Olympus BH-2 (Tokyo, JAPAN) photomicroscope and Image Analyzer software (Digital Prioris, XL-511:IPS 4.02, Alcatel, TITN, Answer). Neural structures, organization of axons, and level of neuroma formation were assessed with H&E, and Toluidine blue while the amount of connective tissue was evaluated with Mason tricrom staining. Nerve cross-sectional area and ratio of neural to connective tissue were calculated using the samples staining with Mason trichrom.

Immunohistochemical Evaluation

Immunohistochemical evaluations were performed to reveal phenotypical differentiation of Schwann cells and level of pain by using antibodies against S100 and CGRP, respectively.
Antibodies against S-100 and Calcitonin Gene Related Peptide (CGRP) were detected to evaluate neural tissues. S-100 identifies phenotypically differentiated Schwann cells. CGRP is a protein that its synthesis increases in painful processes, and its presence is interpreted as the presence of pain. Sections with a thickness of 8 µm were taken on positive scraped slides from each end of the nerve samples, and were fixed in 4% neutral buffered formalin solution for 10 minutes. An automated immunohistochemical stainer (Benchmark XT, Ventana Medical Systems, EMD Millipore Corp., CA, USA) was used in accordance with procedure of the device to stain against S-100 antibody (EMD Millipore Corp, CA, USA) at a dilution of 1:250-1:500, and against CGRP antibody (EMD Millipore Corp, CA, USA) at a dilution of 1:2000-1:4000.

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Real-time PCR Evaluation

Ciliary neurotrophic factor (CNTF) and CGRP-Receptor Component Protein (CRCP) gene expressions were evaluated by real-time PCR. Nerve tissues were subjected to sharp mechanical lysis with a razor and the lysed tissues were subjected to RNA isolation with "RNeasy Plus Mini Kit" (Qiagen, Manchester, UK). Then, the concentration and purity of the isolated RNAs were measured by microdrop spectrophotometer (Thermo Scientific, MultiScan GO). A complementary DNA (cDNA) reaction was established with 250 ng RNA, which was taken from each sample, by using "RT2 First Strand Kit" (Qiagen, Manchester, UK). The reaction product was diluted with 91 µL sterile distilled water and concentration/purity measurements were performed (Thermo Scientific, MultiScan GO). Real-time PCR analysis was performed with the Rotor-Gene Q device according to manufacturer's protocols (Qiagen, Manchester, United Kingdom). Expression profiling of the samples was performed using the proportional expression analysis 2-ΔΔCT method, assessed relative to the rat GAPDH control gene.

Statistical Analysis

The package program SPSS (Statistical Package for Social Sciences for Windows 22.0) was used for statistical analysis. Data were assessed for normality, and values were analyzed by using the Mann-Whitney U test and Kruskal-Wallis test. The findings were evaluated at 95% confidence interval and 5% significance level. In all instances, probability values were two-tailed, with $P<0.05$ were considered significant.

Results

Macroscopic Results

Macroscopic neuroma formation was observed in all groups (Fig. 3). The most prominent neuroma formation was observed in the epineural capping group (Fig. 3E), while "the weakest" neuroma formation was observed in the epineural ligation group (Fig. 3C).

Morphologic Results

Three different parameters were used for morphological evaluation: neuroma formation, ratio of histopathologically chaotic areas to nerve section, and ratio of intraneural connective tissue to nerve section. The experimental groups were compared with the two control groups separately, and in addition, the two control groups were compared within themselves.

Neuroma formation was observed histologically in all the groups, however, neuroma formation levels differed between the groups (Table I), (Fig. 4).

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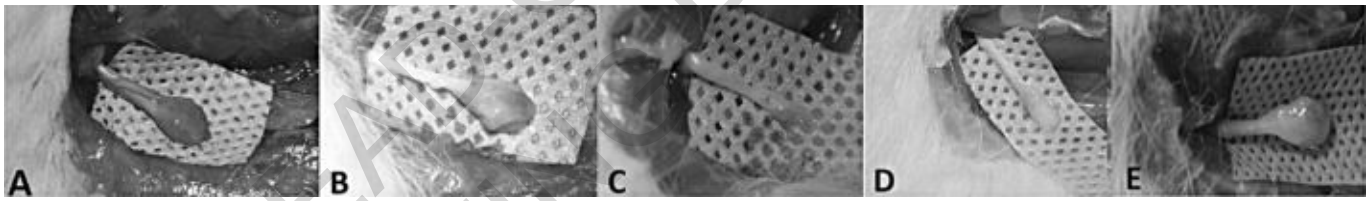

Fig. 3: Macroscopic results of all groups after 6-months follow-up period: A) Free nerve stump group; B) Intramuscular implantation group; C) Epineural ligation group; D) Epineural stripping group; E) Epineural capping group.

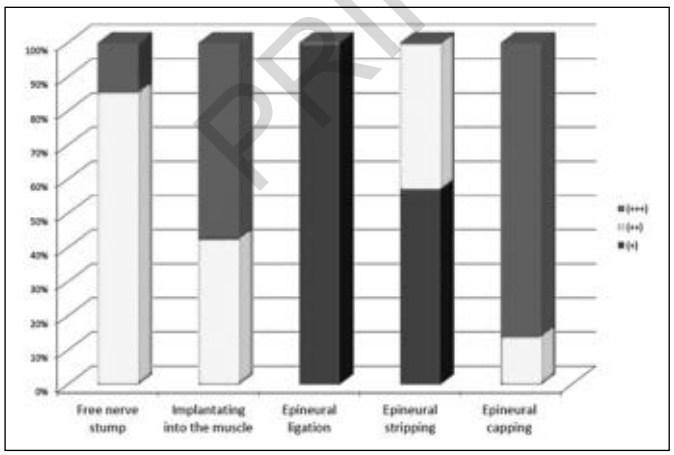

Fig. 4: Graphics showing the distribution of neuroma formation.

Table I - *Data Regarding to Histologic Neuroma Formation*

	Distribution of Histologic Neuroma Formation			
	+	++	+++	Total
Free Nerve Stump Group	0	6	1	7
Intramuscular Implantation Group	0	3	4	7
Epineural Ligation Group	7	0	0	7
Epineural stripping group	4	3	0	7
Epineural capping Group	0	1	6	7
Total	11	13	11	35

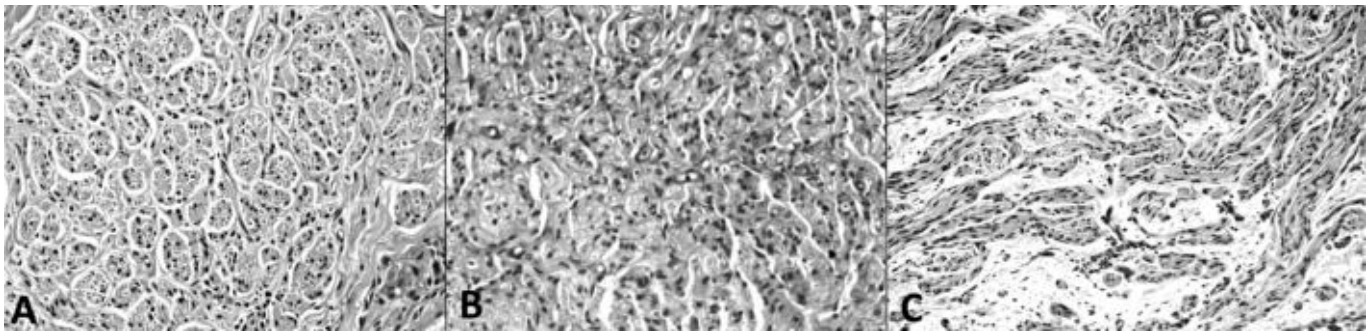

Fig. 5: Histopathologic neuroma formation, Epineural capping group: A) Hematoxilen & Eozin, X400; B) Toluidin Blue, x400; C) Masson Trichrom, x200.

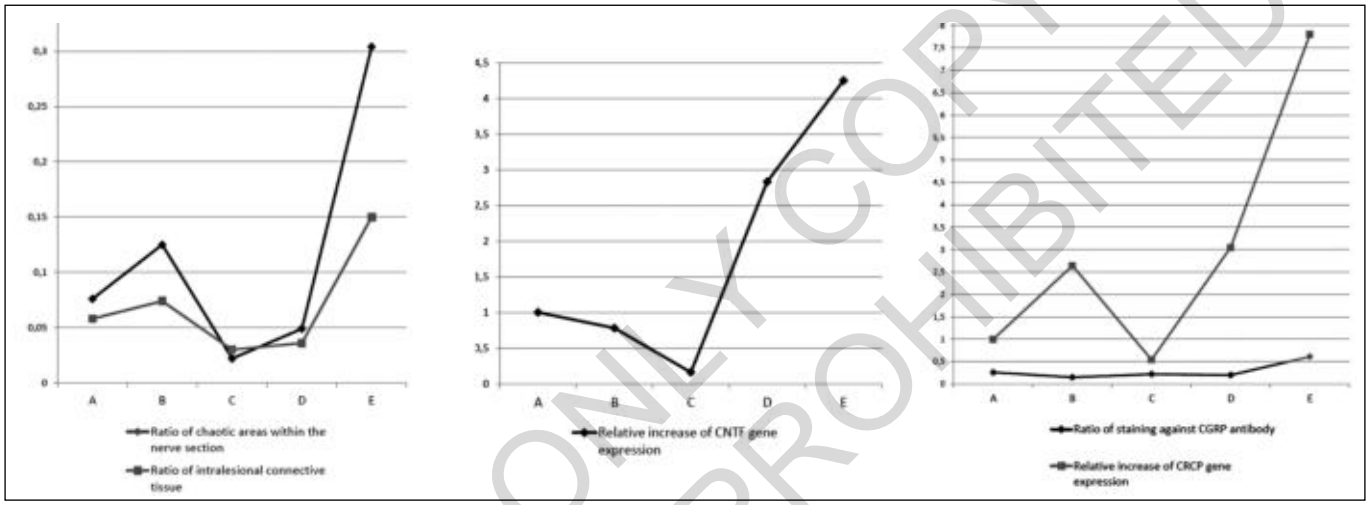

Fig. 6: Graphics showing results of the study: A) Free nerve stump group; B) Intramuscular implantation group; C) Epineural ligation group; D) Epineural stripping group; E) Epineural capping group.

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Statistically significant findings were obtained when the three experimental groups were compared with free nerve stump and intramuscular implantation groups ($p < 0.05$). Group with the weakest neuroma formation was epineural ligation group ($p<0.05$). There was statistically significant increase in neuroma formation in epineural capping group when compared to the free nerve stump group ($p < 0.05$) (Fig. 5).

There was no significant difference between the free nerve stump group and intramuscular implantation group in terms of neuroma formation ($p > 0.05$).

When the ratio of the chaotic areas in the nerve section were examined, there were statistically significant differences between experimental groups and control groups ($p < 0.05$) (Table II), Fig. 6).

It was observed that the chaotic areas in the epineural ligation group were significantly lower than both control groups ($p < 0.05$). There was a significant decrease in epineural stripping group compared to intramuscular implantation group ($p < 0.05$). On the contrary, the proportion of chaotic areas in the epineural capping group was statistically significantly higher than the free nerve stump group ($p < 0.05$).

There was no significant difference between the free nerve stump and intramuscular implantation groups in terms of the ratio of chaotic areas ($p > 0.05$).

When the ratios of intraneural connective tissue to nerve section were examined, it was seen that there was a statistically significant difference between the experimental groups and the control groups ($p < 0.05$).

There was a significant increase in intrinsic connective tissue formation in the epineural capping group compared to control groups ($p < 0.05$) (Fig. 6).

Immunohistochemical Results

Two different parameters were evaluated by immunohistochemical examination. Staining against S-100 antibody and staining against CGRP antibody were determined. The experiment groups were compared with the control groups separately, and in addition, the two control groups were compared with each other.

Statistically significant differences were observed between the experimental groups and the control groups against S-100 antibody ($p < 0.05$) (Table II).

TABLE II - *Results of the study. Histologic, immunohistochemical and real time PCR results and standard deviations were shown*

	Ratio of Chaotic Regions (%)	Ratio of Intraneural Connective Tissue (%)	Staining against S-100 Antibody	Staining against CGRP Antibody	Relative Level of CNTF Gene Expression (%)	Relative Level of CRCP Gene Expression (%)
Free Nerve Stump Group	7.6 ± 6,.7	5,.8 ± 4,1	6,14 ± 0,69	0,26 ± 0,15	100	1
Intramuscular Implantation Group	12.5 ± 5.8	7.4 ± 5.9	5.71 ± 1.11	0.15 ± 0,.14	78,.0 ± 31.0	264.0 ± 114.0
Epineural Ligation Group	2,2 ± 2,9	3,0 ± 4,0	6,86 ± 0,38	0,22 ± 0,21	16,0 ± 26,0	54,0 ± 164,0
Epineural stripping group	4,9 ± 3,5	3.6 ± 2.8	4.86 ± 0.69	0.20 ± 0.23	283.0 ± 108,.0	305.0 ± 109.0
Epineural capping Group	30.4 ± 11.8	15.0 ± 8,.7	5.57 ± 0.53	0.61 ± 0,.16	425.0 ± 61.0	780.0 ± 56.0

It was observed that there was a significant decrease in the epineural stripping group compared to the free nerve stump group ($p < 0.05$).
Statistically significant differences were observed between the experimental groups and the control groups when compared against CGRP antibody ($p < 0.05$).
When the staining rate against CGRP antibody was examined, it was observed that there was a statistically significant increase in the epineural capping group compared to both control groups ($p < 0.05$) (Fig. 6).
There was no statistically significant difference in CGRP antibody staining ($p > 0.05$) between free nerve stump group and intramuscular implantation group.

Real Time PCR Results

The expression of CNTF and CRCP genes was assessed in terms of fold increase relative to the free nerve stump group (Fig. 6).
There was a statistically significant increase in the expression of CNTF gene in the epineural stripping and epineural capping groups compared to the free nerve stump group ($p < 0.05$). In contrast to these two groups, there was a statistically significant decrease ($p < 0.05$) in CNTF gene expression in the epineural ligation group compared to the free nerve stump group (Table II), (Fig. 6).
The expression of CRCP gene in the epineural ligation group was significantly decreased ($p < 0.05$) compared to the free nerve stump group. In contrast, the CRCP gene expression in the epineural capping group was significantly increased compared to the free nerve stump group (Fig. 6).

Discussion

Many methods have been tried to prevent neuroma formation. In this study, the methods used in different studies were used in the same experimental study for the first time and tried to understand their superiority to each other. Because the main purpose of the study was to understand the role of the epineurium layer of the nerve on the formation of neuroma, epineurium-related methods were selected in experimental groups and compared with control groups.
If the proximal stump is left unprocessed after peripheral nerve transection, irregularly and chaoticly regenerated axonal structures are predicted to form a neuroma at the histological dimension [11] and this condition is considered to be a part of normal nerve healing [6].
For this reason, the sciatic nerve was cut and released in the first control group of our study, and this group, which was designed as a negative control group, was called the "free nerve stump group".
In clinical practive, the most common method for the prevention of neuroma formation is implantation of the proximal stump of the transected peripheral nerve into an adjacent muscle tissue. For this reason, in the second control group of the study, proximal stump of the transected nerve was implanted into an adjacent muscle tissue. This group was called the "intramuscular implantation group", and designed as a positive control group.
In our study, 5 groups of animals were used, consisting of 2 control groups and 3 experimental groups, with 11 animals each. The number of animals in the group is mainly designed to be 11 due to differences in assessment methods. The sample size for the data that can be obtained without statistical "bias" in 5 groups for 95% confidence interval and 80% power analysis in order to keep the type alpha and type beta errors statistically low was found as 6 animals in each group. Technically, it is not possible to determine gene expression by real-time PCR in histopathologically examined tissue specimens. For this reason, 4 animals were added to all groups in order to evaluate by real-time PCR. In order to predict that the 6-month follow-up period was quite long for an experimental study, 10% of the animals were operated as an attrition rate in each group so that possible rat deaths would not affect the statistical results. For these reasons, the number of animals in the groups was calculated as 11.
Since the scar tissue in the surgical field may be effective on the formation of neuroma, care was taken not to destroy the tissues during dissections in nerve bed and muscle tissue at the operations. In addition, since peripheral nerve transections using cautery have been reported to be effective on neuroma formation [12], the

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transections were made sharply with a scalpel while creating a nerve defect.

The longest possible defect proximal to the trifurcation of the sciatic nerve was formed (2 centimeters) due to spontaneous regeneration of short nerve defects can be possible in rats [8].

Evaluation methods were histopathological, immunohistochemical and real time PCR. Structurally, the presence of neuroma and "the level of neuroma formation" were assessed with hematoxylin & eosin, and histopathologic chaotic areas within the nerve section were assessed with toluidine blue. Neuromas have connective tissue in the nerve samples, in addition to the regenerated axonal structures. For this reason, nerve samples were stained with masson trichrome which allows discrimination of connective tissue and axonal structures.

CNTF is a neurotrophic factor that plays a role in the maintenance of axonal functions and has been reported to increase in nerve regeneration [13,14]. CNTF binds to the IL-6 receptor [15] and induces cell growth and differentiation through the activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) and mitogen-activated protein kinase (MAP-Kinase) pathways as a result of stimulation of IL-6 receptor. CNTF is an increased protein in increased axonal regeneration and amount of CNTF can be used as an evaluation method for axonal regeneration in experimental studies. For this reason, real-time PCR was used to reveal the expression of CNTF gene.

Levels of CGRP and CGRP receptor were used in the indirect evaluation of the pain levels of the neuromas in our study. CGRP is an increased protein synthesis in painful processes [16-18] expression of its synthesis and its presence is interpreted as the presence of pain. In addition, CRCP, which indicates the level of CGRP receptor, has been reported to be significant on pain pathways with CGRP [19,20]. CGRP levels were assessed by immunohistochemical staining; and CRCP levels at the level of gene expression assessed by real-time PCR in order to understand the pain levels of neuromas formed in our study rats. These two assessment methods have identified the increased pain levels connected to presence of increased CGRP as well as increased receptor levels of CGRP.

At the end of our study, there were significantly less neuroma formation in the epineural ligation and epineural stripping groups compared to the free nerve stump and intramuscular implantation groups. Our results are consistent with the study conducted by Yildirim et al [9], while are contrary to the results of Yuksel et al [10].

In addition, the ratios of intrinsic connective tissue and intrinsic chaotic areas in the epineural ligation and epineural stripping groups were found to be significantly reduced compared to the intramuscular implantation group. In other words, it has been observed that intramuscular implantation of the nerve ending, when compared to epineural ligation and stripping, results in an increased intraneural connective tissue and chaotic areas; and does not inhibit neuroma formation.

When the epineural capping group was compared to the free nerve stump group, significantly higher results were determined in all parameters such as neuroma formation, ratio of intraneural chaotic areas and ratio of intraneural connective tissues. Depending on these, the epineural capping group has been found to have an enhancing effect on neuroma formation. Siemionow et al [21] has described a new surgical technique for neuroma prevention using epineural sheath as a jacket, and they have reported successfull results. However they sutured epineural sheath over the nerve stump using sleeve technique and covered a 7 mm section of the nerve. This difference between the techniques may explain the different results.

When the CNTF gene expression levels were measured by real-time PCR; a significant decrease in expression of CNTF in the epineural ligation group is correlated with a decrease in neuroma formation in the same group. In the epineural capping group, there was a significant increase in the expression of CNTF gene, which correlates with increased neuroma formation in the epineural capping group.

When the pain parameters were examined, no significant difference was found in staining against CGRP antibody between in epineural ligation and epineural stripping groups compared to both control groups. Comparing pain at the receptor level showed less receptor expression in the epineural ligation group than in both control groups. the difference in the level of the receptor level emerged as a consequence of the epinural ligation group being less painful. Epineural capping group was significantly more stained against CGRP antibody compared to both control groups.

It was also observed that there was a significant increase in CRCP gene expression level in the epineural capping group compared to all groups. These parameters leaded a conclusion that epineural ligation group was less painful compared to the release of the nerve endings, or implantation into the muscle. Increase in both CGRP synthesis and CRCP gene expression level in the epineural capping group can be connected to be more painful than both control groups. This finding is in contradiction to the findings of the clinical study conducted by Yüksel et al [10].

When all evaluation parameters are analyzed together, the epineural capping technique which is used epineurium as a graft was seen to increase the painful neuroma formation at the histopathologic and macroscopic levels. In contrast, it has been understood that the epineural ligation technique reduces painful neuroma formation at histopathologic and macroscopic levels. The epineural stripping reduces neuroma formation compared to controls at the histopathological level, but when the pain level is considered, epineural stripping may be more painful than leaving the nerve stump free.

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It was observed that there was no significant difference between release of the nerve stump free and the implantation into the muscle in all evaluation parameters. From here, in clinical practice, implanting the proximal nerve stump into the muscle has resulted in no superiority over the unprocessed endings of a proximal nerve stump. This finding contradicts common clinical practice as well as the conclusion reached by Sinis et al [8]. However, although there is no significant difference between leaving the stump free and implantation the nerve into the muscle, it can be predicted that the muscle tissue can be a protective cushion against external trauma to make feeling pain less.

When we look at the limitations of our study, the most important part of the confrontation is about the evaluation methods of neuroma. Unfortunately, histopathological evaluation of neuroma is frequently performed by subjective methods. Since it is known that neuroma formation usually occur after all peripheral nerve transections, even in the control groups, it is possible to use subjective criteria to decide "less" neuroma and "more" neuroma formation. In order to reduce the weakness of this condition, evaluation was performed with an experienced pathologist on nerve pathology. In addition, all samples were blinded to the groups by the same pathologist and revealed using the neuroma level scoring system. In addition to the determination of neuroma presence, evaluation of chaotic areas, connective tissue and nerve ratios and axonal regeneration parameters in the nerves has been used to strengthen the beforementioned weak sides.

Conclusion

When the data of our study were evaluated, it was found that epineurium as a graft has increased the formation of neuroma compared to native epineurium however, ligation of the epineurium resulted in reducing neuroma formation. Implanting nerve stump intramuscularly, a frequently used method to prevent neruoma formation in clinical practice, had no preventive effect on neuroma formation. However, because of implantation of the nerve stump into the muscle creates an additional physical protective environment around the nerve, the optimal method of preventing neuroma formation may be ligation of epineurium and then implanting it into a adjacent muscle tissue.

Future experimental and clinical studies are needed to reveal the role of epineurium on neuroma formation in more detail.

Riassunto

Il meccanismo di formazione del neuroma che si verifica sul nervo o alla terminazione nervosa dopo precedenti lesioni dei nervi periferici non è stato ancora. Sono stati condotti molti studi per prevenire la formazione del neuroma, che è noto per causare una diminuzione della qualità della vita, soprattutto quando è doloroso. Recenti studi sperimentali includono interventi da realizzare sull'epinevrio. In questo studio sperimentale, i metodi utilizzati in diversi studi sono stati combinati in un unico studio e tutti i gruppi sperimentali sono stati selezionati dai metodi relativi all'epinevrio. In questo modo è prevista la comprensione del ruolo dello strato di epinevrio sulla formazione del neuroma.

Lo studio è stato concepito come uno studio sperimentale su animali. 55 ratti adulti Sprague-Dawley di 12 settimane sono stati divisi in 5 gruppi, 11 ciascuno. Nello studio ci sono tre gruppi sperimentali e due gruppi di controllo. Nei gruppi sperimentali sono state applicate tecniche di legatura epineurale, peeling epineurale e rivestimento epineurale. I gruppi di controllo sono stati progettati come gruppi di controllo negativi e positivi. Nel gruppo di controllo negativo, il nervo periferico è stato tagliato e la terminazione nervosa prossimale è stata liberata. Nel gruppo di controllo positivo, l'estremità prossimale del nervo periferico è stata impiantata nel tessuto muscolare adiacente. Dopo la prima operazione i ratti sono stati seguiti per 6 mesi e alla fine di tale periodo sono stati sacrificati ed è stata avviata la parte di valutazione. Esame istologico con coloranti ematossilina ed eosina, blu di toluidina e tricromia Masson; Esame immunoistochimico con S-100 e peptide correlato al gene della calcitonina (CGRP); Le valutazioni sono state effettuate mediante reazione a catena della polimerasi (PCR) in tempo reale con fattore neurotrofico ciliare (CNTF) e proteina componente del recettore CGRP, ed i risultati sono stati ottenuti dopo i necessari calcoli statistici.

Risultati: Alla fine dello studio, è stato osservato che la legatura dello strato di epinevrio dopo il taglio dei nervi periferici era il metodo di maggior successo nel ridurre la formazione di neuroma tra i gruppi sperimentali ($p<0,05$). Si è concluso che la tecnica di legatura epineurale ha avuto più successo rispetto al rilascio delle terminazioni nervose periferiche o all'impianto intramuscolare ($p <0,05$).

Conclusione: È stato osservato che il rivestimento dell'estremità nervosa con un innesto di epinevrio aumenta la formazione del neuroma piuttosto che diminuirla. Inoltre, si è concluso che lo strato di epinevrio ha un effetto sulla formazione del neuroma e, in presenza di epinevrio, il neuroma si forma a una velocità maggiore. È stato osservato che l'impianto intramuscolare dell'estremità nervosa, che è la misura più frequentemente applicata nella pratica clinica, non ha alcun vantaggio evidente rispetto al rilascio. Tuttavia, poiché l'impianto intramuscolare dell'estremità nervosa fornisce un ambiente protettivo extra dei tessuti molli attorno all'estremità nervosa, si consiglia di collegare l'estremità nervosa periferica e impiantarla per via intramuscolare per ridurre la formazione di neuroma.

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