The neurosurgical comparison of microsuturing with the tissue adhesives

Serap S.İNALÖZ[1], H.Erdem AK[2], Vildan YAYLA[3], Mehmet AKIN[4], Adem ASLAN[5], Ibrahim SARI[5], Yusuf ÇELİK[6], Aydın KETANİ[1]

Depts. of [1]Histology&Embryology, neurosurgery, [3]Neurology, [4]Physics, [5]Pathology, and [6]Biostatics, Medical School of Dicle University, Diyarbakır, TURKEY

The purpose of this study was to investigate and to compare the healing effects of microsuturing to that of tissue adhesives (Tiseef®) in anastomosing peripheral nerve incisions. Assessments of the healing processes were demonstrated by using 1) electromyography (EMG), 2) measuring the electrical responses of the anastomosed nerves after electrical stimuli, 3) histopathologically. In the control group of rats, bilateral sciatic nerves were explored but no incision was made. In the first experimental group, bilateral sciatic nerves were incised and end-to-end anastomosing was performed by microsuturing the epineurium. In the second experimental group, tissue adhesive (Tiseef®) was used in anastomosing the sciatic nerve cuts. After one month, EMG assessments were performed in all rats. After EMG measurements, bilateral sciatic nerves were explored at the site of anastomoses and 2 cm lengths were biopsied at both sites. Immediately after, the electrical responses of the dissected nerves were measured. Finally, histopathological assessment were performed using a light microscope. According to the EMG findings, statistical analysis showed that the Tiseel group was the closest in the healing process to the control group. After applying the electrical stimuli to the dissected nerves, the nearest resistance value to the control group was found to be in the Tiseel group. The histopathological examinations showed higly degenerative nerve bundles and prominent foreign body granuloma at the anastomosed sites of the microsutured group. The granulomatous inflammation was much less observed in the Tiseel group. The healing effect of Tiseel was found to be superior to that of microsuturing as demonstrated by the findings of EMG, electrical responses of the anastomosed nerves and histopathological examination.
[Turk J Med Res 1996; 14(2):42-47]

Key Words: Histopathology, Microsurgery, Sciatic nerve, Tissue adhesives

Peripheral nerve repair has become a specific branch of neurosurgery, with the support of detailed information about the nerve ultrastructure, the advancement in surgical methods using atraumatic techniques, and with surgical instruments which have been specifically designed for nerve repair (1). A great amount of progress has occurred by using microsurgical techniques in the repair of peripheral nerve injuries. In order to obtain a better regeneration in the peripheral nerve cuts, several autogenous materials such as vessel, fascia, durameter and synthetic materials such as collagenous tubes have been used to anastomose the nerve ends (2,3). The common aim of these wrapping techniques was to prevent the formation of connective tissue around the nerve ends (3). However, none of these procedures could prevent the fibrosis of nerve ends.

Important factors such as age, severity of the lesion, degeneration in neural tissues and the position of the lesion, all play a role in the healing of peripheral nerve cuts (4). In gross neural tissue damages, grafting has been preferred to anastomosing because of the complications of increased tension in the healing site. It has been suggested that grafting is more beneficial when the defect is larger than 2.5 cm (1,2,5). Immediate anastomosing is helpful only in smooth nerve cuts. If the injury involves adjacent tissues, primary suturing would not be the choice of treatment. However, only initial suturing can be performed in these kind of injuries with a second operation being performed as early as the 21st day when the infection risk is completely eliminated. The degree of healing in peripheral nerves can be assessed histopathologically and by EMG (1,2,6).

Received: Jan. 23, 1996 Accepted: Feb. 23, 1996

Correspondence: Serap S.INALOZ
Dept. of Histology&Embryology
Medical School of Dicle University
Diyarbakir, TURKEY

The aim of this study was to investigate and to compare the healing effects of microsuturing and tissue adhesives when applied to peripheral nerve cuts. The assessments of the healing process were performed by using 1) EMG, 2) measuring the electrical responses of the anastomosed nerves, 3) histopathological examination.

By using a new assessment method, we plan to measure the currents which flow in the anastomosed nerves after electrical stimuli.

MATERIALS AND METHODS

This collaborative study was performed in the Laboratory of the Histology&Embryology Department, the Micro-

surgery Laboratory of the Neurosurgery Department, the Health&Research Centre of Dicle University, in the EMG Laboratory of the Neurology Department and the Biomedical Laboratory of the Science and Arts Faculty. Forty-five Wistar-Albino rats (200-250 gr) were placed in special cages and separated into three groups (n=15). They were anesthetized with the combination of ketamin hydrochloride (40-50 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The animals were fixed to the surface in the prone position, skin layers and muscles were dissected and bilateral sciatic nerves explored using a surgical microscope. In the control group, bilateral sciatic nerves were only explored. In the first experimental group, bilateral sciatic nerves were cut completely and smoothly near the bifurcation sites and were stitched with silk (10/0). It is very difficult to stitch the fresh nerve cuts in rats, therefore, microsuturing was performed only after treating the tissues with 60% alcohol for 5 minutes.

In the second experimental group, all procedures were done in the same way. The nerve ends were anastomosed with Tiseel® (fibrinogen+factor XIII+fibronectin+aprotinin+plasminogen and thrombin solution) under a surgical microscope.

One month later, all rats were re-anesthetized using the same dosage. The electromyographical assessments were made with an Esaote Biomedica Phasis EMG Instrument. A concentric electrode was used for recording and superficial electrodes were used for nerve stimulation and a fixed stimulus was performed at 40 uA for 100 millisecond (ms). The gastrocnemius muscle and sciatic nerve trace were located. The ground cable was attached to the tail and the concentric electrode was inserted into the gastrocnemius muscle. Pathological activities such as fibrillations and positive spikes were observed during spontaneous activity. After following its trace, the sciatic nerve was stimulated from a proximal distance of 3 cm to the incision. After recording the M-response of the gastrocnemius muscle, its application time and amplitude were recorded in ms and V. This procedure was performed bilaterally to each animal in all groups.

After EMG measurements, bilateral sciatic nerves were explored at the site of the anastomoses and 2 cm lengths were biopsied at both sites. Imediately after, the electrical responses of the dissected nerves were measured in the Biomedical Laboratory. The electrical stimuli (0-8V) were gradually applied to the dissected nerves with a power supply. After each stimulus, the potentials of the nerves were measured at the distal ends by a Voltmeter and in addition, an Ampermeter was used to measure the currents which flowed in the anastomosed nerves.

For histopathological examination, all dissected nerves were placed in a solution of 10% formaldehyde. The tissues were then embedded in paraffin wax, sectioned, and finally stained with Hematoxylen Eosin (H&E). Histological assessments were performed using a light microscope.

RESULTS

In the first few days, bilateral sciatic nerve paralysis was observed in both experimental groups. Subsequently, skin ulcers were noticed on the feet of some rats. However, all wounds and neurological deficits recovered. No neurological deficits were observed in the control group of rats.

According to the EMG findings, average and standard deviation values were found to be 1.45±0.14 ms in the control group. The EMG conduction values compared to those of the control group. The values of the microsutured group were found to be 2.14±0.67 ms and 1.95±0.64 ms in the Tiseel group (Table 1). Statistical analysis using the unpaired-t test was applied, and "p" values were compared in all groups. No significant differences were found between the control and Tiseel groups ($p>0.05$) and between the Tiseel and microsutured groups ($p>0.05$). However, there was a significant difference between the control and microsutured groups ($p<0.02$). As expected, no pathological spontaneous activity was observed with EMG findings in the control group, whereas; the pathological spontaneous activities (fibrillations, positive spikes and denervation potentials) were noteworthy in the microsutured and the Tiseel groups (Figure 1,2,3). There were no significant differences between right and left legs.

In both experimental groups, electrical stimuli (0-8V) were gradually applied to the dissected nerves in the Biomedical Laboratory. The currents which flowed in the anastomosed nerves measured very low when the stimuli were between 0-0.6 V. An increase was observed when the stimuli were more than 0.6 V. The potentials and the currents which flow in the anastomosed nerves were recorded after each stimulus. The currents were found to be directly proportional to the potentials after 0.6V stimuli. The curve between 0.6-8V was almost linear in all groups (Figure 4,5). In each group, the resistances of the anastomosed nerves were found after the calculations described. The resistance values of each group are presented in Table 2. The resistance value of the control group was found to be the highest whereas it was the lowest in the microsutured group. In conclusion, the nearest resistance value to the normal nerve was found to be in the Tiseel group.

The histopathological examination showed highly degenerative nerve bundles and prominent foreign body granuloma were also noticed at the anastomosed sites of

Table 1. Conduction times on EMG of all groups

	Control group	1st group (Suture)	2nd group (Tiseel)
Conduction time (ms)	1.45±0.14	2.14±0.67	1.95±0.64

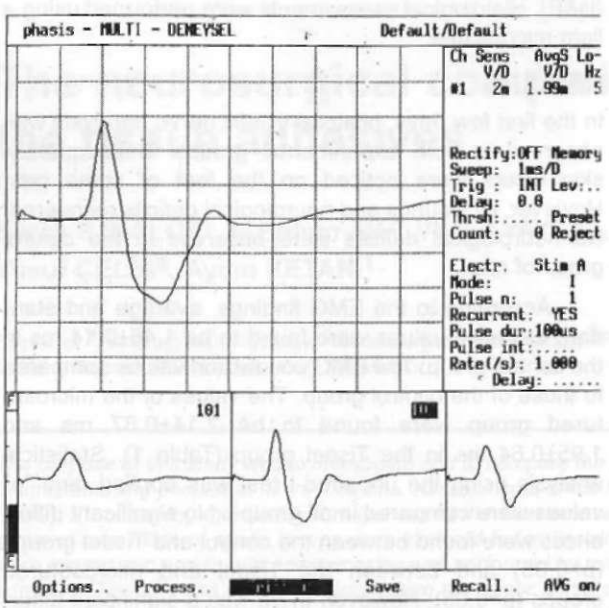

Figure 1. Sample of the EMG findings of the normal sciatic nerve in the control group.

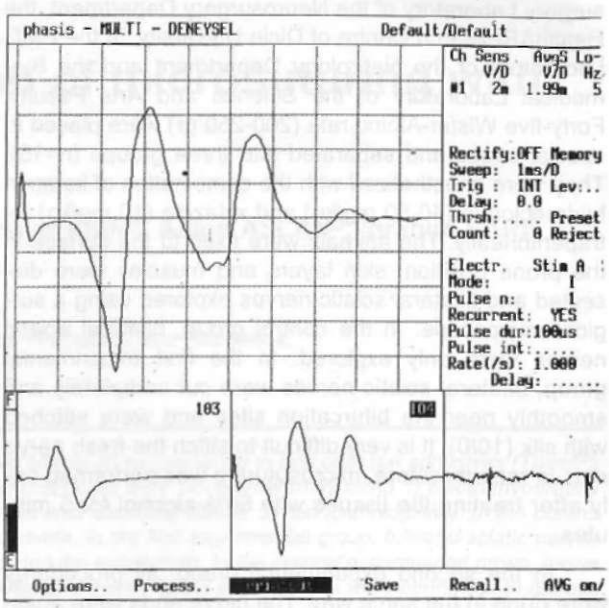

Figure **2.** Sample of the EMG findings of the sciatic nerve in the first experimental group

microsutured group. Foreign body giant cells around the stitch material were seen in most of the fields. Pronounced congestive vessels and conspicious chronic inflammatory cell infiltrate were seen in this group. It was obvious that the stitch material led to prominent foreign body granuloma in the anastomosed sites (Figure 6,7).

In the Tiseel group, very few foreign body giand cells were noticed in the chronic inflammatory cell infiltrate. However, the granulomatous inflammation was not conspicious, as seen in the microsutured group (Figure 8,9). Histologically, the healing effect of Tiseel was found to be superior to the microsuturing in peripheral nerve cuts.

DISCUSSION

Interfascicular suturing under a surgical microscope has been the suggested technique for peripheral nerve repair in several studies (2,3,7-9). However, objectors to the interfascicular suturing are becoming more numerous due to its traumatic and sarcogenic effects. There is a positive correlation between the diameter of the stitch material and the formation of fibrosis (2). The risk of trauma could not be prevented in the nerve reparations even with the use of microsurgical techniques and fine silk (10/0). In recent years, non-allergic tissue adhesives have been commonly used against the traumatic properties of microsuturing, particularly in cranial and peripheral nerve surgery (10-12). However, some poor results regarding with the UL>e of tissue adhesives have been reported (10,13). In a group study, no significant difference was observed between microsurgery and tissue adhesives in the reparation of sciatic nerve cuts (9). In this experi-

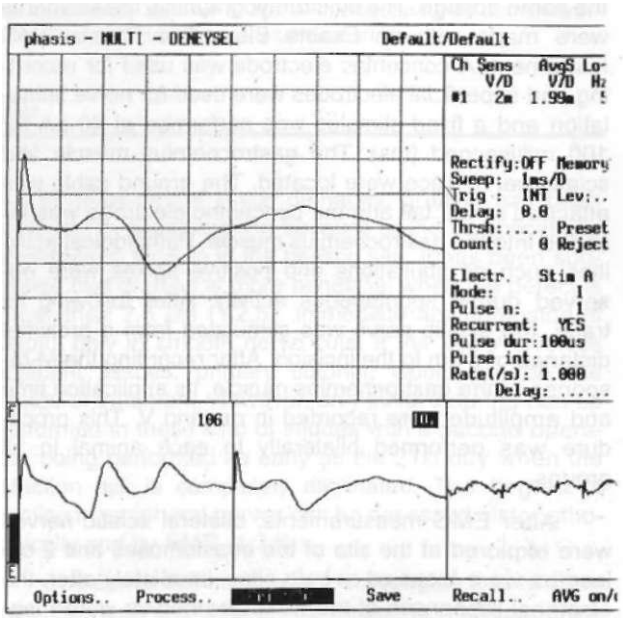

Figure 3. Sample of the EMG findings of the sciatic nerve in the second experimental group.

mental study, histopathological comparison was done on the healing effects of epineural suturing to the tissue adhesives in smooth sciatic nerve cuts. The healing criteria for anastomosed peripheral nerves include anatomical integrity of cut ends and also the recovery of motor and sensorial functions of the nerve (7). In the current study,

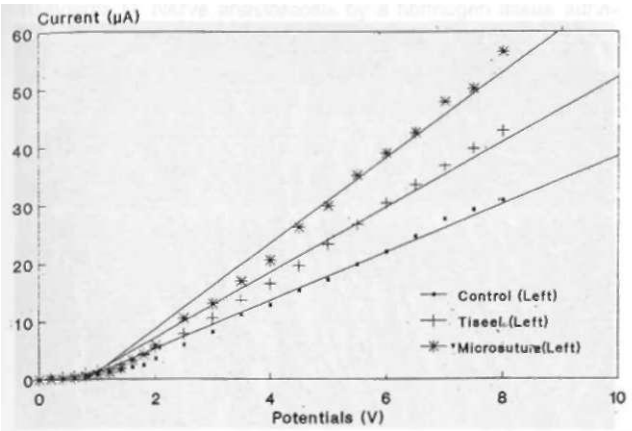

Figure 4. Current-potential graph of the left anastomosed nerves in all groups.

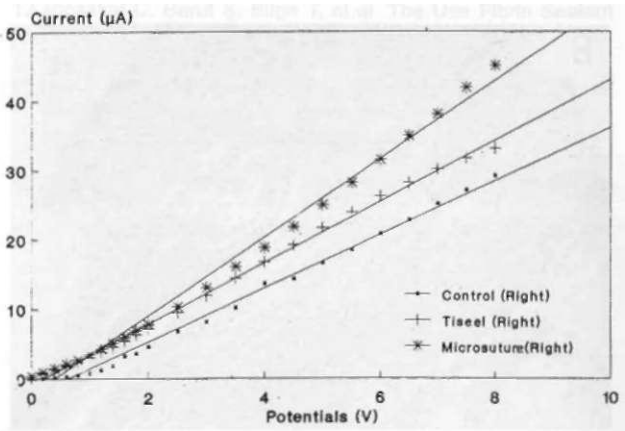

Figure 5. Current-potential graph of the right anastomosed nerves in all groups.

the Tiseel was found to be superior to the microsuturing in all healing criteria.

In a complete nerve cut, a neuroma usually occurs at the proximal end which leads to imperfect healing. As in our study, a complete healing has been observed if an immediate reparation has been done to a smooth nerve cut. However, the surgical method should be altered if there is an excessive loss in neural tissues (2,6,8).

In complete nerve cuts, repair should be aimed to exclude froeign material between the proximal and distal end distal ends of the anastomosed site. This could be performed by using tissue adhesives. In our study, remnants of the foreign material were noticed histologically in only one rat of the Tiseel group, whereas it was seen almost in all rats of the microsutured group. In the process of microsuturing, nerve fibres can be damaged despite the use of interfascicular or epineural microsurgical techniques. This is not a draw-back for the tissue adhesives as their uses have some superior qualities such as short operation time, anastomosing without a trauma and a quick and reliable healing. The only drawback to this technique could be the cost effectiveness.

Table 2. Nerve resistance values in Ohm unit

Sciatic Nerve	1st group Control	2nd group (Suture)	(Tiseel)
Right	$28x10^4$	$15x10^4$	$21x10^4$
Left	$22x10^4$	$11x10^4$	$14x10^4$

In conclusion, we observed that the healing effect of Tiseel was superior to microsuturing, as seen in the findings of EMG, electrical response of the sciatic nerve, and histopathology. We suggest that the use of tissue adhesives is more reliable in anastomosing smooth nerve cuts as demonstrated by the positive effects of minimal fibrosis, quick healing and practical use.

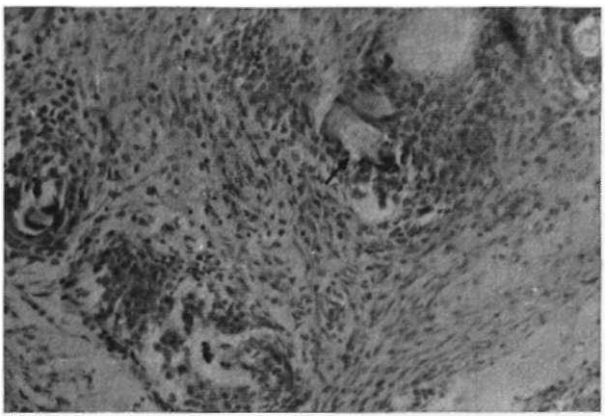

Figure 6. The microsutured group, degenerative nerve bundies, foreign body giant cells and granuloma against the stitch material (arrow) (H&E, original magnificationx82)

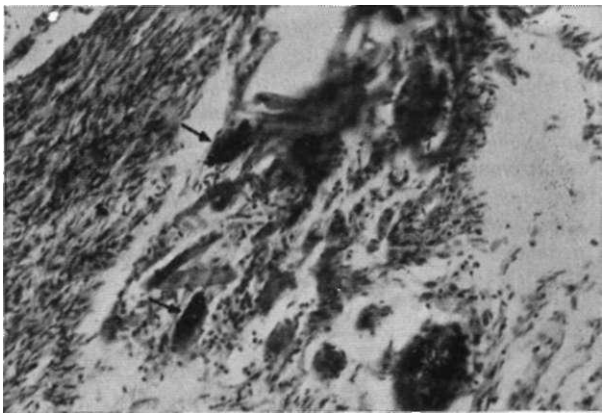

Figure 7. The microsutured group, highly degenerative nerve bundle, foreign body giant cells (arrows), congestive vessels and conspicuous inflammatory cell infiltrate (H&E, original magnificationx82)

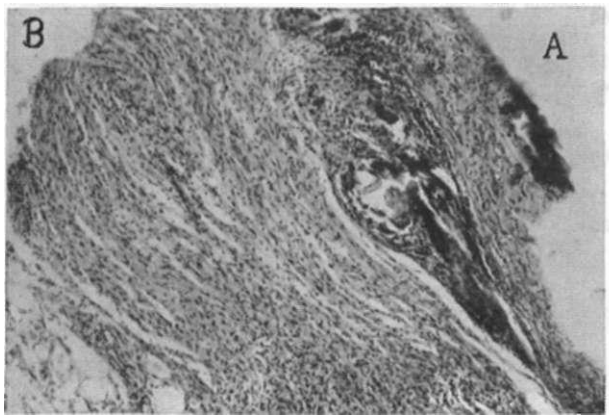

Figure 8. The Tiseel group, granulomatous inflammation with scattered foreign body giant cells (Side A). Uniform pattern of the healed nerve bundles (Side B) (H&E, original magnificationx41)

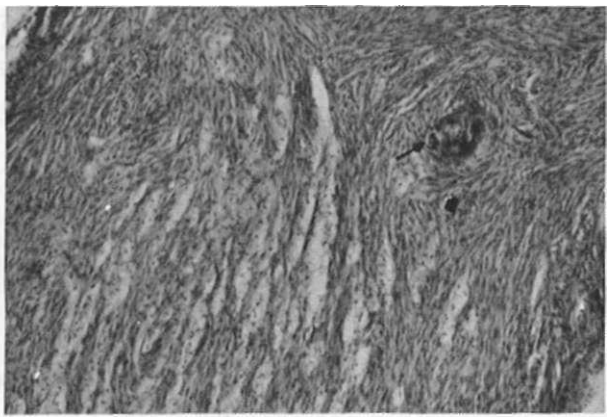

Figure 9. The Tiseel group granulomatous inflammation against foreign body in a small region (arrow). Healed nerve bundles (H&E, original magnificationx41)

Periferik sinir keşi tedavisinde mikrosütür ve doku yapıştırıcıların karşılaştırılması

Bu çalışmanın amacı, periferik sinir kesi anastomozunda mikrosütürün ve doku yapıştırıcısının (tiseel) tedavi edici etkilerini araştırmak ve mukayese etmek. Tedavi olgusunun değerlendirilmesi 1) EMG ölçümü, 2) Anastomoz yapılmış sinirlerin elektriksel uyarıdan sonraki cevabın ölçümü, 3) Histopatolojik olarak yapıldı. Kontrol grubu ratlarda bilateral siyatik sinirler explore edildi fakat kesi yapılmadı. Birinci deneysel grupta, bilateral siyatik sinirler kesildi ve uç uca anastomoz perineriumun mikrosütüre edilmesi ile gerçekleştirildi. İkinci deneysel grupta ise, doku yapıştırıcı (tiseel) siyatik sinir kesi anastomozunda kullanıldı. Bir ay sonra anastomozun olduğu bilateral siyatik sinirler explore edildi ve 2 cm uzunluğunda sinir biyopsisinin alınmasından hemen sonra kesilen sinirlerin elektriksel cevabı ölçüldü. Son olarak, histopatolojik değerlendirme ışık mikroskobu ile yapıldı. EMG bulgularına göre; yapılan istatistiksel analiz sonucunda tiseel grubu, kontrol grubuna en yakın olarak saptandı. Kesilen sinirlere uygulanan elektriksel uyarılardan sonra yapılan ölçümlerde ise; kontrol grubuna en yakın direnç değeri, tiseel grubundaydı. Histopatolojik incelemede ise; oldukça dejeneratif sinir demetleri ve yabancı cisim granulom oluşumu, mikrosütür grubunda gözlendi. Granulomatöz inflamasyon tiseel grubunda çok daha azdı. Sonuç olarak; tiseelin iyileştirici etkisi, 1) EMG bulguları, 2) anastomoz sinirlerin elektriksel cevapları ve 3) histopatolojik değerlendirme ışığında, mikrosütüre göre daha üstündü. [Turk J Med Res 1996; 14(2):42-47]

REFERENCES

1. Sunderland S. Nerve injuries and their repair. In: Sunderland S, ed. A critical Apprasial, 1st ed. Edinburgh: Churchill Livingstone, 1991:361-7.
2. Gökalp HZ, Erongun U. Periferik Sinir Travmaları. Nöroşirurji Ders Kitabı. Ankara: Mars Matbaası, 1988:268-9.
3. Aksoy K, Çordan T, Oğul E, et al. Experimental comparison of the suture technics in peripheral nerve transection. Bursa Tip Fakültesi Dergisi 1979; 4:173-82.
4. Myles LM, Gilmour JA, Glasby MA. Effects of different methods of peripheral nerve repair on the number and distribution of muscle afferent neurons in rat dorsal root ganglion. J Neurosurg 1992; 77(3):457-62.
5. Morris RW, Glasby MA, Gattasuso JM, Bowden RE. Peripheral nerve repair in humans using muscle autograft: A new technique. J Bone&Joint Surg Br 1988; 70(4):530-3.
6. Hudson AR, Hunter D. Timing of peripheral nerve repair: important local neuropathological factors. Clin Neurosurg 1977; 24:391-405.
7. Glasby MA, Gaschmeissner S, Hitchcock RJ, Huang CL. Regeneration of the sciatic nerve in rats: The effect of muscle basement membrane. J Bone&Joint Surg Br 1986; 68(829-33).
8. Levinthal B, Brown WJ, Rand RW. Comparison of fascicular interfascicular and epineural suture techniques in the repair of simple nerve lacerations. J Neurosurg 1977; 47(5):744-50.
9. Maragh H, Meyer BS, Davenport D, et al. Morphofunctional evaluation of fibrin glue versus microsuture nerve repairs. J Reconstr Microsurg 1990; 6(4):331-7.

10-Boedts D. Nerve anastomosis by a fibrinogen tissue adhesive. J Head&Neck Pathol 1982; 3:86-9.

H.Hasegawa H, Bitoh S, Obashi J, Maruna M. Closure of carotid-cavernous fistulae by use of a fibrin adhesive system. Surg Neurol 1985; 24(1):23-6.

12. Topsakal C, Barut Ş, Bilge T, et al. The Use Fibrin Sealant Products in Neurosurgery: Analysis of 6 Cases. Türk Nöroşirurji Dergisi 1993; 3:30-4.

13. Herter T, Windmann D. Choice of thrombin concentration of common fibrin glue systems for nerve anastomosis. Handchir Microchir Plast Chir 1992; 24(6):319-23.