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# An effective technique in nerve defect repair: Analysis of sliding epineural tube graft technique and comparison with autologous nerve graft and turn-over epineural tube graft techniques

Murat Kara[1]
ORCID: 0000-0002-9387-1977

Uğur Koçer[2]
ORCID: 0000-0003-4245-0459

Ramazan Arslan[2]
ORCID: 0000-0002-1075-7416

Koray Gürsoy[2]
ORCID: 0000-0002-7730-7225

Seçil Vural[3]
ORCID: 0000-0001-7736-7802

Ayşegül Fırat[4]
ORCID: 0000-0001-5105-0057

[1]Hacettepe University, Faculty of Medicine, Department Of Plastic Reconstructive and Aesthetic Surgery, Ankara, Türkiye.

[2]Ankara Training and Research Hospital, Divison of Plastic Reconstructive and Aesthetic Surgery, Ankara, Türkiye.

[3]Ankara Training and Research Hospital, Divison of Physical Medicine and Rehabilitation, Ankara, Türkiye.

[4]Hacettepe University Faculty of Medicine, Department of Anatomy, Ankara, Türkiye.

Corresponding Author: Murat Kara
E-mail: mailsmurat@gmail.com

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Objective: Autologous nerve graft (ANG) is the standard of care in the reconstruction of nerve gaps. However, scarification of a donor nerve, donor-site complications (wound complications, sensory dysfunction, neuroma, etc.) and unpredictable results lead surgeons to search for alternative techniques. Epineural tube graft (ETG) is a good option in the repair of nerve gaps. At this point, the present study aims to analyze the utility of the sliding epineural tube graft (SETG)technique in the reconstruction of nerve gaps.

Materials and Methods: Thirty Wistar albino rats were divided into five groups according to the repair technique of a 7 mm nerve defect created on the right sciatic nerve. In Group 1 the defect was left unrepaired as a negative control group. The defect was repaired with ANG in Group 2, with turn-over ETG (TETG) in Group 3, with one-directional SETG (O-SETG) in Group 4 and with bi-directional SETG (B-SETG) in Group 5. On the 12th week of the experiment, electrophysiologic, gross macroscopic and microscopic evaluation of muscle function and microscopic assessments of muscle and nerve samples were performed. The left limb and proximal nerve segment of the defect area were used as the control side.

Results: Electrophysiologic, macroscopic (wet muscle weight) and microscopic (axonal count, muscle fiber thickness was superior in the ANG group compared with TETG and SETG techniques. B-SETG showed poor results in all of the aforementioned findings. TETG and O-SETG techniques showed similar neuromuscular functions.

Conclusion: Although the ANG technique has some disadvantages depending on the sacrification of a donor nerve and donor side, it has significantly superior reconstructive outcomes compared to ETG techniques. However, since the ETG techniques provide acceptable results, they should be in surgeons' treatment repertoire because of the unique features of the microsurgical intervention.

Keywords: Nerve injury, nerve graft, epineural graft.

## INTRODUCTION

Peripheral nerve injuries (PNIs) are commonly associated with traumas with a general incidence of 1% to 3.3% [1-3]. PNIs are frequently seen in the upper extremity with a rate of up to 77%, which causes significant workforce loss [1, 4, 5]. Besides, PNIs are most commonly seen in financially and socially productive ages, between 16 to 35 years [2, 5, 6]. Morbidities due to injury or surgery may affect patients' quality of life and lead this individual clinical situation to a national healthcare problem, not only because of patients' loss of workforce but also high costs of treatment and rehabilitation expenses. Previous studies have reported that PNIs significantly prolong the duration of hospitalization. These aspects of PNIs make these clinical cases and the reconstruction of PNIs considerable.

Although different techniques have been reported in the literature, autologous nerve graft (ANG) is considered the standard of care in PNIs that are not eligible for primary repair [1, 7]. Neurotrophic factors within the nerve graft provide the appropriate microenvironment for axonal regeneration [8, 9]. This is compatible with the concept of "reconstruction of tissue with a similar tissue" in plastic surgery practice. However, because of donor-side morbidity (sensory and wound healing complications, scar, neuroma) and sacrifice of another nerve, previous studies analyzed other types of autologous or synthetic conduits. However, a practical, inexpensive, and minimally morbid surgical technique that provides ideal nerve regeneration and functional recovery has not been widely used in clinical practice yet [1]. On the other hand, some experimental studies have reported remarkable results of epineural sheath grafts and tubes [9-13]. The epineural sheath was used in sleeve or tube formation in some of these studies. Tube formation (epineural tube graft – ETN) was obtained by the pull-out technique [9], turn-over technique [10, 11], or vertical suturing technique [14]. Similar to ANG, the neural origin of ETN can provide superior success of axonal regeneration by secretin neurotrophic agents [8, 9]. This aspect of epineural graft has been confirmed by reported studies in the literature, which reported comparable functional and microscopic results with the ANG technique [10-12, 14, 15]. However, many limitations of the ETG technique in the repair of nerve gaps exist. Firstly, in some studies, ETG was obtained by pulling out the fascicules from a nerve graft [9, 16]. Although the aim of this technique is to demonstrate the effectiveness of ETG on nerve regeneration, the harvesting method of the ETG is not applicable in clinical practice. In the second technique, after harvesting the epineural graft, the tube formation is achieved by longitudinal suturation of the graft [14]. This incision line may cause a significant foreign body reaction, fibrosis and scar block which may prevent the axonal regeneration through the tube [9]. The third technique to obtain ETG is the turn-over technique [10, 11]. In the turn-over ETG (TETG) technique, the outer surface of epineurium becomes the inner surface of ETG. Hypothetically, this may cause fibrosis due to the irregular outer surface of the epineurium. Moreover, in ETG, because of the limited length of ETG, prominent nerve gaps can not be reconstructed with this technique. Eventually, although significant advantages of the ETG technique in the repair of nerve gaps, these prominent limitations prevent its use in clinical cases.

Another technique for harvesting and obtaining ETG is the sliding technique. Instead of turning inside out, the turn-over technique, after circumferential incision of epineurium on the proximal or distal nerve segment, an ETG can be harvested by sliding it into the nerve gap (Figure 1). This technique may prevent the aforementioned limitations. To our knowledge, there is no study analyzing the effect and results of the sliding ETG (SETG) technique in the English literature. Besides, by using the combination of sliding technique from distal and proximal nerve segments, hypothetically, ETG can be used to reconstruct larger nerve gaps. At this point, the aim of the present study is to demonstrate the harvesting of ETG by sliding technique and to analyze its effectiveness on nerve regeneration compared with ANG and previously described TETG techniques. Furthermore, to repair larger gaps, the utility of SETG from both distal and proximal nerve segments will be analyzed.

## MATERIALS AND METHODS

After approval of the institutional ethics committee, 30 Wistar albino rats were divided into five groups
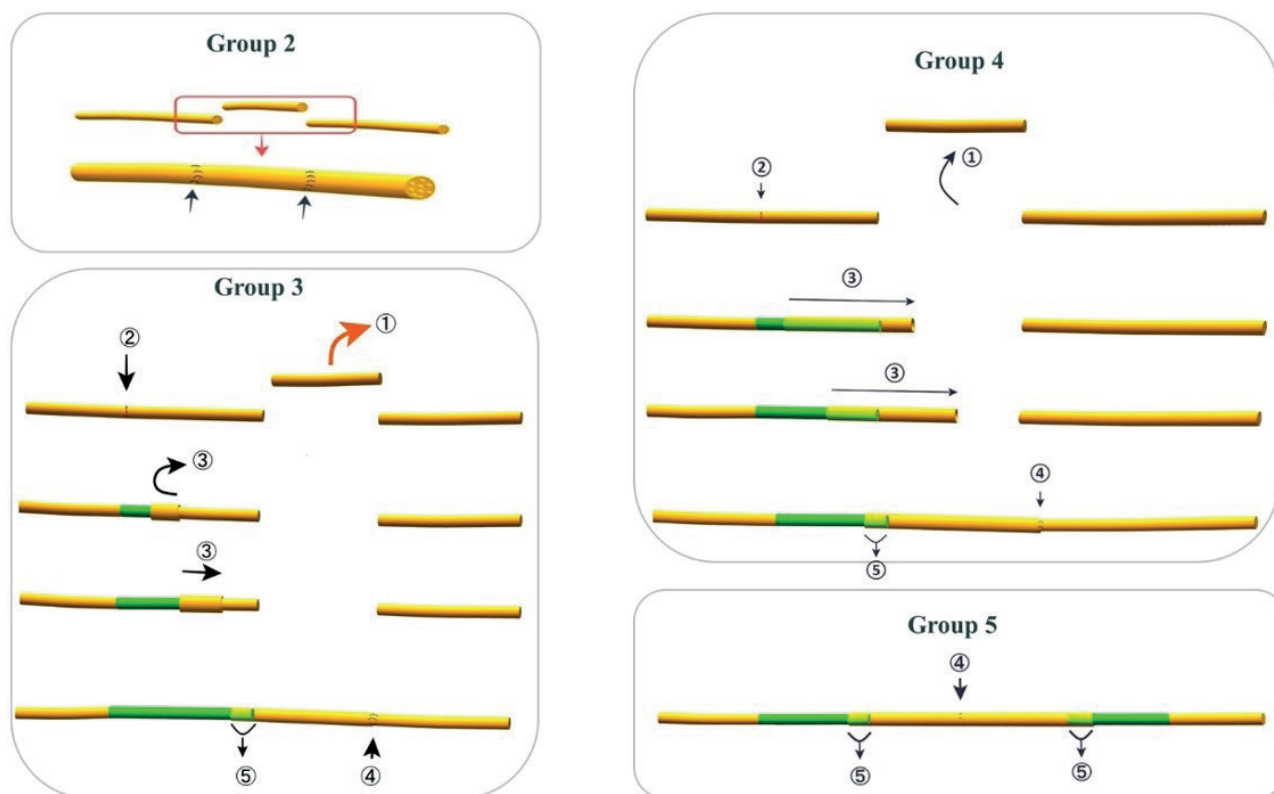
**Figure 1.** Schematic illustration of surgical technique of the groups. **1**: Excision of 7 mm nerve segment to create nerve defect, **2**: Circumferancial epineural incision, **3**: Harvesting the epineural tube graft by turn over technique (Group 3) and by sliding technique in Groups 4 and 5, **4**: Coaptation of ETG to contralateral nerve stump in Group 3 and 4, suturing two ETGs at the middle of the defect in Group 5, **5**: Preserved nerve segments covering with epineural sheath on distal and proximal nerve stumps to prevent the sheath from avulsion.

according to the repair technique. Group 1 was the negative control group without repair of nerve defect, Group 2 was the control group that defects repaired with ANG, Group 3 was the TETG group, Group 4 was the one-directional SETG (O-SETG) group in which ETG was harvested from the proximal nerve segment. Group 5 was bi-directional SETG (B-SETG) group in which ETG was harvested from the proximal and distal nerve segments. On the postoperative 12th week, after electrophysiological evaluation, wet gastrocnemius muscle weight (WGMW) and microscopic nerve and muscle examinations were performed.

### Dissection Technique

In all groups, the right side of the subjects was used as the experimental side and the left side was used as the control side for muscle examination. For nerve samples, proximal nerve segments were used as the control side.

In the prone position, an oblique skin incision was made over the gluteal muscles. Muscle fibers were horizontally dissected with a blunt fashion and the sciatic nerve was exposed from the sciatic notch to the trifurcation point. A 7 mm nerve defect was created on the main trunk of the sciatic nerve, proximal to the branching point [10, 17]. In the B-SETG group, the defect was localized in the middle of the distance between the sciatic notch and trifurcation point, to ensure enough nerve length for proximal and distal sliding epineural tube graft. In other groups, localization of nerve defect was distal 7 mm segment of the main sciatic nerve trunk proximal to the trifurcation point, to ensure enough nerve length for proximal sliding and turn-over ETG.

In Group I, after the excision of the 7 mm nerve segment, the defect was left unrepaired and the wound was closed in layers. In Group 2 (ANG group), the nerve defect was repaired with resected ANG, which was 180 degrees turned (Figures 1 and 2a). In Group 3, the nerve defect was repaired with proximal-based TETG as reported in the literature (Figure 1 and Figure 2b,c and d) [10]. In Group 4 (O-SETG group), a circumferential epineural incision was made on the proximal nerve segment, immediately distal to the sciatic notch (Figure 1
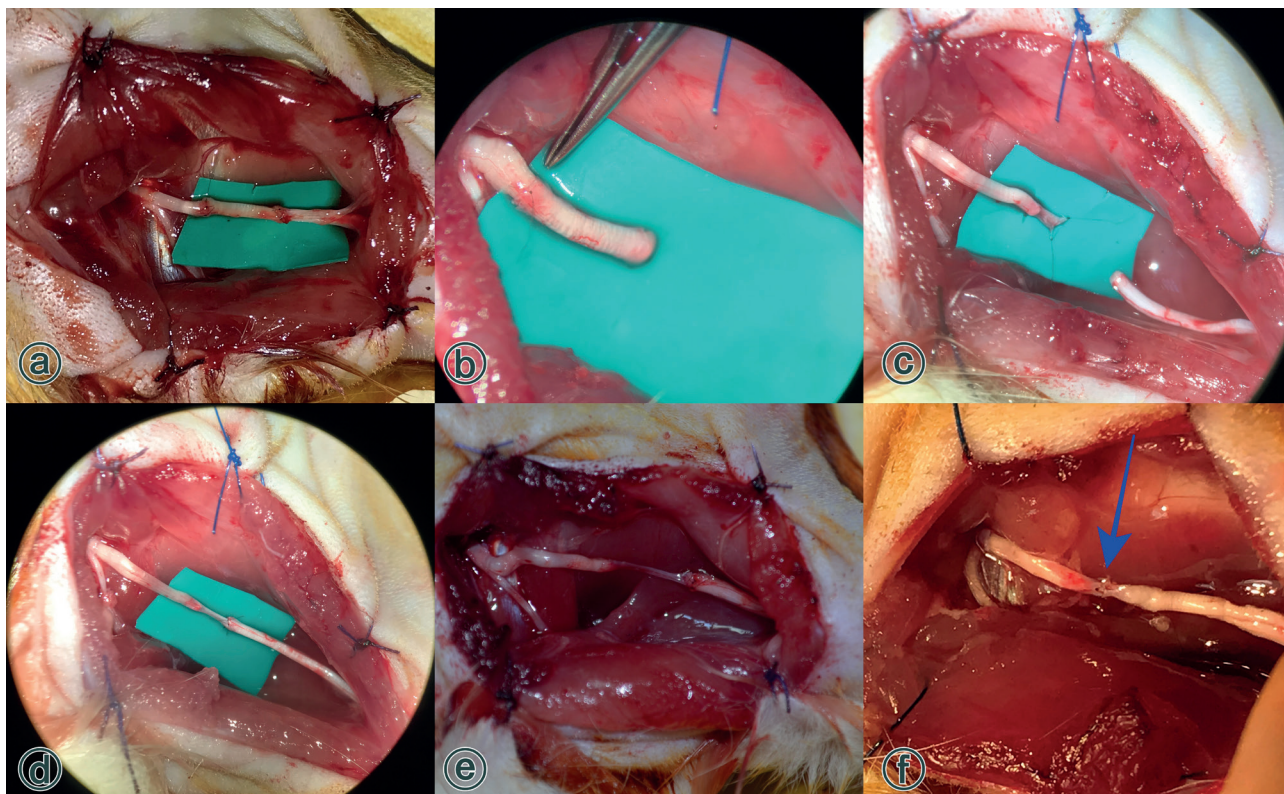
**Figure 2.** Views of the surgical technique of the groups. **a**: Repaired nerve defect with ANG (Group 2), **b**: Circumferential epineural incision pointed with a forceps. Note the fascicular bulging proximal to the epineural incision, **c**: Harvested ETG from the proximal nerve segment. Although it was harvested from a longer nerve segment, because of contraction ETG looks shorter. To prevent the graft from collapsing and facilitate the coaptation, two traction sutures are placed on the graft, **d**: Reconstruction of nerve defect with TETG (Group 3), **e**: Reconstruction with O-SETG, **f**: Reconstruction with B-SETG.

arrow-2, Figure 2b). Later, the epineural sheath was slid by pulling the distal end of the epineurium distally with two microforceps. A three mm length epineurium was left intact over the proximal nerve stump to prevent ETG from being avulsed from the proximal nerve segment (Figure 1 arrow-5). The distal free end of ETG was sutured to the distal nerve stump with three sutures of 120-degree intervals (Figure 2e). In Group 5 (B-SETG), the same surgical technique as Group 4 was applied. Differently, the nerve gap was placed in the middle of the sciatic notch and trifurcation point to leave sufficient nerve length for harvesting two ETGs from proximal and distal nerve segments. Circumferential epineural incisions were made immediately distal to the sciatic notch on the proximal nerve segment and immediately proximal to the trifurcation point on the distal nerve segment. After sliding the epineurium bi-directionally, obtained two ETGs were sutured each other at the middle of the nerve gap, with three sutures at 120 degrees intervals (Figure 1 and Figure 2f). A three mm epineural sheath was left over the proximal and distal nerve

stumps for the stability of the tube graft and to prevent scar formation at the coaptation point (Figure 1 arrow-5, Figure 2-f blue arrow).

## Electrophysiological Assessment

The degree of nerve regeneration and muscle function were evaluated with Electromyogram (EMG) objectively, using the Nihon-Kohden Neuropack M1 device (Tokyo, Japan) under general anesthesia 12 weeks after the nerve repair. Data including compound muscle action potential amplitude (CMAP) and distal latency were recorded [18].

The device's stimulation rate was 1 Hz, sampling time was 100 msec, and filter settings were 5kHz for high-cut and 10 kHz for low-cut. Room temperature was 25 degrees and extremity temperatures measured with a needle thermometer were between 34 to 36 degrees.

After hair removal, a bipolar stimulator needle electrode was placed on the left sciatic nerve, 10 mm proximal to the coaptation point, with the

anode tip distally. The monopolar recording needle electrode was placed as the anode electrode was in the middle of the gastrocnemius muscle and the cathode electrode was on its tendon. The ground electrode was placed on the back of the subject. The degree of stimulation was progressively increased till the supramaximal response was taken from the sciatic nerve

### Gross Muscle Evaluation

After electrophysiological evaluation, the subjects were sacrificed with high-dose thiopental sodium. In the prone position, after taking visual records of a comparative view of the left and right sides, the skin overlying the gluteal region and distal back was removed for a clear macroscopic comparative view of the muscles (Figure 3). Gastrocnemius muscle was detached from its origin and insertion

both from the experimental and control limbs. Wet muscle weight (WMW) was measured with the Sartorius CP225D model analytical scale (Göttingen, Germany) with an accuracy of $10^{-5}$ g.

### Microscopic Evaluation

Two mm cross-sections were obtained from the gastrocnemius muscle belly of experimental and control limbs. Samples were stained with methylene blue and quantitative analyses were performed with a light microscope (Nikon Corporation, Tokyo, Japan) under x100 magnification (Figure 4-c) using semi-automated software (Digimizer 5.4.4, MedCalc Software Ltd., Belgium).

For the nerve samples, 5 mm nerve cross-sections were performed proximal to the proximal nerve stump and distal to the distal nerve stump. After keeping the specimens in 2.5% glutaraldehyde for
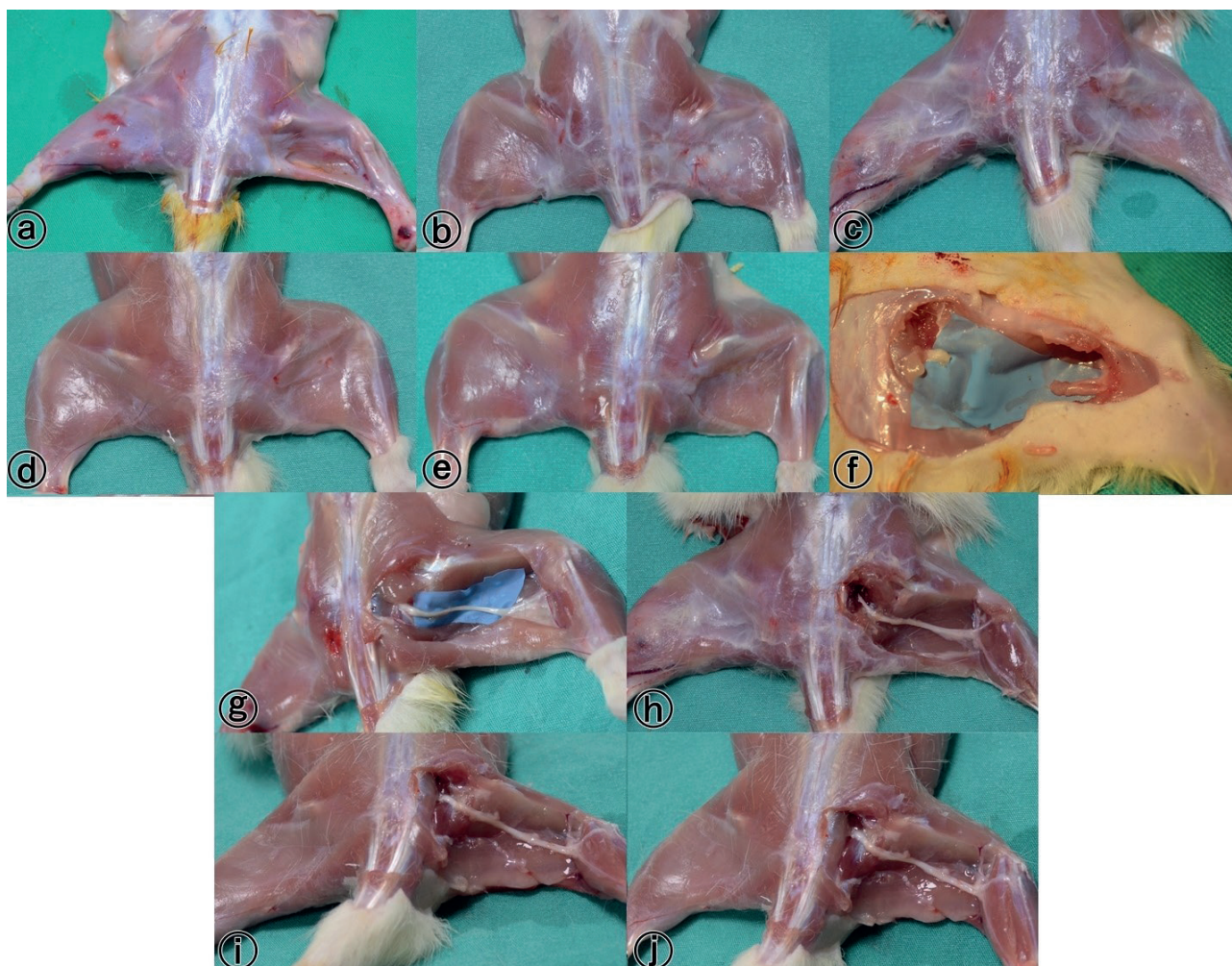


**Figure 3.** Comparative view of muscle atrophy of experimental (right limb) and control limbs (left) and macroscopic view of nerve regeneration. Severe muscle atrophy is seen in Group 1 (**a**) and prominent atrophy is notable in Group 5 (**e**). Acceptable muscle mass is seen in Groups 3 (**c**) and 4 (**d**). Better muscle mass is seen in Group 2 (**b**). Nerve regeneration is not seen in Group 1(**f**). In other groups, macroscopic continuity of the nerve is observed. g: Group 2, **h**: Group 3, **i**: Group 4, **j**: Group 5.
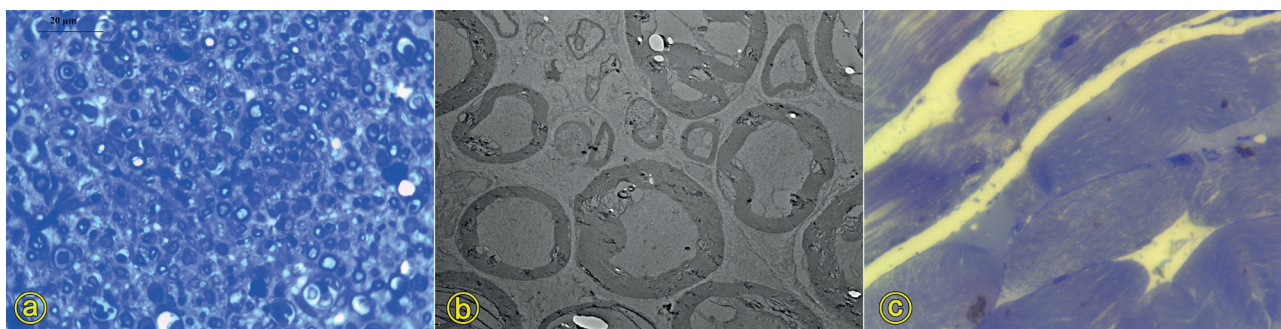
**Figure 4.** Microscopic view of the nerve and muscle samples. **a**: Light microscopic nerve samples under x100 magnification which were used to count the axons (stained with methylene blue), **b**: Transmission electron microscopic view of the nerve samples under x5000 magnification that shows the ultrastructural architecture (stained with uranyl acetate and lead citrate). Myelin sheath thickness, axonal diameter and surface were measured with these sections, **c**: Light microscopic view of the muscle samples under x100 magnification (stained with methylene blue).

24 hours for primary fixation, they were washed with Sorenson's phosphate buffer solution (pH: 7.4) and post-fixed in 1% osmium tetroxide for two hours. The samples were rewashed with the same phosphate buffer solution and dehydrated with increasing alcohol concentrations. Later, tissue samples were washed with propylene oxide and embedded in the mold containing epoxy resin. Embedded specimens were sliced with LKB-Nova ultra-microtome (LKB-Produkter AB, Bromma, Sweden) to obtain two µm thickness sections. These sections were stained with methylene blue for light microscopic (Nikon Corporation, Tokyo, Japan) examination (Figure 4-a) [19-21]. Myelinated axons were counted under x100 magnification with the same semi-automated software [21].

Ultra-thin sections (60 nm) were obtained using the same microtome for electron microscopic examination. After staining these sections with uranyl acetate and lead citrate [19], the ultrastructural examination of the nerve specimens (myelin sheath thickness, the diameter and surface area of the myelinated axons) was performed using an electron microscope (Jeol JEM 1200 EX, Tokyo, Japan) under 5000x magnification (Figure 4-b). The axonal diameter was calculated by taking the average of the long and short axes, which were perpendicularly crossing each other at the center of the sectional nerve area [21]. Myelin sheath thickness was calculated by taking the average thickness of the sheath at its thickest and thinnest points [20, 21].

## Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences for Windows SPSS 23.0 (IBM Corporation, Armonk, New York, United States). The normal distribution of quantitative data was assessed by the Shapiro-Wilk test. The homogeneity of variances was analyzed with Levene's test. The Kruskal-Wallis test was used the analyze quantitative data between the groups. For post hoc pairwise comparison, the Mann-Whitney U test with Bonferroni correction was used. Descriptive statistics of quantitative variables were presented as "mean ± standard deviation" in the text and tables. The variables were examined at a 95% confidence level, and p values <0.05 were considered statistically significant.

## RESULTS

### Electrophysiological Findings

Electrophysiological measurements showed similar distal latency and CMAP between the control limbs of the subjects (Table 1). On the other hand, in the experimental limbs, the lowest distal latency measured in Group 2 was 2.08 ms and the highest value in Group 5 with 4.33 ms following Group 1. There was a statistically significant difference in the distal latency of experiment sites (p=0.023). The post hoc pairwise comparison revealed that the difference between Group 1 and other groups except for Group 5, and the difference between Group 2 and Group 5 (p=0.022) and Group 3 and Group 5 (p=0.008) was statistically significant. The mean CMAP of experimental limbs was statistically similar in all groups.

In the comparison of the experimental limbs with the control limbs within each group, the distal latency of the experimental sides was significantly

**Table 1.** Macroscopic, microscopic and electrophysiologic data of the muscle samples

|  |  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | P Value |
|---|---|---|---|---|---|---|---|
| Muscle Fiber Thickness (μm) | Experimental Limb | 20.65±6.84 | 63.04±1.94 | 61.62±4.65 | 61.08±4.3 | 55.22±6.2 | 0.001 |
|  | Control Limb | 64.74±8.81 | 64.48±3.80 | 63.58±4.45 | 63.20±3.58 | 63.65±5.45 | 0.779 |
| Wet muscle weight (g) | Experimental Limb | 0.87±0.21 | 3.26±0.45 | 3.56±0.12 | 3.42±0.36 | 1.06±0.64 | <0.001 |
|  | Control Limb | 4.58±0.44 | 4.18±0.41 | 4.04±0.60 | 4.82±0.32 | 4.66±0.44 | 0.642 |
| Distal Latency (ms) | Experimental Limb | 5.36±0.18 | 2.08±0.13 | 2.77±0.01 | 2.64±0.35 | 4.33±0.37 | 0.023 |
|  | Control Limb | 1.76±0.71 | 1.73±0.62 | 1,63±0.78 | 1,15±0.30 | 1,76±0.54 | 0.064 |
| Compound Muscle Action Potential | Experiment Site | 6.92±4.64 | 17.58±7.26 | 4,06±23.98 | 21,24±14.27 | 26,58±17.11 | 0.965 |
|  | Control Site | 19.34±5.67 | 17.36±4.77 | 11,96±6.95 | 12,27±4.08 | 31,61±20.42 | 0.748 |

higher compared with their control limbs in all groups (Table 2). However, CMAP was statistically similar in all groups except for Group 1 comparing the experimental and control limbs (Table 2).

## Results of Macroscopic Muscle Evaluation

The intergroup comparison of WMW of control limbs showed a statistically similar distribution of gastrocnemius muscle weight (Table 1). However, in the experimental limbs, the weight difference between the groups was statistically significant (p<0.001). In the post hoc pairwise comparison, WMW was statistically significantly lower in Group 1 and Group 5 compared with the other groups. The WMW was statistically similar in Group 1 and Group 5 (p=0.378). Besides, the difference of WMW in Groups 2,3 and 4 was statistically similar.

In the comparison of the experimental limbs with the control limbs within each group, the level of muscle atrophy was statistically significant in all groups except for Group 2 (Table 2). In group 2, the difference in WMW between experimental and control limbs was statistically similar (p=0.064).

## Microscopic Data of the Samples

The mean axonal counts of proximal nerve segments were statistically similar in all groups. However, in the distal nerve segments, the difference in mean axonal counts was statistically significant between the groups (p=0.013). Post hoc pairwise comparison of the groups revealed a statistically significant difference between Group 2 and Group 4 (p=0.045), Group 2 and gr Group 5 (p=0.008) and Group 4 and 5 (p=045). The difference between Groups 3 and 4 (p=0.810) and Groups 3 and 5 (p=0.128) was not statistically significant. In Group 1, the axon number of distal nerve segments were could not counted due to prominent degeneration of the axons. Ultrastructural architecture (axon surface area, myelin sheath thickness and axon diameter) of the nerve samples was similar in all groups for distal and proximal nerve segments (Tables 2 and 3). In the comparison of the proximal and distal nerve segments within each group, the decrease in axon count was statistically significant in Groups 3,4 and 5 (Table 2). In Group 2, proximal and distal nerve segments' axonal count was statistically similar (p=0.093).

**Table 2.** P values of Comparison of control and experimental sides within each group

|  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Axon Count | None | 0.963 | <0.001 | <0.001 | <0.001 |
| Axon Surface Area | None | 0.330 | 0.147 | 0.981 | 0.879 |
| Myelin Thickness | None | 0.976 | 0.744 | 0.956 | 0.333 |
| Axon Diameter | None | 0.467 | 0.408 | 0.793 | 0.627 |
| Muscle Fiber Thickness | <0.001 | 0.071 | <0.001 | <0.001 | <0.001 |
| Wet Muscle Weight | <0.001 | 0.064 | <0.001 | <0.001 | <0.001 |
| Distal Latency | 0.011 | 0.034 | 0.080 | 0.013 | 0.018 |
| CMAP | 0.031 | 0.925 | 0.356 | 0.149 | 0.632 |

Note. For muscle specimens, the control side was the left limb. For nerve samples, the control side was the proximal nerve segment.

The mean muscle fiber thickness of control limbs was statistically similar between the groups (Table 1). However, in the experimental limbs, the difference between the groups was statistically significant (p=0.001). The pairwise comparison revealed that muscle fiber thickness was statistically similar between Groups 2,3 and 4. However, the mean fiber thickness of Group 1 was statistically significantly lower than the other groups. Furthermore, the mean fiber thickness in Group 5 was significantly lower than Group 2 (p=0.013). This measurement was similar between Group 5 with Groups 3 and 4.

In the comparison of the experimental limbs with the control limbs within each group, mean muscle fiber thickness decreased in experimental limbs compared with their control limbs except for Group 2, similar to WMW (Table 2). Muscle fiber thickness of experimental limbs and control limbs was statistically similar in Group 2 (p=0.071).

## DISCUSSION

Despite advances in microsurgical techniques, the reconstruction of nerve defects is still a challenging field in plastic surgery practice. While the gold standard treatment technique is ANG in the literature, morbidities associated with the sacrifice of the donor nerve and extra surgical field-associated complications are the main challenges of this reconstruction [1, 7]. Although continuing research is to overcome these pitfalls, a practical and applicable technique has not been accepted and routinely used in clinical practice yet [1].

PNI is commonly associated with traumas and its incidence is up to 3.33% [1-3]. The rate of graft needed nerve reconstruction is 5.7% of reported PNIs [6]. Considering both the high incidence of nerve damage and its individual, medical and social consequences, the importance of treatment and follow-up protocols becomes evident. When considered on an individual basis, it can range from tolerable hypoesthesia to severe motor and sensory losses that can interfere with the patient's daily activities. In the literature, it has been documented that injuries involving PNI are associated with longer durations of hospitalization, treatment, rehabilitation, and greater psychosocial impacts compared to traumas without nerve damage [3]. In addition, considering the high treatment costs,

repeated hospital admissions, and loss of workforce due to long rehabilitation periods, the social and national effects of nerve injuries are striking. Moreover, PNIs are frequently seen in young or middle-aged individuals who have a workforce and economic contributions to society [2, 3]. In a study reported by Noble et al., the mean age of nerve damage was 34.6 years, and 59% of them were seen in individuals between the ages of 18 to 35 years [2]. Similarly, McAllister et al. have reported that 57.1% of nerve injuries occur in individuals aged 16 to 35 years. [6] These remarkable data reveal the potential social repercussions of an individual medical problem. In addition, these injuries, which are in productive age, cause loss of function and workforce throughout the life of patients, significantly reducing their quality of life [1].

The ideal treatment method for nerve damage is early, tension-free, end-to-end primary repair, if possible. [6, 10] However, for the nerve gaps not eligible for primary repair, currently, the ANG technique is the standard of care in the literature [1, 7, 22, 23]. The main advantage of this technique is the repair of the nerve defect with a similar tissue that provides the appropriate microenvironment that activates axonal regeneration with neurotrophic factors and mediator cells [24]. However, this technique has some disadvantages such as the sacrifice of a donor nerve and associated anesthesia/hypoesthesia, neuroma, additional surgical area, wound complications (scar, infection, hematoma, dehiscence, etc.), long operation time and two coaptation points on the nerve repair line that may negatively affect the axonal regeneration because of foreign body reaction, fibrosis and scar block [1, 12, 25, 26]. Furthermore, repair with the nerve graft technique is challenging and has unpredictable results [22]. Although various studies reported remarkable outcomes in repairing the nerve gap with different types of conduits (autologous grafts, allografts, synthetic grafts, etc.), they have many limitations that prevent their use in clinical practice. For instance, some of the disadvantages of autologous grafts (vein, muscle, tendon, etc.) are that they are not neural origin and they have suboptimal results in nerve defects longer than 3 cm [10, 27]. In some publications, synthetic conduits were proposed to avoid donor-side morbidity and nerve sacrifice [28, 29]. Although the focus point is very important in minimally invasive surgical notions, high costs and challenges in producing custom-made

conduits make this technique unpractical [10, 30]. Furthermore, similar to vein grafts, long gaps of more than 3 cm are another limitation of this technique [29].

Although promising outcomes of ETG, the literature has many limitations in the ETG technique that prevent its use in clinical practice. First of all, ETG was harvested with an inapplicable technique, pulling the nerve fascicules from the ANG to obtain ETG [9]. Secondly, for obtaining the tube formation, suturing the epineural graft longitudinally is significantly prone to foreign body reaction, scar formation and fibrosis, which reduce axonal regeneration [14, 26, 31]. Besides, ETG was inserted into nerve defects with two coaptation points in the previous studies [9, 14]. On the other hand, TETG is a practical reconstruction option compared to the aforementioned two ETG harvesting techniques [10, 11]. However, in this technique, harvesting the ETG is challenging and requires advanced microsurgical capability. On the other hand, no study was found in which nerve defect reconstruction was performed using the ETG by sliding technique from the nerve proximal or/and distal segments. The sliding technique is more practical and easier to apply compared with the TETG technique. Besides, in the SETG technique, there is only one coaptation point similar to the TETG technique, which is an advantage compared to the ANG technique which has two coaptation points. Furthermore, the inner surface of SETG is smoother than TETG which may hypothetically facilitate axonal regeneration. Considering the promising advantages of the SETG technique, the current study presents remarkable data on ETG techniques. In the present study, the methodology of harvesting the SETG technique is determined, which is practical to apply and it was used to reconstruct a nerve gap, which is not reported in the literature yet. Moreover, the B-SETG technique was performed to reconstruct the larger defects and the SETG technique was compared with TETG and ANG techniques, which are not analyzed in the literature as well.

On the other hand, many studies reporting the results of the ETG technique in the literature analyzed the nerve and muscle function subjectively using the walking track test [10, 11]. This test is prone to subjectivity and may be affected by many conditions depending on the subjects and environmental factors. On the other hand, EMG is an objective and effective technique to assess neuromuscular functions which provides numeric and precise data [18, 32, 33]. This objectivity lets researchers compare data between studies, series, and centers [32, 33]. In the present study, the assessment of neuromuscular functions was performed with EMG to obtain objective results.

For optimal functional results, the ideal nerve conduit should provide anatomical integrity of the nerve, induce minimal inflammation, stimulate axonal regeneration, cause minimal morbidity and have low costs [12]. The epineural sheath is a good candidate that has these advantages. Besides, it is a neural tissue that activates Schwann cell functions and induces axonal regeneration by secreting the laminin, similar to ANG [8, 9]. Therefore, using an epineural sheath has been applied in some studies in the literature, with ETG, sleeve and strip epineural grafts [9-13, 15]. In these studies, ETG techniques have reported similar outcomes with ANG. In a study reported by Ayhan et al., nerve defect repair was performed experimentally by obtaining ETG with the turn-over (TETG) technique [10]. Similar results were observed between ETG and ANG in the analysis of variables such as muscle function, muscle mass and macroscopic structure, diameters of muscle fibers and microanatomical structure of axons. In a study, Luokkala et al. harvested TETG from the distal nerve segment instead of the proximal nerve segment [11]. In addition, Karacaoğlu et al. reported superior results in the ETG technique in their study, in which they examined the results of ETG and vein graft in nerve defect repair and attributed this to the neurotrophic factors provided by the epineural sheath [14]. These studies revealed that the nerve regeneration of the ETG technique is similar to the ANG technique. According to these studies, the main advantage of the ETG technique was emphasized not to need for the sacrification of donor nerve and the absence of donor-side complications (hypoesthesia, hematoma, infection, neuroma, etc.) compared to ANG. In the literature, it has been reported that in cases where the epineural sheath is used as a donor, an epineurium-like layer forms in the donor area on the nerve [10]. In this case, enlargement of the existing nerve defect or morbidity in the nerve donor area is not expected in the SETG or TETG techniques. In addition, since the graft donor is the proximal and/or distal segment of the damaged nerve, a diameter mismatch is not expected. On the other hand, beyond the possible donor-side complications, microscopic

and objective electrophysiological findings of the current study revealed superior outcomes and data of the ANG technique compared with ETG techniques.

In the present study, the electrophysiological finding suggests similar muscle functions between ANG, TETG and O-SETG techniques. However, the functional outcomes of the B-SETG technique were suboptimal compared with other techniques. This is attributed to the prominent collapse of the ETG caused by the coaptation point that is at the middle of the graft (Figure 2-f, blue arrow). Hypothetically, the collapse caused scar formation and eventually blocked the axonal regeneration through the tube. Similarly, findings of muscular atrophy (WMW and fiber thickness) were well tolerated in the ANG technique. However, in ETG techniques, muscle atrophy was prominent compared to their control limbs. In particular, this was significant in the B-SETG technique (Figure 3).

Similar to muscle functions in experimental limbs, the nerve degeneration was well tolerated in the ANG technique, which suggests lower distal latency and better CMAP measurements in EMG compared with control limbs. Due to better axonal regeneration in the ANG technique, the axon count of the distal nerve segment was similar to the proximal (control) nerve segment. In ETG techniques, although different axonal regeneration levels were observed, the decrease in axon counts in distal nerve segments was prominent, which interprets suboptimal axonal regeneration. Although the functional and microscopic findings of TETG and O-SETG techniques were similar, in the B-SETG technique axonal regeneration was prominently impaired compared with other techniques.

The main limiting factor for ETGs is the localization of the injury. In the injury zone, the presence of nerve branching in the epineural sheath donor area will make it difficult to obtain a sufficient length of the epineural graft. This limitation may be overcome by selecting the better donor side, proximal, or distal nerve segment. Another disadvantage of the technique is the necessity of dissection of the nerve to obtain the appropriate epineural sheath length. As this situation could lead to the nerve being skeletonized from the surrounding tissues, it may result in compromised

blood circulation [10]. However, considering the presence of the longitudinal internal vascular network in the peripheral nerves, no circulation problem is expected for the donor nerve segment [34, 35].

## CONCLUSION

The findings of the present study confirm that the ANG technique is a more effective treatment option compared to ETG techniques. In particular, the use of SETG from both sides of nerve segments (B-SETG) causes poor results. On the other hand, O-SETG and TETG techniques revealed acceptable results. Therefore, ETG techniques (TETG or O-SETG) should be in surgeons' treatment repertoire to overcome possible challenges during the management of reconstruction. On the other hand, the present study determined a practical and applicable ETG harvesting technique, the sliding method.

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