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Value of supercharge end to side nerve transfer in peripheral nerve injuries in male albino rats; Experimental study

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Abstract: Background: Peripheral nerve injuries are a significant clinical challenge, often resulting in functional impairments due to the disruption of nerve continuity and the subsequent loss of muscle innervation.

Objectives: The purpose of the study was to assess the impact of supercharge end-to-side (SETS) nerve transfer on peripheral nerve regeneration in male albino rats.

Subjects and methods: This experimental study involving 18 male albino rats, each weighing between 200-350 grams. The rats are allocated into three groups based on the surgical technique performed: Group A: End-to-end (ETE) nerve repair (n=6). Group B: Reverse end-to-side (RETS) neurotomy (n=6). Group C: SETS nerve transfer (n=6). All rats undergone surgery under proper anesthesia, administered as ketamine intraperitoneally. After 12 weeks post-operation, the rats were sacrificed, and the posterior TNs were extracted. A 1 cm segment proximal and distal to the epineurial repair site will be examined histopathologically. The nerves were stained with Toluidine blue to assess myelinated axons number and calculate the neurotization index in all groups.

Results: The study results showed significant variations in the neurotization index and the regenerating nerve fibers number between the groups, particularly in the distal segment, where the SETS nerve transfer (Group C) and RETS neurotomy (Group B) demonstrated superior outcomes compared to traditional ETE repair (Group A).

Conclusion: This study demonstrates the value of SETS nerve transfer and RETS neurotomy in enhancing peripheral nerve regeneration in male albino rats. Both advanced techniques resulted in significantly improved nerve regeneration, particularly in distal segments, compared to the traditional end-to-end repair method. The findings suggest that these techniques hold promise for improving outcomes in clinical settings where robust nerve regeneration is critical for functional recovery.

Keywords: *supercharge end to side, nerve transfer, peripheral nerve injuries.*

Introduction

Preserving the functionality of distal effectors during axonal regeneration is one of the most crucial objectives in the treatment of proximal nerve injury [1].

When treating proximal nerve injuries, traditional end-to-end neurotomy (EEN) frequently produces unsatisfactory functional results. This is mostly due to the lengthy recovery interval that occurs between the lesion and the reinnervation of distal targets, which causes atrophy of the muscles and Schwann cells [2].

In certain instances, such as those with intricate injuries to the upper extremities, end-to-side (ETS) neurorrhaphy is seen as a suitable substitute for neural repair [3]. It assumes that an undamaged neuron can "give" axons to the distal end of a damaged nerve. This approach has garnered special attention when the lesion is proximal [4].

Supercharge ETS (SETS) nerve transmission is an advancement in technology that involves suturing the proximal end of a donor nerve to an epineurial window in the side of a wounded recipient nerve after end-to-end nerve repair [5].

This method seeks to maintain the viability of the target organs while waiting for axonal regeneration from the location of high-level damage occurs [6].

Besides ETS and ETE transfers, the SETS transfer (SETST) is recommended in proximal nerve damage as a strategy for releasing axons distally into the damaged nerve to rapidly innervate and maintain the following organs [2].

The present work aim was to evaluate the effect of SETST in peripheral nerve regeneration in male albino rats.

Subjects and methods

All experimental procedures and protocols for animal research conformed to the rules of the Institutional Animal Care and Use Committee of Zagazig University (IACUCZU) and were conducted at the Zagazig University Hand and Microsurgery Center (ZUHMC), Plastic and Reconstructive Surgery Department, Faculty of Medicine, Zagazig University, Egypt.

This experimental and histo-pathological study included (18) Sprague-Dawley young adult male rats with an average weight of 200-350 grams that were all subjected to the experiment.

All the maneuvers carried on in this experiment concerning the rats were highly ethical and merciful. The subjects of the experiment were (18) Sprague-Dawley young adult male rats with an average weight of 200-350 grams. Healthy male albino rats with average weight 200-350 gm were included.

Surgical procedure

Operations were conducted in aseptic conditions utilizing surgical microscope and microsurgical tools to enable the proper dissection and neurorrhaphy of the nerves. All the rats were anesthetized properly.

Anesthesia:

Anesthesia was administered via an intra-peritoneal injection of a Ketamine / Xylazine cocktail (Ketamine 25mg + Xylazine 10mg per mL) with a dosage of 0.1 mL/100g rat weight then preparing the rats by shaving the hind limb.

The surgical approach:

The operated side was the left side. The skin was marked and incised along the line connecting the knee joint to the ischial tuberosity then dissected bluntly from the underlying muscles after that the gap where the sciatic nerve splits into the peroneal, tibial, and sural nerves was reached via the exposed layers of the gluteal muscles. Gentle and careful separation of the nerve from surrounding tissue by employing suitable microsurgical tools and surgical microscope results in a bloodless field. Then placing a contrast material behind it.

Then divide rats into three equal groups: In group (A) posterior tibial nerve (TN) was transected proximally and repaired immediately with ETE epineurial repair using a poly-propylene 9-0 suture. In group (B) peroneal nerve (PN) was transected and its proximal end connected by end to side neurorrhaphy to the intact posterior TN after making an epineurial window.

In group © posterior TN was transected and repaired with ETE epineurial repair plus ETS neurorrhaphy of the proximal end of PN to the distal part of posterior TN after making an epineurial window and that is called SETST. then approximating the muscles and suturing the skin using a poly-propylene 6-0 suture and painting the skin with povidine iodine 10% solution.

Follow up

The rats were closely monitored during surgery and recovery. Each rat was kept in a separate cage with food and water. They were checked on daily during the first four weeks for feeding, cleaning, antibiotics

administration and wound care. Then every three days during the rest of the experiment up to 12 weeks. The antibiotic was administered only for 7 days as following (Tetracycline PO in drinking water 0.8 mg/100g rat weight/24h). Rats were placed in three separate groups.

Biopsy preparation and histological evaluation

At the end of the 12th week postoperatively, all the surviving rats (18) were humanly euthanized with an overdose of anesthesia (triple the surgical dose). The peroneal and posterior TN was exposed and a 2mm segment proximal and distal to neurotomy harvested then fixated with a 10% formalin solution in a sterile sample collection tube. After 48 hours the samples were washed to remove formalin in distilled water for 30 minutes then processed forming paraffin blocks then cut into histologic sections that are 4-5 microns thick and stained with Toluidine Blue and H&E stains separately [7].

Histologic evaluation was performed using light microscopy where the number of myelinated axons proximal and distal to the epineurial repair site was counted by two separate professional examiners who weren't made aware of the nature of the experiment.

The two observers' average count was computed after counting was done at 400X magnification along the long axis of the fascicles and in a number of neighboring sections.

The mean fibers number in the distal segment/mean nerve fibers number in the proximal segment(s) X 100 was then used to compute a neurotization index as a percentage in G1, G2, and G3.

Statistical Analysis:

Data were gathered and analyzed utilizing SPSS Version 23.0. The Kolmogorov-Smirnov test was employed for normal distribution. Quantitative data were presented as mean \pm SD and (minimum-maximum), f: The Anova test was developed to compare multiple groups of normally distributed variables. If the f-test was significant, the Bonferroni test was utilized to compare the groups. The paired t test was used to compare variables that were not regularly distributed. All tests were two-sided. P-values < 0.05 were significant.

Results

the total number of adult male albino rats used in the present study (18), 6 rat in each group, their body weight ranged from 200-350 (g). (Table 1)

The mean number of regenerate nerve (Proximal Segments) were 161 \pm 8.5 in Group (A), 203.8 \pm 17.6 in Group (B), and 197.7 \pm 17.2 in Group (C). (Table 2)

The mean number of degenerate nerve (Distal Segments) were 142 \pm 8.6 in Group (A), 291.3 \pm 10.8 in Group (B), and 254.2 \pm 14.8 in Group (C). (Table 3)

The mean neurotization index were 88.2 \pm 2.5 in Group (A), 143.55 \pm 9.6 in Group (B), and 128.89 \pm 5.05 in Group (C). The results showed a significant increase in Neurotization Index (P < 0.001) when compared to that of group A. Group B was substantially high when compared to that of group A (P < 0.001) and was substantially lower than group B (P= 0.004). The obtained percentage represents the axons number that efficiently passed the repair site from the proximal to the distal segment, better in group B, then group C, and finally group A. (Table 4)

The mean values of proximal segment were 161 \pm 8.5 in Group (A), 203.8 \pm 17.6 in Group (B), and 197.7 \pm 17.2 in Group (C). The results of group B showed a significant increase in proximal segment (P =0.001) when compared to that of group A. The mean value in group C was significantly elevated when compared to that of group A (P = 0.002), while, it was found non-significant when compared to group B (P= 0.98). The resulting percentage indicates the proximal better in group B, Then group C, after that group A. The mean values of distal segments were 142 \pm 8.6 in Group (A), 291.3 \pm 10.8 in Group (B), and 254.2 \pm 14.8 in Group (C). The results of group B showed a substantial elevation in distal segment (P < 0.001) when compared to that of group A. mean value of group C was substantially elevated when compared to that of group A (P < 0.001) and was substantially lower than group B (P<0.001). The resulting percentage indicates the distal better in group B, Then group C, after that group A. (Table 5)

There was a substantial reduction in the number of renewing nerve fibers distal to the repair in group A (142) compared to the proximal segments of the same group (161) (P<0.001). The number of regenerated nerve fibers distal to the repair increased in group B (291.3) compared to the proximal portions (203.8), with substantial variations (P < 0.001). There was a considerable increase in the number of regenerating nerve fibers distal to the repair in group C (254.2) compared to the proximal segments (197.7), with P<0.001. (Table 6)

Weight(gm)	Group A	Group B	Group C	f	p
Mean ±SD	254.0±51.42	269.17±37.74	257.5±45.8	0.181	0.836
Range	200-350	230-340	200-330		
sex	male	male	male		
Total number	6	6	6		
F: Anova test, p≥0.05 no significant					

Proximal end nerve regeneration	Group A	Group B	Group C
1	150	191	174
2	172	227	218
3	160	180	180
4	155	200	200
5	159	205	210
6	170	220	204
Min.	150	180	174
Max.	172	227	218
Mean	161	203.8	197.7
SD	8.5	17.6	17.2
Min: minimum, max: Maximum, SD: standard deviation			

Distal end nerve regeneration	Group A	Group B	Group C
1	130	278	232
2	152	305	273
3	135	290	245
4	140	280	250
5	145	295	260
6	150	300	265
Min.	130	278	232
Max.	152	305	273
Mean	142	291.3	254.2
SD	8.6	10.8	14.8
Min: minimum, max: Maximum, SD: standard deviation			

Table (4): Comparison of the Neurotization Index (mean of distal count/mean of proximal count X 100) between three Groups

Neurotization index	Group A	Group B	Group C
1	86.67	145.55	133.33
2	88.37	134.36	125.23
3	84.38	161.11	136.11
4	90.32	140	125
5	91.19	143.9	123.81
6	88.24	136.36	129.90
Min.	84.38	134.36	123.81
Max.	91.19	161.11	136.11
Mean	88.2	143.55	128.89
SD	2.5	9.60	5.05
F	119.6		
P	0.0001		
P1	<0.001		
P2	<0.001		
P3	0.004		

F: Anova test, P <0.05 significant, p≥0.05 no significant, P1: Compare group A&B, P2: Compare group A&C, P3: Compare group B&C

Table (5): comparison of the means in studied groups regarding the Proximal and Distal Segments respectively

	Proximal segment			Distal segment		
	Group A	Group B	Group C	Group A	Group B	Group C
Mean	161	203.8	197.7	142	291.3	254.2
SD	8.5	17.6	17.2	8.6	10.8	14.8
F	14.22			256		
P	0.0003			0.0001		
P1	0.001			<0.001		
P2	0.002			<0.001		
P3	0.98			<0.001		

F: Anova test, P <0.05 significant, P1: Compare group A&B, P2: Compare group A&C, P3: Compare group B&C

Table (6): Within -group comparison of the Proximal segments and Distal segments nerve regeneration count

	Group A		Group B		Group C	
	Proximal segment	Distal segment	Proximal segment	Distal segment	Proximal segment	Distal segment
Mean	161	142	203.8	291.3	197.7	254.2
SD	8.5	8.6	17.6	10.8	17.2	14.8
Paired t	11.63		17.92		22.97	
p	<0.001		<0.001		<0.001	

paired t-test: Statistically highly significant at p< 0.001

Discussion

Peripheral nerve injuries (PNI) are a common yet complex clinical challenge, often resulting in serious functional disability due to the loss of nerve continuity and subsequent muscle denervation. The ability to restore function through nerve regeneration has been a primary focus of both clinical and experimental research, leading to the development of various nerve repair techniques. Traditional methods, such as ETE nerve repair, have been the mainstay in clinical practice; however, their limitations in promoting consistent and robust nerve regeneration have prompted the exploration of more advanced techniques [8].

SETST is one of these cutting-edge techniques that has drawn a lot of attention for its potential to improve nerve regeneration, especially in situations where more conventional treatments might not be sufficient. Through ETS connection, donor nerve fibers are transferred to a recipient neuron in SETS procedure, therefore "supercharging" the recipient nerve with extra axonal input. This method is thought to increase damaged nerves' ability to regenerate, particularly when the receiving nerve is unable to do so entirely on its own [9]. The current study was designed to investigate and compare the potency of supercharge end to side nerve transfer technique in a controlled experimental setting. PNI present a significant challenge in both clinical and experimental contexts, as the restoration of function often depends on the ability to effectively regenerate nerve fibers across the injury site.

In this study, we explored the outcomes of three distinct nerve repair strategies: traditional ETE nerve repair (Group A), RETS neurorrhaphy (Group B), and SETST (Group C). By measuring the nerve fibers regeneration in both the distal and proximal segments of the injury site, we aimed to elucidate the relative advantages of these techniques and provide insights into their potential applications in clinical practice.

The discussion that follows will contextualize our findings within the broader body of research, comparing the results of our study with recent advancements in the field. We will examine the implications of the observed differences in nerve fiber regeneration and functional recovery, particularly focusing on the effectiveness of the supercharge and RETS techniques in promoting superior outcomes. This analysis will contribute to a deeper understanding of the value and limitations of these advanced nerve repair strategies in the management of PNI.

In our study, a total of 18 adult male albino rats were utilized, allocated equally into three groups. The body weight of the rats ranged from 200 to 350 grams. This sample size and the specific range of body weights are carefully chosen to ensure that the experimental conditions are consistent and that the findings are reliable and reproducible.

Rats are a good choice for animal models because of their extensively researched morphological anatomy, low care expenses, availability of several strains, infection-resistant and surgical problems.

The study by Öksüz et al. [10] also used rodent models with similar characteristics to investigate nerve grafting techniques, ensuring that their findings could be generalized across similar biological models.

Likewise, the Abaskhron et al. [11] study on SETST also employed a controlled sample size and weight range to accurately assess the functional outcomes of nerve repair techniques. These methodological choices contribute to the validity of our findings, ensuring that any observed differences in outcomes between the groups are due to the surgical interventions rather than extraneous variables.

However, for motor axons to produce sprouting, the donor nerve must be purposefully injured (e.g., crushed or neurotomy) [12]. The term RETS refers to the transfer of motor nerve into the side of a damaged nerve [1,5]. To simplify the RETS terminology, we prefer the term SETS, which is identical to the vascular description [13]. Isaacs et al., [5] reported that in animal model they compared the first repair of a transected PN to the RETS transfer of the TN to the PN's side, with and without PN transection. Muscle force testing exhibited identical results between the peroneal initial repair group and the RETS tibial-to-PN transfer group in which the PN was transected (RETS alone).

When the PN was not damaged, the TN transfer produced no muscular force. The researchers determined that a RETS nerve transfer might innervate a denervated muscle [5].

Moreover, Isaacs et al., [1] examined the force produced by the gastrocnemius muscle in two groups: one that underwent TN transection and initial repair, and the other that underwent both TN transection and primary repair in addition to SETST of the PN to the TN. Once a putative regenerative pathway had been ruled out, proximal stimulation was carried out. Rerinnervation via both axonal pathways direct repair and SETST was notably accomplished.

"Finding the axons origins that produced effective motor reinnervation" proved to be challenging for Isaac et al. [1]. to detect these nerve fibers origin more precisely. The green fluorescent protein is expressed in the neural tissues of Thy-1GFP transgenic rats, which have been employed in other literature. These animals offered verifiable visual proof that, following epineurotomy, donor axons may be moved from the side of a recipient nerve into the recipient's nerve and that the axons would regenerate distantly in the direction of a new muscle target.

Additionally, according to comparable axonal counts on histomorphometry, this study demonstrated that SETST was just as successful as standard ETE transfer in donating axons to a damaged nerve [14].

The usage of Thy1-GFP rats indicated that the regeneration of donor axons happen in both distal and proximal orientations within the recipient nerve after traversing a SETS coaptation [15].

These investigations also indicated the rapidity of axonal development across a SETS coaptation: entrance into a recipient rat TN by day 7, and diffused spread across the nerve's cross-sectional region by day 10 [16].

In addition, Balik and Menderes [17] demonstrated an identical axon number and proportion of myelinated to unmyelinated fibers on both sides of a modified SETST. Each pathway in the recipient neuron terminated in a distinct muscle.

Isaacs et al. [1] showed that investigation was made to figure out if donor axons that regenerate in a proximal direction following a SETS coaptation become active motor units. There was no noticeable difference in muscle force between stimulation and a greater proximal transection of the recipient nerve, indicating that proximally developing donor axons do not contribute significantly to functional motor units. Notably, their force testing data had substantial standard deviations, showing that their experimental model was inaccurate. In conclusion, donor axons do not seem to develop selectively in either direction after passing a SETS coaptation, and there is inadequate information to identify the fate and influence of proximally developing donor axons.

In the current study which was done at Hand and Microsurgery Center (ZUHMC). The total number of adult male albino rats utilized in this study (18), allocated into 3 groups, each contributing 6 rats. Group A: ETE nerve repair group (posterior TN). Group B: RETS neurorrhaphy group (peroneal proximal end to intact posterior TN). Group C: SETS group (peroneal proximal end to distal end of repaired posterior TN).

The peroneal and posterior TN was exposed at the end of the study period and a 1 mm segment proximal and distal to neurorrhaphy harvested, processed into paraffin blocks then cut into histologic sections, stained, histologically evaluated using light microscopy where the number of myelinated axons proximal and distal to the epineural repair site were counted using 400X magnification then the average count was calculated.

In our experiment, the mean value of nerve regenerate in proximal segment of neurorrhaphy, in group A, it was 161 ± 8.5 . while in group C, it was found that mean value was 197.7 ± 17.2 which was remarkably higher when compared to that of group A ($P = 0.002$).

The resulting percentage indicates that proximally there was increased axonal growth that was more evident in supercharge group, then end to end group. This could be because of the SETS coaptation as mentioned by other studies.

In comparison to the axons number remaining following ETE repair and crush injury without SETS protection, it has been demonstrated that the inclusion of a SETST increases axons number in the distal recipient nerve [18,19].

On the other hand, nothing is known about how donor axons affect native axon regrowth. Double retrograde labeling has been utilized in two investigations to measure the effect of a SETS translocation on native neuron regeneration by identifying the source of neurons distal to a SETS coaptation.

Fujiwara et al. [20] observed that the quantity of native motor or sensory neurons renewal was unaffected by the inclusion of a SETST.

Nevertheless, the scientists acknowledged that there were significant differences in the quantity of marked neurons in each of their rats. Using a sophisticated retrograde labeling method, Nadi et al. [19] demonstrated that donor axons might hinder native motor axon regeneration (AR) in the context of a crush injury.

According to the group, by 10 weeks following an injury managed with a SETST, just 4% of native recipient motor axons had regenerated, while 12% had done so after an injury managed without a SETST.

Additional research from the literature on peripheral nerves could shed light on how the existence of donor axons affects native AR. Gordon et al. [21] looked at the possibility of using denervated distal nerve stumps to stimulate nerve regeneration. They estimated motor AR from a donor's nerve into a recipient's nerve, even though their primary objective was not the SETST. All native recipient motor axons grow into a distal recipient stump following the proximal recipient nerve's delayed healing [21].

The same researchers demonstrated that following protection by a conventional ETS transfer, approximately half of native motor axons renewed into a distal recipient stump, and around 40% did so following delayed ETE restoration without protection. They suggested that the quantity of donor axons in the receiving nerve correlates with the degree of native recipient motor AR, with more donor axons translating into more recipient motor regeneration [21].

The production of growth factors from the donor axons may operate as a mediating mechanism for this impact. Schwann cells return to their mature myelinating phenotype within 8 weeks of restoring axonal contact, indicating that Schwann cells may not be responsible for the recipient nerve's continued presence of an enriched regeneration environment after that time [22].

After ETE repair and 12 weeks of donor axon preservation via a cross-bridge graft, Gordon et al. [21] observed significant native AR into a distal nerve stump. They postulated that a portion of donor axons in the recipient nerve show a persistent discharge of growth factors that enhances native AR, even though these axons are not associated with Schwann cells.

We calculated the mean value of nerve regenerate in distal segment in all groups, in group A the mean value was 142 ± 8.6 While In group C, it was found that mean value 254.2 ± 14.8 was substantially elevated when compared to that of group A ($P < 0.001$). The resulting percentage indicates the distal count was better in supercharge group, after that ETE group.

Counting the nerve regenerate within group and comparison of the proximal segments and distal segments nerve regeneration count showed that a decline was detected in the nerve fibers renewal number distally to the repair than proximally group A (142), these differences were highly remarkable, ($P < 0.001$).

While in the supercharge group an increase was detected in the nerve fibers renewal number distally to the repair than proximally in group C (254.2), these differences were highly remarkable ($P < 0.001$).

So, the Neurotization Index was found as follows, in group A, mean value of the neurotization index was 88.2 ± 2.5 while in group C, it was found that mean value was 128.89 ± 5.05 which was significantly higher when compared to that of group A ($P < 0.001$).

The resulting percentage indicates axons number that efficiently transverse the repair site from the proximal to the distal segment is better in group C than that in group A.

There has been debate over whether the SETST is ready for broad clinical application in light of the significant gaps in our knowledge of its mechanics [23].

It is agreed that strong donor nerve regrowth takes place throughout a SETS coaptation. When these donor axons reach the receiving nerve, they seem to develop in both directions, producing motor units and slowing down muscle atrophy.

The ideal connective tissue window depth and other technical factors need more research. It is unclear how donor fiber type, donor axons, and muscle end organ affect the ability of native recipient axons to regenerate. Unlocking the clinical potential of this transfer requires a deeper comprehension of its mechanisms and subsequent technical optimization. The effects and indications of SETSTs in clinical settings will be better

defined with the use of prospective clinical research and improved animal models with trustworthy outcome evaluation [2].

Conclusion

The study underscores the importance of selecting appropriate nerve repair technique based on the specific context and injury type. The substantial variations observed between the groups highlight the value of the SETST, particularly when traditional methods may be insufficient to achieve optimal recovery. Overall, this experimental study provides strong evidence supporting the clinical application of SETSTs as effective strategy for improving outcomes in PNI. These findings contribute to the growing body of research advocating for the refinement and adoption of advanced nerve repair techniques in clinical settings.

References

1. Isaacs JE, Cheatham S, Gagnon EB, Razavi A, McDowell CL. Reverse end-to-side neurotization in a regenerating nerve. *J Reconstr Microsurg.* 2008;24:489–96.
2. Von Guionneau N, Sarhane KA, Brandacher G, Hettiaratchy S, Belzberg AJ, Tuffaha S. Mechanisms and outcomes of the supercharged end-to-side nerve transfer: a review of preclinical and clinical studies. *Journal of Neurosurgery.* 2021;134:1590–8.
3. Cederna PS, Kalliainen LK, Urbanchek MG, Rovak JM, Kuzon WM. “Donor” muscle structure and function after end-to-side neurotization. *Plast Reconstr Surg.* 2001;107:789–96.
4. Johnson EO, Soucacos PN. Nerve repair: Experimental and clinical evaluation of biodegradable artificial nerve guides. *Injury.* 2008;39:30–6.
5. Isaacs J, Allen D, Chen LE, Nunley J. Reverse end-to-side neurotization. *J Reconstr Microsurg.* 2005;21:43–8; discussion 49–50.
6. Gesslbauer B, Furthmüller GJ, Schuhfried O, Roche AD, Sporer M, Aszmann OC. Nerve grafts bridging the thenar branch of the median nerve to the ulnar nerve to enhance nerve recovery: a report of three cases. *J Hand Surg Eur Vol.* 2017;42:281–5.
7. Abdel-Wahhab KG, Ashry M, Hassan LK, Gadelmawla MHA, Elqattan GM, El-Fakharany EM, et al. Nano-chitosan/bovine lactoperoxidase and lactoferrin formulation modulates the hepatic deterioration induced by 7,12-dimethylbenz[a]anthracene. *Comp Clin Pathol.* 2023;32:981–91.
8. Elfar JC. Priming the stump in peripheral nerve injury (Commentary on Zhang et al. (2017)). *Eur J Neurosci.* 2017;45:748–9.
9. Vanhove W. Nerve Surgery. *Acta Chir Belg.* 2016;116:268.
10. Öksüz S, Eren F, Cesur C, Acikel Elmas M, Şirvancı S. Loop nerve graft prefabrication for peripheral nerve defect reconstruction. *Ulus Travma Acil Cerrahi Derg.* 2022;28:1043–51.
11. Abaskhron M, Ezzat M, Boulis AG, Safoury YE. Supercharged end-to-side anterior interosseous nerve transfer to restore intrinsic function in high ulnar nerve injury: a prospective cohort study. *BMC Musculoskelet Disord.* 2024;25:566.
12. Ray WZ, Kasukurthi R, Yee A, Mackinnon SE. Functional recovery following an end to side neurotization of the accessory nerve to the suprascapular nerve: case report. *Hand (N Y).* 2010;5:313–7.
13. Kim CY, Kim YH. Supermicrosurgical reconstruction of large defects on ischemic extremities using supercharging techniques on latissimus dorsi perforator flaps. *Plast Reconstr Surg.* 2012;130:135–44.
14. Moore AM, Borschel GH, Santosa KB, Flagg ER, Tong AY, Kasukurthi R, et al. A transgenic rat expressing green fluorescent protein (GFP) in peripheral nerves provides a new hindlimb model for the study of nerve injury and regeneration. *J Neurosci Methods.* 2012;204:19–27.
15. Isaacs J, Patel G, Mallu S, Ugwu-Oju O, Desai A, Borschel G, et al. Effect of Reverse End-to-Side (Supercharging) Neurotization in Long Processed Acellular Nerve Allograft in a Rat Model. *J Hand Surg Am.* 2019;44:419.e1-419.e10.
16. Kale SS, Glaus SW, Yee A, Nicoson MC, Hunter DA, Mackinnon SE, et al. Reverse end-to-side nerve transfer: from animal model to clinical use. *J Hand Surg Am.* 2011;36:1631-1639.e2.
17. Balik O, Menderes A. A successful neurotization of two different muscles using a single intact motor nerve: experimental study on rats. *Ann Plast Surg.* 2011;66:172–8.
18. Placheta E, Wood MD, Lafontaine C, Liu EH, Hendry JM, Angelov DN, et al. Enhancement of facial nerve motoneuron regeneration through cross-face nerve grafts by adding end-to-side sensory axons. *Plast Reconstr Surg.* 2015;135:460–71.
19. Nadi M, Ramachandran S, Islam A, Forden J, Guo GF, Midha R. Testing the effectiveness and the contribution of experimental supercharge (reversed) end-to-side nerve transfer. *J Neurosurg.* 2019;130:702–11.
20. Fujiwara T, Matsuda K, Kubo T, Tomita K, Hattori R, Masuoka T, et al. Axonal supercharging technique using reverse end-to-side neurotization in peripheral nerve repair: an experimental study in the rat model. *J Neurosurg.* 2007;107:821–9.
21. Gordon T, Hendry M, Lafontaine CA, Cartar H, Zhang JJ, Borschel GH. Nerve cross-bridging to enhance nerve regeneration in a rat model of delayed nerve repair. *PLoS One.* 2015;10:e0127397.
22. Hendry JM, Alvarez-Veronesi MC, Snyder-Warwick A, Gordon T, Borschel GH. Side-To-Side Nerve Bridges Support Donor Axon Regeneration Into Chronically Denervated Nerves and Are Associated With Characteristic Changes in Schwann Cell Phenotype. *Neurosurgery.* 2015;77:803–13.
23. Mackinnon SE. In reply. *J Hand Surg Am.* 2013;38:618–9.