Inside-out and Standard Vein Grafts, with or without Muscle Filling, in Peripheral Nerve Repair: A Histomorphometric Study

Domingos D. Roque¹, Karina T. Pominì², Rogério L. Buchaim²*, Daniela V. Buchaim¹, Jesus C. Andreo², José S. Roque³, Antonio C. Rodrigues², Geraldo M. Rosa Júnior⁴, Luis Henrique R. Moraes⁵ and Fausto Viterbo⁶

¹Medical School, Discipline of Human Morphophysiology, University of Marilia (UNIMAR), Marilia, R. Hygino Muzy Filho, 17525-902 Marília, SP, Brazil.
²Department of Biological Sciences (Anatomy), Bauru School of Dentistry, University of São Paulo, Al. Dr. Octávio Pinheiro Brisola 9-75, 17012-901 Bauru, SP, Brazil.
³Department of Anatomy, Northern Paraná State University (UENP), Av. Manoel Ribas 215, 86400-970, Jacarezinho, PR, Brazil.
⁴Department of Health Sciences, University of Sacred Heart, R. Irmã Arminda 10-50, 17011-160 Bauru, SP, Brazil.
⁵Department of Structural and Functional Biology, Institute of Biology, State University of Campinas, UNICAMP, Cidade Universitária Zeferino Vaz - Barão Geraldo, 13083-970 Campinas, SP, Brazil.
⁶São Paulo State University, Julio de Mesquita Filho, UNESP, Via Domingos Sartori, s/n - Distrito de Rubião Junior, 18607-621 Botucatu, SP, Brazil.

Authors’ contributions

This work was carried out in collaboration between all authors. Authors RLB, JCA, DVB, ACR, JSR, DDR and FV participated in concept and design, data collection and data analysis/interpretation. Authors KTP, LHRM and GMRJ participated in manuscript creation involving critical writing and revising of the content. All authors read and approved the final version of this manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/23294

Editor(s):
(1) Vijay K. Sharma, Division of Neurology, Yong Loo Lin School of Medicine, National University of Singapore, National University Hospital, Singapore.
(2) Masahiro Hasegawa, Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu City, Mie, 514-8507, Japan.

Reviewers:
(1) Somchai Amornyotin, Mahidol University, Bangkok, Thailand.
(2) Dror Robinson, Tel Aviv University, Israel.
Complete Peer review History: http://sciencedomain.org/review-history/12779

Received 23rd November 2015
Accepted 14th December 2015
Published 24th December 2015

*Corresponding author: E-mail: rogerio@fob.usp.br;
ABSTRACT

Aim: The aim of this study was to compare two tubulization techniques, inside-out veins and standard veins, both filled with skeletal muscle or not, in sciatic nerve by morphological and histomorphometric study.

Methodology: Seventy Wistar rats were divided in 4 experimental groups (IOVNF - inside-out vein with no filling; IOVSM - inside-out vein filled with skeletal muscle; SVNF - standard vein with no filling; and SVSM - standard vein filled with skeletal muscle) and a control group (Sham). The left external jugular vein was sectioned into about 14 mm segments to be used as autologous vein grafts. A 10 mm gap was then created in the sciatic nerve and the vein graft was inserted into the vein with or without filling of the right caudal tibial muscle. The animals were euthanized 12 weeks after surgery.

Results: Myelinated and unmyelinated nerve fibers were observed in the histological analyses for all groups, as well as neoformation of the perineurium and intraneural organization of fascicles and blood vessels. In the morphometric analysis of the distal stump, regarding the myelin sheath area, all groups had a significant difference. The IOVNF group had the highest means for fiber, axon and myelin sheath areas. The SVSM group had the lowest means in all features measured, except for the axon area (4.95±1.72 graft; 3.71±0.90 distal stump).

Conclusion: These results show that sciatic nerve repair with inside-out veins and no filling (IOVNF) had the best results, in the majority of measured variables, when compared to the other groups.

Keywords: Autologous graft; nerve repair; nerve regeneration; peripheral nerve; sciatic nerve.

1. INTRODUCTION

Peripheral nerve lesions are frequently found in clinical practice and have great impact on morphofunctional alterations [1]. Despite continuous research and surgical innovation, peripheral nerve lesion repair with function recovery remains as a clinical challenge [2,3] that is influenced by several factors, such as: patient's age; and duration, degree and extent of the lesion [4].

For lesions with gaps above 20 mm and no signs of neurorrhaphy [5], can be applied the tubulization technique, in which autogenous, biological exogenous structures and artificial materials with different manufacturing methods and physical properties can be used [6-14].

Some studies have shown that tubulization techniques with standard and inside-out veins allow dissemination of neurotrophic factors, prevent the infiltration of fibrous tissue [15,16] and are composed of layers of laminin and collagen that are similar to the basal layers of nerve fibers, creating a microenvironment for axon growth [17]. However, other studies have shown that the tunica adventitia in inside-out vein grafts creates a more favorable environment for nerve repair [18-21].

The use of skeletal muscle as grafts in the tubulization technique started with the observation of the regular longitudinal alignment of muscle fibers [22,23], and the presence of extracellular matrix components that direct and increase the regeneration of nerve fibers [24-26]. Despite these advantages of using skeletal muscle as grafts for repairing nerve defects, few clinical studies have been reported to date [14]. Due to controversy in the literature about the use of standard or inside-out veins, the development of new research is necessary to contribute to this technique, which has great clinical applicability.

The importance of these methods of repairing peripheral nerve injury, and the small number of studies with morphometric analysis emphasizing their combination with muscle filling, led to the development of this study. The objective was to compare tubulization technique using inside-out veins (filled with skeletal muscle or not) with that using standard veins (filled with skeletal muscle or not) in the regeneration of the sciatic nerve, by means of morphological and histomorphometric analyses of graft sites and distal stumps.

2. MATERIALS AND METHODS

2.1 Animals and Surgical Procedures

Animals were previously selected, and comprised 70 Wistar rats (Rattus norvegicus), adults (60 days of age), with an average weight of 195 grams, provided by the Central Animal
The animals were randomly divided into five groups: a Sham group \((n=10)\) with no grafts, and four groups \((n=15\) each) submitted to a 10-mm gap in the right sciatic nerve and repaired with tubulization by means of a graft taken from the left external jugular vein filled with skeletal muscle or not.

Animals were first weighed and then anesthetized by intramuscular injection of 50% tiletamine and 50% zolazepam at 10 mg/kg (Telazol®; Fort Dodge Laboratories, USA). Aseptic techniques were adopted in all surgical procedures involving the animals.

All surgical procedures were performed with the help of a stereo microscope (MC/M9 - DF Vasconcellos S/A, São Paulo, Brazil). After anesthesia and trichotomy on the left side of the neck region, a 20-mm longitudinal incision was made in the skin. The jugular vein was isolated and ligature of its confluents was done; then a periodontal probe was introduced and both ends were sectioned (Fig. 1a). In Groups IOVNF and IOVSM, vein was reversed with the aid of a periodontal probe. The option to use the external jugular vein was due to its larger diameter and the absence of valves, which presented fewer obstacles to axonal regeneration [18]. This segment of the vein had an average diameter of 1.6 mm to 2.1 mm and was 14 mm in length [27], and was kept in physiological serum until its placement, as a graft, between the injured nerve stumps. The skin of the donating site was sutured with 4-0 monofilament nylon thread (Ethicon Inc., Somerville, NJ, USA).

2.2 Euthanasia and Collection of Nerve Segments

Euthanasia of all animals was done after 12 weeks by means of an intraperitoneal injection of a high dose of pentobarbital (Nembutal®, 30-50 mg/kg, Abbott Laboratory, Quebec, Canada). Afterwards, two fragments were collected, one from the middle third of the vein graft, and the other from the middle third of the distal stump of the right sciatic nerve (Fig. 1f).

2.3 Histological Processing of Sciatic Nerve Samples

Histological processing of samples collected from the graft sites and the distal stumps of the sciatic nerves was performed at the Microscopic and Experimental Anatomy Laboratory of the Institute of Biosciences - UNESP Anatomy Department, Botucatu campus, São Paulo, Brazil.

The histological fixative used was modified Karnovsky liquid (Electron Microscopy Sciences® Hatfield, Pennsylvania, United States) diluted in sodium cacodylate buffer at 0.1 M, pH 7.3.

After this period of fixation, the sciatic nerves were sectioned crosswise into two segments and left in the same fixative solution that was previously used for a period of one to two hours, and they were post-fixed for two hours in osmium tetroxide at 1% [29] in sodium cacodylate buffer 0.1 M pH 7.3. Subsequently, they were dehydrated in ethanol and propylene oxide and embedded in resin for histological examination.
Cross sections of 0.5 µm were obtained with a microtome (RM2265, Leica Biosystem® Germany), colored with toluidine blue at 1% in borax aqueous solution at 1% and osmium tetroxide for morphological and histomorphometric analysis.

The images of histological sections of grafts and distal stumps were captured with 400 times magnification (40x lens), by means of a video camera coupled to an optical microscope (Axiophot 2 Zeiss KS – 300, Germany) and a computer.

2.4 Morphological and Histomorphometric Analyses

In the analysis of the sciatic nerves in the experimental groups, the neuronal morphology and the entire set of structures were observed, namely, the epineurium, perineurium, adipose tissue, intraneural and extraneural blood vessels and myelin sheath.

For the Sham group, assessment of the area and minimum diameter of nerve fibers was performed, as well as the area and minimum diameter of axons, the area and thickness of the myelin sheath; for the other four groups (IOVNF, IOVSM, SVNF and SVSM), the assessment was made on the graft and the distal stump. These measures were obtained in four randomly selected fields by using the Image-Pro Plus™ program, version 6.0 (Media Cybernetics, Rockville, MD, USA). For the histological slides, the assessment was made with SigmaScan Pro version 5.0 (San Jose, CA, USA).
2.5 Statistical Analysis

Data obtained were submitted to ANOVA, followed by Scheffé's test. For all analyses, p<0.05 values were considered as statistically significant.

3. RESULTS

Myelinated and unmyelinated nerve fibers were observed in the histological analyses of the middle third of the grafts (Fig. 2) and the middle third of the distal stumps (Fig. 3), for all groups, as well as neoformation of the perineurium and intraneural organization of fascicles and blood vessels. The pattern of constitution of nerve fibers in most grafts of the experimental groups was similar to that of the sciatic nerve of the Sham group.

It was possible to observe the axons being regenerated and placed outside the epineurium on the site of the grafts (Fig. 2) and the distal stump (Fig. 3) in all experimental groups. It was also possible to see the thickening of the perineurium within the experimental groups when compared to the Sham group.

In the IOVSM and SVSM groups, on the graft site (Fig. 2), the nerve fibers appeared in well-organized microfascicles and the presence of remains of muscle fibers.

Fig. 2. Graft site (40X): (a) Sham group; (b) IOVNF group; (c) IOVSM group; (d) SVNF group; (e) SVSM group; Perineurium (PE) and blood vessel (black arrow)
The histomorphometric results for the graft sites are shown in Table 1. All experimental groups showed significant differences regarding values obtained from the fiber area. The IOVNF group had the highest means for fiber area (16.98±1.01), fiber and axon diameters (5.15±1.52 and 2.28±0.77, respectively) and thickness of the myelin sheath (1.43±0.58). However, there was no statistically significant difference between IOVNF and SVNF groups regarding the values of axon area (5.01±1.01 and 5.56±0.65, respectively) and myelin sheath area (11.97±1.36 and 12.77±1.33, respectively). The SVSM group showed the lowest means regarding fiber area and diameter (8.19±1.45 and 2.29±0.35, respectively), axon diameter (1.14±0.29), and area and thickness of the myelin sheath (3.25±1.18 and 0.58±0.08, respectively).

The histomorphometric results for the distal stump sites are shown in Table 2. Regarding the myelin sheath area, all groups had a significant difference. The IOVNF group had the highest means for fiber, axon and myelin sheath areas (15.54±0.67, 3.87±0.65 and 11.68±0.75, respectively). As for fiber diameter and thickness of the myelin sheath, the IOVNF group (2.63±0.45 and 0.64±0.13, respectively) had no significant difference when compared to the SVNF (2.65±0.76 and 0.62±0.32, respectively) and IOVSM (2.89±0.86 and 0.69±0.43, respectively) groups. The SVSM group had the lowest means in all features measured, except for axon area (3.71±0.90).
are rich in collagen and laminin to better guide This phenomenon occurs because vein grafts form structures that favor the migration of axons in standard venous grafts or inside-out grafts without filling (IOVNF and SVNF), the vein acted as a support for axon regeneration through the presence of nerve fibers at the graft site, as it was also observed by Ferrari and collaborators [42]. This statement is based on histological analysis, which verified the presence of regenerating axons in standard venous grafts or inside-out grafts without filling with skeletal muscle. Thus, the vein allowed axonal growth with no collapse. This can be explained by the formation of hematomas and the presence of thrombin, which keeps venous lumen, favoring nerve regeneration [43].

In contrast, the IOVSM and SVSM groups had muscle fragments at the graft site and the lowest means for the analyzed variables. We tested the axonal growth to the distal end of the injured nerve and have lower inflammatory response [34-36].

The absence of muscle filling in the vein graft can make the vessel collapse, due to the action of adjacent tissues [37-39]. This occurs in gaps that are wider than 5 cm [40,41]. The present study showed that, in groups without filling (IOVNF and SVNF), the vein acted as a support for axon regeneration through the presence of nerve fibers at the graft site, as was also observed by Ferrari and collaborators [42]. This statement is based on histological analysis, which verified the presence of regenerating axons in standard venous grafts or inside-out grafts without filling with skeletal muscle. Thus, the vein allowed axonal growth with no collapse. This can be explained by the formation of hematomas and the presence of thrombin, which keeps venous lumen, favoring nerve regeneration [43].

In contrast, the IOVSM and SVSM groups had muscle fragments at the graft site and the lowest means for the analyzed variables. We tested the

### Table 1. Morphometry of nerve fibers on the graft site

| Groups       | Fiber area (µm²) | Axon area (µm²) | Fiber diameter (µm) | Axon diameter (µm) | Myelin sheath area (µm²) | Myelin sheath thickness (µm) |
|--------------|-----------------|----------------|---------------------|-------------------|------------------------|-----------------------------|
| Sham        | 42.81±2.80⁺     | 9.76±1.75⁺     | 6.24±0.91⁺          | 2.70±0.83⁺        | 33.05±2.21⁺           | 1.77±0.08⁺                   |
| IOVNF       | 16.98±1.01⁰     | 5.01±1.04⁰     | 5.15±1.52⁰         | 2.28±0.77⁰        | 11.97±1.36⁰           | 1.43±0.58⁰                   |
| IOVSM       | 14.01±1.67ᶜ     | 3.72±0.96ᶜ     | 3.13±0.64ᶜ         | 1.60±0.37ᶜ        | 10.18±1.46ᶜ           | 0.76±0.21ᶜ                   |
| SVNF        | 15.53±0.59ᵈ     | 5.56±0.65ᵈ     | 3.55±1.56ᵈ         | 1.60±0.60ᵈ        | 12.77±1.33ᵈ           | 0.98±0.56ᵈ                   |
| SVSM        | 8.19±1.45⁺⁰     | 4.95±1.72⁺⁰    | 2.29±0.35⁺⁰        | 1.14±0.29⁺⁰       | 3.25±1.18⁺⁰           | 0.58±0.08⁺⁰                  |

Groups: Sham (Control Group); IOVNF (Inside-Out Vein with No Filling); IOVSM (Inside-Out Vein filled with Skeletal Muscle); SVNF (Standard Vein with No Filling); and SVSM (Standard Vein filled with Skeletal Muscle).

Different lower-case letters (a, b, c, d, e) indicate significant differences between groups in each measurement performed (vertical columns) by means of ANOVA, followed by Scheffé’s test (p<0.05).

### Table 2. Morphometry of nerve fibers in the distal stump

| Groups       | Fiber area (µm²) | Axon area (µm²) | Fiber diameter (µm) | Axon diameter (µm) | Myelin sheath area (µm²) | Myelin sheath thickness (µm) |
|--------------|-----------------|----------------|---------------------|-------------------|------------------------|-----------------------------|
| Sham        | 42.81±2.80⁺     | 9.76±1.75⁺     | 6.24±0.91⁺          | 2.70±0.83⁺        | 33.05±2.21⁺           | 1.77±0.08⁺                   |
| IOVNF       | 15.54±0.67⁺⁰    | 3.87±0.65⁺⁰    | 2.63±0.45⁺⁰        | 1.35±0.25⁺⁰       | 11.68±0.75⁺⁰         | 0.64±0.13⁺⁰                  |
| IOVSM       | 11.97±1.93ᶜ     | 3.14±0.54ᶜ     | 2.89±0.86ᶜ         | 1.50±0.32ᶜ        | 8.40±1.88ᶜ           | 0.69±0.43ᶜ                   |
| SVNF        | 11.39±1.70ᶜ     | 3.23±0.13ᶜ     | 2.65±0.76ᶜ         | 1.41±0.52ᶜ        | 5.77±1.39ᶜ           | 0.62±0.32ᶜ                   |
| SVSM        | 4.32±0.87ᵈ      | 3.71±0.90ᵈ     | 1.15±0.17ᶜ         | 0.82±0.13ᶜ        | 0.62±0.18ᵈ           | 0.17±0.06ᵈ                   |

Groups: Sham (Control Group); IOVNF (Inside-Out Vein with No Filling); IOVSM (Inside-Out Vein filled with Skeletal Muscle); SVNF (Standard Vein with No Filling); and SVSM (Standard Vein filled with Skeletal Muscle).

Different lower-case letters (a, b, c, d, e) indicate significant differences between groups in each measurement performed (vertical columns) by means of ANOVA, followed by Scheffé’s test (p<0.05).

4. DISCUSSION

The objective of this study was to compare the tubulization technique using inside-out veins (filled with skeletal muscle or not) to that using standard veins (filled with skeletal muscle or not) in the regeneration of the sciatic nerve. Our results indicate that sciatic nerve repair with inside-out vein grafts and no filling (IOVNF) has the best results, in the majority of measured variables, when compared to the other experimental groups.

In recent decades, much progress and innovation have taken place in techniques of surgical repair of injured peripheral nerves aiming to reestablish their function [2,3,6,30]. In this study, it was possible to observe the axons being regenerated and placed outside the epineurium in all experimental groups. Vein grafts form structures that favor the migration of axons to distal stumps and provide a favorable microenvironment of extracellular matrix and growth factors for the maturation of cells [31-33]. This phenomenon occurs because vein grafts are rich in collagen and laminin to better guide
hypothesis that the use of muscle fibers could increase axonal sprouting, due to availability of a longitudinally oriented basal lamina and extracellular matrix components [6,22], however the presence of muscle resulted in disorganized growth, and could even make it difficult for axons to pass through the graft.

Of all the variables analyzed, on the graft site, there was a significant difference between the IOVNF group and the other groups in the variables: area and fiber diameter, axon diameter and thickness of the myelin sheath, confirming that the inversion of the vein in the tubulization technique contributed to nerve regeneration through the microenvironment, which was high in collagen, lamini, and the presence of Schwann cells in the tunica adventitia [20,44].

The SVSM group had the lowest means, due to the probable resistance of the muscle to the growth of axons and the absence of the tunica adventitia [6]. The application of this knowledge for routine clinical practice are based on studies showing that the venous grafts, combined or not with muscle tissue in clinical cases, usually have good results, improvement of up to 85% of our cases with a minimum follow-up of 14 months [45].

There were some limitations on this study. We could have performed the functional tests and use of post-surgery techniques for evaluating possible functional improvement, such as laser therapy [46], electrotherapy [47], or physical exercise [48]. However, this study was focused on better understanding of the recovery of the peripheral nerve using two grafting techniques with veins filled or not with muscle, focusing on the morphometry of the nerve fibers.

5. CONCLUSION

Based on the results of the present study, we concluded that sciatic nerve repair performed by the technique of inside-out veins and no filling had the best results (IOVNF group), in the majority of measured variables, when compared to the other experimental groups.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by the ethics committee of the University of Marília (Marília, São Paulo, Brazil).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huang YC, Huang YY. Biomaterials and strategies for nerve regeneration. Artif Organs. 2006;30:514-522.
2. Siemionow M, Bozkurt M, Zor F. Regeneration and repair of peripheral nerves with different biomaterials: Review. Microsurgery. 2010;30:574-588.
3. Yegiyants S, Dayicioglu D, Kardashian G, Panthaki ZJ. Traumatic peripheral nerve injury: A wartime review. J Craniofac Surg. 2010;21:998-1001.
4. Terzis JK, Konofaos P. Radial nerve injuries and outcomes: Our experience. Plast Reconstr Surg. 2011;127:739-751.
5. Francel PC, Smith KS, Stevens FA, Kim SC, Gossett J, Gossett C, Davis ME, Lenaerts M, Tompkins P. Regeneration of rat sciatic nerve across a LactoSorb bioreorbable conduit with interposed short-segment nerve grafts. J Neurosurg. 2003;99:549-554.
6. Konofaos P, Ver Halen JP. Nerve repair by means of tubulization: Past, present, future. J Reconstr Microsurg. 2013;29:149-164.
7. Abernethy DA, Rud A, Thomas PK. Neurotropic influence of the distal stump of transected peripheral nerve on axonal regeneration: Absence of topographic specificity in adult nerve. J Anat. 1992;180:395-400.
8. Brushart TM. Motor axons preferentially reinnervate motor pathways. J Neurosci. 1993;13:2730-2738.
9. Yutaka M. Comparison of neurotropic effect on motor and sensory nerve regeneration. J Jpn Soc Surg Hand. 1994;468-471.
10. Nakamura T, Inada Y, Fukuda S, Yoshitani M, Nakada A, Itoi S, Kanemaru S, Endo K, Shimizu Y. Experimental study on the regeneration of peripheral nerve gaps through a polyglycolic acid-collagen (PGA-collagen) tube. Brain Res. 2004;1027:18-29.
11. Strasberg SR, Mackinnon SE, Genden EM, Bain JR, Purcell CM, Hunter DA, Hay JB. Long-segment nerve allograft regeneration in the sheep model: Experimental study and review of the
literature. J Reconstr Microsurg. 1996; 12:529-537.

12. Williams LR. Rat aorta isografts possess nerve regeneration-promoting properties in silicone Y chambers. Exp Neurol. 1987;97:555-563.

13. Benito-Ruiz J, Navarro-Monzonis A, Piquerases A, Baena-Montilla P. Invaginated vein graft as nerve conduit: An experimental study. Microsurgery. 1994; 15:105-115.

14. Geuna S, Tos P, Titolo P, Ciclamini D, Beningo T, Battiston B. Update on nerve repair by biological tubulization. J Brachial Plex Peripher Nerve Inj. 2014;9(1):3.

15. Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. Clin Plast Surg. 1999;26:617-628.

16. Ahmed FJ, Junior GM, Shinohara AL, De Souza Melo CG, Buchaim RL, Andreo JC, De Castro Rodrigues A. Comparison of results obtained with standard and inside out vein graft techniques and their implication on neurotrophin expression in repair of nerve defect: An experimental study. Microsurgery. 2015;35(3):227-34.

17. Thanos PK, Okajima S, Terzis JK. Ultrastructure and cellular biology of nerve regeneration. J Reconstr Microsurg. 1998; 14:423-36.

18. Wang KK, Costas PD, Bryan DJ, Jones DS, Seckel BR. Inside-out vein graft promotes improved nerve regeneration in rats. Microsurgery. 1993;14:608-618.

19. Geuna S, Tos P, Battiston B, Guglielmone R, Giacobini-Robecchi MG. Morphological analysis of peripheral nerve regenerated by means of vein grafts filled with fresh skeletal muscle. Anat Embryol (Berl). 2000;201:475-482.

20. Wang KK, Costas PD, Bryan DJ, Eby PL, Seckel BR. Inside-out vein graft repair compared with nerve grafting for nerve regeneration in rats. Microsurgery. 1995; 16:65-70.

21. Ide C, Tohyama K, Yokota R, Nitatori T, Onodera S. Schwann cell basal lamina and nerve regeneration. Brain Res. 1983; 288:61-75.

22. Meek MF, Varejão AS, Geuna S. Use of skeletal muscle tissue in peripheral nerve repair: Review of the literature. Tissue Eng. 2004;10:1027-1036.

23. Riccio M, Pangrazi PP, Parodi PC, Vaientti L, Marchesini A, Neuendorf AD, Bottegoni C, Tos P, Geuna S. The amnion muscle combined graft (AMCG) conduits: A new alternative in the repair of wide substance loss of peripheral nerves. Microsurgery. 2014;34(8):616-22.

24. Lundborg G, Dahlin L, Danielsen N, Zhao Q. Trophism, tropism, and specificity in nerve regeneration. J Reconstr Microsurg. 1994;10:345-354.

25. Couturier CA, Dauge MC, Henin D, Alnot JY, Masmejean EH. Nerve repair using a composite graft of vein and denatured skeletal muscle: Morphologic analysis. J Reconstr Microsurg. 2002;18:681-688.

26. Raimondo S, Nicolin S, Tos P, Battiston B, Giacobini-Robecchi MG, Perroteau I, Geuna S. Schwann cell behavior after nerve repair by means of tissue-engineered muscle-vein combined guides. J Comp Neurol. 2005;489:249-259.

27. Glasby MA, Gschmeissner 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(5):829-33.

28. Di Scipio F, Raimondo S, Tos P, Geuna S. A simple protocol for paraffin-embedded myelin sheath staining with osmium tetroxide for light microscope observation. Microsc Res Tech. 2008;71(7):497-502.

29. Friedman AH. An eclectic review of the history of peripheral nerve surgery. Neurosurgery. 2009;65:A3-8.

30. Friedmar AH. An eclectic review of the history of peripheral nerve surgery. Neurosurgery. 2009;65:A3-8.

31. Guo Y, Chen G, Tian G, Tapia C. Sensory recovery following decellularized nerve allograft transplantation for digital nerve repair. J Plast Surg Hand Surg. 2013;47(6):451-3.

32. Walton RL, Brown RE, Matory WE Jr, Borah GL, Dolph JL. Autogenous vein graft repair of digital nerve defects in the finger: A retrospective clinical study. Plast Reconstr Surg. 1989;84:944-949.

33. Manoli T, Schulz L, Stahl S, Jaminet P, Schaller HE. Evaluation of sensory recovery after reconstruction of digital nerves of the hand using muscle-in-vein conduits in comparison to nerve suture or nerve autografting. Microsurgery. 2014; 34(8):806-15.

34. Millesi H. Progress in peripheral nerve reconstruction. World J Surg. 1990; 14(6):733-47.
35. Brunelli GA, Vigasio A, Brunelli GR. Different conduits in peripheral nerve surgery. Microsurgery. 1994;15(3):176-8.

36. Fornaro M, Tos P, Geuna S, Giacobini-Robecchi MAG, Battiston B. Confocal imaging of Schwann-cell migration along muscle-vein combined grafts used to bridge nerve defects in the rat. Microsurgery. 2001;21:153-155.

37. Eren F, Öksüz S, Kıcıkodaci Z, Kendiril MT, Cesur C, Alarçın E, İrem Bektas E, Karagöz H, Kerimoğlu O, Kösse GT, Ulkür E, Gorantla V. Targeted mesenchymal stem cell and vascular endothelial growth factor strategies for repair of nerve defects with nerve tissue implanted autogenous vein graft conduits. Microsurgery. 2015. (Epub ahead of print).

38. Nijhuis TH, Brzezicki G, Siemionow M. Isogenic venous graft supported with bone marrow stromal cells as a natural conduit for bridging a 20 mm nerve gap. Microsurgery. 2010;30(8):639-45.

39. Moore AM, Kasukurthi R, Magill CK, Farhadi HF, Borschel GH, Mackinnon SE. Limitations of conduits in peripheral nerve repairs. Hand (NY). 2009;4(2):180-6.

40. Ristano G, Cavallaro G, Mernino T, Coppolino S, Ruggeri F. Clinical results and thoughts on sensory nerve repair by autologous vein graft in emergency hand construction. Chir Main. 2002;21:194-197.

41. Ulkür E, Yüksel F, Ağikel C, Okar I, Çeliközk B. Comparison of functional results of nerve graft, vein graft, and vein filled with muscle graft in end-to-side neurorrhaphy. Microsurgery. 2003;23:40-48.

42. Ferrari F, De Castro Rodrigues A, Malvezzi CK, Dal Pai Silva M, Padovani CR. Inside-out vs. standard vein graft to repair a sensory nerve in rats. Anat Rec. 1999;256(3):227-32.

43. Tseng CY, Hu G, Ambro RT, Chiut DT. Histologic analysis of Schwann cell migration and peripheral nerve regeneration in the autogenous venous nerve conduit (AVNC). J Reconstr Microsurg. 2003;19:331-340.

44. Ide C. Nerve regeneration and Schwann cell basal lamina: Observations of the longterm regeneration. Arch Histol Jap. 1993;46:243-257.

45. Battiston B, Tos P, Cushway TR, Geuna S. Nerve repair by means of vein filled with muscle grafts I. Clinical results. Microsurgery. 2000;20(1):32-6.

46. Buchaim RL, Andreo JC, Barraviera B, Ferreira Junior RS, Buchaim DV, Rosa Junior GM, de Oliveira AL, de Castro Rodrigues A. Effect of low-level laser therapy (LLLT) on peripheral nerve regeneration using fibrin glue derived from snake venom. Injury. 2015;46(4):655-60.

47. Kao CH, Chen JJ, Hsu YM, Bau DT, Yao CH, Chen YS. High-frequency electrical stimulation can be a complementary therapy to promote nerve regeneration in diabetic rats. PLoS One. 2013;8(11):e79078.

48. Brandt J, Evans JT, Mildenhall T, Mulligan A, Konieczy A, Rose SJ, English AW. Delaying the onset of treadmill exercise following peripheral nerve injury has different effects on axon regeneration and motoneuron synaptic plasticity. J Neurophysiol. 2015;113(7):2390-9.