INSIDE-OUT VEIN GRAFT AND INSIDE-OUT ARTERY GRAFT IN RAT SCIATIC NERVE REPAIR

ALÉCIO SANTOS BARCELOS, Ph.D.,[1]
ANTONIO CASTRO RODRIGUES, Ph.D.,[2]*
MAELI DAL PAI SILVA, Ph.D.,[3] AND
CARLOS R. PADOVANI, Ph.D.[4]

Although veins and arteries present similar wall structures, there are differences which may be relevant in peripheral nerve reconstruction. Inside-out vein grafts (IOVG) have been satisfactorily used to repair both motor and sensitive nerves. However, the inside-out artery graft (IOAG) is a new technique and not fully investigated. Our study presents comparative morphological data on nerve regeneration achieved with IOVG and IOAG in the repair of Wistar rat sciatic nerves. Jugular veins and aorta arteries were harvested from donor animals and used "inside-out" to bridge a 10-mm gap. Animals were sacrificed at 10 weeks to evaluate nerve regeneration. Both techniques presented great variability in nervous tissue, though some animals showed satisfactory results. Different intensities of scarring processes might have interfered with nerve regeneration. Although IOVG and IOAG techniques showed similar morphometric results, in general, IOVG presented a closer-to-normal nerve organization than IOAG.

MICROSURGERY 23:66–71 2003

Peripheral nerve reconstruction must restore tension-free nerve continuity to improve axonal regeneration.[1–7] Therefore, in the presence of important nerve tissue loss, a conduit between the proximal and distal stumps is necessary. Although autologous nerve grafting is still considered the best method for bridging peripheral nerve injuries with substance defects,[8–11] this technique does not provide fully satisfactory recovery, and several other conduits have been studied.[12]

Veins and arteries have been experimentally used as grafting tubulus.[13–18]

Some authors found that in standard vein grafting studies, there is minimal scar-tissue invasion inside the graft.[13–15] Others reported that contact between vein graft endothelial cells and regenerating axons stimulates connective tissue development and fibrosis which causes nerve constriction, impairing axon regeneration.[16,17]

Standard artery tubulization did not show positive results and should not be applied in peripheral nerve repair. Artery endothelial basement membrane provides a large diameter conduit, therefore, the contact surface for regenerating axons or Schwann cells becomes very small. According to the contact guidance theory, axonal outgrowth and Schwann-cell differentiation require not only contact with nerve fibers but also contact with a connective tissue matrix. Furthermore, basal membrane laminin may be insufficient to accelerate regeneration.[18]

Inside-out vein grafts have been used in motor and sensitive nerve repair. In this technique, a vein graft is pulled inside-out, inverting the normal orientation and exposing adventitia to direct contact with injured axons. The vein wall presents neurotrophic substances also found in Schwann-cell basal membrane: endothelial basal membrane and muscular layer are rich in laminin, and adventitia is rich in collagen. In contrast to the standard technique, an inside-out vein graft provides an adequate microenvironment for axonal regeneration.[19–21]

An artery graft presents large quantities of laminin and some collagen, while veins are especially rich in collagen. These substances are also found in Schwann-cell basal membrane and are reported as axonal outgrowth factors.[18–24] Laminin, one of the main basal membrane components,[25] stimulates neurite outgrowth,

[1]Department of Anatomy-IB and Medical School, UNESP/Botucatu, São Paulo, Brazil

[2]Department of Anatomy, IB/UNESP/Botucatu, São Paulo, Brazil

[3]Department of Morphology, IB/UNESP/Botucatu, São Paulo, Brazil

[4]Department of Biostatistics, IB/UNESP/Botucatu, São Paulo, Brazil

Grant sponsor: FAPESP; Grant number: 00/11021-9.

*Correspondence to: A.C. Rodrigues, Ph.D., Department of Anatomy, IB/UNESP/Botucatu, 18618-000 Rubião Júnior, São Paulo, Brazil.
E-mail: acastro@ibb.unesp.br

Received 17 September 2002; Accepted 11 December 2002

Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/micr.10083

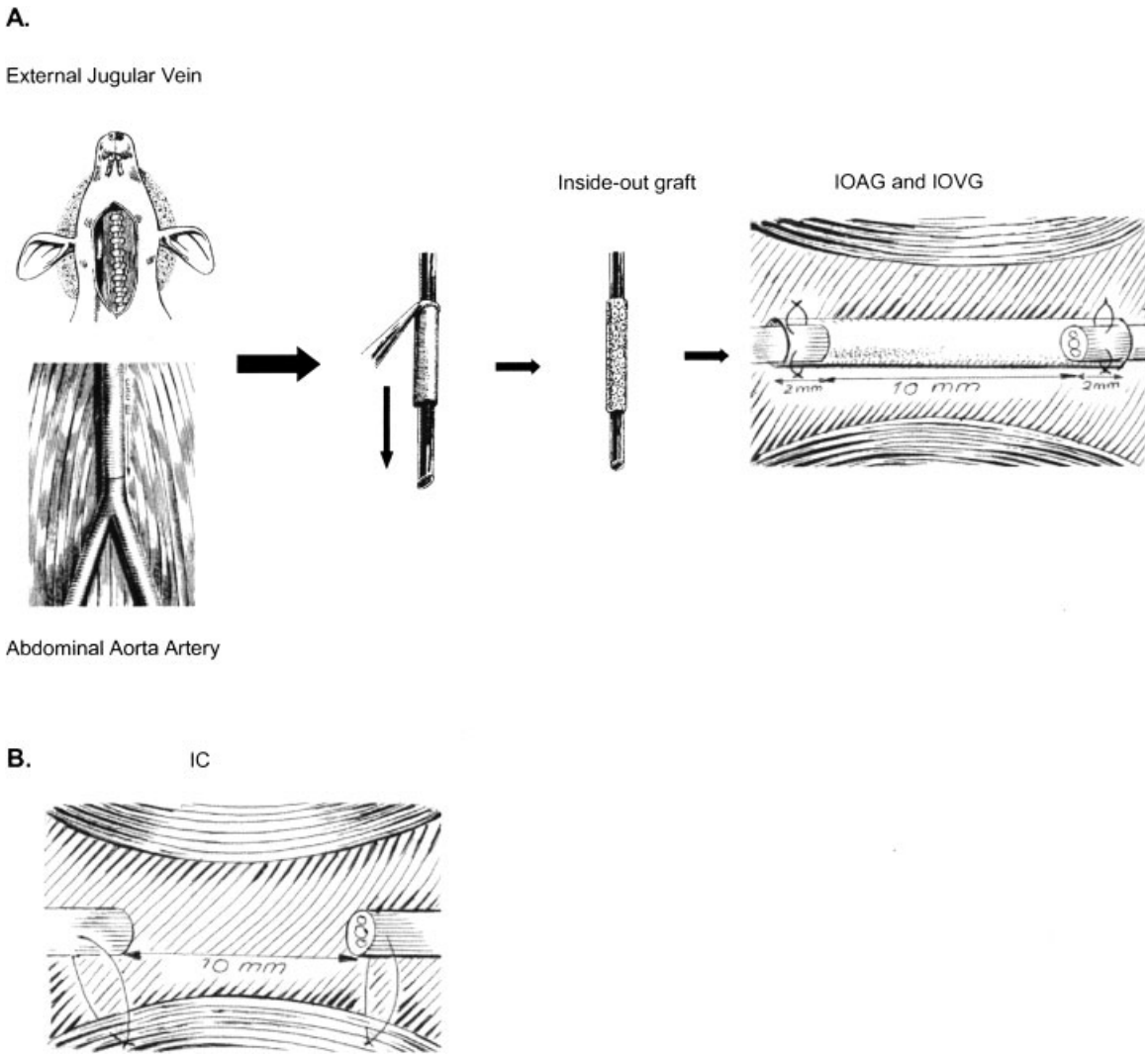

Figure 1. Experimental designs of IOAG, IOVG, and IC. ***A:*** *IOAG and IOVG. Abdominal aorta artery and right external jugular vein were harvested from donor animals, turned inside-out, and used to bridge a 10-mm gap created in right sciatic nerve.* ***B:*** *IC. A 10-mm right sciatic nerve segment was removed, and proximal and distal stumps were fixed in adjacent musculature; no conduct was placed between stumps.*

induces Schwann-cell mitosis, and plays a fundamental role in peripheral nerve regeneration.[26,27] As well as requiring nerve fiber contact, normal Schwann-cell differentiation requires contact with a connective tissue matrix or some associated material such as collagen.[28]

Although veins and arteries present similar wall structures, there are differences such as muscular layer and adventitia thickness, wall rigidity and permeability, and inner diameter, which may be relevant in peripheral nerve reconstructive surgery.

In this study, we present comparative morphological data on nerve regeneration between an inside-out vein graft (IOVG) and inside-out artery graft (IOAG) in the repair of experimentally sectioned rat sciatic nerves.

MATERIALS AND METHODS

Experimental Design

Twenty-four male Wistar rats weighting 200–250 g were divided into three experimental groups (n = 8): inside-out vein graft (IOVG), inside-out artery graft (IOAG), and injured control (IC). Eight male Wistar rats weighting 300–350 g were used as graft donors. Six male Wistar rats weighting 400–450 g were used as normal controls (NC). Intraperitoneal sodium pentobarbital, 30 mg/kg, was used as anesthetic. Aseptic technique was observed in all surgical procedures. All procedures on animals were in accordance with the Brazilian Society of Animal Experimentation (COBEA).

The abdominal aorta artery was exposed through a median abdominal incision and canulated, and then a

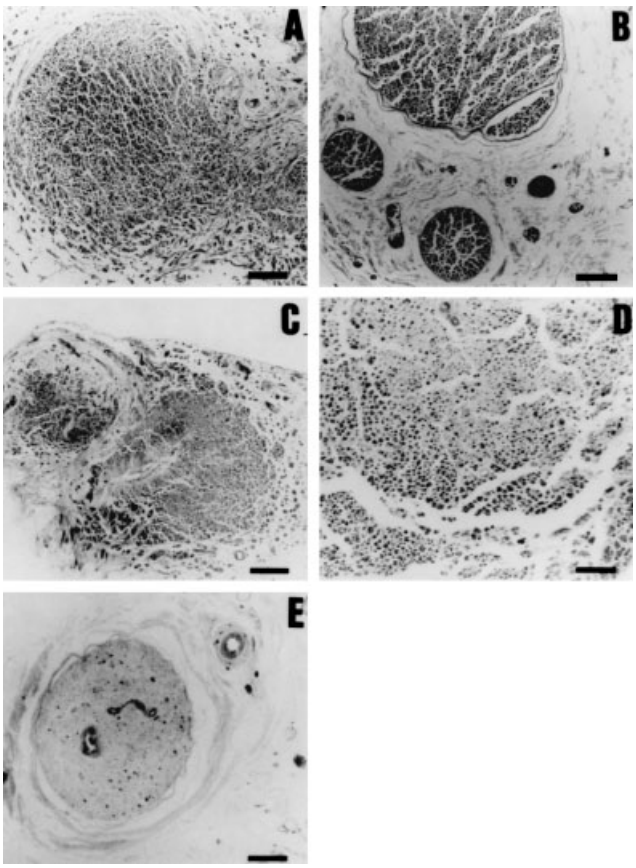

*Figure 2. Light micrographs of IOVG, IOAG, and IC nerve segments at 10 weeks. IOVG middle cable (**A**) nerve-fiber organization was closer to normal nerve than was IOAG (**C**). Nerve fibers reached distal stump in both experimental groups, IOVG (**B**) and IOAG (**D**). Note difference between a degenerated distal stump in IC (**E**) and experimental-group regenerated distal stumps. Bars: A, C, 200 μm; B, E, 100 μm; D, 50 μm.*

15-mm segment was harvested on the tube. Then the right external jugular vein was exposed through a paramedian neck incision, and a 15-mm segment was removed the same way. Donor animals were sacrificed after graft harvest with high-dose anesthetic. Grafts were washed in physiological solution and left at room temperature for 30–40 min. A subtle retraction of 1 mm was already expected. Allografts did not receive preliminary treatment to reduce their antigenicity. Each graft was inverted inside-out by pulling it down the cannula with microsurgery tweezers.

In the experimental groups, the right sciatic nerve was exposed through median-tight and muscle-splitting incisions, and a 10-mm nerve segment proximal to its trifurcation was removed. In IOVG and IOAG, proximal and distal stumps were each inserted 2 mm into the graft, and two 10-0 nylon stitches were placed at each end of the cuff to fix the graft in place and leave a 10-mm gap between the stumps (Fig. 1A). In IC, proximal

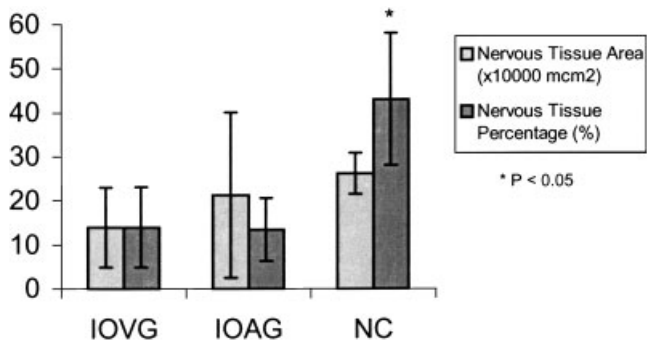

Figure 3. IOVG and IOAG grafts and NC nervous tissue areas and percentages are shown. Nervous tissue area: IOVG = IOAG = NC. Nervous tissue percentage: IOVG = IOAG < NC.

and distal stumps were fixed in the adjacent muscle 10 mm away from each other with one 7-0 nylon stitch. No conduit was placed between the stumps (Fig. 1B). The surgical incision was closed in layers, muscular and cutaneous, with 4-0 nylon sutures.

Postoperation, animals were housed in groups of four per cage in a temperature- and humidity-controlled environment with 12-hr light-dark cycles. They had food and water ad libitum. Ten weeks postoperation, the animals were sacrificed with a high dose of anesthetic to evaluate the extent of nerve regeneration. Perfusion with glutaraldehyde (2.5%) was performed by the abdominal aorta artery. Animals of the same age, without lesions or repairs, were used as normal controls (NC).

Histological Preparation and Studies

Graft middle cable (IOVG and I OAG), distal stump (IOVG, IOAG, and IC), and normal sciatic nerve (NC) were excised and fixed in 2.5% glutaraldehyde solution and 1.0% osmium tetroxide. Thereafter, they were embedded in paraplast paraffin and cut in 7-μm transverse sections with a microtome. Fragments from IOVG and IOAG middle cables were submitted to transmission electron microscopy.

Six external jugular veins and abdominal aorta arteries were harvested from adult male Wistar rats. The vessels were fixed in 10% formalin, embedded in paraplast paraffin, cut in 7-μm transverse sections with a microtome, and then stained with hematoxylin-eosin and Caleja Tricrome.

Analysis of cross sections was performed using light microscopy (Axiophot 2, Zeiss) with a software image analyzer (KS-300, Zeiss) connected to a computer.

Morphological evaluation of nervous tissue area and percentage was performed under ×60 magnification. At this magnification, myelinated axons appear as dots. Using an image analyzer application, for each nerve

Table 1. Quantitative Analysis Results of Diameter and Myelin Sheath Thickness of Nerve Fibers*

Variable	IC ds	IOAG g	IOAG ds	IOVG g	IOVG ds	NC
Fiber diameter	3.99 ± 0.56 (a)	5.07 ± 0.98 (a)	4.02 ± 0.54 (a)	5.24 ± 1.31 (a)	4.89 ± 0.69 (a)	9.15 ± 0.73 (b)
Myelin thickness	0.89 ± 0.13 (a)	1.12 ± 0.22 (a)	0.80 ± 0.14 (a)	1.10 ± 0.34 (a)	0.80 ± 0.10 (a)	1.81 ± 0.22 (b)

**a < b (P < 0.05). ds, distal stump; g, graft. Fiber diameter: IC = IOVG = IOAG < NC. Myelin thickness: IC = IOVG = IOAG < NC.*

segment, a black scale was determined that best represented its nerve fibers, and then undesirable structures were excluded such as vessels and dense connective tissue (perineurium and epineurium). After that, myelinated-axon total areas were measured automatically and designated "nervous tissue areas." This morphological analysis allowed the evaluation of nerve tissue graft invasion, although the criteria cannot be considered as precise as in fiber number counting. IOVG and IOAG middle cables, and NCG segments, were analyzed.

Semiautomatic morphometry was performed at ×800 magnification to obtain nerve fiber diameter and myelin sheath thickness.

Morphometry of vein and artery adventitia thickness and lumen diameter was performed at ×60 magnification.

Statistical analysis was performed with a parametric ANOVA (Tukey test) and nonparametric ANOVA (Kruskal-Wallis test).[29]

RESULTS

Histological Analysis

All inside-out vein graft animals showed nerve fibers at distal stumps 10 weeks after surgery (Fig. 2A, B). However, nerve tissue areas ranged from tenuous to similar to those found in normal nerves. In contrast, two IOAG animals did not present regeneration at the graft, and three did not present regeneration at the distal stump. Curiously, in two IOAG animals, axon outgrowth occurred over the graft external surface, and a few fibers reached the distal stump. In two IOAG animals, regeneration was very satisfactory, and nerve tissue areas were similar to normal nerves (Fig. 2C, D). In general, although there was great variability in regeneration among the animals of both groups (IOVG and IOAG), nervous tissue areas did not show significant differences when compared to NC data. However, connective tissue was much more abundant in experimental groups than in NC; this is clearly noticeable in nerve tissue percentages (Fig. 3). In nerve tissue area and percentage analysis, IOAG animals with regeneration outside the conduit were not considered. In IC, with no conduit between the proximal and distal stumps, five animals presented a few nerve fibers at distal stumps. The other animals showed degenerated distal stumps (Fig. 2E).

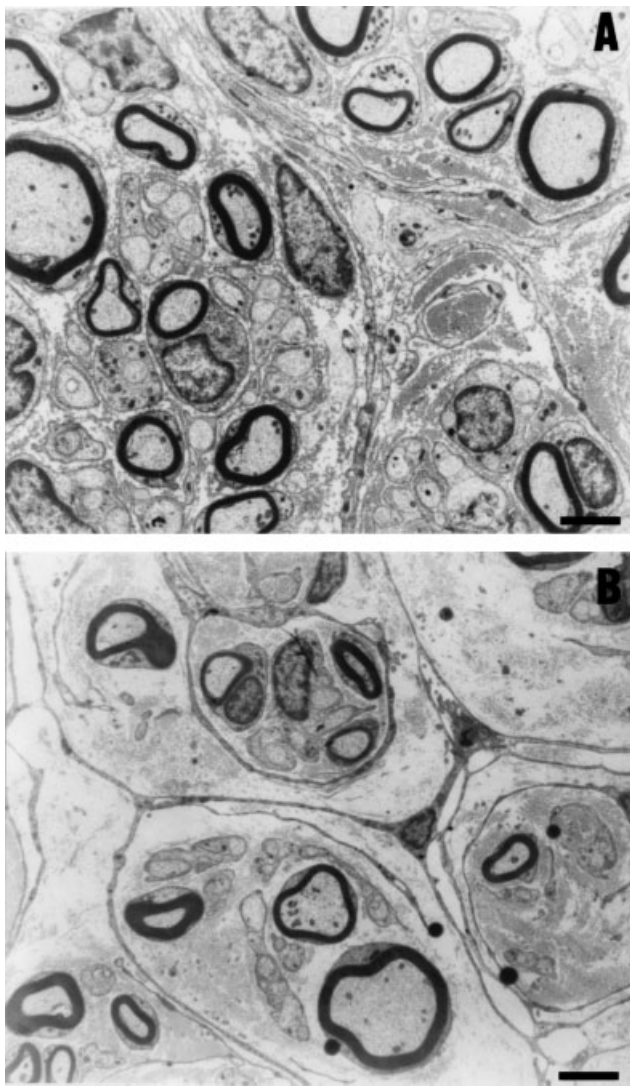

*Figure 4. Electron micrographs of IOVG (**A**) and IOAG (**B**) grafts at 10 weeks. IOVG showed thicker perineurium, more rounded myelinated fibers, greater quantity of unmyelinated fibers, and better nerve fiber organization than IOAG. Bars: A, 2 μm; B, 2.5 μm.*

Normal controls presented significantly greater nerve fiber diameters and myelin sheath thickness compared to inside-out vein and artery grafts and injured controls (Table 1). Although both experimental groups presented good regeneration patterns, IOVG presented a nerve organization closer to normal nerves than IOAG (Fig. 4A, B).

Abdominal aorta artery inner diameter (AoD) was 2.7 times larger than the right external jugular vein (VD) (AoD = 865.40 ± 140.39 μm^2; VD = 322.04 ± 90.52 μm^2). Furthermore, the artery muscular layer was thick and dense, while veins presented just a few muscle fibers. Artery wall is more rigid and therefore has a tendency to keep the lumen patent. On the other hand, veins present a considerably flexible wall which, associated with a thick adventitia (1.8 times thicker than artery; AoD = 132.68 ± 20.64 μm^2; VD = 233.42 ± 61.82 μm^2), results in an inside-out vein graft with a connective matrix-filled lumen.

DISCUSSION

Inside-out vein and artery allografts in the repair of Wistar rat sciatic nerves presented controversial results. The presence of abundant connective tissue was expected, as the repair process involves an inflammatory reaction with some scarring. Great variability in nerve tissue area was observed in both experimental groups. Different scarring-process intensities might have interfered in nerve regeneration; grafts did not receive any treatment to reduce antigenicity. Nerve fiber diameter and myelin sheath thickness were also lower in experimental groups and the injured control group than in the normal control group. Regenerating axonal sprouting tended to be smaller than for uninjured axons.

Artery walls, as mentioned before, are quite rigid, and we felt that in some animals the graft might have caused excessive pressure on the nerve diameter and hypoxia, thus impairing regeneration. Other authors also suggested the importance of this phenomenon.[30,31] In contrast to vein wall, an artery thick muscle layer was still present 10 weeks after the operation, and probably resulted in longer exposure to alloantigens. It is also possible that artery grafts suffered more immunological influences than veins, as Wistar rats are not isogenic and grafts did not receive any treatment to reduce immunogenicity. Furthermore, some authors claim that the standard artery graft basal membrane tube diameter is large, and the contact surface for migrating Schwann cell or axonal outgrowth cone adhesion becomes very small.[20] Nevertheless, in inside-out artery grafts, this negative effect might be diminished once the adventitia provides a permissive matrix which increases the contact surface for axons. Although some negative events took place in some animals, the inside-out aorta artery graft can provide a balanced microenvironment: some IOAG animals presented very satisfactory nerve regeneration. The presence of an artery wall middle layer until 10 weeks after the operation may have resulted in a gradual and continuous discharge of laminin, which probably contributed to nerve recovery.

In IOVG, although all animals presented nerve fibers at the distal stump 10 weeks after the operation, nerve tissue presence was also very variable. Previous studies concerning the standard vein technique showed both good and frustrating results. Some authors found minimal scar tissue invasion inside the graft,[15–17] while others reported that contact between endothelial cells and regenerating axons gave rise to graft constriction, secondary to abundant scar connective tissue development.[18,19] Inside-out autogenous vein grafts in the repair of rat sciatic nerves presented satisfactory results.[21,22] In our study, although we observed tenuous nerve regeneration in some animals, others presented very satisfactory results. Probably a subtle to intense scarring process, perhaps due to immunological responses, impaired regeneration in some IOVG animals. Histopathological inside-out vein and artery graft approaches at different postoperative times will be necessary to clarify the progress of nerve regeneration processes.

In IC, in which no conduit was placed between proximal and distal stumps, five animals presented a few nerve fibers at distal stumps. This agrees with classical studies[32] that claim that substances from degenerating distal stumps attract terminal axonal sproutings from proximal stumps, and the axons that do not spread reach the distal stump (neurotropism); however, intense fiber spreading and lack of neurotrophic factors might have impaired nerve recovery.

CONCLUSIONS

Although inside-out vein and artery graft techniques gave similar morphometric results and good regeneration patterns, in general, IOVG presented a closer-to-normal nerve organization than did IOAG. An important advantage of the vein graft over the artery graft is that in experimental studies it can be harvested from the animal itself with minimal injury to the donor area and thus is autologous, while the artery cannot.

Both techniques can serve as conduits for axonal regeneration. However, further experimental studies must be performed to further investigate which graft characteristics and related biological events impair regeneration in some animals. We need to investigate the possibility of controlling those features to achieve better nerve regeneration.

ACKNOWLEDGMENTS

The authors thank Mrs. Maria Helena Spadoto de Lima and Mr. Gelson Rodrigues for their technical assistance, Mr. Benedito Vinicio Aloise for his great illustrations, and Prof. Ramón Takeno for his generous support.

REFERENCES

1. Brooks D. The place of nerve grafting in orthopedic surgery. J Bone Joint Surg [Am] 1955;37:299.
2. Terzis J, Faibisoff B, Williams HB. The nerve gap: suture under tension versus graft. Plastic Reconstr Surg 1975;56:166–170.
3. Sunderland S. Nerves and nerve injuries. Edinburgh: Churchill Livingstone; 1968. 1161 p.
4. Millesi H. The nerve gap: theory and clinical practice. Hand Clin 1987;2:651.
5. Millesi H. Reconstruction of the peripheral nerve. In: Terzis J, Smith K, editors. The peripheral nerve. New York: Raven Press; 1990. p 116–131.
6. Mackinnon S. Update on peripheral nerve microsurgery. Adv Plast Reconstr Surg 1995;2:75–79.
7. Zhang F, Inserra M, Richards L, Terris DJ, Lineaweaver WC. Quantification of nerve tension after nerve repair: correlations with nerve defects and nerve regeneration. J Reconstr Microsurg 2001;17:445–451.
8. Hudson AR, Hunter D, Kline DG, Bratton BR. Histological studies of experimental interfascicular graft repair. J Neurosurg 1979;51:333–340.
9. Millesi H. Peripheral nerve injuries. Nerve sutures and nerve grafting. Scand J Plast Reconstr Surg [Suppl] 1982;19:25–37.
10. Terzis JK, Sun DD, Thanos PK. Historical and basic science review: past, present and future of nerve repair. J Reconstr Microsurg 1997;13:215–225.
11. Evans GR. Challenges to nerve regeneration. Semin Surg Oncol 2000;19:312–318.
12. 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.
13. Chiu DTW, Janecka I, Krizec TJ, Wolf M, Lovelace RE. Autogenous vein graft as a conduit for nerve regeneration. Surgery 1982;91:226–233.
14. Suematsu N, Atsuta Y, Hirayama T. Vein graft for repair of peripheral nerve gap. J Reconstr Microsurg 1988;4:313–318.
15. Chiu DTW, Strauch B. A propective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr Surg 1990;86:928–934.
16. Heike GC, Klopper PJ, Dutrieux RP. Vein graft conduits versus conventional suturing in peripheral nerve reconstructions. Microsurgery 1993;14:594–588.
17. Guda CMH, Pieter JK, Richard PD. Vein graft conduits versus conventional suturing in peripheral nerve reconstructions. Microsurgery 1993;14:584–588.
18. Itoh S, Shinomiya K, Samejima H, Ohta T, Ishizuki M, Ichinose S. Experimental study on nerve regeneration through the basement membrane tubes of the nerve, muscle, and artery. Microsurgery 1996;17:525–534.
19. 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.
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. Ferrari F, Rodrigues AC, Malvezzi CK, Silva MDP, Padovani CR. Inside-out vs. standard vein graft to repair a sensory nerve in rats. Anat Rec 1999;256:227–232.
22. Ide C, Tohyama K, Yokota R, Nitatori T, Onodera S. Schwann cell basal lamina and nerve regeneration. Brain Res 1983;288:61–75.
23. Lander AD, Fujii DK, Reichardt LF. Laminin is associated with the ''neurite outgrowth-promoting factors'' found in conditioned media. Proc Natl Acad Sci USA 1985;82:2183–2187.
24. Valentini RF, Aebischer P, Winn SR, Galletti PM. Collagen- and laminin-containing gels impede peripheral nerve regeneration through semipermeable nerve guidance channels. Exp Neurol 1987;98:350–356.
25. Tohyama K, Ide C. The localization of laminin and fibronectin on the Schwann cell basal lamina. Arch Histol Jpn 1984;47:519–532.
26. Wang GK, Hirai K, Shimada H, Taji S, Zhong S-Z. Behavior of axons, Schwann cells and perineural cells in nerve regeneration within transplanted nerve grafts: effects of anti-laminin and anti-fibronectin antisera. Brain Res 1992;583:216–226.
27. Bryan DJ, Miller RA, Costas PD, Wand KK, Shekel BR. Immunocytochemistry of skeletal muscle basal lamina grafts in nerve regeneration. Plast Reconstr Surg 1993;92:927–939.
28. Bunge RP, Bunge MB. Evidence that contact with connective tissue matrix is required for normal interaction between Schwann cells and nerve fibers. J Cell Biol 1978;78:943–950.
29. Streiner DL, Norman GR. Biostatistics: the bare essentials. St. Louis: Mosby-Year Book; 1994. 260 p.
30. Lehman RA, Hayes GJ. Degeneration and regeneration in peripheral nerve. Brain 1967;90:285–296.
31. Causey G, Palmer E. The effect of pressure on nerve conduction and nerve fibre size. J Physiol (Lond) 1949;109:220–231.
32. Ramón Y Cajal S. Degeneration and regeneration of the nervous system. London: Oxford University Press; 1928.