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Inside-Out vs. Standard Vein Graft to Repair a Sensory Nerve in Rats

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ABSTRACT

Nerve regeneration in a sensory nerve was obtained by the application of different techniques: inside-out vein graft (IOVG group) and standard vein graft (SVG group). These techniques provide a good microenvironment for axon regeneration in motor nerves, but their efficiency for regeneration of sensory nerves is controversial.

The saphenous nerve was sectioned and repaired by the inside-out and standard vein graft techniques in rats. After 4, 12, and 20 weeks the graft and the distal stump were observed under electron microscopy. In each studied period, the pattern, diameters, and thickness of the myelin sheaths of the regenerated axons were measured in the graft and distal stump. A comparative study about the regenerated nerve fibers by these two different techniques was performed.

Regenerated nerve fibers were prominent in both vein grafts 4 weeks after the surgical procedures. On the other hand, in the distal stump, regenerated nerve fibers were observed only from 12 weeks. In both inside-out vein graft and standard vein graft statistical difference was not observed about the diameters and thickness of the myelinated fibers after 20 weeks. On the other hand, the inside-out group had greater regenerated axon number when compared to the standard group.

There is a capillary invasion in both graft and distal stump, especially in the IOVG group. The regenerated axons follow these capillaries all the time like satellite microfascicles. After 20 weeks, the diameters of regenerated fibers repaired by the standard vein graft technique were closer to the normal fibers compared to the inside-out vein graft. On the other hand, the pattern of these regenerated axons was better in the IOVG group. Anat Rec 256:227–232, 1999.

Key words: nerve regeneration; vein graft; rat

Nerve injuries have been recognized for centuries, and the degeneration of the axon in the distal stump following the transection of the nerve fibers was first described in the last century.

Schwann cells deprived of axons remain alive. They become elongated with slender processes, which are interconnected with one another, to form a cell column called the Schwann cell column or band of Büngner. It should also be observed that the Schwann cell basal lamina remains almost unaffected during the process of Wallerian degeneration, followed by the formation of Schwann cell columns. Therefore, every Schwann cell column is located within its own basal lamina tube.

Peripheral nerve repair has been studied using biological and nonbiological materials as a conduit in different techniques (Williams et al., 1983; Fields et al., 1989; Abernethy et al., 1994; Madison and Archibald, 1994).

Some authors report that the vein graft could be used for nerve repair since it is easily obtained without damage for

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the animal (Chiu et al., 1982; Godard et al., 1984; Risitano et al., 1989). On the other hand, vein graft functionality has been discussed. Guda et al. (1993) observed fibrous obstructive tissue invasion into the vein graft. They affirm that the contact between the endothelial cells of the transplanted venous segment and nervous tissue initiates the development of connective tissue and constriction of the nerve outside, which begins before axons can regenerate.

Many authors have reported the beneficial effects of laminin and collagen in the enhancement of peripheral nerve regeneration (Lander et al., 1985; Valentini et al., 1987; Eppley and Delfino, 1988; Muller, 1988).

The vein wall has three layers: the endothelial layer contains a laminin-rich basal lamina, the media is a thin muscle layer that is also rich in laminin, and the adventitia is rich in collagen. By pulling the vein graft through itself and then turning it inside-out, these layers become reversed. The resulting conduit exposes regenerating axons directly to the adventitia. This conduit is nonimmunogenic, permeable to external factors, and lined with abundant trophic and neurite-promoting factors; therefore, it may provide a superior microenvironment for peripheral nerve regeneration (Wang et al., 1993, 1995).

In this study, we have conducted a comparative study about the efficiency of autologous inside-out and standard vein grafts for the repair of a sensory nerve experimentally transected in rats.

MATERIALS AND METHODS

Twenty-four adult male Wistar rats weighing 150 to 200 g were used. The animals were divided into two groups according to the different techniques employed, the inside-out (IOVG) group and the standard vein (SVG) group. The animals were killed 4, 12, and 20 weeks postsurgery. In all groups the left saphenous nerve was used as the experimental subject and the right saphenous nerve as the control. The control nerves were sham dissected, not divided and biopsied in the same periods as the experimental groups.

Intraperitoneal sodium pentobarbital (30 mg/kg) was used as an anesthetic. Aseptic technique was observed in all surgical procedures.

The right external jugular vein was used in both groups as a conduit to repair the experimental nerve injuries. The skin was incised at the right side of the neck and the external jugular vein was exposed. Then a segment of about 15 mm in length was dissected, canulled, and harvested. In the inside-out vein graft group, the venous segment was pulled through itself by inserting jeweler's tweezers inside it, grasping the proximal end, and pulling the distal end down the tweezers, using other tweezers to create the inside-out orientation. The harvested vein was washed with saline solution and left at room temperature.

A second incision at the medial part of the left thigh was made and the saphenous nerve was exposed. A segment of about 10 mm in length was resected resulting in a gap of the same size (Fig. 1).

Finally, with a DF Vasconcelos operating microscope, the vein graft was positioned between the proximal and distal stumps. Each nerve stump was inserted 2 mm into the vein graft. Two sutures with 10–0 nylon were placed at each end of the cuff, 2 mm away from the exposed nerve stumps, to prevent formation of a neuroma at the suture line. The skin was closed with 5–0 nylon separated stitches. In the standard group, similar procedures were used although the vein was harvested and used in normal position.

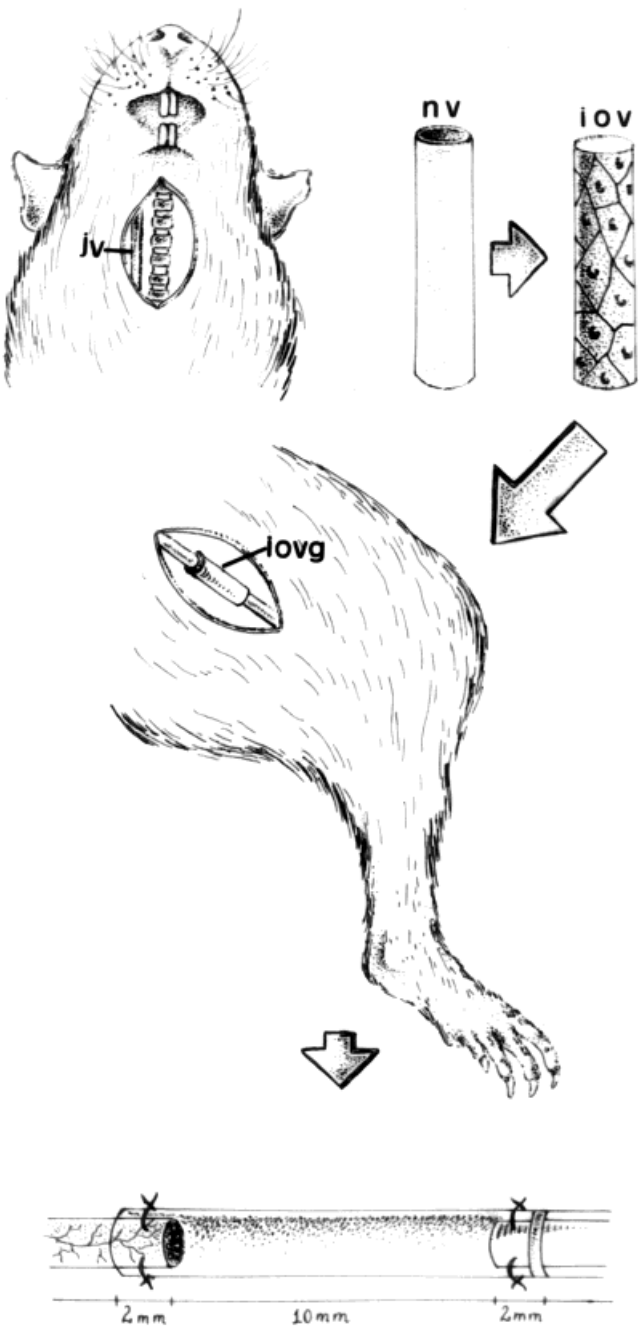

Fig. 1. Schematic illustration of the steps of the IOVG technique. JV, jugular vein; NV, normal vein; IOV, inside-out vein; IOVG, inside-out vein graft.

The animals were housed in temperature and humidity controlled environment with 12-hr on-off light cycles. They received food and water ad libitum.

The perfusion with glutaraldehyde (2.5%) was performed by the abdominal aortic artery 4, 12, and 20 weeks after the surgical procedures. Fragments from the middle of the graft and distal stump were submitted to transmis-

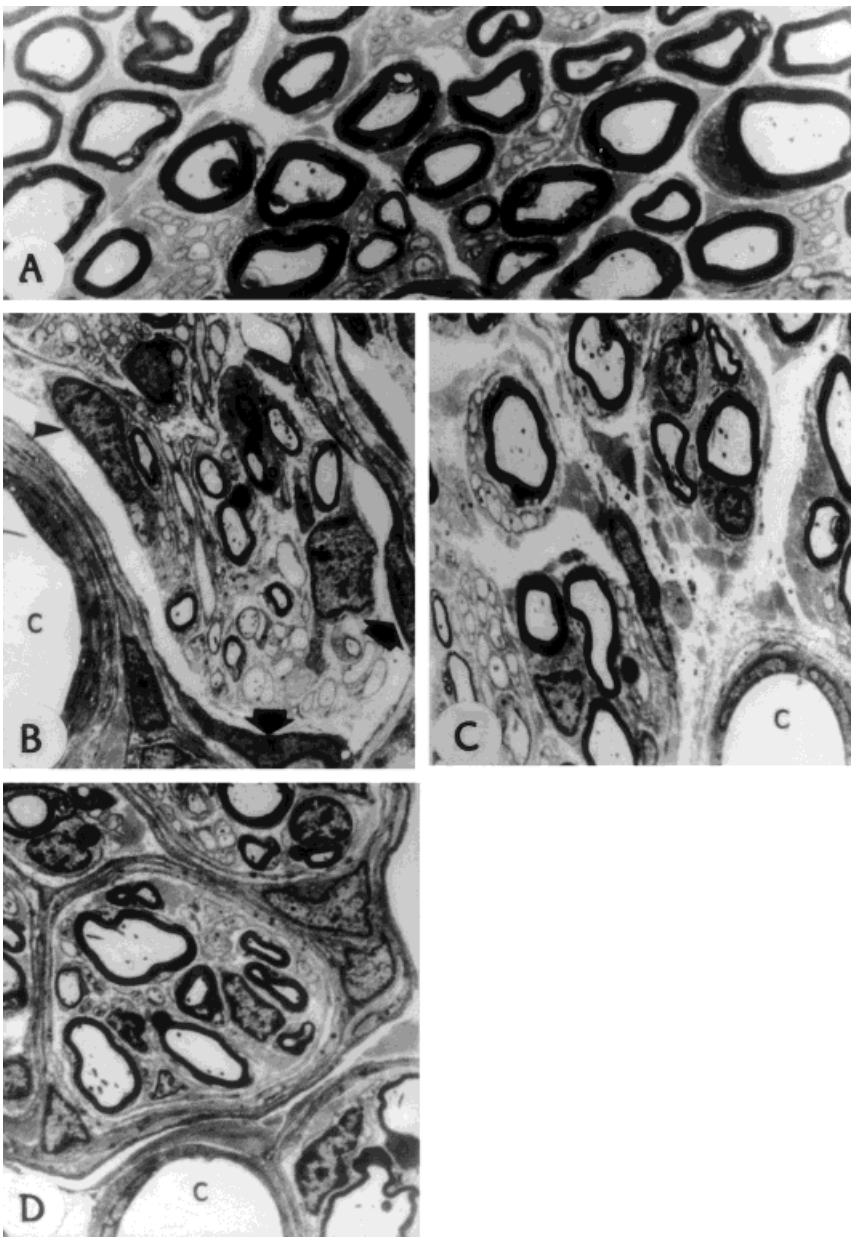

Fig. 2. Electron micrographs of the graft in the IOVG group. **A:** Normal saphenous nerve (×3,600). **B:** Four weeks after surgery. Note fibroblasts (arrows) beginning to involve myelinated and non-myelinated nerve fibers just beside the capillar (c); Arrowhead indicates Schwann cells (×3,600). **C:** Twelve weeks after surgery. Note the increased size of the myelinated axons compared to the anterior period (×3,600). **D:** Twenty weeks after surgery. The organization of the regenerated nerve fibers is different compared to the normal nerve. Note, the arrangement of the grouped microfascicles, forming fascicles, beside the capillary (c), and the minimal amount of conective tissue between them (×3,600).

sion electronic microscopy. Thick sections for electron microscopy were used for morphometric analyses.

Morphometric analyses were made using a software image analyzer (OPTMAS 4.10, Image Corporation of Edmonds, Washington, USA). The data were analyzed by Tukey's test.

RESULTS

We have not observed ulcer formation in any animal after denervation during the experiment.

The normal saphenous nerve has 1,000 myelinated axons with 3.9 µm in diameter and 0.58µm of sheath myelin thickness on an average (Fig. 2A) and (Table 1). This sensory nerve is placed in the medial side of the thigh beside the saphenous vessels.

After 4 weeks, the regenerated axons in the grafts exhibited a disorganized distribution in small fascicles compared to the normal group. These small fascicles were located around the new capillaries like satellite structures, especially in the IOVG group. The capillary density was more pronounced in the IOVG group than in the SVG group. At this stage, fibroblasts could be seen initiating the formation of microfascicles ensheathing the regenerated

TABLE 1. Morphometric data of the saphenous normal nerve

Animal	Diameter (µm)	Myelin thickness (µm)
1	3.80 ± 1.11	0.57 ± 0.17
2	4.43 ± 1.01	0.58 ± 0.14
3	4.58 ± 1.21	0.67 ± 0.22
4	2.85 ± 0.67	0.57 ± 0.20
Mean	3.91 ± 0.78	0.58 ± 0.05

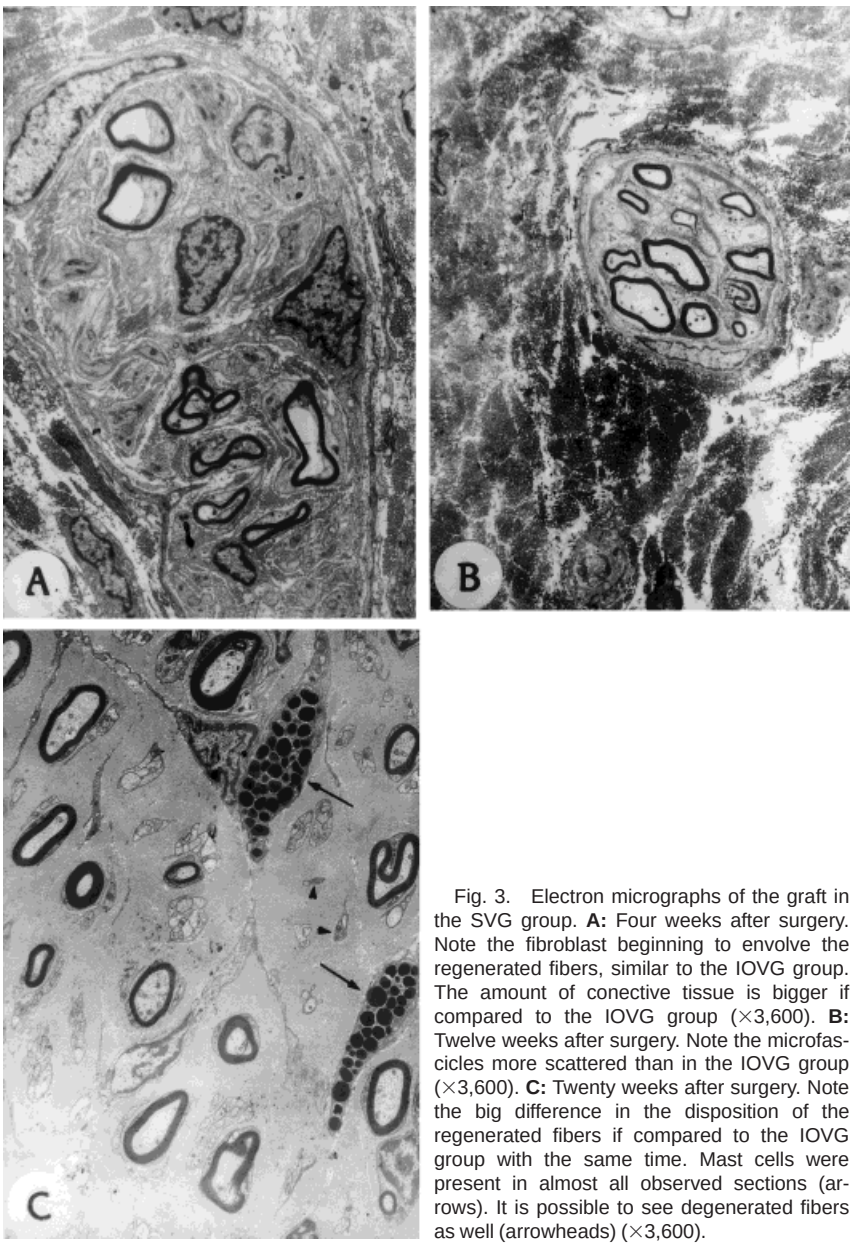

Fig. 3. Electron micrographs of the graft in the SVG group. **A:** Four weeks after surgery. Note the fibroblast beginning to envolve the regenerated fibers, similar to the IOVG group. The amount of conective tissue is bigger if compared to the IOVG group (×3,600). **B:** Twelve weeks after surgery. Note the microfascicles more scattered than in the IOVG group (×3,600). **C:** Twenty weeks after surgery. Note the big difference in the disposition of the regenerated fibers if compared to the IOVG group with the same time. Mast cells were present in almost all observed sections (arrows). It is possible to see degenerated fibers as well (arrowheads) (×3,600).

axons. The collagen fibers were arranged in a disorganized pattern in the graft 4 weeks after surgery. During this period, regenerated axons were not observed in the distal stump (Figs. 2B and 3A).

After 12 weeks, the axons were more organized in microfascicles in the IOVG group, while in the SVG group the regenerated axons were surrounded by abundant connective tissue. These microfascicles were concentrated around the capillaries in the IOVG group, but not in the SVG group (Figs. 2C and 3B). During this period, an arrangement of the regenerated nerve fibers closer to the normal pattern could be seen in the distal stump of the IOVG group (Fig 4A).

Finaly, after 20 weeks, the microfascicles were reorganized in fascicles with a minimal connective tissue between them in the IOVG group (Fig. 2D). On the other hand, in the SVG group, the regenerated axons were scattered inside the graft with a larger amount of connective tissue around them (Fig. 3C). The nerve segments within the distal stump were always more regular in pattern with more rounded fibers in the IOVG group. Regarding the diameters and the myelin sheath thickness of the regenerated fibers, no statistical difference was observed between both techniques. On the other hand, statistical difference was observed between the IOVG group and the normal group in relation to the diameters and the myelin sheath thickness (Tables 2 and 3).

DISCUSSION

Today, experimental peripheral nerve repair has been intensely studied by different techniques. In almost all

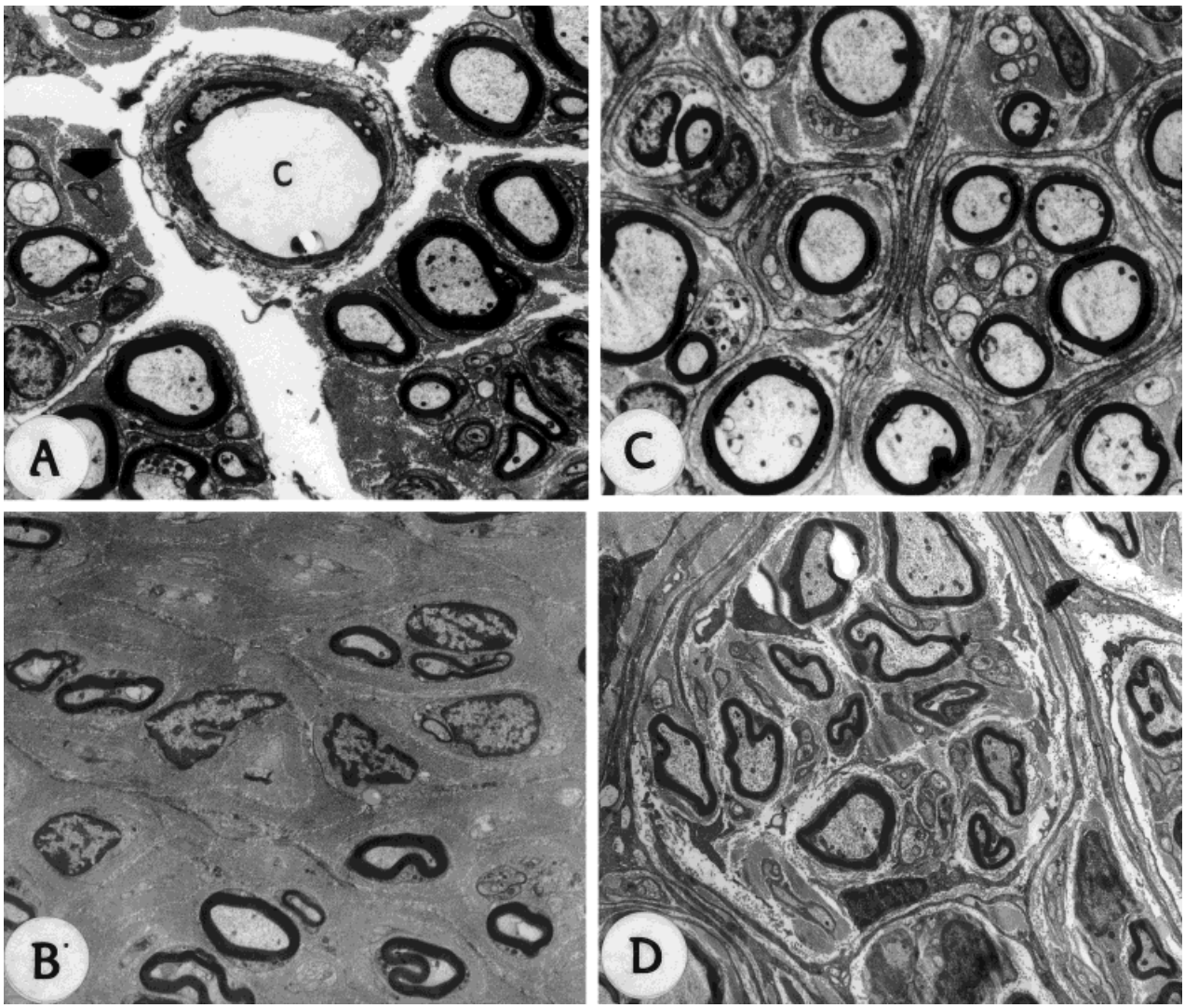

Fig. 4. Electron micrographs of the distal stumps: A and C in the IOVG group; B and D in the SVG group. Panel **A** shows 12 weeks after surgery (×4,500) and panel **C** shows 20 weeks after surgery (×4,500). The disposition of the microfascilcles is similar to the graft in the IOVG group at same time. Panel **B** shows 12 weeks after surgery (×4,000) and panel **D** shows 20 weeks after surgery (×4,000). The regenerated fibers are more rounded in the IOVG group.

TABLE 2. Diameter and myelin sheath thickness of the regenerated fibers after statistical comparative study in the grafts*

Variable	IOVG	SVG	NG[a]	DMS (5%)	CV (%)
Diameter	2.045a	2.525ab	3.920b	1.776	31.60
Thickness	0.291a	0.299a	0.588b	0.103	13.31

[a]NG, normal group.
*Conclusion: diameter, IOVG = SVG, IOVG ≠ NG, SVG = NG; thickness: IOVG = SVG, IOVG ≠ NG, SVG ≠ NG.

TABLE 3. Diameter and myelin sheath thickness of the regenerated fibers after statistical comparative study in the distal stumps*

Variable	IOVG	SVG	NG[a]	DMS (5%)	CV (%)
Diameter	2.017a	2.404ab	3.920b	1.642	29.90
Thickness	0.295a	0.263a	0.588	0.075	9.92

[a]NG, normal group.
*Conclusion: diameter, IOVG = SVG, IOVG ≠ NG, SVG = NG; thickness, IOVG = SVG, IOVG ≠ NG, SVG ≠ NG.

cases, a motor nerve has been chosen for the experiment. Since, very little is known about the behavior of a sensory nerve after an injury and its vein repair through vein graft, we decided to study the saphenous nerve (a sensory nerve), using two different vein graft techniques.

Some authors claim that the vein graft technique provides very good results in nerve regeneration after a nerve experimental section because the scar tissue invasion is minimal inside the graft (Chiu et al., 1982; Suematsu, 1988; Chiu and Strauch, 1990). On the other hand, Heijke et al. (1993) studied the saphenous nerve regeneration using a vein graft in rabbits. They report that the contact of endothelial cells with the regenerating axons can give rise to a constriction caused by the connective tissue

invasion. Opposite results were observed by Smith and Browne (1998), who studied the effects of an angiogenic factor, endothelial cell growth factor (ECGF), on regenerating nerves.

We decided to compare the inside-out vein graft technique with the standard vein graft technique for the repair of a sensory nerve in the rat. Wang et al. (1993, 1995) obtained good results studying the inside-out technique, in a motor nerve. Heijke et al. (1993) studied the regeneration of the saphenous nerve in rabbits, using the vein graft and epineural suture, with no difference between the two techniques.

In this study, we were also successful in achieving regeneration of the rat saphenous nerve using both techniques, which shows that probably vein grafts really provide a better microenvironment for the regenerating axons, probably due to the balance between collagen, laminin, fibronectin, and other substances in the vein wall.

We did not find regenerated fibers in the distal stump in the first studied period (4 weeks). On the other hand, Wang et al. (1993) found regenerated nerve fibers in the distal stump 4 weeks after surgery. These authors used the inside-out vein graft and the same vein (external jugular vein), but not the same nerve, studying a motor nerve. Janecka (1987) studied the regeneration in the sciatic nerve, using a standard vein graft. The regenerated nerve fibers in the distal stump could be seen only 8 weeks after surgery.

The difference in these results is probably due to the nerve type (sensory or motor), the technique (inside-out vein graft or normal vein graft), and the vein used (with or without valves) are important factors in the nerve regeneration.

Immediately after nerve transection, there is a great activity of the perikaryon with an increase in the production of proteins and other substances for the axon regeneration.

We conclude that regeneration of a sensory nerve by the inside-out and standard vein graft techniques consists of three steps: 1) there is an invasion of new capillaries into the vein graft, which come mainly from the proximal stump; 2) the neurites follow the vessels and are arranged around them; 3) the connective tissue decreases between the new microfascicles in the different postsurgical times. The amount of connective tissue was minimal between these microfascicles 20 weeks after surgery when compared to 4 weeks after surgery.

The diameters and the myelin sheath thickness of the fibers were not different in both techniques; however, they were smaller in the experimental groups than in the normal group.

In conclusion, under morphological criteria IOVG and SVG proved to be good techniques for the repair of sensory peripheral nerves in rats since they allow nerve regeneration in a good morphological pattern.

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LITERATURE CITED

Abernethy DA, Thomas PK, Rud A, King RHM. 1994. Mutual attraction between emigrant cells from transected denervated nerve. J Anat 184:239–249.

Chiu DTW, Janecka I, Krizec TJ, Wolff M, Lovelace RE. 1982. Autogenous vein graft as a conduit for nerve regeneration. Surgery 91:226–233.

Chiu DTW, Strauch B. 1990. A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr Surg 86:928–934.

Eppley BL, Delfino JJ. 1988. Collagen tube repair of the mandibular nerve: a preliminary investigation in the rat. J Oral Maxillofac Surg 46:41–47.

Fields RD, Le Beau JM, Longo FM, Ellisman MH. 1989. Nerve regeneration through artificial tubular implants. Prog Neurobiol 33:87–137.

Godard J, Coulon G, Monnier G, Rouillon D, Jacquet G, Bourghli A, Steimle R. 1984. Régénérescence nerveuse a travers un greffon veineux chez le rat: résultats préliminaires. Neurochirurgie 30:407–416.

Heijke GC, Klopper PJ, Dutrieux RP. 1993. Vein graft conduits versus conventional suturing in peripheral nerve reconstructions. Microsurgery 14:584–588.

Janecka IP. 1987. Peripheral nerve regeneration: an experimental study. Laryngoscope 97:942–950.

Lander AD, Fujii DK, Reichardt LF. 1985. Laminin is associated with the "neurite outgrowth-promoting factors" found in conditioned media. Proc Natl Acad Sci USA 82:2183–2187.

Madison RD, Archibald RJ. 1994. Point sources of Schwann cells result in growth into a nerve entubulation repair site in the absence of axons: effects of freeze-thawing. Exp Neurol 128:266–275.

Muller H. 1988. Further evaluation of the effects of laminin, testosterone, ganglioside GM1, and catalase on early growth in rat nerve regeneration chambers. Exp Neurol 101:228–233.

Risitano G, Cavallaro G, Lentini M. 1989. Autogenous vein and nerve grafts: a comparative study of nerve regeneration in the rat. J Hand Surg 14:102–104.

Smith MS, Browne JD. 1998. The effect of endothelial cell growth factor on peripheral nerve regeneration. Otolaryngol Head Neck Surg 118:178–182.

Suematsu N, Atsuta Y, Hirayama T. 1988. Vein graft for repair of peripheral nerve gap. J Reconstr Microsurg 4:313–318.

Valentini RF, Aebischer P, Winn SR, Galletti PM. 1987. Collagen and laminin gels impede peripheral nerve regeneration through semipermeable nerve guidance channels. Exp Neurol 98:350–356.

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

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

Williams LR, Longo FM, Powell HC, Lundborg G, Varon S. 1983. Spatial temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. J Comp Neurol 218:460–470.