POLYGLYCOLIC ACID TUBE ASSOCIATED WITH GM1 IN REGENERATION OF PERIPHERAL NERVES

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ABSTRACT

Introduction: Nerve allografting is regarded as a treatment of choice in large neural tissue losses preventing repair by primary anastomosis. In these cases, a synthetic polyglycolic acid tube is an alternative for nerve grafting. On the other hand, several studies have emphasized the importance of neurotrophic factors on neural regeneration, including substances with potential to optimize neural regeneration, especially the GM1, an neurotrophic enhancer factor. Objective: to compare, in rats, the neural regeneration degree using histological analysis, regenerated myelinized axons count, and functional analysis with the use of neurotube and GM1. Methods: This assessment was performed by interposing allograft (group A), polyglycolic acid tube (group B) and polyglycolic acid tube associated to GM1 (group C) on 5-mm sciatic nerve defects. Results: Neuroma formation was found only on group A. Groups A and C showed similar histological patterns, except for the regenerated axons on group C, which were shown to be better organized and myelinized than in group A. Conclusion: on functional recovery, no statistically significant difference was found for the three groups, despite of qualitative and quantitative histological differences found.

Keywords: *Peripheral nerves/surgery. Nerveregeneration. Polyglycolic acid/therapeutic use. Immunosuppressive agents therapeutic use.*

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INTRODUCTION

Trauma and tumor resections frequently result in extensive peripheral neural tissue loss, preventing repair by means of primary anastomosis. In these cases, nerve autografting has been the most commonly employed repair method and, despite of appropriate surgical treatments, functional deficits are seen.[1]

The interposition of tube conductors as a bridge between the stumps of the sectioned nerve has been shown to be an alternative technique offering some advantages: it provides a better confinement of the regenerating fibers, reduced inflammatory response and cicatricial tissue formation at the repair site; furthermore, it avoids sequels associated to the use of autogenous material.[1]

Recent studies established synthetic polyglycolic acid tube (PGAt) as an alternative for nerve grafting. On the other hand, many studies have emphasized the importance of neurotrophic factors on neural regeneration: one of the key glycosphyngolipids of mammalian nervous tissue - the GM1 - is regarded as an enhancer of the effects of these factors.[2] However, little has been studied about the effects of local administration of GM1 in the regeneration of peripheral nerves, and no reports exist on the use of this substance associated to absorbable neural prosthesis in literature.

The objective of this study is to compare, by histological and functional analysis, the degree of neural regeneration achieved by interposing an autologous graft, PGAt and PGAt with GM1 for fixing defects on rats' sciatic nerves.

MATERIALS AND METHODS

Fifteen isogenic 8-week old Lewis rats weighting 200g - 300g were used in this study. Using a microsurgical technique, 5mm-long defects have been produced on the sciatic nerve of the right paw. The animals were divided into 3 groups of five animals each according to the treatment approach.

For surgical procedure, the animals were anesthetized with sodium pentobarbital (5m/kg) intraperitoneal injection. Through dorsal access to the right paw, sciatic nerve was dissected and a 5-mm nerve segment was removed. (Figure 1)

On group A, the removed segment was sutured in its normal position with 4 epineural isolated stitches of mononylon 10.0. (Figure 2)

On group B, the tube was anchored in its position by one "U"-shaped stitch crossing each end as follows: from inside out at the tube, crossing the epineurum of the nerve stump and returning to the tube from inside out, so that about 2.5mm of the inserted stump was left. (Figures 5, 6, 7 and 8) Once one of the ends was fixated with the stitch, the tube was filled with heparin saline solution (10 units/ cc). The second end was fixated using the same technique and again filled with the heparin solution (10 units/ cc). (Figures 3 and 4)

On group C, a tube segment with the same characteristics was interposed associated to a GM1 solution (Sygen*, distributed by TRB PARMA- Brazil) at a final concentration of 100 mg/ml

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by tube injected inside the tube and sealed with Vaseline gel. (Figures 5 and 6)
The animals received water and food *ad libitum* being sacrificed

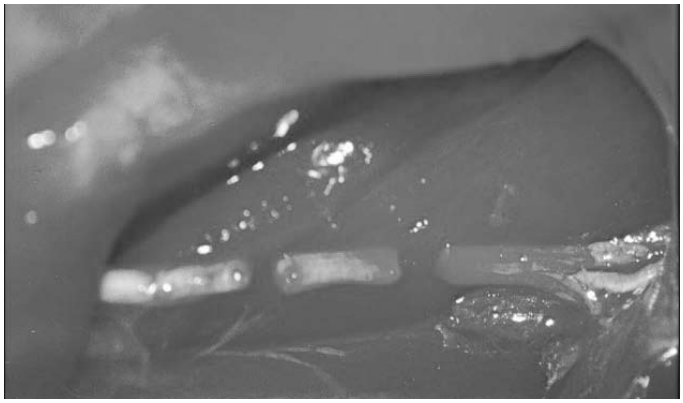

Figure 1 - *Sciatic nerve resection*

Figure 2 - *Group A: autografting*

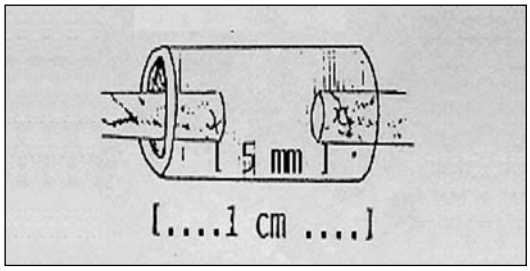

Figure 3 - *Group B: schematic illustration PGAt*

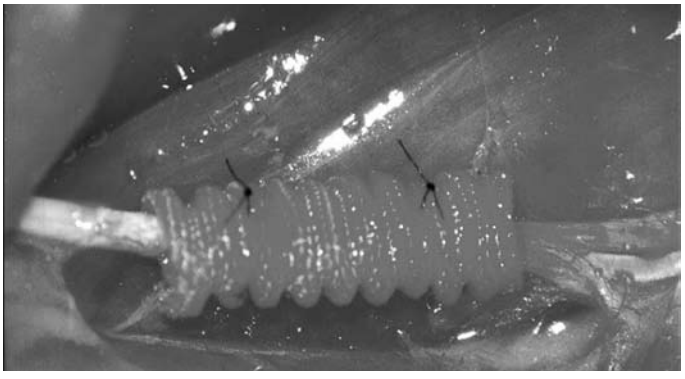

Figure 4 - *Group B: PGAt*

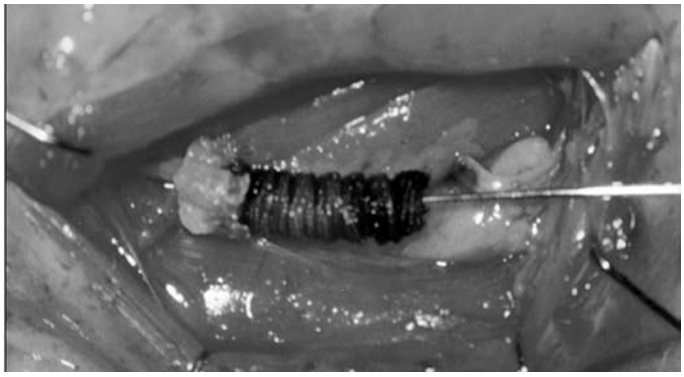

Figure 5 - *Group C: PGAt + GM1*

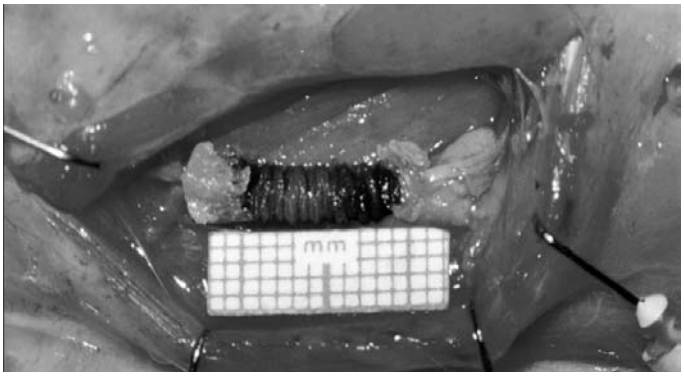

Figure 6 - *Group C: sealed with Vaseline gel*

6 weeks after surgery for histological analysis and count of the number of regenerated myelinized axons. The evaluation of functional recovery was made by the technique assessing posterior paws' footprints (walking track analysis) pre- and postoperatively, on the third week and at the moment of sacrifice (6 weeks).

RESULTS

During the six weeks of the study, all animals remained healthy, with no infection at surgical wound being found, as well as neurodistrophic plantar ulcers.
At the moment of sacrifice, Group A (autografting), under gross analysis, showed intact grafts, with small neuromas at the level of suture lines.
On Groups B (PGAt) and C (PGAt + GM1), the presence of a thin layer of fibrous tissue was observed externally involving the tubes, which were shown to be almost intact, still on a initial phase of the reabsorption process. No neuroma was seen in these groups.

QUALITATIVE HISTOLOGY

Group A (control, autografting): microscopic analysis of the slides showed the presence of a large number of fibers with variable diameters, reasonably myelinized, dispersed throughout neural stroma, sometimes grouped as small fascicles. Tissue response around the graft was stronger when compared to the other groups. Regenerated fibers' escape was detected out of the boundaries of the epineurum in 3 animals. (Figures 7 and 8)
Group B (PGAt): presence of a thin loose connective tissue cord, with neural stroma pattern, with satisfactory neoangiogenesis, but with a lower number of smaller dispersed and little myelinized fibers. (Figures 9 and 10) The presence of several multinucleated giant cells was detected with tube fragments on cytoplasm sug-

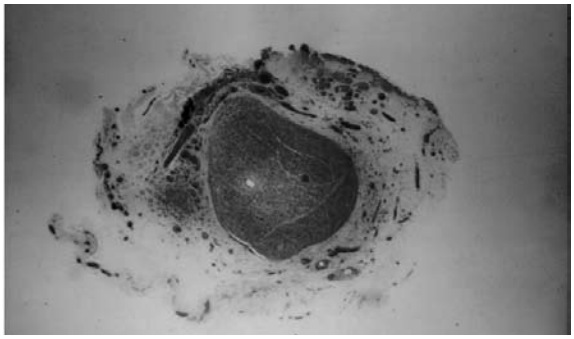

Figure 7 - *Group A: autografting – tissue response out of the graft (40X)*

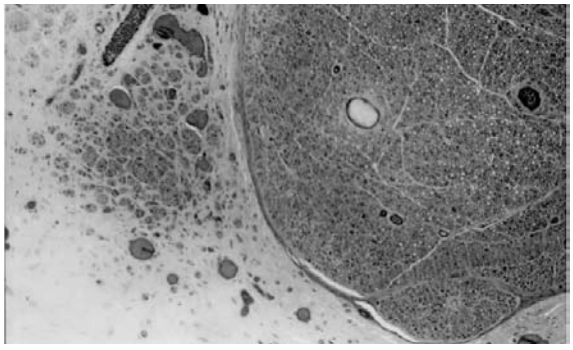

Figure 8 - *Group A: autografting - escape (100X)*

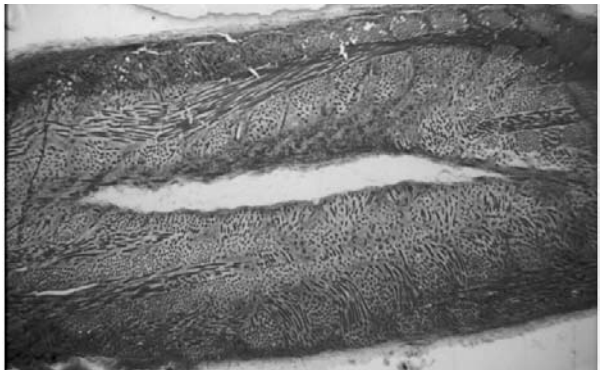

Figure 9 - *Group B: PGAt within*

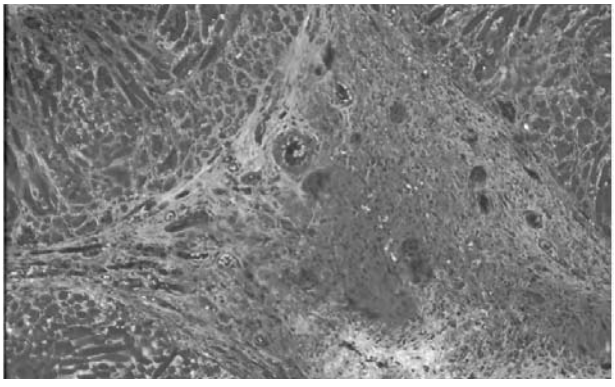

Figure 10 - *Group B: myelinized fibers on narrower tube (100X)*

gesting early reabsorption process. Fibrosis response around the tubes was clearly weaker when compared to Group A (autografting). No escapes were found.

Group C (PGAt + GM1): within the tubes, fibroblasts concentrically distributed on the periphery outlined an area of loose connective tissue taking almost all the intra-lumen space, with a stronger neoangiogenesis process than previous groups. At a more central portion of that newly-formed stroma, a concentration of a good number of nervous fibers of medium width, moderately myelinized and distributed as fascicles was noticed. Overall, the area occupied by the regenerated fibers in this group was smaller than the one seen on control group, larger than group B (PGAt), but with a stronger concentration of fibers in the space and higher myelinization. (Figures 11 and 12) A thin layer of fibrous tissue was seen involving the tubes, but smaller if compared to group A (autografting) and similar to group B. No fiber escape was seen out of the tube.

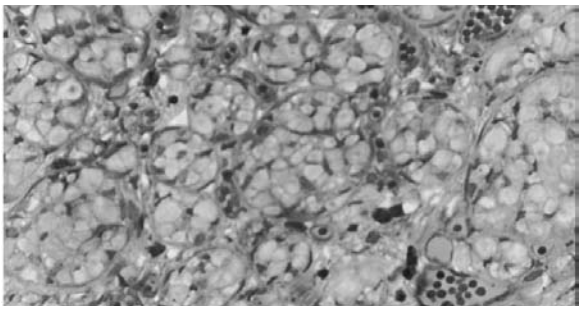

Figura 11 - *Grupo C: tAPG+ GM1: padrão faz-quantidade cicular (400X)*

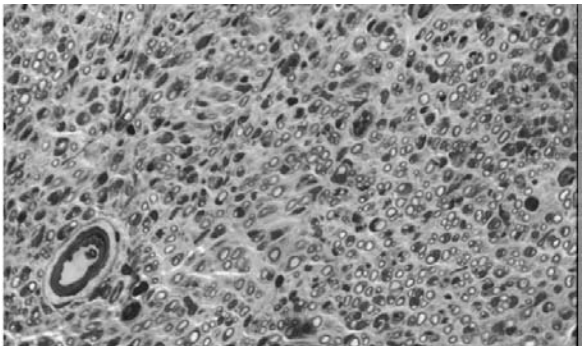

Figure 12 - *Group C: PGAt + GM1: larger number of myelinized fibers (400X)*

FUNCTIONAL STUDIES

Figure 13 shows the appearance of rats' footprints pre- and postoperatively for the 3 groups.

The mean values of the SFR (sciatic function rate) calculated for each group are shown on Figure 14.

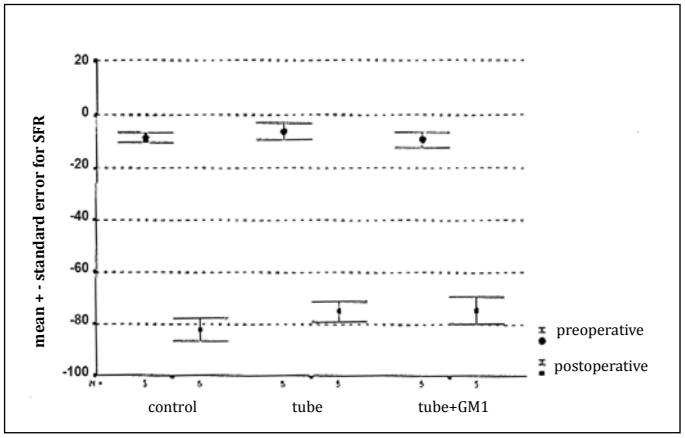

Figure 14 - *Mean SFR calculated for each group*

The mean value of the preoperative sciatic function rate for each group was: Group A (autografting) -8.4 +/– 4.38, Group B (PGAt) 6.34 +/– 7 and Group C (PGAt+GM1) -9.47 +/– 6.34. No significant difference was found between groups.

Concerning the mean value of the postoperative sciatic function rate for each group, the following was detected: Group A (autografting) -82.12 +/- 9.77, Group B (PGAt) -75.19 +/- 8.07 and Group C (PGAt+GM1) -74.71 +/- 11.19.

The average variation of the sciatic function rate after 6 weeks of surgery are summarized on Figure 15.

In the statistical analysis (Kruskall- Wallis test, with $p<0.05$), in average, no significant difference was found ($p<0.05$) between the 3 groups concerning postoperative SFR values when compared to normal baseline values preoperatively.(Figure 15)

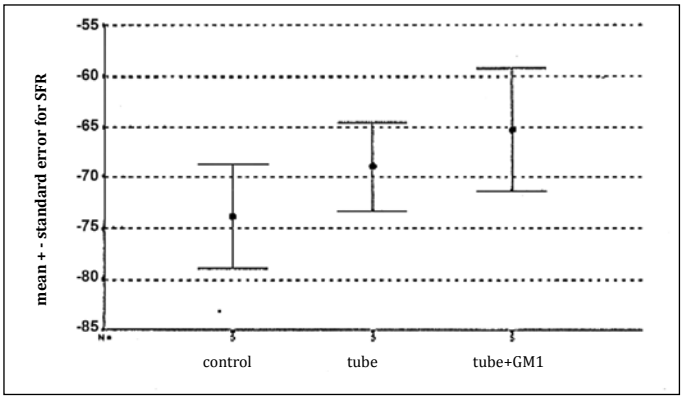

Figure 15 - *Mean SFR variation after 6 weeks*

DISCUSSION

In cases of peripheral nerve injuries with substance loss where the defect extension prevents stumps to directly reapproximate to each other, the best repair method seems to be autografting.[1]

However, there are factors that lead us to pursue a new kind of conductor for axonal growth: 1. the removal of autologous grafting material always produce morbidity to the donor site; 2. large defects demand removal of extensive portions of autogenous tissue; 3. the use of artificial material saves the time for removing autologous tissue.[1,3]

The interposition of tube conductors as a bridge between the stumps of a sectioned nerve has presented encouraging experimental and clinical results. For fixing small defects, where the distance between stumps is not big enough to cause problems to chemotaxis and chemotrophic attraction exerted by distal stump on axonal growth cone, the results are comparable to those obtained with autografting.[1-7]

The tubing technique also offers additional theoretical advantages over traditional grafting methods: it provides good coaptation of both stumps with less manipulation trauma; enables a better confinement of growing fibers inside the tube, isolating the repair site from surrounding inflammatory response; guides fiber growth towards distal stump, enabling local neurotrophic factors concentration; reduces neuroma formation and fibers escape to out of the conductor; enables the flow of regeneration enhancer substances.[5,6]

Several materials have been employed for building tubes, which may be absorbable[8] or non-absorbable.[1]

Many recent studies established polyglycolic acid (PGA) while an absorbable artificial material for clinical use as an alternative for nerve grafting.[7] Synthetic tubes made of absorbable materials have been shown to provide better late functional results when compared to non-absorbable tubes.[1] Once these are biotolerable, they cause a less intense fibrosis response and, after reabsorbed, they do not prevent a regenerating nerve from growing wider[1], as opposite as it occurs with non-absorbable conductors.

A successful process of sectioned peripheral nerve repair also depends on the existence of a favorable "micro environment" to regeneration.[9] This essential neural substrate is locally present at the moment of nerve lesion, and is composed by factors such as Schwann cells, perineural fibroblasts[10], components of extracellular matrix (laminin, fibronectin, adhesion molecules)[1,11] and molecules produced by the ends of a sectioned nerve that stimulate regeneration[12] , the so-called neurotrophic factors.

GM1 is a key glycosphyngolipid of mammalian nervous tissue, constituting the major subclass of brain gangliosides with effects on neural differentiation and repair processes, acting on the burgeoning and growth of *in vivo* and *in vitro neurites.*[13-18]

Exogenous GM1 is incorporated to neuronal membranes and would have a enhancing action on neurotrophic factors.[13-18] Recent studies have demonstrated its effectiveness in helping functional repair in cases of diabetic peripheral neuropathy, stroke and acute rachimedullary trauma in humans and animals.[13-18]

Previous studies have evidenced that the local administration of gangliosides at the repair site of a peripheral nerve injury produces an increased number of regenerated myelinized fibers.[13-18] Its action would preferentially focus the regeneration of motor fibers, since GM1 has been shown to account for about 15% of all gangliosides in motor nerve myelin, in contrast to accounting for less than 5% of the myelin in sensitive nerves.[13-18]

Rats are frequently used as an experimental model in literature[1,19], being regarded as a classic model in peripheral nerves study. In addition to this fact, this animal enables us to assess func-

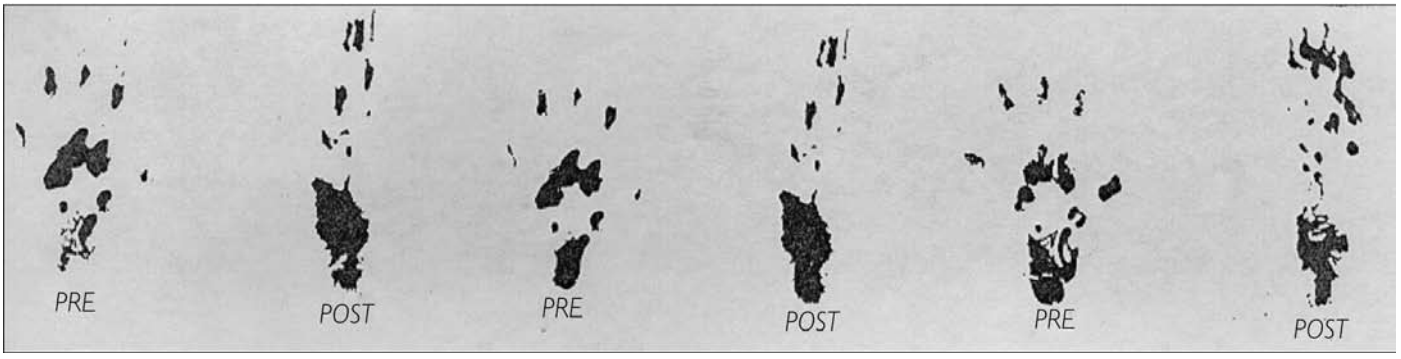

Figure 13 - *Appearance of rats' pre- and postoperative footprints for all 3 groups.*

tional recovery of an injured sciatic nerve by the walking track analysis, which is a methodology described by De Medinaccelli and modified by Bain et al.[19], based on the footprint pattern of these animals.

The sciatic nerve was used to provide a peripheral nerve injury model, since its functional repair pattern can be assessed[19], and this is an important fact to compare the best technique when repairing neural tissue defects.

The surgical technique employed for accessing sciatic nerve, autografting, and polyglycolic acid tubing is the same employed by several authors[3,9], as well as the standard gap size used.[3]

The technique for preparing histological sections using fixation with osmium tetroxide and toluidine blue stain is the one that best preserves myelin sheath, being widely employed in peripheral nerve studies.[1,20]

Macroscopically, neuroma formation was found only in rats submitted to autografting. This data is consistent to a previous study in literature.[20]

When slides are analyzed, we can see that neural regeneration showed different patterns from group to group. Concerning fascicle organization, Group C (PGAt+GM1) axons found in smaller number, but wider in diameter, were shown to be more uniformly grouped than in Group A (autografting). A higher level of myelinization was also found on fibers of Group C. This trend to a stronger fiber gathering within PGA+ GM1 tubes was also noticed by Keeley et al.[3], but, in that study, the tubes were filled only with plasma.

Tissue response and fibrous tissue deposition seen on the group with autografting was regarded as stronger on the other groups. In literature, there are no data available comparing tissue response associated to PGA tubes and autografts.

In autografts, the presence of fibers out of the boundaries of the epineurum and neuroma formation on suture lines were seen. These changes are also reported by other studies.[20] No fiber escaping was found in the groups with tubes.

Concerning functional recovery evaluation by the walking track analysis technique, there were no statistically significant differences (Kruskall- Wallis test, $p<0.05$) between the results achieved on the three groups. Currently, many authors have stressed the importance of the accuracy of connections reestablished by regenerated axons at the target organ if compared to the total number of fibers versus functional recovery.[19] Therefore, histological characteristics provide a reliable picture of trophic conditions of a regenerated nerve, but do not correlate with the degree of functional recovery.[19] Accordingly, the degree of functional recovery achieved with PGAt and PGAt + GM1 was not significantly different from that obtained with autografting, despite of the different histological patterns found on the three groups.

CONCLUSIONS

1. The groups presented different histological patterns. The one using autografting (control) showed a stronger tissue response when compared to the others, as well as the presence of fiber escaping, a phenomenon that was not seen in other groups. The area occupied by regenerated fibers on Group C (PGAt + GM1) was smaller than on control group, larger than group B (PGAt), but with a higher concentration of fibers in the space and a stronger myelinization degree.

2. The groups did not show significant differences concerning functional recovery.

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