UNIVERSITÀ DEGLI STUDI DI TORINO

TUBULIZATION WITH CHITOSAN GUIDES FOR THE REPAIR OF LONG GAP PERIPHERAL NERVE INJURY IN THE RAT

F. GONZALEZ-PEREZ, M.Sc.,[1] S. COBIANCHI, Ph.D.,[1] S. GEUNA, M.D., Ph.D.,[2] C. BARWIG, Ph.D.,[3] T. FREIER, Ph.D.,[3] E. UDINA, M.D., Ph.D.,[1] and X. NAVARRO, M.D., Ph.D.[1]*

Biosynthetic guides can be an alternative to nerve grafts for reconstructing severely injured peripheral nerves. The aim of this study was to evaluate the regenerative capability of chitosan tubes to bridge critical nerve gaps (15 mm long) in the rat sciatic nerve compared with silicone (SIL) tubes and nerve autografts (AGs). A total of 28 Wistar Hannover rats were randomly distributed into four groups (n 5 7 each), in which the nerve was repaired by SIL tube, chitosan guides of low (2%, DAI) and medium (5%, DAII) degree of acetylation, and AG. Electrophysiological and algesimetry tests were performed serially along 4 months follow-up, and histomorphometric analysis was performed at the end of the study. Both groups with chitosan tubes showed similar degree of functional recovery, and similar number of myelinated nerve fibers at mid tube after 4 months of implantation. The results with chitosan tubes were significantly better compared to SIL tubes (P < 0.01), but lower than with AG (P < 0.01). In contrast to AG, in which all the rats had effective regeneration and target reinnervation, chitosan tubes from DAI and DAII achieved 43 and 57% success, respectively, whereas regeneration failed in all the ani-mals repaired with SIL tubes.

Peripheral neurons have the ability to regenerate and reinnervate target organs when there is a suitable envi-ronment to do so, thus allowing some degree of recovery of the lost functions, depending upon severity of the injury and quality of the repair.[1,2] On the other hand, after complete nerve transection, surgical repair is man-datory to reunite the two stumps to facilitate regenera-tion. When the distance to be bridged does not allow direct suture between stumps, the interposition of a nerve graft is used as the gold standard technique in clinical practice. The use of autografts (AGs) has some disadvan-tages, such as the sacrifice of a healthy nerve of the sub-ject affected, the mismatch between the injured nerve and the grafts, and the limited source of donor nerves. As an alternative to AGs, the use of biogenic conduits[3,4] or artificial guides has been proposed.[5,6] However, the success of regeneration when using artificial nerves guides is limited by the length of the gap (less than 15 mm in the rat).[7] Strategies focused on altering the char-acteristics of the guidance tubes to increase the ability to sustain axon regeneration, have been attempted to over-come the gap limitation.[5,8]

The cross-sectional dimensions of the tube[9,10] and the materials, in which the tubes are constructed are other factors that also affect the final outcome. Initial studies used nondegradable silicone (SIL) tubes[11] but now-a-days it is considered that the ideal material should be biocompatible, have sufficient mechanical stability, be flexible, be porous to facilitate the incorporation of nutrients, and degrade into nontoxic products to prevent long-term body reaction.[12] Indeed, nerve guides made from collagen (NeuraGen[TM]),[13] polyglycolic acid (Neuro-tube[TM]),[14] and polylactide caprolactone (Neurolac[TM])[15] have been approved for clinical use. However, for the moment nerve guides have been approved and tested in humans only for the repair of relatively short gap injuries.

Among the different materials experimentally tested to improve the results obtained by available nerve guides, chitosan is a promising alternative.[16,17] Chitosan is a polymer derived from chitin, a molecule obtained from the exoskeleton of arthropods, shellfish, and cell wall of fungi,[18] and fulfills the characteristics above indicated to construct nerve guides. Some in vivo studies reported the benefits of chitosan scaffolds and guides in peripheral nerve repair. However, most of these studies combined the chitosan-based guide with internal fillers, such as neurotrophic factors,[19,20] molecules or peptides from the extracellular matrix[21,22] and supporting cells,[23,24] in non-critical peripheral nerve gaps. Recent technological improvements overcame the poor mechanical strength of chitosan tubes, which was one of the main factors limit-ing their use as single material for nerve guides. In this study, we aimed to evaluate the capabilities of hollow chitosan tubes to sustain regeneration when used to repair a critical 15 mm sciatic nerve gap in rats, and compare their outcome to standard SIL tubes and the ideal nerve AG. We tested two types of chitosan conduits with dif-ferent degrees of acetylation, whose

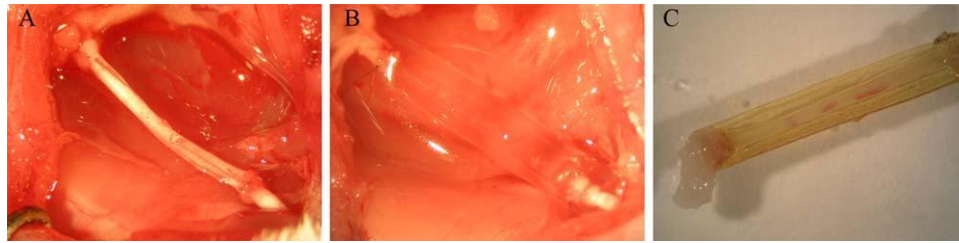characteristics in terms of biocompatibility and adequacy for nerve repair have been recently described.17

Figure 1. Representative images of a sciatic nerve resected and repaired with an autograft (AG) of 15 mm (A), or with a hollow DAII chito-san tube of 19 mm leaving a 15 mm gap (B). Regenerated nerve found 4 months after repairing the sciatic nerve with a hollow DAII chito-san tube (C).

MATERIALS AND METHODS

Animals

A total of 28 female Wistar Hannover rats, aged 3 months were used in the experiment. The animals were housed in plastic cages, maintained at 22 C with a 12 hour light/dark cycle and allowed free access to water and food. The experimental procedures were approved by the Ethical Committee of our institution and followed the rules of the European Communities Council Directive.

Experimental Design and Surgical Procedure

Animals were randomly distributed into one of four experimental groups according to the type of repair: SIL repaired animals (n 5 7), chitosan of low degree of acet-ylation (2%) (DAI) (n 5 7), chitosan of medium degree of acetylation (5%) (DAII) (n 5 7), and AG (n 5 7). The chitosan tubes manufacturing and characteris-tics were the same as reported in a previous study.17

All surgical procedures were performed with aseptic operating conditions and under anesthesia with ketamine/ xylazine (90 mg/kg and 10 mg/kg i.p., respectively). Chi-tosan tubes were immersed in saline solution 20 minutes before implantation to reduce the strength of the tube. Under a dissecting microscope, the sciatic nerve was exposed and cut 6 mm distal to the exit of the gluteal nerve, and a nerve segment of 6 mm was resected. The distal and proximal stumps were fixed by two epineural 10-0 sutures into the ends of the implanted tube leaving a 15 mm gap (Fig. 1). All the tubes used had a length of 19 mm, and an internal diameter of 2 mm. Once implanted, the tubes were filled with sterile physiologic saline solution. For the AG group, the sciatic nerve was cut at the same level explained above and 15 mm dis-tally. The nerve segment resected was flipped and sutured to bridge the gap with two 10-0 sutures at each side. The muscle plane was then sutured with reabsorbable 5-0 sutures, the skin with 2-0 silk sutures, and the wound was disinfected. Animals were treated with amitriptyline for preventing autotomy.25

Electrophysiological Tests

Functional reinnervation of target muscles was assessed at 7, 30, 60, 90, and 120 days postoperation (dpo). Animals were anesthetized with pentobarbital (40 mg/kg i.p.). The sciatic nerve was stimulated by transcu-taneous electrodes placed at the sciatic notch, and the compound muscle action potential (CMAP) of tibialis anterior and plantar muscles was recorded using monopo-lar needle electrodes, placing the active one in the mus-cle belly and the reference in the fourth toe.26 During the tests, the rat body temperature was maintained by means of a thermostated warming flat coil. The amplitude and the latency of the M-wave were measured. The contralat-eral limb was used as control.

Functional Evaluation of Sensory Recovery

The threshold of nociceptive responses to mechanical and thermal stimuli was evaluated on both hindpaws by means of algesimetry tests at 7, 21, 45, 60, 90, 92, and 120 dpo. For both tests, the lateral area (innervated by tibial and sural nerves, both being branches of the sciatic nerve) of the plantar surface was tested.27 The contralat-eral paw of each rat was tested as control each day, to overcome possible variations between testing conditions. Sensibility to mechanical stimuli was measured by means of an electronic Von Frey algesimeter (Bioseb, Chaville, France). Rats were placed on a wire net platform in plas-tic chambers 30 minutes before the experiment for habit-uation. The mechanical nociceptive threshold was taken as the mean of three measurements per paw region, and expressed as the force (in grams), at which rats withdrew their paws in response to the stimulus. A cutoff force was set to 40 g, at which stimulus lifted the paw with no response. Thermal sensibility was assessed by using a plantar algesimeter (Ugo Basile, Comerio, Italy). The beam of a projection lamp was focused onto the hindpaw plantar surface pointing at the lateral side. The thermal nociceptive threshold was taken as the mean of three trials, and expressed as the latency (in seconds) of paw with-drawal response. A cutoff time was set at 20 seconds to pre-vent tissue damage. All the values are presented as percentage of response with respect to the contralateral non-injured paw.
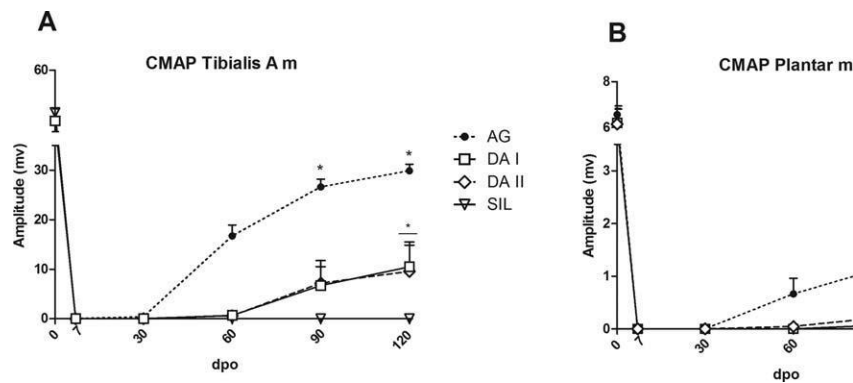
Figure 2. Mean amplitude of the compound muscle action potential (CMAP) of tibialis anterior (A) and plantar muscles (B) of the injured hind limb of the rats during 4 months after sciatic nerve lesion and repair. *P < 0.05 AG vs. DAI, DAII, and SIL; *P < 0.05 DAI and DAII vs. SIL.

Histology and Morphometry

Four months after the injury, animals were deeply anes-thetized and perfused transcardially with 4% paraformalde-hyde in phosphate-buffered saline solution (0.1M, pH 5 7.4). After perfusion, the regenerated nerves were harvested and postfixed in 3% paraformaldehyde and 3% glutaralde-hyde phosphate-buffered solution. The nerves were post-fixed in osmium tetroxide (2%, 2 hours, 4 C), dehydrated through ascending series of ethanol, an embedded in Epon resin. Nerves were sectioned using an ultramicrotome (Leica). Semithin sections (0.5 lm) were stained with tolui-dine blue and examined by light microscopy. Images of the whole sciatic nerve were acquired at 103 with a digital camera, while sets of images chosen by systematic random sampling of squares representing at least 30% of the nerve cross-sectional area were acquired at 1003 from mid and distal parts of the tube or graft. Measurements of the cross-sectional area of the whole nerve, and counts of the num-ber of myelinated fibers, were performed by using Image software (National Institutes of Health). Morphometrical analysis was made using a protocol previously described28 to obtain measurement of regenerated fibers and axons diameter, myelin thickness, and the g-ratio.

Muscle Weight

Once the animals were perfused, tibialis anterior and gastrocnemius muscles were dissected. The muscles were kept in a tube placed in an incubator at 37 C for 2 days to allow them to dry, and then weighted.

Statistics

Results are expressed as mean 6 SEM. Statistical comparisons between groups and intervals for algesimetry and electrophysiological tests results were made by two-way ANOVA for repeated measurements, followed by Bonferroni post hoc test. Statistical analysis of histo-logical results was made by one-way ANOVA followed by Bonferroni post test. Differences were considered sig-nificant if P < 0.05.

RESULTS

Muscle Reinnervation

Nerve conduction tests performed 1 week after sciatic nerve injury demonstrated complete denervation of the hindlimb muscles. At 30 dpo, four of six rats in the AG group had evidence of reinnervation in the tibialis anterior muscle, whereas in the groups with chitosan tubes, the first

CMAPs were recorded at 60 dpo in two of seven animals. The CMAPs progressively increased in amplitude and were recorded in higher number of animals over time. At the end of follow up (120 dpo), reinnervation of the tibialis anterior muscle was observed in all the animals in the AG group, in three of seven rats repaired with the chitosan tubes and in none of the rats repaired with SIL tube. When comparing the mean CMAP amplitude between groups at the end of follow up (all the animals included in the analysis), the AG group showed a mean amplitude (29.90 ± 1.40 mV) signifi-cantly higher than SIL (0 ± 0 mV; $P < 0.01$), DAI (10.50 ± 5.00 mV; $P < 0.01$), and DAII (9.54 ± 5.75 mV; $P < 0.01$) groups (Fig. 2A). Significant differences were also found between the two groups repaired with chitosan guides compared to SIL group ($P < 0.05$), but no differences ($P > 0.05$) were observed between DAI and DAII groups.

At the more distal interosseous plantar muscle, the first CMAPs were recorded as small polyphasic potentials at 60 dpo in all the animals of the AG group, and in two animals of the DAII group, whereas the first signs of plantar reinnervation appeared at 90 dpo in rats of the DAI group. After 4 months follow-up, plantar muscle reinnervation was detected in all the animals in the AG group, in three of seven rats in group DAI, in two of seven rats in group DAII, and in none of group SIL. Mean CMAP amplitude in group AG was significantly higher (2.65 ± 0.49 mV) compared to SIL (0 ± 0 mV, $P < 0.01$), DAI (0.212 ± 0.10 mV, $P < 0.01$), and DAII (0.716 ± 0.53 mV, $P < 0.01$) groups (Fig. 2B). Significant differences were not observed within the tube-repaired groups ($P > 0.05$). When CMAPS were recorded, the latency of the waves was considerably lon-ger than normal during the first stages of reinnervation and tended to shorten with time toward normal values. At the end of follow-up, latencies were 1.81 ± 0.03 ms in the AG group, 2.63 ± 0.31 ms in group DAI and 2.89 ± 1.38 ms in group DAII for the tibialis anterior muscle, and 3.69 ± 0.18 ms in group AG, 5.44 ± 0.89 ms in group DAI, and 4.43 ± 0.25 ms in group DAII for the plantar muscle (Fig. 3B).
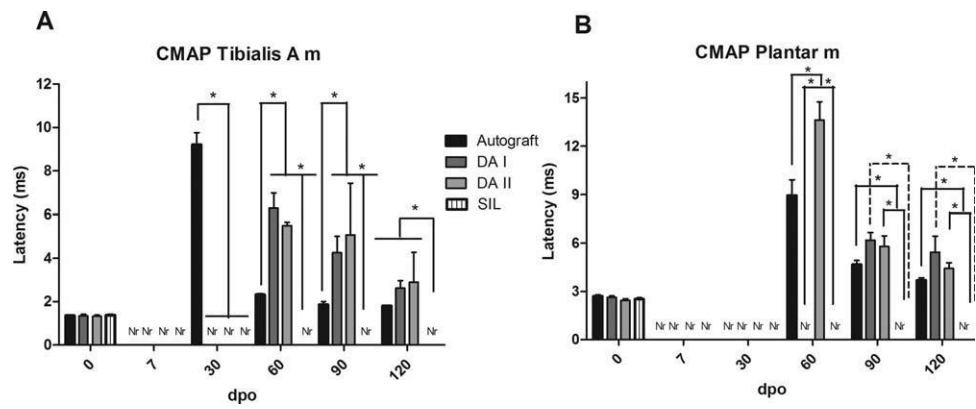
Figure 3. Mean latencies of the tibialis anterior (A) and plantar muscles (B) CMAP recorded in the regenerated rats during the 4 months follow-up. *P < 0.05.

Recovery of Nociceptive Sensibility

Withdrawal responses to mechanical stimuli, eval-uated by means of the Von Frey test, showed that ani-mals had no responses in the denervated paw until 30 dpo, and therefore they were penalized with a cut off value of 40 g. From 60 to 90 dpo most rats showed with-drawal responses at lower stimulus intensity than in the contralateral side. After elimination of the saphenous nerve at 90 dpo, measurements made at120 dpo showed that all the rats of the AG group had withdrawal responses to mechanical stimuli (17.35 6 7.16 g at 120 dpo) at lower intensity than in the contralateral paw (27.28 6 1.76 g, P < 0.05). Mean values in the chitosan tubes groups were higher (34.12 6 4.34 g, P < 0.05 for DAI; 31.45 6 4.26 g, P < 0.05 for DAII), due to failed regeneration of some animals that did not respond to mechanical stimuli. Values of the subset of rats that had reinnervated were similar to the values observed in AG rats. None of the animals of the SIL group responded (40.0 6 0.0 g, P < 0.05) indicating absence of sensory reinnervation of the hindpaw (Fig. 4A).

Withdrawal responses to heat stimulation in the plan-tar test showed similar results than the ones observed for the Von Frey test. Denervated paws did not respond to the hot stimuli on the sciatic lateral region until 45 dpo. At 120 days withdrawal latencies in the AG group (12.75 6 1.38 seconds) were similar to the contralateral paw (12.36 6 0.76 seconds, P > 0.05). After saphenous nerve cut, some of the animals of the chitosan groups did not respond to heat stimuli, due to lack of reinnervation in the sole, thus resulting in slightly higher mean values (16.80 6 2.19 seconds, P > 0.05 for DAI; 16.87 6 1.66 seconds, P > 0.05 for DAII). None of the rats repaired with SIL tubes withdrew the paw when applying the heat stimuli (20.0 6 0.0 seconds, P < 0.05) (Fig. 4B).

Histological Results

Macroscopic examination of the injured nerves after the 4 months follow-up showed that all the AG repaired nerves had good regeneration, whereas three of the seven rats in the DAI group and four of the seven rats in the DAII group presented a regenerative cable inside the tube. The regenerated nerve had a compact appearance and occupied the center of the tube lumen (see Fig. 1C). The nerves were surrounded by a thick, homogeneous connec-tive layer, with no signs of inflammatory reaction. The size of the regenerated sciatic nerve found in the AG group was larger than the regenerated cables found in both chitosan groups. There was no regenerative cable in any of the rats of the SIL group.

Transverse sections of the regenerated nerves taken at the midpoint of the graft of the tube and at the distal segment were analyzed under light microscopy (Figs. 5 and 6). To compare the absolute number of myelinated fibers, regenerated and non-regenerated rats were included in the statistical analysis. Non-regenerated ani-mals were given null value. The mean number of
myelinated fibers at the midpoint of the graft or the tube was higher in the AG group (14,409 6 1,564; P < 0.01) compared to both chitosan tube groups (DAI: 2,275 6 1,159; DAII: 2,265 6 1,534). Significant differences were observed between the chitosan-repaired groups and the SIL group (P < 0.01) (Fig. 7A). These differences were also observed at 3 mm distal to the end of the graft or the tube, where the estimated number of myelinated fibers in the AG group (6,865 6 295; P < 0.01) was sig-nificantly higher than in both chitosan tube groups (DAI: 1,971 6 1,013; DAII: 1,644 6 984). Significant differen-ces were again observed between chitosan groups and the SIL group (P < 0.01) (Fig. 7B). When taking into account only the animals, in which a regenerated nerve was found after 4 months postinjury, the number of myelinated fibers was still higher in the AG group com-pared to both chitosan groups at the mid level (DAI: 5,308 6 1,161; DAII: 6,218 6 2,328) and at the distal level (DAI: 4,598 6 1,070; DAII: 3,837 6 1,602), but differences were not significant (P > 0.05).
The regenerated nerves at the mid level had a larger cross-sectional area in the AG group than in the chitosan tube repaired animals (AG: 0.427 6 0.173 mm2; DAI: 0.089 6 0.056 mm2; DAII: 0.149 6

0.095 mm2) (P < 0.05). However, the density of the myelinated fibers was similar in the three groups (AG: 40,714 6 4,538 axons/ mm2; DAI: 51,886 6 2,530 axons/mm2; DAII: 38,877 6 3,371 axons/mm2) (P > 0.05). At the distal level, the AG group presented a higher density of myelinated fibers per section (32,805 6 1,686 axons/mm2) compared to both chitosan groups (DAI: 16,865 6 2,506 axons/mm^2; DAII: 18,399 6 4,636 axons/mm^2) (P < 0.05).

Morphometrical analysis performed at the midpoint of the graft or tube showed that the mean values of the diam-eter of the regenerated axons were not significantly differ-ent (P > 0.05) between the AG group (2.12 6 0.16) compared to DAI (1.9 6 0.08) and DAII (2.27 6 0.19) groups. The myelin thickness of the regenerated axons showed no differences (P > 0.05) between the AG group (0.59 6 0.03) and DAI (0.49 6 0.01) and DAII (0.49 6 0.03) groups. Regarding the g-ratio of the regenerated axons, no significant differences (P > 0.05) were observed between the AG group (0.63 6 0.01) and the DAI (0.64 6 0.01) and DAII (0.68 6 0.03) groups (Fig. 8).
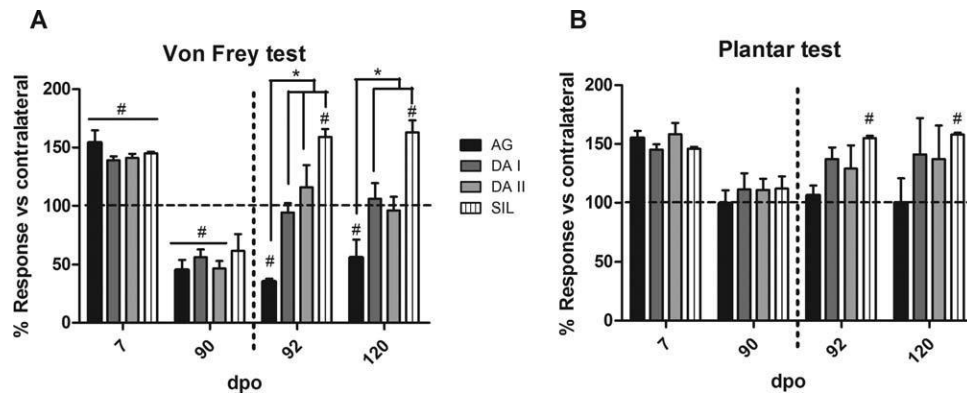

Figure 4. Mechanical (A) and thermal (B) algesimetry test results. Values were expressed as percentage of withdrawal response to mechanical stimulus (A) and thermal stimulus (B) applied to the lateral side of the injured paw versus the withdrawal response in the unin-jured paw. *P < 0.05 for differences between groups. #P < 0.05 for differences between groups and the baseline. Horizontal dotted lines represent the normalized baseline values. Vertical dotted lines indicate when the saphenous nerve was cut.
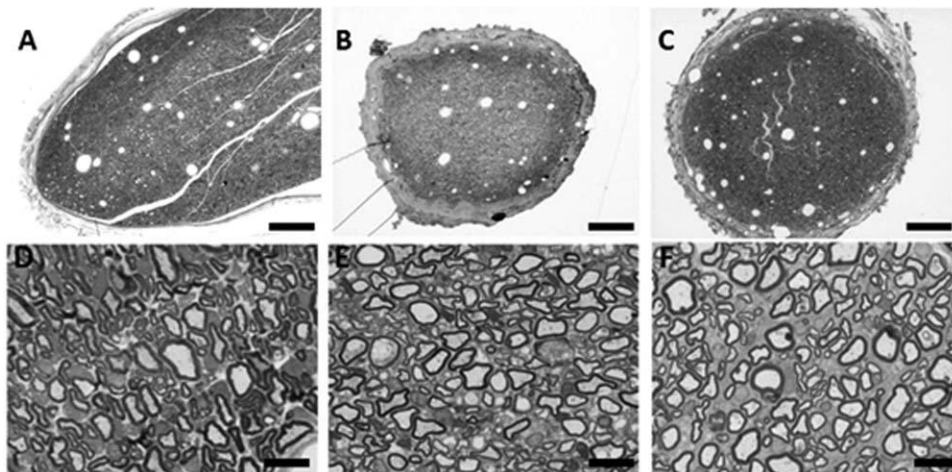

Figure 5. Micrographs of semithin sections of the regenerated nerve taken at the midpoint of the graft or tube 4 months after sciatic nerve resection and repair from a representative animal of group AG (A, D), and one of the animals that regenerated in group DAI (B, E), and DAII (C, F). General appearance of the regenerated nerves (A–C); bar 5 100 mm. Higher magnification of the regenerated nerves (D–F); bar 5 10 mm.
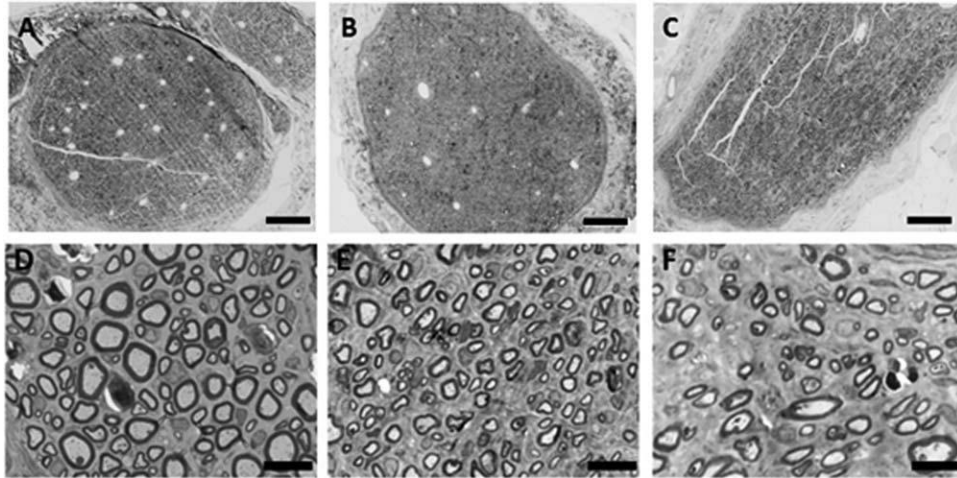
Figure 6. Micrographs of semithin sections of the regenerated nerve taken 3 mm distally to the graft or tube 4 months after sciatic nerve resection and repair from a representative animal of group AG (A, D), and one of the animals that regenerated in group DAI (B, E) and DAII (C, F). General appearance of the regenerated nerves (A–C); bar 5 100 mm. Higher magnification of the regenerated nerves (D–F); bar 5 10 mm.
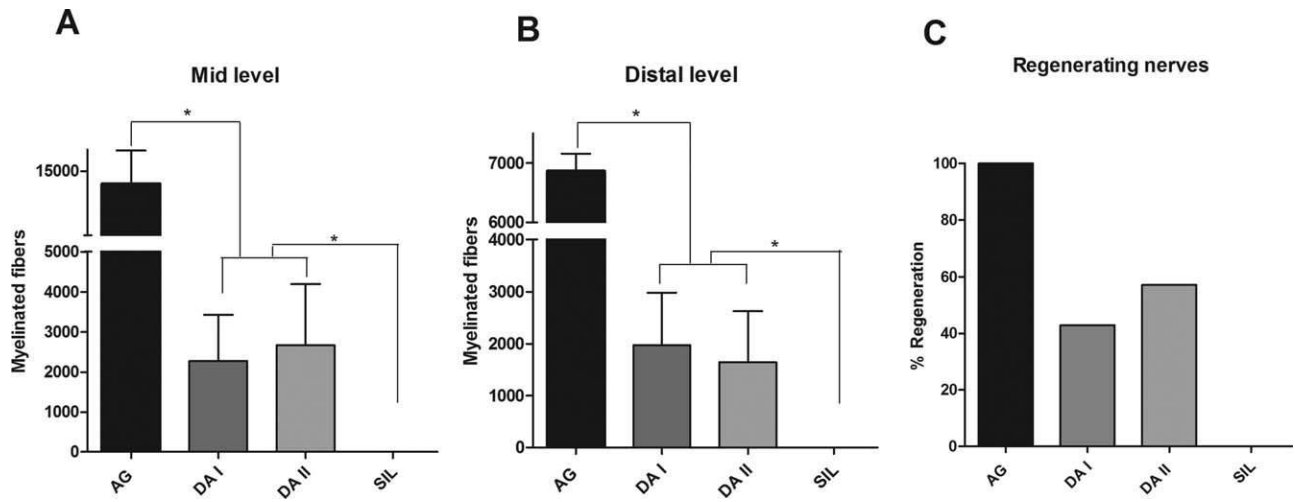
Figure 7. Number of regenerated myelinated fibers found in the tibial nerve at the mid-tube or graft (A) and 3 mm distal (B) in AG, DAI, DAII, and SIL groups. Animals with no regenerated nerve were also included (with values of 0) in the calculation. *P < 0.05. Percentage of regenerated nerves found at 120 dpo (C).

Muscle Weight

Both tibialis anterior and gastrocnemius muscles of the AG group had higher weight (56.3 6 4.66; 62.82 6 3.57 g; P < 0.05) than in animals repaired with chitosan tubes of the two degrees of acetylation (DAI: 35.89 6 1.56; 45.94 6 0.72 g; DAII: 35.49 6 4.37; 52.98 6 3.79 g, respectively), corroborating a lower degree of reinnervation.

DISCUSSION

In this study, we have investigated the capability of hollow chitosan tubes to sustain axonal regeneration when used to repair a critical 15 mm gap resection of
the sciatic nerve in rats, compared with repair by stand-ard SIL tubes and the gold standard autologous graft. In contrast to AGs, in which all the cases presented effec-tive regeneration and target reinnervation, only three of seven and four of seven animals of groups DAI and DAII, respectively presented a regenerated nerve inside the guide at the end of follow up, whereas regeneration failed in all the animals repaired with SIL tubes. These failures were expected, since the main limitation of nerve guides is the distance between the stumps that may be bridged. As the distance increases, regeneration and func-tional outcome decrease and eventually fail.29 In rats, the limiting distance, at which a simple nerve guide cannot sustain regeneration is considered 15 mm.7 With SIL tubes, no axons reached the distal segment in a 15 mm defect, whereas axons readily crossed a gap up to 10 mm. Over time, the characteristics and the quality of the nerve guides have been improved by research on bioma-terials with the aim of sustaining regeneration over such critical long-gap8. The advantages of these guides are that, being artificial, there is no need to sacrifice a healthy donor nerve from the patient, and they reduce surgical time for repair.30 In addition, nerve guides may
be advantageous since they may reduce the fibrous entrapment of the injured nerve at the suture site31,32 and
the problems related to noncorrect alignment of nerve fascicles.

Among other biomaterials, chitosan has emerged as an interesting polymer for peripheral nerve bridging. Chi-tosan has been proved as a suitable biomaterial for medi-cal and pharmaceutical applications because of its compatibility, nontoxicity, and biodegradability.17,33 Fur-thermore, chitosan-based tubes are easy to handle and their transparency facilitates surgical manipulation and suturing of the nerve stumps in place. Chitosan has also been used as an scaffold, in the form of freeze-dried sponge34 and micro/nanofiber mesh,35 serving as an inter-nal filler of the lumen of the tube that can also be com-bined with other biomaterials or grafted cells.36 Furthermore, chitosan tubes offer the possibility of modi-fying their inner surface to mimic the nerve-guiding basal lamina present in nerve grafts, by coupling small
peptides derived from extracellular matrix components, such as laminin and fibronectin. By influencing cell adhe-sion and migration, axonal growth and vascularization of the regenerating cable,37 these extracellular matrix mole-cules can potentiate the role of tubes in repairing long peripheral nerve defects.38,39 Chitosan-based materials have already been used for repairing long gap nerve inju-ries in rats. In previous studies a mix of polypyrrole/chi-tosan composite40 or the combination of chitosan tubes with cross-linked peptides38 resulted in enhancement of nerve regeneration, but the efficacy of the chitosan mate-rial alone to improve nerve regeneration has not been previously reported.

In the current study, we used hollow chitosan tubes of two different degrees of acetylation, controlled during the manufacturing process that may affect the degrada-tion process of the tube once implanted. We chose these degrees of acetylation since higher ones have been shown to be affected by faster degradation and lower mechani-cal stability.17 Although, the success of regeneration was

lower when using chitosan tubes than when using AGs, these tubes showed considerably better results than the standard control SIL tube. Indeed, the rate of successful regeneration and the levels of reinnervation achieved are among the highest reported for a hollow nerve guide alone over the critical 15 mm long gap in the rat sciatic nerve model.

To evaluate the success of regeneration and target reinnervation, we used functional and electrophysiologi-cal techniques that allowed us to follow the evolution of motor and sensory recovery over time. By means of algesimetry tests, we evaluated the responses to mechani-cal and thermal stimuli of the denervated hind paw. Since confounding responses can be due to collateral sprouting of the intact saphenous nerve, we cut this nerve after tests performed at 90 dpo and repeated the measure-ments at 92 and 120 dpo to guarantee that the responses observed were exclusively due to reinnervation by the regenerated sciatic nerve.27 We found withdrawal responses in all the animals of the AG group and some of the chitosan tube groups. In fact, all the animals with evidence of paw reinnervation displayed a mechanical withdrawal threshold lower than the control, indicative of hyperalgesia.41 All the SIL tube repaired animals failed to respond to noxious stimuli. We also performed serial electrophysiological tests to evaluate the degree of mus-cle reinnervation by regenerating motor axons. Muscle reinnervation started earlier and achieved higher levels in the rats repaired by AG than in those repaired with chito-san tubes. These differences are not unexpected, since regeneration in tube repair depends on the initial forma-tion of a new extracellular matrix bridge, over which fibroblasts and Schwann cells migrate and form a new nerve structure.42 This implies a delay in onset of axonal elongation, and failure of regeneration if the nerve stumps do not provide enough promoting elements inside the tube, as occurred in the long gap repaired with SIL tubes. The histological study corroborated the functional findings explained above. Only in the animals with evi-dence of reinnervation a regenerated nerve was found inside the tube. The regenerated sciatic nerves in the AG group were larger and had a higher number of myelin-ated fibers than in the chitosan tubes groups. Although, the mean size of the myelinated fibers was similar between animals repaired with AG and with the chitosan guides, the myelin sheath was slightly thicker in axons of the AG group. This could be due to the faster onset of regeneration in AGs compared to tubulization, where the formation of the fibrin cable slows the initial phase of regeneration.43
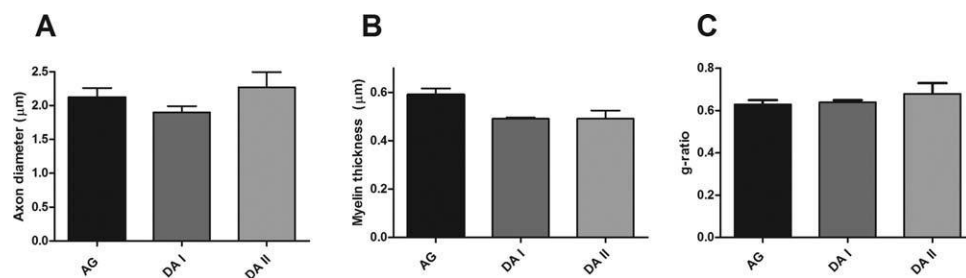

Figure 8. Morphometrical results of axon diameter (A), myelin thickness (B), and g-ratio (C) of the regenerated nerves found at the mid-point of the graft or the tube in groups AG, DAI, and DAII. No significant differences were found between groups.

CONCLUSION

The current study provides novel proof that chitosan-based tubes are good candidates for an artificial nerve guide, allowing nerve regeneration across a critical long gap in a significant number of cases.

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