Research in Veterinary Science 102 (2015) 27–33

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc

Functional and regenerative effects of local administration of autologous mononuclear bone marrow cells combined with silicone conduit on transected femoral nerve of rabbits

Anelise Bonilla Trindade [a,*], Pedro Schestatsky [b], Vítor Félix Torres [b], Cristiano Gomes [a], Giordano Cabral Gianotti [a], Ana Helena da Rosa Paz [c], Paula Barros Terraciano [c], Janete Maria Volpato Marques [a], Karina Magano Guimarães [a], Dominguita Lühers Graça [d], Elizabeth Obino Cirne-Lima [c], Emerson Antonio Contesini [e]

[a] Programa de Pós-graduação em Ciências Veterinárias, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[b] Neurology Department, Eletroneuromyography, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil
[c] Laboratory of Embryology and Cell Differentiation, CPE, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[d] Laboratory Animal Pathology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[e] Department of Animal Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 3 June 2015
Accepted 9 July 2015
Available online xxxx

Keywords:
Femoral nerve regeneration
Autograft
Autologous mononuclear cell
Cell therapy
Microsurgery

ABSTRACT

The inoculation of cells into injury sites can accelerate and improve the quality of nerve regeneration. This study aimed to evaluate the functional and regenerative effects of mononuclear autologous bone marrow cells (MABMC) combined with silicon conduit grafting in rabbit femoral nerves. Twenty-eight animals were allocated to one of two groups: treatment group (TG) or control group (CG), divided according to the time of evaluation, at either 50 or 75 days. After neurotmesis of the femoral nerve, surgical repair was performed with nerve autografts in silicon conduits, leaving a 5 mm gap in both groups. The TG received MABMC in silicon conduits, and CG received a sham saline inoculum. Histological, clinical and electrophysiological analyses detected no differences between groups, but analysis of leg diameter showed that TG diameters were larger. This cell therapy did not improve regeneration of the femoral nerve, but there was a tendency for better functional recovery.

1. Introduction

The peripheral nerves are susceptible to all of the same types of traumas that other tissues can suffer, but when the continuity of a nerve structure is interrupted, transmission of nervous impulses is stopped and functional activities are disrupted (Mattar and Azze, 2008) causing considerable disability and/or permanent physical incapacity (Ignatiadis et al., 2007). This is why there are scientists all over the world conducting research designed to increase the understanding of the nerve regeneration process and employing a variety of treatment approaches in attempts to find the ideal method with which injured nerves can be restored to full functionality (Santos and André, 2007).

There are reports of successful surgical repair of peripheral nerves in literature going back to the nineteenth century (Ignatiadis et al., 2007), when suggested that direct repair of nerves could be achieved by drawing together the epineurium. However, not all nerve injuries provide the conditions for direct anastomosis and significant nerve tissue losses demand grafts or conduits allowing communication between the two extremities (Mimura et al., 2004; Ignatiadis et al., 2007). These conduits or regeneration guides are made from biological or synthetic materials and are considered viable alternatives, for grafting techniques, performing the function of helping to guide axonal growth from a sectioned nerve, containing the diffusion of neurotrophic and neuroprotective factors with regenerative functions produced by the nerve stumps (Murakami et al., 2003; Yin et al., 2007; Zhang et al., 2008; Salomone et al., 2013). Even using these techniques, regeneration is still a slow process and one that is not always completed, since the silicone remains within the body and can cause foreign body reactions and may compress the nerve, leading to loss of function and formation of a neuroma (Yin et al., 2007). In recent attempts to achieve recuperation of both nerve continuity and function, a treatment combining cell therapy and trophic factors with the well-established technique of nerve repair using tubes has been tested (Chen et al., 2007; Colomé et al., 2008; Wang et al., 2011; Costa et al., 2013; Mohammadi et al., 2013) with encouraging results in terms of nerve regeneration.

* Corresponding author at: Veterinary Hospital, Universidade Federal do Rio Grande do Sul, Rua Bento Gonçalves, 9090, CEP 91540-000 Porto Alegre, RS, Brazil.
E-mail address: anelisebt@yahoo.com.br (A.B. Trindade).

http://dx.doi.org/10.1016/j.rvsc.2015.07.008
0034-5288/

The objective of this study was to evaluate the functional and histological effects of a combination of silicone tube conduit and inoculation of autologous mononuclear bone marrow cells on rabbit femoral nerves after neurotmesis.

2. Materials and methods

2.1. Animals

A total of 28 New Zealand rabbits (*Oryctolagus cuniculus*) of both sexes (17 females and 11 males), aged 4 ± 1 months and with body mass of 3.52 kg ± 0.64 were obtained from the Universidade Federal de Santa Maria (UFSM) central animal house.

The animals were given a period of at least 5 days to acclimatize at the Hospital de Clínicas de Porto Alegre (HCPA) animal experimentation unit where they were fed on a commercial rabbit food in pellets and water ad libitum and housed in individual cages at a controlled temperature of 18.9 °C with mean air humidity of 68.2% and a 12 + 12 light–dark cycle.

The animals were allocated to one of two equal sized groups at random: Control (CG, $n = 14$) or Treatment (TG, $n = 14$), which were then further subdivided into two equal subsets for evaluation at either 50 or 75 days (CG50, $n = 7$; TG50, $n = 7$; CG75, $n = 7$; TG75, $n = 7$).

This study was reviewed by the HCPA Animal Research Ethics Committee for compliance with the principles and standards regulating the use of experimental animals and was approved under protocol number 07672.

2.2. Harvesting and processing autologous mononuclear bone marrow cells

Bone marrow was harvested in advance of the surgical procedure, under sterile conditions and all samples were handled separately.

Autologous mononuclear bone marrow cells were extracted from the greater tubercle of the humerus of both limbs or until a minimum bone marrow aspirate volume of 5 mL had been collected. Animals were anesthetized with ketamine hydrochloride (20 $mg \cdot kg^{-1}$), midazolam (0.5 $mg \cdot kg^{-1}$) and pethidine hydrochloride (5 $mg \cdot kg^{-1}$) via intramuscular (i.m) injection and then isoflurane vaporization in 100% oxygen was initiated. All of the animals were given intravenous (i.v) enrofloxacin (5 $mg \cdot kg^{-1}$) at the point of anesthetic induction.

Bone marrow aspirate was homogenized and washed twice with D-MEM culture medium containing 10% fetal bovine serum and 1% penicillin and then added to conical tubes containing Ficoll-hypaque. The resulting cell suspension was centrifuged for 5 min at 160 ×*g* (Eppendorf®, USA).

The cell pellet was resuspended in 3 mL of complete D-MEM medium. Another conical tube was prepared by adding 3 mL of Ficoll-hypaque (proportion 1:1) and then the cell suspension was pipetted onto the gradient, down the side of the tube, before the tube was centrifuged for 20 min at 110 ×*g* and 18 °C. After centrifugation, the mononuclear cells at the interface were removed once more, placed in another conical tube and centrifuged for a further 5 min at 200 ×*g*. The cell pellet was then resuspended in 1 mL of PBS. Cells were quantified and tested for viability using trypan blue vital stain.[1]

2.3. Cell transplantation

A total of 1×10^6 viable cells in a volume of 0.2 mL were transplanted the same day, into the silicone prosthesis, which was itself attached to the right femoral nerve that had been transected. For cell transplantation, it was used a needle 12.7 mm × 33 mm (29 G) attached in a syringe and administered through the distal end of the silicone tube.

[1] Trypan blue, Acros Organic, Geel, Belgium.

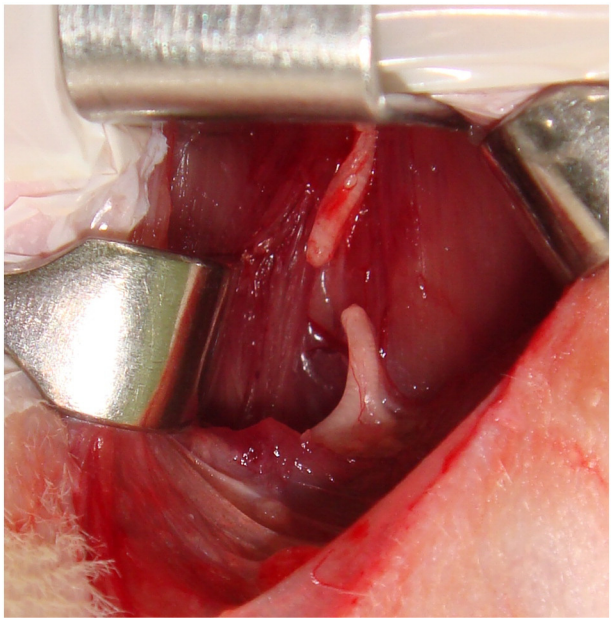

Fig. 1. Nerve femoral of rabbit. Location of the femoral nerve and nerve section.

2.4. Surgical procedure and transplantation of autologous mononuclear bone marrow cells

With the animal positioned in decubitus dorsal and under general anesthetic, a transverse incision was made at the right groin. The femoral nerve was located and completely sectioned, without removing any portion of the nerve (Fig. 1). A 7.5 mm long section of hollow cylindrical silicone tubing,[2] with internal diameter of 1.5 mm and external diameter of 2.42 mm was fitted over the extremities of the sectioned nerve. This was achieved by placing the tube between the nerve stumps and then sliding the proximal stump into the tube and suturing it to the epineural tissues with a single simple stitch using 6–0 nylon monofilament thread. The procedure was repeated for the distal stump, leaving a gap of approximately 5 mm between the nerve extremities. A surgical microscope[4] was used to aid suturing with 40× image magnification.

In the TG, a volume of 0.2 mL containing 1×10^6 mononuclear autologous cells, previously harvested from bone marrow aspirate was inoculated into the space within the silicone tube. In the CG, 0.2 mL of 0.9% NaCl solution was used instead (Fig. 2).

At the end of surgery and for the 2 following days, all rabbits were given ketoprofen[3] (1.0 $mg \cdot kg^{-1}$ IM, SID). They were also given tramadol hydrochloride[4] (2.5 $mg \cdot kg^{-1}$ IM, SID) for 5 days and enrofloxacin[5] was used as systemic antibiotic therapy at a dosage of 5 $mg \cdot kg^{-1}$ IM, SID, for the first 5 postoperative days. The surgical wound was cleaned using 0.9% NaCl solution every 24 h until healed.

2.5. Clinical assessment

After the date of the surgical procedure (day zero) animals were assessed clinically every 10 days. The area innervated by the femoral nerve was tested for sensitivity using a needle and animals were also tested for conscious proprioception. The thickness of both pelvic limbs was measured using tape measure. Finally, the animals' ambulatory ability was assessed before and after the nerve section procedure,

[2] Medicone, Cachoeirinha, RS, Brazil.
[3] Ketofen, Rhodia, Mérieux, Paulínia, SP, Brazil.
[4] Tramadol, Cristália Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brazil.
[5] Flotril, Indústria Química e Farmacêutica Schering-Plough S/A, Rio de Janeiro, RJ, Brazil.

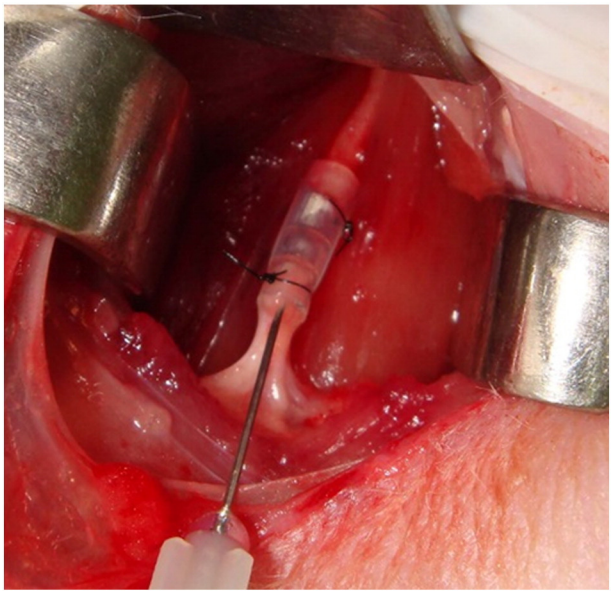

Fig. 2. Placing the silicone tube between the nerve stumps and suturing it to the epineural tissues with a single simple stitch. The procedure was repeated for the distal stump, leaving a gap of approximately 5 mm between the nerve extremities. In the therapy group, the mononuclear cell of the bone marrow was inoculated into the space within the silicone tube. In the CG, 0.9% NaCl solution was used instead.

both on a smooth and a rubberized surface. Ambulation was graded either as zero – with no apparent claudication – or as 1 — if claudication was evident.

For the last assessment of each animals' gait, the plantar surface of the pelvic limbs was stained with nontoxic tempera paint, making it possible to assess its gait by analyzing the tracks it left (Fig. 3).

All clinical assessments were conducted on all animals in both groups sequentially, before the surgical procedure and every 10 days during the entire postoperative evaluation period.

2.6. Electrophysiological assessment of the femoral nerve

The animals were tested for femoral nerve conductivity after anesthesia following the same anesthetic protocol described for the surgical procedure. The velocity of nerve conduction was assessed in both limbs of 16 rabbits, comparing per individual the operated limb and the healthy contralateral limb. Nine of these animals were from TG and seven were from CG.

The nerve conductivity tests were conducted by trained neurophysiologists who were blind to the animals' data. Animals underwent this test on the day they were to be euthanized, either 50 or 75 days after surgery.

With the animal positioned in decubitus dorsal, the femoral nerve was stimulated electrically by inserting intradermal needles into the medial section of the iliac spine, lateral to the femoral artery, which was located by digital palpation. The mean intensity of the electrical current was 10 $\pm$ 5.2 mA. Compound motor action potential (CMAP) was recorded using 2 cm superficial electrodes arranged longitudinally along the vastus medialis muscle, on the medial face of the pelvic limb (active electrode), and patella (reference electrode). Stimuli lasted 0.2 ms and reached 1 Hz using constant voltage from a power supply.

Compound motor action potentials were recorded using pairs of disc electrodes with a 9 mm conducting surface. Responses were recorded at 10 second intervals, with gain of 0.2 to 1.0 mV and frequency of 0.1 Hz to 0.5 kHz. Motor latency, amplitude, the distance between electrodes and spontaneous muscle activity were all assessed bilaterally.

2.7. Euthanasia

At the end of the evaluation periods (either 50 or 75 days), all animals were euthanized. They were first sedated with ketamine hydrochloride

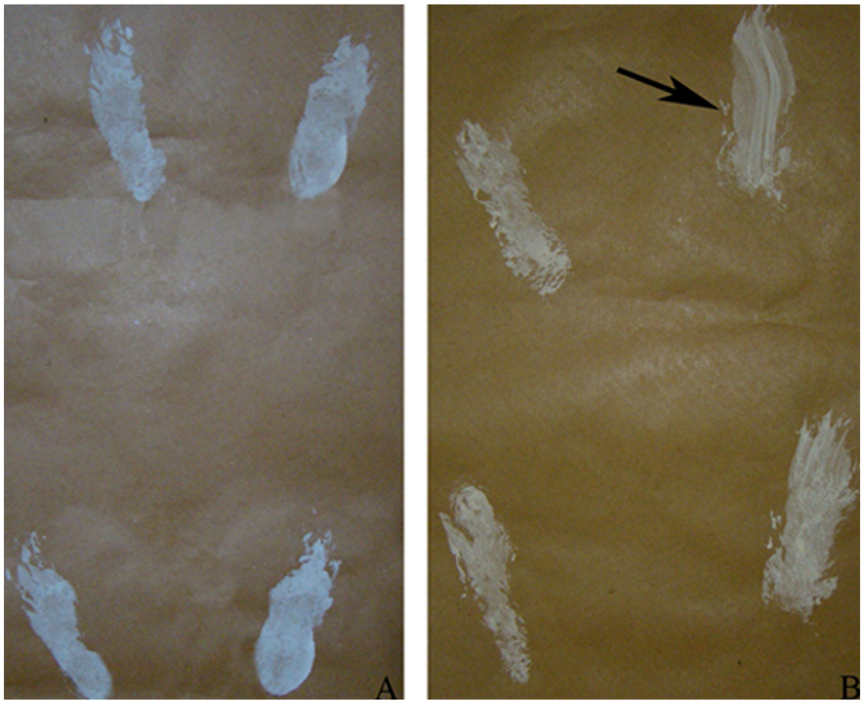

Fig. 3. Clinical assessment of ambulation of the rabbits by dyeing the plantar region of the hind limbs. (A) Animals with no claudication. (B) Animals showing lameness, seen through the disproportionate support of the right pelvic limb operated (black arrow) with respect to the contralateral limb.

(20 mg·kg^{-1}) combined with midazolam (0.5 mg·kg^{-1}) and morphine (0.5 mg·kg^{-1}), via IM injection, and then a sodium thiopental[6] overdose was administered via IV injection until cardiorespiratory arrest.

2.8. Macroscopic assessment

After euthanasia, the operated femoral nerve was assessed directly for nerve recovery at the site of tube placement, on the basis of whether there was a nerve bridge between the stumps and on the basis of presence or absence of signs of infection. After direct observation, the nerve segment was removed surgically and immersed in a 10% buffered formalin solution % (17 samples) or 2.5% glutaraldehyde (10 samples) for histological assessment, depending on whether they would be observed with optical or electronic microscopy.

2.9. Microscopic assessment

The 17 samples that had been fixed in 10% buffered formalin were set in paraffin blocks and sectioned into 3 μm slices. They were later stained for microscopic assessment using hematoxylin–eosin (HE), or periodic acid-Schiff (PAS), or Masson's trichrome and picrosirius.

Ten samples were fixed in 2.5% glutaraldehyde with 0.1 M sodium cacodylate buffer at a pH of 7.4. Two of these samples (one each from the CG and TG) were washed once more in a sodium cacodylate buffer solution and then dehydrated using ascending concentrations of ethanol (30, 50, 70, 85, 90, 95 and 100%) and acetone P.A. (100%), maintaining the samples in each concentration for 15 min, with the exception of the 100% concentration, in which samples were immersed three times for 15 min each time.

The samples were then dried in a critical point dryer, using liquid carbon dioxide, fixed to specimen holders with conductive carbon-glue tape and metallized with a 35 nm gold and palladium coating in an ion sputter coater for 2 min. The samples were then analyzed in a scanning electron microscope, operating at 15 kV and magnifications between 35 and 55 times.

When analyzing the samples stained with HE, the following were recorded if present in the regeneration site or the nerve segments cranial and caudal to it: inflammatory cells (eosinophils), digestion chambers (Wallerian degeneration), hemosiderin granules, and granulomas or giant cells.

When analyzing the samples stained with PAS, the following observations were recorded: orientation of fibers (longitudinal, disordered or presence of neuroma) and presence of digestion chambers or Wallerian degeneration (graded as zero if absent, 1 if present). The presence of collagen (blue fibers) was investigated using Masson trichrome stained samples, observing the entire slice, in the center and the periphery, and grading 0 for absent, 1 for weak, 2 for average, and 3 for accentuated. Picrosirius was used to test for the presence of collagen type I (red-orange tinged fibers), graded as zero when absent and 1 when present.

The histological slides were graded for the variables listed above, in sequence, by the same pathologist who was blind to which group each sample came from.

2.10. Statistical analysis

The chi-square test (SPSS version 18.0 for Windows) was used for statistical analysis of the results of the clinical assessments of conscious proprioception, patellar reflex and sensitivity to needle prick. The same test was used to compare gait at 3 weeks with gait at 7 weeks. Generalized estimating equations (GEE) were used to evaluate differences in degree of claudication between assessment weeks. Histological analysis results for HE, PAS, Picrosirius and Masson's trichrome were analyzed using the chi-square test. Electroneuromyography results and pelvic limb circumference measurements were analyzed using GEE. The significance level adopted was 5% ($p < 0.05$) for all tests.

[6] Sodium thiopental, Cristália Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brazil.

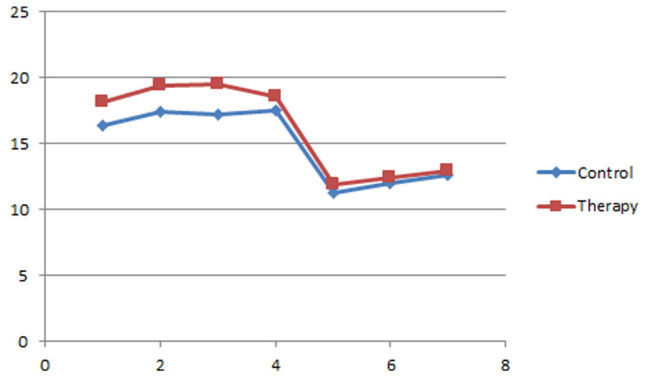

Fig. 4. Graph showing average values of circumference (cm) of the operated limb of the CG and TG animals in seven week assessments.

3. Results

3.1. Clinical assessment

There was no statistical difference between groups in the results for conscious proprioception, patellar reflex or local sensitivity to needle prick. However, the circumference measurements of the operated pelvic limb were statistically smaller for the CG than the TG (Fig. 4) up until the fourth week (first week: $p < 0.001$; second week: $p < 0.001$; third week: $p < 0.001$; fourth week: $p = 0.038$).

After three weeks, 92.9% of CG animals and 78.6% of TG animals exhibited some degree of claudication during the gait assessments. After seven weeks, 57.7% of the CG and 28.6% of the TG exhibited some degree of gait impairment. Notwithstanding, the differences in gait assessment results were not statistically significant for week 3 or week 7 ($p = 0.596$ and $p = 0.592$ respectively).

3.2. Electrophysiological assessment

There were no statistically significant differences between the TG and CG groups' electrophysiological assessment results, according to GEE (Fig. 5). Mean amplitudes and latencies for healthy and operated limbs for both TG and CG are listed in Table 1.

3.3. Macroscopic assessment

Direct observation of the site where the femoral nerve had been bridged by the silicon tubing demonstrated the viability of the surgical technique, since all of the animals in both groups and after both 50 and 75 days exhibited nerve regrowth bridging the gap between the sectioned nerve stumps. There were no occurrences of dehiscence of the sutures holding the silicon prosthesis to the nerve nor was there any evidence of infection in any of the animals.

3.4. Microscopic assessment

There were no statistical differences between the CG and TG in terms of the results for HE, PAS, Picrosirius, Masson trichrome or Toluidine blue at either of the assessment times (Fig. 6). The samples stained with HE showed that, in general, both CG and TG animals had organized nerve fibers. Wallerian degeneration at the distal end of the regenerated segment, inflammatory reactions and/or hemosiderin deposits and granuloma were all observed rarely.

The CG50 and TG50 animals exhibited greater Wallerian degeneration than the 75-day groups; but the difference did not attain statistical significance. Two animals in TG50 and one in CG50 exhibited signs of

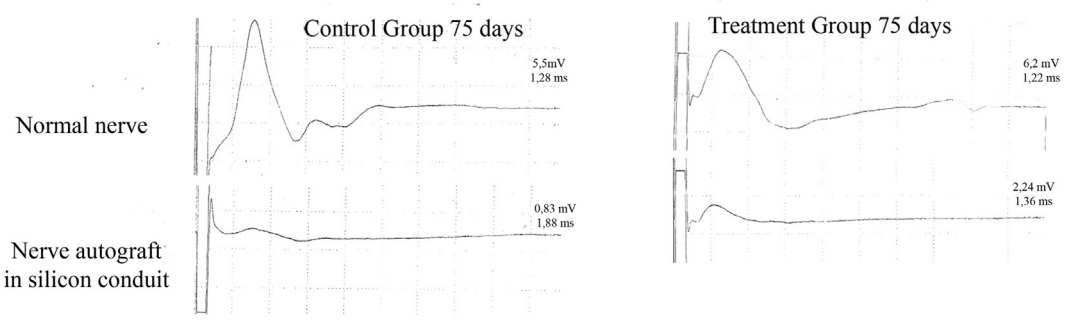

Fig. 5. Graphical analysis of the electrophysiology of both hind limbs of rabbits and therapy control group at 75 days postoperatively.

Table 1
Comparison of mean values and standard deviations of the latency and amplitude of the healthy femoral nerve compared with the femoral nerve of the operated animals CG and TG.

		Latency (ms)	Amplitude (mV)
Control group	Operated nerve	1.70 ± 0.45	4.38 ± 4.20
	Healthy nerve	1.37 ± 0.13	10.16 ± 3.63
Therapy group	Operated nerve	2.51 ± 1.69	3.57 ± 2.80
	Healthy nerve	1.45 ± 0.49	10.82 ± 3.01

recent hemorrhage in tissue sections, in the form of hemosiderin granules revealed by HE staining (Fig. 6A).

The PAS results revealed no statistically significant differences between groups for any of the variables studied (Fig. 6B). Four CG animals ($n = 9$, 44.4%) and two TG animals ($n = 6$, 33.3%) exhibited Wallerian degeneration ($p = 0.67$). In the CG, analysis of fiber orientation ($n = 9$) detected just one animal (7.1%) with disordered fibers and three (21.4%) with neuroma. In the TG ($n = 6$), just two animals (14.3%) exhibited neuroma ($p = 0.57$).

There was no statistically significant difference in presence or absence of collagen type I at the nerve regeneration site, tested using Picrosirius staining. All CG animals ($n = 9$) exhibited a weak presence of collagen type I and just one TG animal ($n = 6$) did not ($p = 0.30$).

Collagen was assessed in Masson trichrome-stained samples (Fig. 6C). Four CG animals ($n = 6$) did not exhibit collagen throughout the nerve (66.7%), while in the TG group ($n = 8$), seven animals (87.5%) did not ($p = 0.30$). Five CG animals (83.3%), and six TG animals (75%) did not exhibit collagen at the center of the nerve ($p = 0.71$) and three CG animals (50%) and four TG animals (57.1%) did not exhibit collagen at the nerve periphery ($p = 0.69$).

The toluidine blue results revealed digestion chambers and rare metachromatic mast cells in some sections in all animals (Fig. 6D).

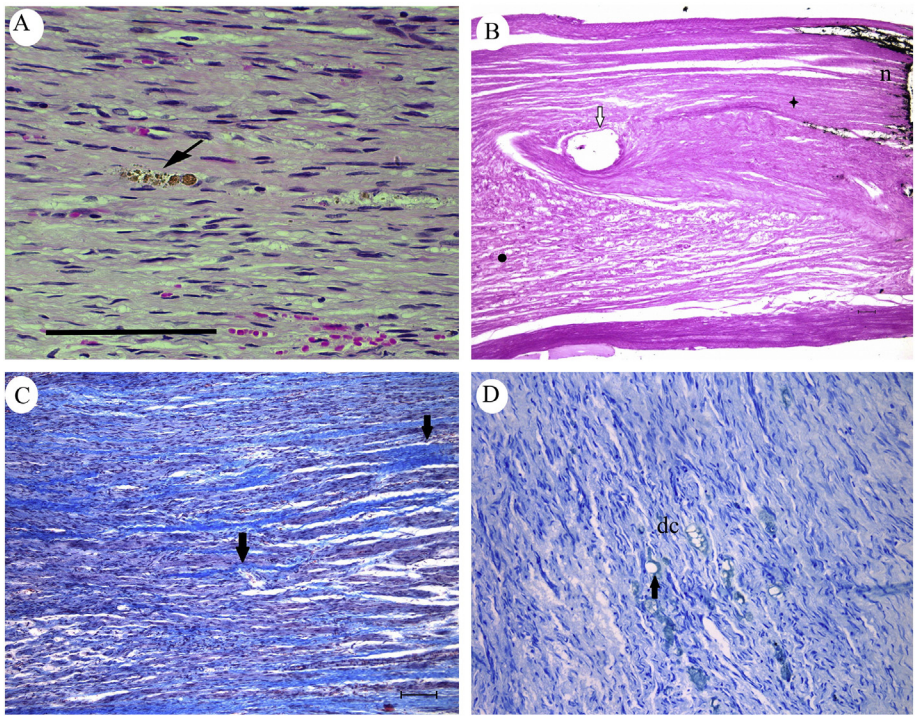

Fig. 6. Neuronal tissues of rabbits underwent neurotomy with immediate neurorrhaphy of the right femoral nerve. A) Hemosiderin granules indicated by the black arrow. Control group 50 days. Hematoxylin–eosin. Bar 100 μm. B) Disclosure of aligned nerve fibers (*), digestion chambers (Wallerian degeneration) (•), continuity solution allowed by the suture (arrow). PAS. Control group 50 days. Bar 300 μm. C) Thin collagen fibers in bright blue (arrows) between nerve fiber ashes. Masson's trichrome. Treatment group 75 days. Bar 300 μm. D) The nerve fibers are indicated by gray coloring, the myelin sheaths highlighted in dark blue color. Are views of digestion chambers (dc) and blood vessels (arrow). Control group 50 days. Toluidine blue. Bar 300 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

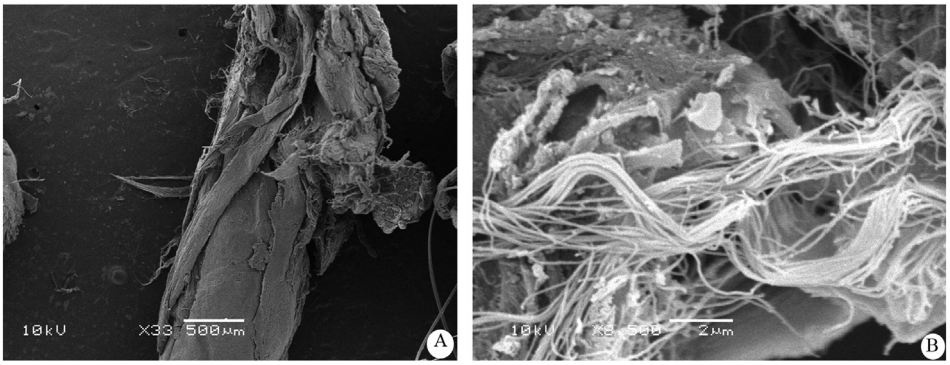

Fig. 7. Scanning electron microscopy of the femoral nerve. A) Surface of the femoral nerve of treatment group 75. B) Longitudinal image showing continuity of fibers in the central region of the nerve of animals of treatment group 75.

Scanning microscopy revealed no differences between the CG animal and the TG animal in terms of continuity or orientation of nerve fibers (Fig. 7).

4. Discussion

Regeneration techniques employing tubes are considered viable alternatives to grafting since the latter suffers from certain limitations, including achieving a good fit in terms of both length and diameter of the nerve; the need for subsequent surgery to the same patient, with loss of donor nerve function; formation of neuromas and greater post-operative pain (Yin et al., 2007; Mohammadi et al., 2013). In the present study, use of a silicone tube to guide regeneration of rabbit femoral nerves subjected to neurotmesis facilitated union of the sectioned nerve stumps, guided nerve fiber growth and acted as a scaffold to maintain the mononuclear fraction of bone marrow at the injury site. The silicone tube interposed between the stumps provided adequate support for regeneration of the femoral nerve of rabbits, as confirmed by analysis of specimens prepared with several different types of histological stains.

The marrow aspirate was harvested from the greater tubercle of the humerus, as described by Grindem et al. (2002), Zago (2006) and Colomé et al. (2008), who suggest that this is one possible location for obtaining bone marrow samples. Choice of this site was important to avoid harvesting traumas interfering with animals' claudication assessment results.

Although an injured femoral nerve exhibits distinctive clinical characteristics, the neurological clinical assessment of animals involved a certain degree of difficulty because some animals sometimes took a long time to respond to the stimuli. This might be explicable by the fact that in the wild these animals have many predators, meaning they become stressed easily and will often become agitated or immobile during clinical examinations, as described by Vernau et al. (2007).

The gait analysis was important to describe evaluations of forms of locomotion such as walking and running. The rabbits' gait was examined neurologically on the basis of strength and coordination (Chrisman, 1985). Although this was a subjective analysis, it was of real importance because it provided the opportunity to observe and compare the clinical evolution of the group given cell therapy against that of the group treated with tubing only. Additionally, all analyses were conducted by the same evaluator in order to reduce variations. Since rabbits do not have fused digits, they increase the length of their stride in order to increase the size of the limb and duration of movements, increasing the power with which they can push against the ground and the degree to which they extend and flexion the spinal column (Fostowicz-Frelik, 2007). These characteristics justify impregnating the plantar region of the pelvic limbs with paint in order to investigate claudication, since, when they push harder against the ground in order to jump, both of the stained limbs should leave well-defined marks, which is what was observed with the majority of animals in both groups, suggesting functional recovery of the nerve. Therefore, the animals who were only treated with silicone guides and those who had both the tube procedure and cell therapy exhibited the same quality of gait at all analysis points, according to GEE.

In contrast, although the animals had normal gait, the electrophysiological test showed that there had been a reduction in amplitude and an increase in latency, which indicate axonal injury and myelin sheath injury, respectively, in all of the animals assessed. The explanation that was found for this results is that neither the velocity of nerve conductivity nor the peak motor action potential asses the whole nerve function, but a fraction of the population of nerve fibers (Kanaya et al., 1996). This explains the lack of a significant correlation between post-operative nerve conductivity velocity and the number of axons regenerated, since histological, clinical neurological assessment and gait assessment results all demonstrated nerve regeneration and functional recovery. Similar results have been reported by Chen et al. (2007) and Sandrini et al. (2007).

A number of different staining techniques were used for the histological analysis, including hematoxylin–eosin (HE), picrosirius, Masson trichrome and periodic acid-Schiff (PAS) and also scanning electron microscopy. No statistically significant differences were observed in any of the variables studied histologically.

The hemosiderin observed in HE histological analysis of some animals signified the presence of recent hemorrhage, caused by the iatrogenic injury to vessels close to the nerve when the specimens were collected. Hemosiderin is a pigment that is formed by degradation of hemoglobin and, like ferritin, it contains iron and can be deposited in excessive quantities in tissues in a localized or systemic manner. Localized deposits are found after hemorrhages, when hemosiderin can be observed in adjacent macrophages some hours after bleeding begins (Costa-Val et al., 2006).

The finding that control group animals had eosinophils and plasmacytes but treatment group animals did not is the result of the fact that cell therapy promotes faster resolution of the immunoresponse, which facilitates removal of myelin and axonal fragments, favoring production of neurotrophic factors, which in turn benefit axonal regeneration (Colomé et al., 2008). Immunoresponse would therefore have declined or ceased in the treated group by the time of assessment.

Animals in the CG and animals in TG exhibited aligned nerve fibers at both assessment times, using PAS staining. This can be explained by the fact that the silicone tube technique reduces manipulation of the nerve and the quantity of suture material required at the anastomosis site, in addition to providing sufficient support for new regeneration, which makes it more likely that axons will be aligned in the direction of the distal stump (Gibson and Daniloff, 1989; Rodrigues et al., 2012).

The weak presence of collagen type I and type IV observed in CG animals and TG animals, seen on picrosirius and Masson trichrome-stained specimens, could be considered a beneficial factor, since

collagen is needed for formation of the normal extracellular matrix and plays an important role regulating the function of Schwann cells; but after an injury, collagen production often exceeds the ideal and impedes growth of the axon, which delays nerve regeneration (Koopmans et al., 2009).

Macroscopically, all animals exhibited regeneration tissue interconnecting the nerve stumps and there were no statistical differences between the groups. This can be explained by the fact that surgery was conducted respecting the principles of microsurgery and neurosurgery and with the aid of magnification. These results are similar to results reported by Chen et al. (2007), Colomé et al. (2008), Costa et al. (2013) and Mohammadi et al. (2013). In general, histological findings indicated good nerve regeneration, in agreement with the gait assessments of all animals, since at the last assessment all of them had low claudication indices.

Several different studies have demonstrated that cell therapy offers advantages for neuronal regeneration, when compared with individuals who did not receive it (Chen et al., 2007; Hu et al., 2007; Costa et al., 2013), suggesting that the mononuclear fraction of bone marrow secrete biologically active molecules and attracts neurotrophic factors, providing an appropriate environment early on and contributing to an acceleration of nerve regeneration (Chen et al., 2007). This mechanism of action of MSC it is known as paracrine effect (Chen et al., 2008; Linero and Chaparro, 2014). Since the survival and differentiation of MSC at the site of the lesion is limited, this mechanism starts in the first hours after transplantation of cells and, it is proposed that paracrine signaling is the primary mechanism of their therapeutic effects (Horie et al., 2012; Linero and Chaparro, 2014).

In this study, there was a tendency for animals which underwent cell therapy to exhibit better functional recovery and maintenance of a large proportion of the original diameter of the treated limb, which was a statistically significant difference. The advantages of combining cell therapy with the silicon guide tube technique would have been more evident if a larger number of cases had been evaluated.

5. Conclusions

In this study, local administration of the mononuclear fraction of autologous bone marrow together with fitting of a silicone conduit did not improve regeneration of transected femoral nerves of rabbits, but the limb diameter of animals given cell therapy was significantly greater than in the control group, demonstrating a tendency towards better functional recovery.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Acknowledgments

We are grateful to the Hospital de Clínicas de Porto Alegre-Fundo de Incentivo a Pesquisa (www.hcpa.ufrg.br) for providing financial support for the study and for paying for the translation of this article. The article was translated by Robert Coulthard for Scientific Linguagem Ltda.

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