ORIGINAL ARTICLE

Effect of laser therapy (660 nm) on recovery of the sciatic nerve in rats after injury through neurotmesis followed by epineural anastomosis

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Abstract The aim of this study was to analyze the influence of aluminum gallium arsenide (AlGaAs) laser (660 nm) on the myelin sheath and functional recovery of the sciatic nerve in rats. The sciatic nerves of 12 Wistar rats were subjected to injury through neurotmesis and epineural anastomosis, and the animals were divided into two groups: group 1 was the control and group 2, underwent low-level laser therapy (LLLT). After the injury, AlGaAs laser at 660 nm, 4 J/cm^2, 26.3 mW and beam area of 0.63 cm^2 was administered to three equidistant points on the injury for 20 consecutive days. In the control group the mean area of the myelin impairment was 0.51 (± 0.11) on day 21 after the operation, whereas this value was 1.31 (± 0.22) in the LLLT group. Student's t-test revealed a P value=0.0229 for the mean area values of the myelin sheath between the LLLT and control groups. Comparison of the sciatic functional index (SFI) showed that there was no significant difference between the pre-lesion value in the laser therapy group and the control group. The use of AlGaAs laser (660 nm) provided significant changes to the morphometrically assessed area of the myelin sheath, but it did not culminate in positive results for functional recovery in the sciatic nerve of the rats after injury through neurotmesis.

Keywords Nerve regeneration · Low-level laser therapy · Sciatic nerve

Introduction

The peripheral nerves are the constant target of injury of a traumatic origin, such as crushing and total cutting, resulting in a diminishing or loss of sensitivity and motor activity in the nerve territory, the severity of which depends on the extent to which the structures have been compromised. The negative effect on the daily activities of patients with a peripheral nerve injury is a determinant factor in establishing the goals of early recovery [1].

The recovery of a peripheral nerve following injury has long intrigued researchers, and there are a large number of scientific studies on the most varied repair methods, phenomena involved in regeneration and assessment methods regarding results. Although there is a certain degree of recovery in most nerve injuries, the process is slow and often incomplete [2]. It is estimated that the incidence of traumatic injury in some countries is more than 500.000 new cases annually, and that 2.8% of patients acquire lifelong disabilities [1, 3]. This justifies the continuity of the production of therapies that allow the minimization of the degree of injury and disability.

To evaluate the levels of nerve injuries in experimental situations, the functional assessment of gait has been shown

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to be a reliable and reproducible method [4, 5]. In 1982, De Medinaceli, Freed and Wyatt [5] proposed the use of a method of assessment named the sciatic functional index (SFI), based upon measurements of the rear feet of rats. This method was used with a normal control group and in experimental groups, after the sectioning and crushing of the sciatic nerve. The experiment consisted of obtaining images of the animals' footprints while they walked on a track specifically built for this purpose, and the images were recorded and analyzed.

It is a common practice in physiotherapy to use therapeutic instruments with a regenerative finality. For peripheral nerve injuries, electrical stimulation, ultrasound and low-level laser have been used to accelerate regenerative processes, seeking an early return of functionality for the patient [6, 7, 8]. Low-level laser therapy (LLLT) began to be used in the regeneration and functional recuperation process of peripheral nerves in the 1970s, with a number of reports and divergences regarding the results [8].

From an analysis of studies, it was determined that, in the past, the helium–neon (He-Ne) laser, emitted in the red region of the electromagnetic spectrum, was the most studied wavelength in the biomodulation of biological response in the repair process [7, 9]. At the present time, new wavelengths are being employed, as in lasers emitting radiation in wavelengths from 650 nm to 1000 nm [10, 11]. In many studies, descriptions of the irradiation parameters, such as dose, average power, time and application methods, are expressly varied, which hampers methodological comprehension for the reproduction of results and hinders comparisons between studies.

Owing to the small number of studies that use laser diodes in nerve lesions by neurotmesis and little use of two methods of analysis, the investigation of an experimental model linked to a histological and functional analysis may provide relevant data for the basis of future clinical applicability in the treatment of nerve injuries.

Materials and methods

Animals

Twelve [12, 13] adult male Wistar rats (3 months old) were used, with body weights ranging from 300 g to 350 g. The animals were provided by the Central Bioterium of the Universidade para o Desenvolvimento do Estado e da Região do Pantanal (UNIDERP) and kept under controlled light and temperature conditions, six animals to a cage, with standard chow and water ad libitum. All experimental procedures were carried out in compliance with the norms of the Brazilian College of Animal Experimentation (COBEA). The study received approval from the Research

Ethics Committee of the Universidade do Vale do Paraíba (UNIVAP) under process number L185/2005/CEP.

The animals were randomly divided into two experimental groups, according to the procedure to be carried out:

- Group 1, the control ($n=6$). The animals were subjected to injury through unilateral neurotmesis of the sciatic nerve, with epineural anastomosis and no irradiation.
- Group 2, – the group given LLLT ($n=6$). The animals were subjected to injury through unilateral neurotmesis of the sciatic nerve, with epineural anastomosis and subsequent laser irradiation to the injured region for 20 consecutive days.

Surgical procedure

After being weighed, each animal was given a pre-anesthetic of butorphanol (Turbogesic, 2 mg/kg) in combination with acepromazine (Acepran, 1 mg/kg), both in a single intramuscular dose. After 15 min, zolazepam and tiletamine (Zoletil 50, 40 mg/kg) were administered. Once anesthetized, each animal was positioned in the ventral decubitus position, with the front and hind paws maintained in abduction. Anti-sepsis was performed with iodated alcohol, followed by trichotomy and incision on the lateral face of the right thigh from the trochanter major to the knee. The sciatic nerve was approached and, with a standardized aid of a magnifying glass, we injured the nerve approximately 3 mm distal to the tendon of the internal obdurator [14] by a complete resection of the sciatic nerve. Epineural anastomosis of the sciatic nerve was then performed with three simple stitches of nylon monofilament (Mononylon 10–0, Polysuture®). The soft tissues were also sutured with simple stitches of nylon monofilament (Mononylon 5/0, Ethicon®). Following surgery, each animal was given a single dose of fentanyl intra-peritoneally (0.032 mg/kg) for infection prophylaxis and analgesia. Analgesic was administered at 12 h intervals for two consecutive days.

Laser therapy

Aluminum gallium arsenide (AlGaAs) laser (KLD®; Endophoton model) was used with a wavelength of 660 nm, a power of 26.3 mW, a beam area of 0.63 cm^2, in continuous mode. The form of application was based on the contact point transcutaneous method, with an energy density of 4 J/cm^2, power density of 0.0413 W/cm^2 and 96.7 s per point. Average energy of the equipment was measured prior to the experiment with the aid of an energy meter (2-Watt Broadband Power/Energy Meter, Model 13 PEM 001/J, Holland). Three points on the surgical incision were irradiated: one point at each extremity and one central

point. Laser therapy was initiated on the first postoperative day and was performed for 20 consecutive days. The animals in the control group were subjected to the same procedure, but with the device turned off.

Acquisition of samples and histological analysis

To obtain the sciatic nerve for histological analysis on day 21 of the postoperative period, we gave each animal a pre-anesthetic solution of butorphanol (Turbogesic, 2 mg/kg) together with acepromazine (Acepran, 1 mg/kg), both in a single intramuscular dose. After 15 min the animals were anesthetized with a combination of zolazepam and tilet-amine (Zoletil 50, 40 mg/kg). We killed the rats with a lethal dose of potassium chloride (0.4 ml/100 g of weight) to the heart. Prior to removing the sciatic nerve for analysis, we soaked it in a 25% glutaraldehyde solution in sodium cacodylate at 0.025 M (pre-fixed for 2 min and fixed for 12 h in a refrigerator at 6°C). After fixation of the segment, it was treated with 2% osmium tetroxide and 0.2% sodium cacodylate for 8 h. The segments were then washed in an isotonic sodium cacodylate buffer and dehydrated in increasing solutions of ethanol and propylene oxide. The samples were embedded in a plastic resin (EPON 812) for 48 h at 60° in an oven. The blocks were cut transversely to the sciatic nerve (2.0 μm in thickness). The nerve fibers in the injured region were imaged with the Leica Q-Win® program.

Histomorphometric analysis

The histomorphometric analysis was performed with the aid of the Image Pro Plus program (version 4.5.0.29). The first step of the analysis consisted of capturing the entire fascicle image. The next step was to capture sequential inner areas of the fascicle, with the examiner selecting five quadrants per fascicle–four peripheral and one central (122,500 μm^2). In each quadrant a square of 350 μm × 350 μm was selected, in which the average area of myelin sheath was measured [15].

Functional analysis

The animals' footprints were obtained before and 7 days, 14 days and 21 days after surgery through the use of millimetered paper strips that were held in a gait track built in accordance with the proposal by Dijkstra et al. [15]. After the initial gait training for 5 min, the animal's paws were painted with Nanjing ink and their footprints were recorded for the analysis of the sciatic functional index (SFI).

We measured the distances between the second and fourth distal phalanges, between the first and fifth distal phalanges, and between the proximal edge of the foot and the third distal phalanx. After these variables values had been obtained, they were introduced into a mathematical equation where the results represented the percentage of the deficit in the harmed side compared with the normal side. The normal function, or the absence of injury, was indicated by an index of 0%, while −100% represented the complete loss of function and total nerve injury

Data analysis

The values obtained from the histomorphometric analysis were subjected to Student's t-test, with the rejection of the null hypothesis set at 5% ($P<0.05$). Statistical analysis was performed with the GraphPad Prism® 4.00 statistical package for Windows.

Results

Histomorphometric analysis

The data obtained through histomorphometric analysis of the general averages of the area of myelin impairment obtained from the five quadrants analyzed in each sample allowed the comparison of the control group and LLLT group (Fig. 1). In the control group the mean area of myelin impairment on day 21 of the postoperative period was 0.51 μm^2 (± 0.11) (Table 1). In the LLLT group, mean area of myelin impairment on day 21 of the postoperative period was 1.31 μm^2 (± 0.22) (Table 2). Student's t-test revealed a P value of 0.0229, indicating a significant difference in the mean area of myelin impairment between the control and LLLT groups (Fig. 2).

Functional analysis

The SFI of the control group was, on average, −35.9± 48.0 of the data collected prior to the injury; −88.8± 23.2 on the seventh day after surgery; −101.9±25.9 on the 14th day after surgery; and −98.3±34.3 on the 21st day after surgery. For the laser therapy group, the mean values were −7.3±18.3 before injury; −98.2±18.7 on the seventh day after surgery; −87.4±9.1 on the 14th day after surgery; and −79.0±11.2 on the 21st day after surgery. analysis of variance (ANOVA) of the control group, for the different days of the SFI collection, showed that only the pre-injury values were statistically significant ($P<0.001$) when compared with the values from the other days. For the laser therapy group, the pre-injury values compared with those of the seventh, 14th, and 21st days after surgery showed statistically significant differences ($P<0.001$). However, there were no

Fig. 1 Microphotographs of the third quadrant of the fascicle of the sciatic nerve, in which **a** and **b** correspond to group 1 (control) and **c** and **d** correspond to group 2 (laser therapy). *Ep* epineuron, *Pn* perineuron, *BhM* myelin sheath. Note the difference in area of the myelin sheath between groups. ×400
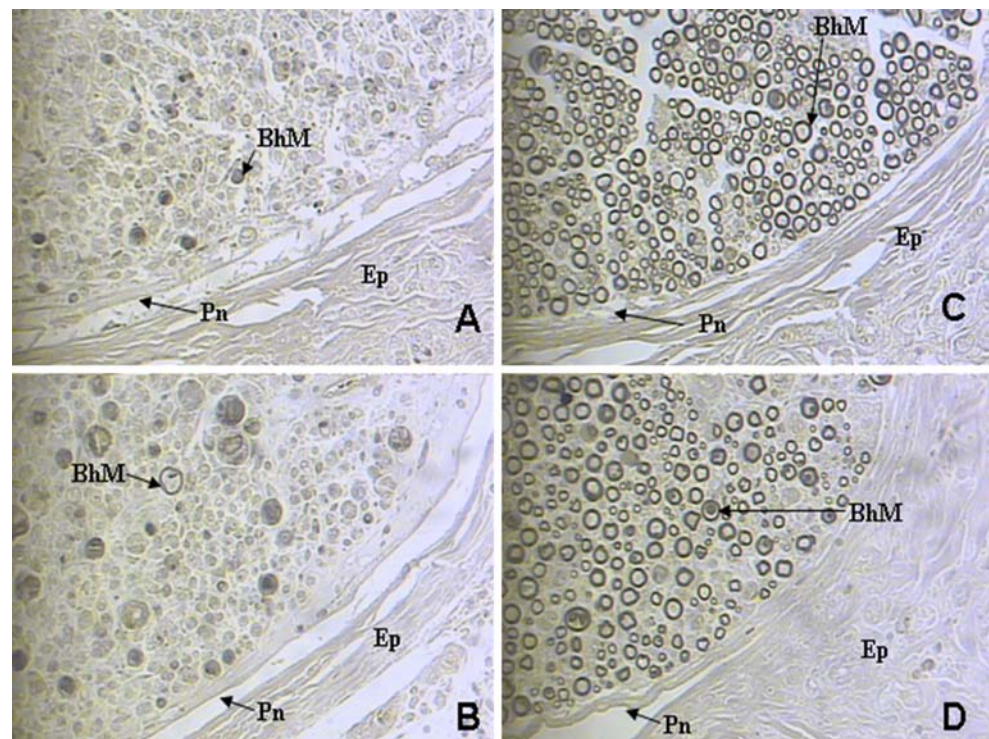
difference between the seventh, 14th, 21st post-surgery days ($P>0.05$).

In a comparison between the two experimental groups (control and laser) it was noticed that there were no significant differences in the values between the four periods of evaluation ($P>0.05$).

Discussion

There is evidence from clinical and experimental studies that the effects of laser include an increase in nerve function, a reduction in the formation of wounds, increased metabolism of neurons and an increased capacity for the production of myelin [10]. As laser therapy is non-invasive, it offers the advantage of being able to irradiate injured nerves without surgical intervention.

The aim of our study was to determine whether AlGaAs laser (660 nm) influenced the regeneration of the sciatic nerve in rats subjected to injury through complete resection followed by epineural anastomosis and assessed through histomorphometry. Rats were chosen for the experimental because they are easy to obtain and handle and have a low operational cost. The similarity in the distribution of their nerve trunks with those in humans, and the adequate anatomical characteristics for the surgical procedures, were considerations that also led us to choose this animal [1]. Pathological conditions that affect the sciatic nerve are very common in clinical practice, including herniated disc, stenosis of the medullary canal and piriform syndrome, but isolated injuries of the sciatic nerve such as neurotmesis are very rare. Therefore, this model provides a test bank for injuries involving pluri-fascicular nerves with axons of different

Table 1 Individual values of the impaired areas of myelin sheath in the five quadrants analyzed in each sample from group 1 (control) on postoperative day 21, with respective means and standard deviations (*Q* quadrant)

| Sample number	Q1	Q2	Q3	Q4	Q5	Mean	SD
1	0.86	0.68	0.26	0.36	1.07	0.65	0.34
2	0.42	0.16	0.19	0.50	0.25	0.30	0.15
3	0.87	0.66	0.79	0.52	0.77	0.72	0.14
4	0.46	0.30	0.29	0.44	0.49	0.40	0.10
5	0.44	0.28	0.38	0.42	0.49	0.40	0.08
6	0.28	1.00	0.52	0.35	0.64	0.56	0.29
					Group	0.51	0.11

Table 2 Individual values of the impaired areas of myelin sheath in the five quadrants analyzed in each sample from group 2 (LLLT) on postoperative day 21, with respective means and standard deviations (Q quadrant)

Sample number	Q1	Q2	Q3	Q4	Q5	Mean	SD
1	2.50	1.59	2.39	1.78	1.62	1.98	0.43
2	0.73	0.46	0.41	0.60	0.51	0.54	0.12
3	2.12	3.04	2.03	1.46	1.88	2.11	0.58
4	0.72	0.46	0.43	0.35	0.67	0.53	0.16
5	0.55	0.96	1.12	1.10	1.15	0.98	0.25
6	1.04	1.89	2.64	1.26	1.83	1.73	0.62
					Group	1.31	0.22

types and sizes competing in the reach for distal endoneural tubes and re-enervation targets [1].

Injury by complete resection was preferred to injury through crushing in this study. Crushing preserves the sustention structure of the nerve, increasing axonal extension, as the neural tubes remain in continuity, thereby facilitating regeneration. However, the principal aim of our study was to determine whether laser therapy influences nerve regeneration without any type of interference or help. Endo states that injury through neurotmesis introduces a series of variables that are difficult to control and standardize [16]. However, we did not encounter any great difficulties in our study, as the injury was standardized on all the animals in both experimental groups. Another important factor in the choice of this type of injury was the scarcity of studies using laser on an injury by resection, which justifies carrying out studies that clarify the action mechanisms of laser therapy on this type of injury.

A diversity of surgical modalities has been used in the repair of peripheral nerves, including epineural and perineural repair, autogenous grafts, vein grafts and entubulation, with or without associated neurotrophic factors [17].
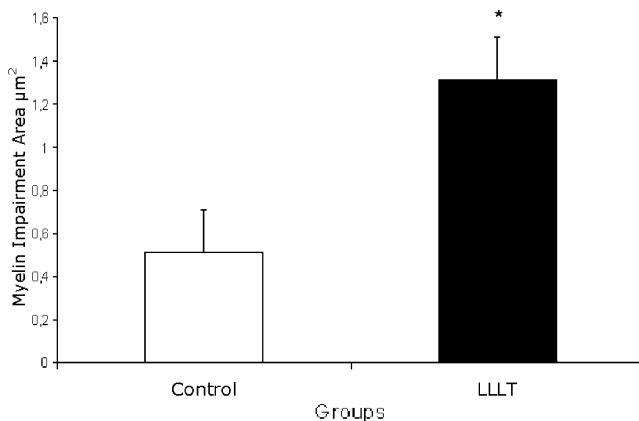
Fig. 2 Comparison of means obtained through histomorphometric analysis of the impaired areas of myelin sheath. The *bars* are means ± standard errors of the means (SEMs) of the values from six animals. *$P<0.05$ compared with the control group using the Student's t test

In our study, the simple epineural anastomosis method was adopted, due to its ease of execution and for having demonstrated a high biomechanical resistance to traction, according to Temple et al. [18].

The He-Ne laser (632.8 nm) in the red emission region of the electromagnetic spectrum has been the wavelength most studied in the biomodulation of the biological response in the repair process [19]. Other wavelengths are currently being employed and studied, as in lasers emitting at 650–830 nm (AlGaAs) and 904 nm (GaAs) [9, 20, 21]. The AlGaAs laser at 660 nm was adopted in our study due to its low-level intensity and the fact that this wavelength is very often employed in clinical practice. Moreover, there has not been a large number of previous studies regarding the effects of this wavelength on peripheral nerve regeneration. In clinical practice, low-level laser therapy employs energy densities from 1 J/cm^2 to 4 J/cm^2, associated with an output ranging from 10 mW to 90 mW, and it is widely used in diverse musculoskeletal injuries as well as algic and inflammatory processes [9]. This justifies the 4 J/cm^2 density used in our study. It is important to point out that this parameter is extremely variable in laser therapy studies on nerve regeneration.

Rochkind and Quaknine [2] report that the effects of low-power laser depend upon the dose, as low doses cause the regulation of oxy-reduction of the cell metabolism and high doses cause photodynamic damage. This affirmation was made possible after the authors analyzed different wavelengths and energy doses administered to fibroblasts and determined that 630 nm obtained the greatest number of mitoses than did 360 nm and 780 nm, whereas peak mitosis was achieved with a dose of 15 J/cm^2, but there was a reduction in cell reproduction above 60 J/cm^2.

The use of LLLT as a therapeutic method still generates discussion, and its biomodulatory effect on peripheral nerves remains unclear. Some studies have presented positive results, whereas other indicate that laser exercises no influence whatsoever on peripheral nerves [9, 21, 22].

The molecular basis to justify the effectiveness of laser therapy on nerve regeneration remains unclear. Karu [21] found that the irradiation of isolated mitochondria induced

positive alterations in cell homeostasis. The author suggests that components of the respiratory chain (cytochromes, flavins and dehydrogenase) are capable of absorbing light of a particular wavelength. Thus, this absorption may result in an increase in the synthesis of adenosine triphosphate (ATP), affecting the hydrogen levels in the cells and activating the ionic balance (sodium, potassium, calcium).

Manteifel and Karu [22] report that cytochrome-C oxidase, a terminal enzyme in the respiratory chain, is a photoreceptor of light from the red range, which is the same region of the wavelength used in our study. The absorption of light by this enzyme is thought to accelerate the trans-membrane electric potential of the mitochondria, thereby activating ATP synthesis and, consequently, cell metabolism.

The results of our study regarding the repairing action of laser were confirmed through the statistical analysis of the data obtained from the histomorphometric assessment. There were clear signs of improved recovery of the nerves treated with laser in comparison with those of the control group. The histomorphometric analysis following the experimental traumatic lesions allowed the reliable quantification of the trophic conditions of the regenerated nerves, but it did not assist in the understanding of function.

The use of the gait track is a very common method of assessment [23] that has a wide applicability to experimental research for its easy execution and low cost. Some researchers are trying to modernize this data collection with the use of digital cameras, making possible a more dynamic evaluation [4]. However, the purpose of this research better fits in with the use of the conventional method with a track of wood and the use of Nanjing ink. The values obtained in this study, related to the SFI, showed that, after the nerve injury by complete transection, there was a severe functional loss in both experimental groups on the seventh day after surgery; however, in the control group, the functional index had decreased even more on the 14th and 21st days, while, in the same period, the laser therapy group showed functional improvement when it was compared with that on the seventh day, although the statistical analysis did not show significant results between groups.

A probable explanation for the low SFI of the irradiated group on the seventh day after surgery was the fact that, in the first hours after the axon has ruptured, the cell body starts to show alterations (chromatolysis), histologically characterized by cell ingurgitation, degeneration of Nissl substances (neuron rough endoplasmic reticulum) and nucleus migration from the center to the periphery. These alterations, in spite of the production of neurotransmitters, aim to increase protein synthesis (actin and tubulin), which are related to the regeneration of the axon's cytoskeleton, and they affect intracellular transportation and growth cone movement [24]. It is probable that the 7-day period after

injury is marked by these events, but the use of laser therapy within 24 h of injury could reduce the immediate functional loss, confirming the claim by Dahlin [24].

De Medinaceli et al. [5] found that, 1.5–2 months after a crushing injury, functional recovery reaches its plateau, although there is no morphometrically significant change. An important fact to be mentioned is the functional level previous to the injury, noting that, in both groups during the evaluation period, there were no significant statistical differences, thus showing the uniformity of our research sample.

Further studies should be carried out with various energy densities as well as other wavelengths or even with new tissue repair methods in order to clarify the effects of laser therapy on this type of injury.

Conclusion

The use of aluminum gallium arsenide LLLT (660 nm) provided significant changes to the morphometrically assessed areas of the myelin sheath, but it did not culminate in positive results for functional recovery in the sciatic nerve of rats after injury through neurotmesis.

References

1. Rodríguez FJ, Valero-Cabré A, Navarro X (2004) Regeneration and functional recovery following peripheral nerve injury. Drug Discov Today Dis Models 1:177–185. doi:10.1016/j.ddmod.2004.09.008
2. Rochkind S, Quaknine GE (1992) New trend in neuroscience: low-power laser effect on peripheral and central nervous system (basic science, preclinical and clinical studies). Neurol Res 14:2–11
3. Noble J, Munro CA, Prasad VSSV (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma 45:116–122. doi:10.1097/00005373-199807000-00025
4. Varejão AS, Meek MF, Ferreira AJA, Patrício JAB, Cabrita AMS (2001) Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis. J Neurosci Methods 108:1–9. doi:10.1016/S0165-0270(01)00378-8
5. De Medinaceli L, Freed WJ, Wyatt RJ (1982) An index of the functional conduction of the rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 77:634–643. doi:10.1016/0014-4886(82)90234-5
6. Mendonça AC, Barbieri CH, Mazzer N (2003) Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods 129:183–190. doi:10.1016/S0165-0270(03)00207-3
7. Gigo-Benato D, Geuna S, Rochkind S (2005) Phototherapy for enhancing peripheral nerve repair: a review of the literature. Muscle Nerve 31:694–701. doi:10.1002/mus.20305
8. Raso VVM, Barbieri CH, Mazzer N, Fasan VS (2005) Can therapeutic ultrasound influence the regeneration of the peripheral nerves. J Neurosci Methods 142:185–192. doi:10.1016/j.jneumeth.2004.08.016

9. Basford JR (1995) Low intensity laser therapy: still not an established clinical tool. Lasers Surg Med 16:331–342. doi:10.1002/lsm.1900160404

10. Bagis S, Comelekoglu U, Sahin G, Buyukakilli B, Erdogan C, Kanik A (2002) Acute electrophysiologic effect of pulsed gallium-arsenide low energy laser irradiation on configuration of compound nerve action potential and nerve excitability. Lasers Surg Med 30:376–380. doi:10.1002/lsm.10057

11. Rochkind S, Nissan M, Alon M, Shamir M, Salame K (2001) Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats. Lasers Med Sci 28:216–219. doi:10.1002/lsm.1041

12. Campolat L, Kükner A, Campolat I, Ozan E (1999) Ultrastructural and morphometric analysis of peripheral nerve regeneration within silicone tubes. Turk J Med Sci 29:203–209

13. Zuo J, Neubauer D, Graham J, Krekoski CA, Ferguson TA, Muir D (2002) Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. Exp Neurol 176:221–228. doi:10.1006/exnr.2002.7922

14. De Sá JMR, Mazzer N, Barbieri CH, Barreira AA (2004) The end-to-side peripheral nerve repair functional and morphometric study using the peroneal nerve of rats. J Neurosci Methods 136:45–53. doi:10.1016/j.jneumeth.2003.12.018

15. Dijkstra JR, Meek MF, Robinson PH, Gramsbergen A (2000) Methods to evaluate functional nerve recovery in adult rats: walking track analysis, video analysis and the withdrawal reflex. J Neurosci Methods 96:89–96. doi:10.1016/S0165-0270(99)00174-0

16. Endo C (2002) Estudo dos efeitos do tratamento com laser num modelo experimental de lesão nervosa por esmagamento do nervo ciático em ratos [Dissertação – Mestrado] Universidade de São Paulo

17. Byrnes KR, Waynant RW, Ilev IK, Wu X, Barna L, Smith K et al (2005) Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. Lasers Surg Med 99:1–15

18. Temple CLF, Ross DC, Dunning CE, Johnson JA (2004) Resistance to disruption and gapping of peripheral nerve repairs: an in vitro biomechanical assessment of techniques. J Reconstr Microsurg 20:645–650. doi:10.1055/s-2004-861525

19. Snyder SK, Byrnes KR, Borke RC, Sanches A, Anders JJ (2002) Quantitation of calcitonin gene-related peptide mRNA and neuronal cell death in facial motor nuclei following axotomy and 633 nm low power laser. Lasers Surg Med 31:216–222. doi:10.1002/lsm.10098

20. Chelyshev IA, Kubitskii AA, Plakseichuk A (1996) Regeneratsiia nervnykh volokon pri obluchenii nizkointensivnymi lazerami. Morfologiia 110:47–50

21. Karu TI (1988) Molecular mechanisms of the therapeutic effect of low-intensity laser irradiation. Lasers Life Sci 2:53–74

22. Manteifel VM, Karu TI (2005) Structure of mitochondria and activity of their respiratory chain in successive generations of yeast cells exposed to He-Ne laser light. Izv Akad Nauk Ser Biol 32:556–566

23. Koka R, Hadlock TA (2001) Quantification of functional recovery following rat sciatic nerve transection. Exp Neurol 168:192–195. doi:10.1006/exnr.2000.7600

24. Dahlin LB (2004) The biology of nerve injury and repair. J Am Soc Surg Hand 4:143–155. doi:10.1016/j.jassh.2004.06.006