

## **Morphometric analysis of low level laser therapy (780 nm) in transected sciatic nerve regeneration after end to end neurorrhaphy in rabbit**

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### **Abstract**

Peripheral nerve transections represent common clinical occurrence. There are two main requirements for a good clinical outcome after these lesions. The first is surgical treatment so as to make possible the regeneration of the transected nerve fibers toward their original periphery. The second important requirement for successful recovery after a nerve lesion is postoperative rehabilitation treatment. In fact, although the surgical techniques have proved to lead almost always to the regeneration of nerve fibers, the degree of recovery can be highly variable among patients. The objective of this study was to investigate whether 780 nm low level lasers could stimulate the healing process in the sciatic nerve regeneration in rabbits submitted to complete transection through morphometric analysis. Twenty adult white New Zealand male rabbits were used, where the injury of the type neurotmesis of the right sciatic nerve under general anesthesia (intramuscular ketamine/xylazine and atropine sulfate) was approximated using prolene 6-0. The rabbits were randomly distributed in 2 groups with 10 rabbits each. In group I, Arsenate of Gallium Laser was used with the extension of wave of 780 nm, 10 mW powers with 1 J/cm<sup>2</sup> irradiation with 10 seconds for each Cm<sup>2</sup>, in the pulsed form. The laser therapy was initiated on the post-surgical first day with application 10 minutes for each rabbit in group I, once a day for 15 days. Rabbits in group II had not given treatment (control group). The samples of transected nerves were collected and prepared for morphometric analysis on the 90 days, they were analyzed the average number of total counted fibers and the mean fiber density on proximal, intermediate and distal segments. On group I and II, the average number of total counted fibers and the mean fiber density on proximal, intermediate and distal segments was significant in each group. The differences found for total number of counted fibers and for fibers density between proximal segments of groups I and II were not significant (p=0.31 and p=0.17, respectively). On intermediate segments (injury site), the total number of counted fibers was significantly higher in group I (p=0.05). On distal segments, both the total number and the density of fibers were significantly higher in group I (p=0.03 and 0.001, respectively). The irradiation with low level laser (780 nm) influenced positively the regeneration of the sciatic nerve in rabbits after being injured and end to end anastomosis, becoming the nerve recovery more rapid and efficient.

**Keywords:** Low level laser therapy; morphometric analysis; nerve regeneration; sciatic nerve; rabbit

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### **Introduction**

Nerves can be damaged either through disease or trauma. Trauma to the nerves can occur as a

consequence of motor vehicle accidents, severe falls, lacerations and typing. Traumatic injury, such as falls and motor vehicle accidents can lead to the severing of nerves. Several diseases can damage nerves.

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Several diseases can damage nerves. They include multiple sclerosis, diabetes, spina bifida, and polio.

Once the peripheral nerves are damaged, myelin sheaths and axons distal to the damaged area undergo inevitable Wallerian degeneration. This process of myelin phagocytosis incorporates Schwann cells and macrophages in its segmentation (Hirata and Kawabuchi 2002). Following a partial denervation of nerve, the intact and surviving motor axons in a peripheral nerve may go through distal collateral sprouting and enlargement of their motor unit size in a compensatory fashion (Rafuse et al., 1992). Methods of facilitating regeneration by maximizing the number of regenerated fibers are essential for a functional recovery. Surgical intervention of anastomosis (Ozkan et al., 2002) or titanium staples (Payne et al., 2002) (for a completely transected nerve) and laser irradiation (Khullar et al., 1995) (for a partially injured nerve) are becoming increasingly popular for therapeutic purposes. However, despite advances in the medical and surgical management of peripheral nerve injuries, recovery is often incomplete. Therefore it would be clinically beneficial to develop new treatments to accelerate and improve the recovery process.

Several experimental and clinical studies have been conducted to analyze the regeneration process and functional recovery of the nerve with the assistance of therapeutic resources, such as electrical stimulation (Mendonça et al., 2003), therapeutic ultrasound (Monte-Raso et al., 2005) and low level laser therapy (Rochkind et al., 2007; Rochkind et al., 2007; Endo et al., 2008). Laser affects tissues differently according to the wave-length, pulse duration, pulse/energy, energy density and delivery system. Thus, finding the optimal conditions for laser irradiation is essential in the application of laser in medicine.

The present study aimed to determine whether Ga-As (Gallium-Arsenide, wave-length: 780 nm) infrared laser irradiation could stimulate the healing process of experimentally injured sciatic nerves in rabbit.

## Materials and Methods

All rabbits of the present research were cared according to the norms of the Islamic Azad University Faculty of Specialized Veterinary Science Tehran Iran laboratory of animal experimentations. This investigation was approved by the Committee of Ethics in Research with animals in Islamic Azad University. Therefore 20 male adult white New Zealand rabbits were utilized, with body weight varying between 2.5 to 3 kg. The rabbits were maintained in individual stainless cages in adequate sanitary conditions, and kept them in the temperature-controlled environment (20°C), provided with water and standardized food *ad libitum*. Animals were divided randomly into two experimental

groups of 10 rabbits each: group treatment (group I) and group control (group II). The rabbits were anesthetized intramuscular whit solutions of ketamine hydrochloride 10% (50 mg/kg), xylazine hydrochloride 2% (5 mg/kg) and atropine sulfate (0.05 mg/kg). They were maintained in the left lateral recumbency. The site of the surgery was prepared on the caudal region of the right femoral area which was clipped and followed by local anti-sepsis. The 5 cm incision was given longitudinally to that of semi-membrane and semi-tendinous muscles. The sciatic nerve was exposed by longitudinal intramuscular separation semi-membranous and semi-tendineous and quadriceps muscles were carried out allowing the complete vision of the nerve (Fig. 1). With the help of scalpel blade, nerve was cut in to 2 pieces and was re-anastomosed using prolene 6-0 threads. Then, the surgical closure of the region was down with nylon 3-0 threads.

In group I, Arsenate of Gallium Laser (Ga-As Mustang 2000) (Fig. 2) was used with the extension of wave of 780 nm, 10 mW power, 1 J/cm<sup>2</sup> with irradiation of 10 seconds for each cm<sup>2</sup>, in the pulsed form (Fig. 3). Rabbits in group II were not treated (control group).

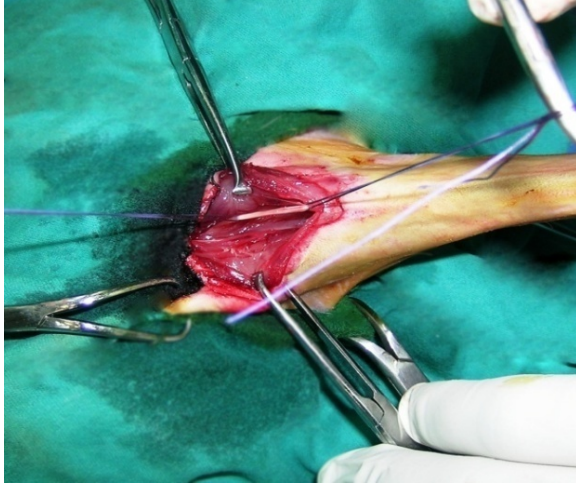
In group I, the laser therapy was initiated on the first day of surgery, where all the rabbits were treated once a day for 15 days. On the 90 days, all rabbits were submitted to euthanasia by an anesthetic overdoses, after that the 8 mm nerve specimen was harvested from each sciatic nerve, 4 mm proximal and 4 mm distal to the repair site. These segments were fixed in Karnovsky's solution and postfixed with osmium tetroxide, dehydrated in a series of alcohol solutions and finally embedded in epoxy resin. Thin transverse sections (1 µm thick) were cut and stained with toluidine blue for examination under the light microscope. For each nerve segment, images were analyzed with a digital image analysis system linked to a histomorphometry software (Sigma Scan Pro 5.0).

Once the number of total counted fibers and fiber density on proximal, intermediate and distal segment was counted, 5 sampling fields were selected by a random sampling technique.

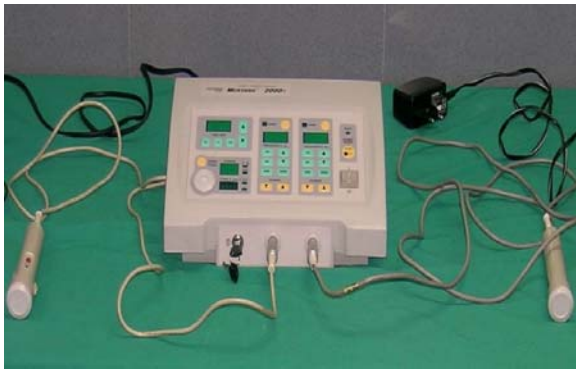
Statistical analyses were carried out using SPSS statistical software (version 16). Results were expressed as the mean ± standard deviation. The ANOVA with the Turkey's post-test were employed to analyze two groups consecutively. Values less than 0.05 were considered as statistically significant.

## Results

All the rabbits tolerated operation and survived until the final study period. Figure 4 illustrates representative photomicrograph of histological slices of intermediate segments (injury site) of sciatic nerves on group I and group II.



**Fig. 1: Dissection of rabbits sciatic nerve.**



**Fig. 2: GaAs laser equipment, extension of wave of 780nm.**



**Fig. 3: Transcutaneous method of laser application.**

Morphometric results are summarized in Table 1. In group I, the average numbers of fibers were 10016, 11378 and 10409 on proximal, intermediate and distal segment, respectively. The mean fiber density on proximal, intermediate and distal segments was 22417, 20532 and 23071 fibers/mm<sup>2</sup>, respectively, with a

significant difference between these segments ( $P < 0.05$ ). In group II, the average number of counted fibers was 7936, 6203 and 5085 on proximal, intermediate and distal segment, respectively. The mean fiber density on proximal, intermediate and distal segments was 19094, 15969 and 13210 fibers/mm<sup>2</sup>, respectively, with a significant difference between these segments ( $P < 0.05$ ). The difference found in total number of counted fibers and fibers density between proximal segments of groups I and II were not significant ( $P = 0.17$  and  $P = 0.31$ , respectively). On intermediate segments (injury site), the total number of counted fibers was significantly higher in group I ( $P = 0.05$ ), but not for fibers density ( $P = 0.86$ ). On distal segments, both the total number and the density of fibers were significantly higher in group I ( $P = 0.03$  and  $0.001$ , respectively).

## Discussion

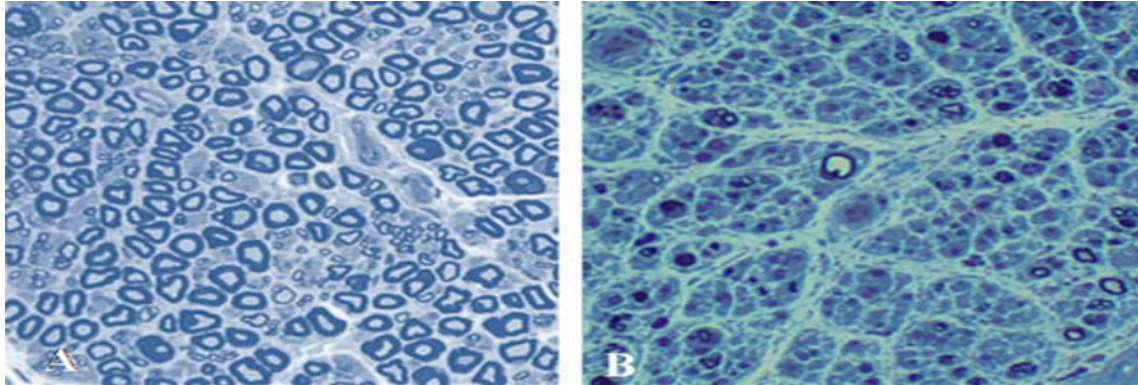
Low level laser is one of the types of biostimulants used most often in rehabilitation at the present time, which has contributed to a better understanding of its principles and range of applicability (Schindl et al., 2000). According to Enwemeka (Enwemeka et al., 2004) low level laser therapy is being widely used in situations of healing processes, aiming to obtain faster tissue healing. Its success is suggested by the particularities of responses induced in the tissues, such as decrease of the inflammatory process, increase of phagocytosis, edema reduction, increase of collagen synthesis and epithelization. Low level laser irradiation has also been indicated due to its ability to accelerate the formation of new vessels after tissue injury (Agaiby et al., 2000).

Various preliminary studies, both clinical and experimental, demonstrated that low level laser therapy has positive effects on the regeneration of peripheral nerve injuries (Rochkind et al., 2007; Endo et al., 2008; Reis et al., 2009; Belchior et al., 2009). Bagis et al. (2003) did not observe beneficial effects of the use of low level laser (904 nm) on nerve injuries. Therefore, finding the optimal wave-length, pulse duration, pulse/energy, energy density and delivery system for laser irradiation is essential in the application of laser in medicine.

In literature, there is a considerable number of studies with low level laser, yet there is no standardization of the parameters employed and the shortage of data in the studies found hindered the comparison of results and the understanding of some mechanisms involved. Accordingly, new surveys are necessary to verify the importance and the dependence between each one of the laser parameters, as well as the possible influences on the biological responses.

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**Fig. 4: Representative photomicrograph from intermediate segments (injury site). Toluidine blue staining. Myelin is stained in blue. The nerve tract is filled with ring-shaped structures, indicating a transverse section of myelinated nerve fibers. A: group I. B: group II, diffuse loss of large myelinated axons. Compare this with a section of group I.**

**Table 1: Total number and density of nervous fibers on proximal, intermediate and distal segments of the sciatic nerve for group I and II.**

	Group I			Group II		
	prox.*	interm.**	dist.***	prox.	Interm.	dist.
Total number of fibers(mean $\pm$ SD)	10016 $\pm$ 1973	11378 $\pm$ 3827	10409 $\pm$ 4002	7936 $\pm$ 2861	6203 $\pm$ 4093	5085 $\pm$ 2498
Fibers density(mean $\pm$ SD)	22417 $\pm$ 2328	20532 $\pm$ 6718	23071 $\pm$ 6120	19094 $\pm$ 7653	15969 $\pm$ 11002	13210 $\pm$ 4361

\*The differences found for total number of counted fibers and for fibers density between proximal segments of groups I and II were not significant ( $p=0.17$  and  $p=0.31$ , respectively);\*\* On intermediate segments (injury site), the total number of counted fibers was significantly higher in group I ( $p=0.05$ ), but not for fibers density ( $p=0.86$ ); \*\*\* On distal segments, both the total number and the density of fibers were significantly higher in group I ( $p=0.03$  and  $0.001$ , respectively).

standardization of the parameters employed and the shortage of data in the studies found hindered the comparison of results and the understanding of some mechanisms involved. Accordingly, new surveys are necessary to verify the importance and the dependence between each one of the laser parameters, as well as the possible influences exercised on the biological responses, in order to thus improve the specificity of laser therapy as well as the preparation of protocols with safer and more effective treatments.

In the present research, morphometric analysis in the laser-treated group showed an increased total number of axons and an increased number of large-diameter axons, compared to the control group. The study suggested that postoperative 780 nm low level laser therapy enhanced the regenerative processes of peripheral nerves after complete transection.

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