

In-Vivo Experimental Study of Facial Nerve Repair by Diode Laser (980 nm.) Welding Vs Microsuturing: Functional and Histopathological Evaluations

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ABSTRACT

Regaining nerve function after nerve injury is still a clinical problem inspite of the great advents of the techniques of nerve repair. Nerve repair by laser welding is one of these new techniques, which has many advantages over the microsuturing nerve repair. For this reason, the aim of the present study is to evaluate the effectiveness of diode laser for facial nerve repair from the functional and histopathological points of view. Forty facial nerves from twenty adult mature Velandar rabbits were divided into two equal treatment groups. Group (A), is the diode laser facial nerve coaptation group and group (B), is the microsutured facial nerve coaptation group. Eight weeks latter, the repairs were evaluated by electrophysiologic, light microscopic and transmission electron microscopic evaluating parameters. The favorable results of diode laser for facial nerve repair at all the evaluating parameters denoted its superiority over the microsuturing technique. Diode laser was found to be effective for a rapid, accurate and safe technique for facial nerve repair at minimal side effects.

INTRODUCTION

Re-establishment of nerve function after nerve injury remains a puzzling matter. In spite of the advent of the surgical microscope and fine suture materials for fascicular, group fascicular repairs and nerve grafting, none of them has approached the ideal goal to approximate each severed axon to its appropriate distal counterpart [1]. Laser nerve coaptation is a new technique at its early footsteps. It has a group of advantages, which include reduction of the surgical time, extreme precision, repair nerves especially those of small caliber, deep positions and at important anatomical relationship. It minimizes tissue manipulation for lesser tissue damage and greatly limits the possibility of gapping that occurs with microsuturing which allows for the exit of axon sprouts and the en-

trance of scar tissue with a higher incidence of neuroma formation [2].

There are many types of laser nerve repair. They are mainly the minicuff of blood and the heat-induced fusion of the epineurium. In the first type, a coagulum is produced around single or multiple fascicles to adhere and realign both ends of the injured nerve. In the second type, a coagulum is formed all over the adjacent structures for a true bonding of both ends of the injured nerve [3,4].

Carbon dioxide (CO₂) laser (wavelength 10600 nm.), is a non-selective laser that uses water as a chromophore. Any tissue will absorb it almost completely. CO₂ milliwatt laser has been previously tested [5] with several ongoing studies [6,7] to evaluate its effect on nerve repair. These studies reported a favorable response on sciatic nerve repair, which are recently applied for facial nerve repair [8]. CO₂ laser produces a limited amount of energy, which is absorbed at the superficial layer of the nerve to seal the adjacent margins of the coapted nerve ends [6-8]. On the other hand, Neodymium: Yttrium Aluminum Garnette (Nd:YAG) laser (wavelength 1064 nm.) has more theoretical advantages. It is more tissue-selective and can be delivered through an easy fiber-optic system. It has a specific affinity for the collagen fibrils which loose their periodicity, increase in caliber and then split into fine fibrillary substructures. These substructures become more amorphous, homogeneous with neither foreign body reaction nor carbonaceous deposits at the surroundings that was reported during CO₂ laser nerve welding [9]. However, the reported unfavorable response

with both CO₂ and Nd:YAG lasers was the escapement of some axon fibers at the site of nerve injury with a higher incidence of neuroma formation [10,11].

The favorable results with Nd:YAG laser nerve welding encouraged the introduction of diode laser at different wavelengths. It was used for welding of various tissue types [12]. Diode lasers are tissue-specific and have the ability to be delivered through an easy fiberoptic system. Diode lasers could be applied with different solders; either dye-enriched or not which were carried out in-vitro experimentally with lack of in-vivo evaluation [2,12-16].

Albumin solders from different species have been used for laser welding with inconsistent success rates [17]. This can be attributed to many variants namely; laser irradiance, exposure time, as well as, solder composition and chromophore type and their concentrations. Diode laser tissue welding by using dye enriched protein solder was applied topically to the tissue surface and denatured by laser [18]. This was achieved either as a premixed or as a separate dye-solder technique [19]. The depth of energy absorption, heat generation and the tensile strength of the mould were predominantly determined by protein solder and Indocyanine (ICG) dye concentrations [20,21]. ICG dye acts as a chromophore to limit the area of heat generation at the solder-tissue interface for selective tissue damage. Some authors claimed that the mechanism of welding is wavelength-dependant, while others considered the photo-thermal rather than photochemical theory [6,7,15]. This idea depends upon that temperature at which welding occurs is ranging between 60-100°C which is higher than that needed for collagen denaturation at 60°C.

The type, amount and concentration of the protein solder contribute to the weld strength. Excess solder blocks the sprouting axons and increases scar formation, while, thick solder coagulates superficially resulting into a weak bond. It acts as a homogeneous cast for collagen fibrils to embed forming an exogenous and endogenous glue. There are many types of solder material including albumin suspension of different forms, fibrin glue, fibrinogen suspension, red blood corpuscles and egg white. The immunologic reaction to the protein solder is practically of no value since the protein solder denatures and loses its antigenicity. However, egg

white is used only for experimental studies despite of its stronger welds due to the higher potential of contamination [22]. Moreover, ICG dye concentration could affect the bond strength, as low ICG dye concentration increases the penetration depth of the laser energy into the solder for better mould [13].

It is to be mentioned that the reported studies for welding were lacking for the use of diode laser (wavelength 980 nm.). For this reason, it was found interesting to evaluate the histopathological and functional changes of the facial nerves after diode laser (wave length 980 nm.) nerve coaptation in vivo in rabbits in comparison to the traditional microsuturing technique.

MATERIAL AND METHODS

Animal model:

The present study was carried out at the National Institute of Laser Enhanced Sciences (N.I.L.E.S.), Cairo University. Twenty adult mature Veldner rabbits (body weight of 3.0-3.5 kg. and age of 6-7 months) were anaesthetized by a single intramuscular injection of Ketamine hydrochloride (35 mg/kg body weight) and Xylazine hydrochloride (5 mg/kg body weight). Facial nerves bilaterally (n = 40 nerves) were exposed by transverse incisions in the cheeks below the eyes (Fig. 1). The nerves were identified electro-physiologically by measuring the nerve action potential and excitation curves before and immediately after nerve transection (Fig. 2: A,B and C). This was carried out by inserting two electrodes of the biotech data acquisition device in the nerve substance (Fig. 1). The device parameters were kept constant at 500 pulses per second, a fixed time of 30 seconds and pulse speed of 2 seconds. These parameters can detect the nerve current equal to 90 millivolt. The nerves were then divided into two equal groups, 20 facial nerves for each group.

Group A: Diode laser nerve coaptation technique:

Twenty right facial nerves were transected almost at the same level and were immediately repaired by use of diode laser (wave length 980 nm.), (Premier Laser Systems, model LM-2500). Diode laser was delivered at a power output of 500 mW, continuous wave, through a 600 µm core bare fiber, 0.38 NA fiber, fitted with a SMA-905 fiber connector. A combination of 20% human albumin as a solder and ICG dye at a

concentration of 0.25 mg/ml as a chromophore by a separate dye-solder technique were applied drop-wisely at the site of the repair at the time of application of diode laser. These parameters have previously been shown to produce the strongest bonding strength in vitro during laser nerve coaptation technique [17]. The surface temperature at which welding was achieved as denoted by the visual change of the protein solder, was measured by a Thermocouple (Fluke 52 K/J thermometer). By the end of the procedure, one-to-two stay micro-sutures may be added to maintain the proper coaptation of both ends of each repaired nerve.

Group B: Microsuturing nerve coaptation technique:

Twenty left facial nerves were transected almost at the same level and were immediately repaired with microsuturing by approximation the epineurium with 3-4 micro-sutures using 0/9-0/10 prolene sutures on round needles. The procedure was carried out with the aid of an operating microscope (ZEISS OPMIFC, Germany) under X 40 magnification.

After wounds closure, all the rabbits were returned back to their cages and were fed ad libitum with addition of a prophylactic antibiotic to their drinking water.

Evaluating parameters:

I- Functional evaluation:

Eight weeks later, all the facial nerves bilaterally (n = 40 nerves) were exposed again. Each facial nerve was examined for electrophysiological evaluation namely action potential and excitation curves by the same procedure previously mentioned.

II- Histopathological evaluation:

After electrophysiological evaluation, the nerves were removed and marked as a proximal-to-distal orientation. From each treated group, randomly selected 10 nerve specimens were examined with light microscopy while the other 10 specimens were examined with transmission electron microscopy (TEM).

A- Light microscopy:

Specimens were trimmed and immediately fixed in 10% formaldehyde for 2 days, then washed by distilled water and left in 70% ethylalcohol overnight at room temperature. Dehy-

dratation of the specimens was completed by 96% followed by absolute ethylalcohol for an hour. Subsequently, the specimens were immersed in 1% celloidin methyl benzoate overnight at room temperature then embedded in paraffin. From each paraffin block, 5 sections of 5 µm thickness were obtained at the longitudinal plane. Sections were subjected to Haematoxylin and Eosin (Hx & E) and silver stain, in addition to Mallory staining for the connective tissue. Ten fields were examined in each specimen using an objective lens of X 40 magnification.

B- Transmission electron microscopy (TEM):

Specimens were immediately fixed in 3% glutaraldehyde solution buffered with cacodylate, postfixed with 1% osmium tetroxide, stained with uranyl acetate, dehydrated in acidified 2,2-dimethoxypropane and embedded in epoxy resin. After hardening, cross-sections of 1.25 µm thickness were cut with an ultramicrotome (Ultra-cut E, Reichert-Jung, Germany) and stained with toluidine blue and basic fuschine and examined in a transmission electron microscope (Philips E.M. 420, Eindhoven, Netherlands) at X 7500 magnification.

RESULTS

On re-exposure of the facial nerves, by their gross appearance, the nerves had apparently normal looking at both groups. This gave a false impression of a normal morphology of the repaired facial nerves.

Evaluating parameters:

I-Functional evaluation:

In group A, of diode laser nerve coaptation, the action potential and excitation curves showed almost the same normal pattern and with higher amplitude of nerve excitation curve (Fig. 3: A and B) in relation to those obtained before cutting the nerves. This indicates the regaining of the electric conductivity of the diode laser welded facial nerves at a normal pattern.

On the other hand, in group B, of microsutured nerve coaptation, electro-physiologic curves showed noisy bizarre-shape pattern with higher amplitude at the excitation curve (Fig. 4: A and B) in relation to those obtained before cutting the nerves. This denoted the regaining of the electric conductivity of the microsutured facial nerves but in an abnormal pattern.

II- Histopathological evaluation:

A- Light microscopy:

Regarding group A, of diode laser nerve coaptation, Fig. 5 (A and B) showed well-organized healing pattern with little fibrous tissue inbetween the two cut ends of the nerves. There are regenerating axon sprouts in the areas previously filled with the protein solder. Those axons appeared thin, less wavy and less myelinated. The regenerating axons appeared to be partially originating from the proximal end of the injured nerve and partially from the subepineural axons just below the epineurium. None of the laser-welded nerves showed any disorganized bulbs or outgrowths of neuroma formation. Moreover, there was no apparent increase in the inflammatory cellular infiltration at the site of the healing.

On the other hand, at group B, of microsutured nerve coaptation, Fig. 6 (A and B) showed apparent discontinuity between both ends of the injured nerves. Fibrous tissue did not fill completely the area between both ends of the nerve with formation of gaps. The regenerating nerve axons appeared relatively few with poor alignment and no subepineural regenerating axons with excess connective tissue interposition. These findings collectively could explain the higher tendency for neuroma formation at its early stage. Finally, there was no apparent increase in the mononuclear cells at or nearby the area of healing.

B- Transmission electron microscopy (TEM):

TEM of nerve specimens of group A, of diode laser nerve coaptation (Fig. 7) revealed regenerating myelinated nerve fibers of high density with peripherally located degenerated nerve fibers and blackening of their myelin sheath. There were different diameters of unmyelinated regenerated nerve fibers with others at sequential degrees of myelination (Fig. 8). Neither inflammatory cellular infiltration, nor Schwann cell excitation for mitotic activity was detected.

TEM of nerve specimens of group B, of microsutured nerve coaptation (Fig. 9) revealed regenerating myelinated nerve fiber at low density. Easily distinguishable macrophages with numerous numbers of phagosomes were detected. Other field shows inflammatory cellular infiltration mainly lymphocytes (Fig. 10). Figs. (11 & 12) revealed Schwann cells hyperactivity, which was manifested by active rough endoplasmic

reticulum and actively mitotic dividing Schwann cell nucleus respectively.

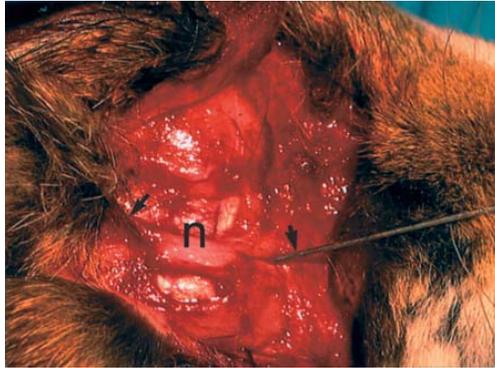


Fig. (1): Dissected facial nerve was exposed (n). Two electro-physiological electrodes were inserted into the nerve (arrowhead).

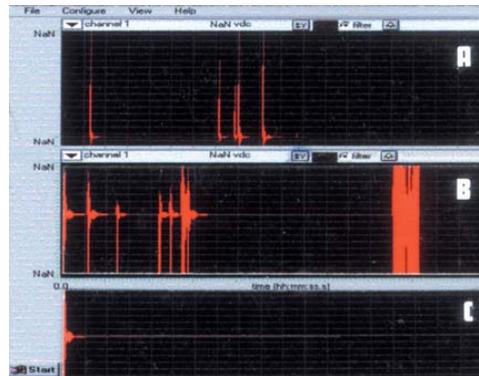


Fig. (2): Normal electrophysiological examination of the facial nerve. (A) Normal action potential curve before cutting the nerve. (B) Normal nerve excitation curve before cutting the nerve. (C) Flat curve of lost nerve conductivity after cutting the nerve.

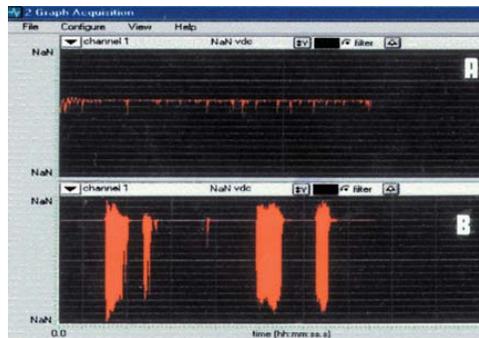


Fig. (3): Electrophysiological examinations of facial nerves of group A (diode laser nerve coaptation). (A) Action potential curve. (B) Nerve excitation curve.

Fig. (4): Electrophysiological examination of facial nerves of group B (micro-sutured nerve coaptation). (A) Action potential curve. (B) Nerve excitation curve.

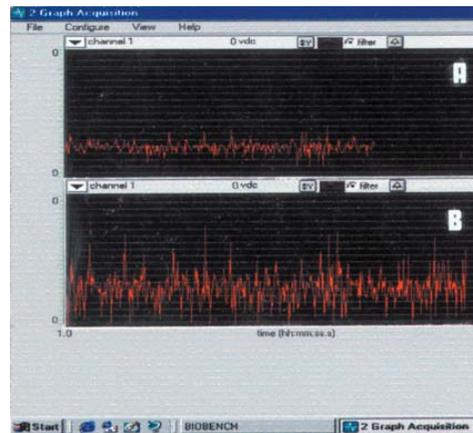


Fig. (5): Micrograph of light microscopic examination of facial nerve specimens of group A (diode laser nerve coaptation) stained with:

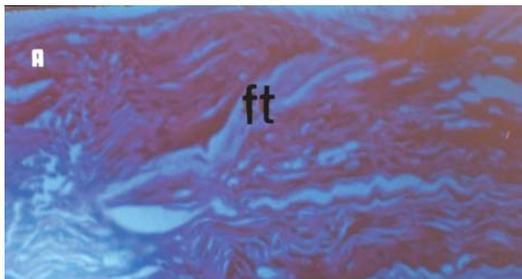


Fig. (5-A): HX and E stain: revealed well-organized arrangement of nerve axons. No prominent fibrous tissue (ft). Regenerating axon sprouts are observed. No apparent inflammatory cellular infiltration.

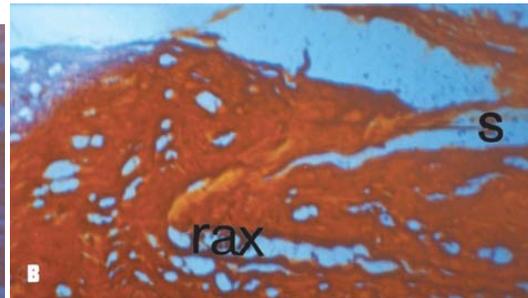


Fig. (5-B): Silver and Mallory trichrome stain: revealed thin, wavy, less myelinated axon sprouts. The regenerating axons (rax) originate partially from the proximal end of the cut nerve and partially from the subepineural axons (s).

Fig. (6): Micrograph of light microscopic examinations of facial nerve specimens of group B (micro-sutured nerve coaptation) stained with:



Fig. (6-A): HX and E stain: revealed apparent discontinuity and gapping (g) between both end of the cut nerve. No detected increase at the inflammatory cellular infiltration.

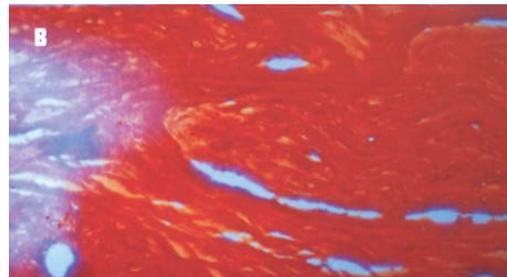


Fig. (6-B): Silver and Mallory trichrome stain: revealed few regenerating nerve axons with poor alignment and gaps. No subepineural regenerating axons and excess connective tissue element at the site of the cut of the nerve. There was no apparent increase in the mononuclear cells at or nearby the area of healing.

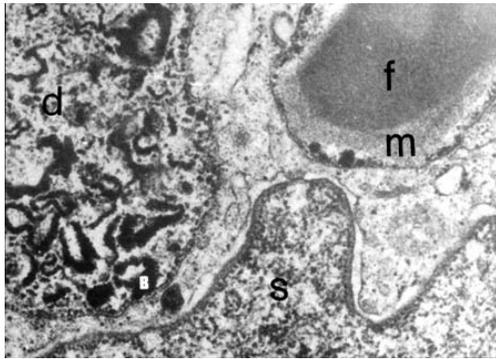


Fig. (7): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group A (diode laser nerve coaptation) shows regenerating myelinated (m) nerve fiber (f) of high density and degenerated nerve fiber (d) with a blackening of the myelin sheath around (B) surrounded by Schwann cell cytoplasm (s).

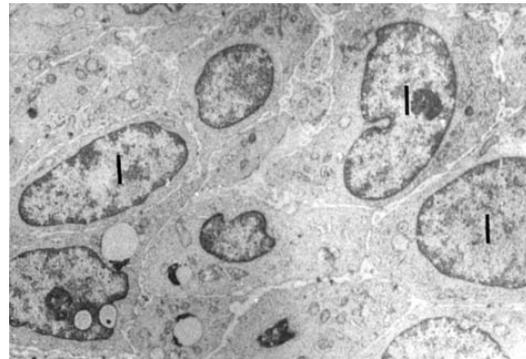


Fig. (10): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group B (microsutured nerve coaptation) shows numerous inflammatory cells mainly lymphocytes (l) which denotes starting an inflammatory process.

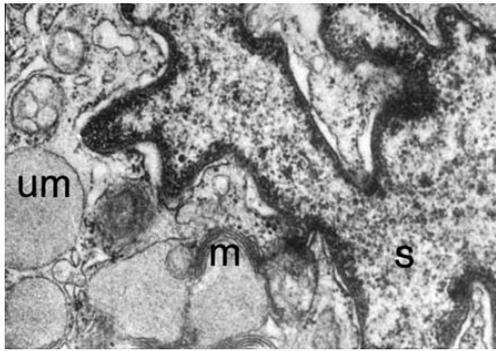


Fig. (8): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group A (diode laser nerve coaptation) shows regenerating unmyelinated (um) and myelinated (m) nerve fibers. The latter exhibits an outer collar of myelin filaments. They are embedded into Schwann cell cytoplasm (s).



Fig. (11): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group B (microsutured nerve coaptation) shows active rough endoplasmic reticulum (rer) with numerous ribosomes on their surface.

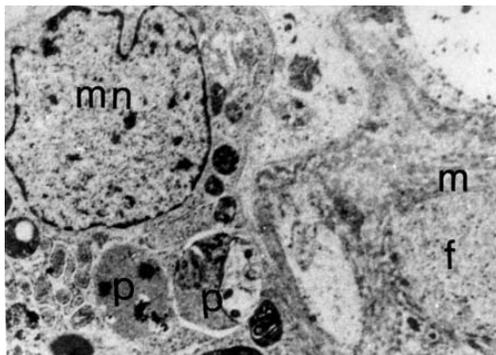


Fig. (9): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group B (microsutured nerve coaptation) shows regenerating myelinated (m) nerve fibers (f) of low density with adjacent macrophage with its nucleus (mn) with numerous phagosomes (p) at its cytoplasm.

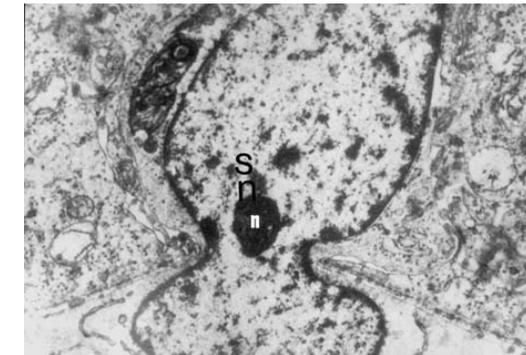


Fig. (12): Micrograph of TEM examination (original magnification X 7500) of facial nerve specimens of group B (microsutured nerve coaptation) shows activity of Schwann cell as was denoted by active mitosis at its nucleus (sn).

DISCUSSION

The choice of the facial nerve in rabbits for the present study was based on its approximate caliber to that of the human being. However, most of the reported experimental studies were carried out on the sciatic nerve due to its relatively more easily exposure [6,7]. Despite of the relative small number of the present materials, it seemed quite obvious to express the superiority of diode laser (wavelength 980 nm.) nerve repair over microsuturing. These results are in good agreement with those obtained with diode lasers of wavelengths 808 and 830 nm., as well as, with Nd:YAG laser (wavelength 1064 nm.) [11,15,21].

The type, amount and concentration of both the protein solder and the dye as a chromophore are important factors that can contribute to the success of the laser nerve coaptation technique [14,17,20,21]. ICG dye was used by the separate dye-solder technique [19]. The dye, by this technique, is more stable than the premixed dye-solder method, which is simpler and faster. The separate dye-solder method increases the durability of the solder because the dye is mixed at the time of the experiment, thus conserving its spectral absorbency properties.

The recorded temperature at which welding occurred in the present work was found to be $64 \pm 3.2^\circ\text{C}$ which supports the hypothesis of photothermal theory [6,7,15]. The only opponent concept was by those who reported a welding temperature below 60°C by using argon laser [3]. The most accepted theory is that the laser beam acts on the extra-cellular proteins, collagen, exogenous solder and on the leaked intracellular proteins after cellular damage, which will undergo a photothermal degradation of the bonds and formation of new molecular bonds [4,9,15].

Regarding the evaluating parameters in the present study, first; the electro-physiological examination of the facial nerves, showed almost normal action potential and excitation curves among group A. This denotes the regaining of normal nerve conductivity after diode laser repair, which could be attributed to the precise action of the laser beam for better alignment of the regenerating axons and less gaps. The same basis could explain, on the other hand, the noisy bizarre shaped action potential and nerve excitation

curves among group B, denoting the regaining of the nerve electric conductivity but in an abnormal pattern after microsutured nerve repair inspite of the apparently normal morphology of the specimens. This histopathological-based explanation was previously interpreted about the mechanism of laser nerve welding to diminish the incidence of neuroma formation [4,6-8,10,11].

Secondly, regarding the histopathological examination, an interesting observation of high number of the extrafascicular regenerating nerve fibers within the epineurium was noticed at specimens of group A. This was previously explained by the watertight sealing of the epineurium by the protein solder, which prevented the sprouting nerve fibers to grow outside the epineurial tube. These fibers remain extrafascicular within the epineurium resulting into better functional results as a higher number of the nerve fibers reached the target organ [4]. The amount, shape and density of the regenerating axons and their origin, as well as, the amount of the connective tissue interposition with gap formation could explain the stimulatory effect of diode laser for axon regeneration.

Finally regarding the neuroma formation, previous investigations [10,11] suggested three conditions for its formation. These conditions are axonal growth by Schwann cells proliferation at the proximal and distal nerve stumps, damaged perineurium and open endoneurial tubules. These three adverse events can be successfully avoided by use of diode laser welding for nerve repair. During laser nerve repair, Schwann cells proliferation is prevented most likely due to the photothermal effect on the cells as was denoted in the present study by the presence of degenerating nerve fibers with blackening of their covering myelin sheaths. Moreover, intact epineurium and endoneurium could be re-established after coagulation and watertight sealing of the nerve. These events contribute for the better histopathological and functional results after diode laser nerve coaptation technique. On the contrary, in group B, TEM showed early Schwann cell hyperactivity as was denoted by their mitotic activity and rough endoplasmic activity. These findings globally could strongly explain the higher incidence of neuroma formation after nerve repair by microsuturing especially with the existence of high inflammatory cellular infiltration.

Conclusion:

Diode laser (wavelength 980 nm.) welding could be used safely for rapid, accurate and effective nerve repair at minimal side effects. It was found to be more superior to the traditional microsuturing technique for nerve repair at all the evaluating parameters. Diode laser welding for nerve repair expressed not only the normal electrophysiological readings which denote the regaining of normal nerve conductivity for better nerve function but also normal histopathological findings with both light and transmission electron microscopic studies.

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