Background: Posttraumatic nerve repair and prevention of muscle atrophy represent a major challenge in restorative medicine. Considerable interest exists in the potential therapeutic value of laser phototherapy for restoring or temporary preventing denervated muscle atrophy as well as enhancing regeneration of severely injured peripheral nerve.

Methods:
I-Muscle and Peripheral Nerve Experimental Studies: low power laser irradiation (laser phototherapy) was applied for treatment of rat denervated muscle in order to estimate the biochemical transformation at cellular and tissue levels, as well as in a rat sciatic nerve model after crush injury, direct anastomosis and neurotube reconstruction.
II- Intraoperative Clinical Study: following microsurgical release and neurolysis the nerve was subjected to direct laser irradiation using fiber optics.
III- Clinical double-blind, placebo-controlled randomized study: based on the outcome of the animal trials, a clinical trial, which measured the effectiveness of 780-nm laser phototherapy on patients suffering from incomplete peripheral nerve injuries for 6 months up to several years, was designed.

Results: Experimental model of denervated muscle: the animal study suggested that function of denervated muscles was partially preserved by temporary prevention of denervation–induced biochemical changes. The function of denervated muscles was restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury.
Experimental peripheral nerve injury: laser phototherapy had an immediate protective effect, maintained functional activity of the injured nerve, decreased scar tissue formation at the injury site, decreased degeneration in corresponding motor neurons of the spinal cord and significantly increased axonal growth and myelinization.
Intraoperative clinical study: direct laser irradiation was shown to improve functional activity of the surgically treated nerve.
Clinical, double-blind, placebo-controlled randomized pilot study: in patients with incomplete long-term peripheral nerve injury, it was demonstrated that 780-nm laser irradiation progressively improved peripheral nerve function, which lead to significant functional recovery.

Conclusions: Laser phototherapy temporarily preserves the function of a denervated muscle and accelerates and enhances axonal growth and regeneration after peripheral nerve injury or reconstructive procedures. Animal and clinical studies have both demonstrated the promoting action of phototherapy on peripheral nerve regeneration, which makes it possible to suggest that the time for broader clinical trials has come.

Key Words: denervated muscle, peripheral nerve, laser phototherapy
Peripheral Nerve Injury

When muscles are denervated, in cases of complete peripheral nerve injury, they deteriorate progressively. Although some muscle regeneration does occur, it is at a level insufficient to replace the degenerative loss. There is a need to find effective methods for muscle preservation and nerve regeneration enhancement, especially after surgical nerve repair. Surgical repair is the preferred modality of treatment in case of a complete or severe peripheral nerve injury. In most cases, the results can be successful if the surgery is performed in the first six months after injury, in comparison to long-term cases where surgical management is less successful. Nonetheless, there are several publications in the relevant literature on the surgical treatment of long-term injuries (most of which were severe, incomplete and with minimal or partial preservation of muscle activity) of the brachial plexus and peripheral nerves. The reason for early surgical intervention has to do with the fact that between 1 and 3 years post-injury, denervated muscle undergoes necrotic degeneration, which leads to loss of muscle fibers and their replacement with fat and fibrous connective tissue. For most patients who suffer from long-term peripheral nerve injuries, spontaneous recovery is often unsatisfactory. The usual results after such an injury are degeneration of the axons and retrograde degeneration of the corresponding neurons of the spinal cord, followed by a very slow regeneration. Recovery may eventually occur, but it is slow and frequently incomplete. The secondary effects of peripheral nerve injury are wasted muscles and a high incidence of pressure sores. Understandably, therefore, numerous attempts have been made to enhance and/or accelerate the recovery of injured peripheral nerves and decrease or prevent atrophy of the corresponding muscles. Among the various proposed methods for enhancing nerve repair, phototherapy has received increasing attention over the last two decades. The term phototherapy refers to the use of light for producing a therapeutic effect on living tissues, but in an athermal and atraumatic manner. Although a pioneering report on the effects of laser phototherapy on the regeneration of traumatically injured peripheral nerves was published by Rochkind in 1978, it is only since the late 1980s that scientific interest was kindled in this therapeutic approach for neural rehabilitation, leading to the publication of a number of studies that have shown positive effects of phototherapy on peripheral nerve regeneration. A possible mechanism of action of phototherapy on the nervous tissue with respect to peripheral nerve regeneration has been provided by in vitro studies which showed that phototherapy induces massive neurotrophic sprouting and outgrowth in cultured neuronal cells, as well as Schwann cell proliferation. Also, it has been suggested that phototherapy may enhance recovery of neurons from injury by altering mitochondrial oxidative metabolism, and guide neuronal growth cones in vitro, perhaps due to the interaction with cytoplasmic proteins and, particularly, to the enhancement of actin polymerization at the leading axon edge. Phototherapy alters nerve cell activity, including upregulation of a number of neurotrophic growth factors and extracellular matrix proteins known to support neurite outgrowth.

A possible molecular explanation was provided by demonstrating an increase in growth-associated protein-43 (GAP-43) immunoreactivity in early stages of rat sciatic nerve regeneration after phototherapy. Another study showed that application of phototherapy upregulates calcitonin gene-related peptide (CGRP) mRNA expression in facial motor nuclei after axotomy. By altering the intensity or temporal pattern of injury-induced CGRP expression, phototherapy may optimize the rate of regeneration and target innervation and neuronal survival of axotomized neurons.

The purpose of this review is to summarize data on how laser phototherapy affects long-term denervated muscles and show the effectiveness of laser phototherapy in treating severely injured peripheral nerve, based on our 30 years of research.

I. PHOTOTHERAPY IN DENERVATED MUSCLE PRESERVATION

Using the denervated rat gastrocnemius muscle (in vivo) as a study model, we investigated the influence of low incident levels of laser irradiation on creatine kinase (CK) activity, which is an important enzyme for supplying a source of energy to the muscle, and the level of acetylcholine receptors (AChR), which play a special role in neuromuscular transmission, in denervated muscle in order to estimate their biochemical transformation at cellular and tissue levels. Much of the literature on the effects of long-term denervation of mammalian skeletal muscle has focused on experimental studies of total sciatic section in rats. In our study, rats were chosen for investigation in the vast majority of cases due to their availability, good survival record and ease of treatment. For the surgical proce-
dure Wistar rats were anesthetized and complete denervation of the gastrocnemius muscle was done (cut and removal of a 1 cm segment of the sciatic nerve). The rats were postoperatively divided into 4 groups: Group I – denervated non-irradiated group (15 rats); Group II – denervated laser treated group (15 rats); Group III – intact non-irradiated group (15 rats), and Group IV – intact irradiated group (15 rats). The rats underwent laser treatment (HeNe laser, 35 mW, 30 minutes) every day, for 14 days. Laser and light irradiation at low intensities was delivered transcutaneously to the gastrocnemius muscle of denervated Group II and intact Group IV. Under general anesthesia, the rats were sacrificed and the gastrocnemius muscle was homogenized.

**CK activity** was measured by the specific spectrophotometric method [28] [29] using a spectrophotometer at 340 nm and a Sigma kit at 7, 30, 60 and 120 days after denervation in both denervated and intact muscles.

*Internal and membrane inserted AChR* was quantitated using $^{125}$I-alpha-bungarotoxin on the same homogenates [30] [31] at 7, 30, 60 and 120 days after denervation in both denervated and intact muscles. The data obtained were evaluated as the counts per minute (cpm) of bound $^{125}$I-a-BuTX/mg protein. Radioactivity was assessed with an Auto-Gamma Counter in denervated and intact muscles.

A. Creatine Kinase (CK) Activity in Intact and Denervated Rat Gastrocnemius Muscle

Muscle contraction and relaxation require the action of CK. Phosphocreatine, formed by the reaction of this enzyme, constitutes a reservoir of high energy phosphate which is available for quick resynthesis of ATP. This high concentration of ATP is then accessible for muscle contraction. Following muscle denervation, the level of CK and muscle weight decreases. [32] As in previous reports [33] we found that in the control non-irradiated group, denervation of the gastrocnemius muscle reduced creatine kinase activity. [25] In that study, the decrease in CK activity in both groups (non-irradiated and laser-treated) progressed to a similar value for 7 days after denervation and was followed by a sharp fall in the non-laser treated group in comparison to the delayed and attenuated decrease of the CPK activity in the laser-irradiated group. Thus, in the control non-irradiated group, 30 days after denervation, the amount of CK decreased markedly to 41% of the normal value (intact muscle). At the same time delayed and attenuated decrease in the CK activity was observed in the laser treated group. The CK activity of the laser treated denervated muscle decreased only to 83% of the normal value. The analysis of CK activity in the denervated laser treated group, compared to the control denervated group showed a statistically significant difference ($p = 0.008$). After the 30-day period the CK activity gradually began to decrease in both groups and 4 months after denervation it reached similar levels (Fig. 1). In denervated muscle the protein degradation rate is recognized as being accelerated. [32] The temporary prevention of denervation-induced biochemical changes may be prompted by a trophic signal for increased synthesis of CK, thus preserving a reservoir of high energy phosphate available for quick resynthesis of ATP. This data supports the results from Bolognani (1991), [34] which showed that laser irradiation increased ATP production in the mitochondria.

B. Acetylcholine Receptors (AChR) Synthesis in Intact and Denervated Rat Gastrocnemius Muscle

Acetylcholine receptors (AChRs), which play a special role in neuromuscular transmission, are concentrated at the neuromuscular junction. A nerve impulse triggers the release of acetylcholine, producing a much larger end-plate potential, which excites the muscle membrane and leads to muscle contraction. The amount of AChRs in neuromuscular transmission appears to increase and to cover the entire extra-junctional area following muscle denervation. In the denervated muscle, the amount of AChRs increase prior to muscle degeneration. [35]

In the control non-irradiated group, 7 days after

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**Fig. 1** Graph illustrating the results of a study evaluating creatine kinase (CK) activity (unit/mg protein) in intact and denervated rat gastrocnemius muscle. The graph shows CK levels (unit/mg protein) at 7, 30 and 120 days in intact and denervated muscles with and without laser treatment.
muscle denervation, as expected, the amount of AChR increased to 161% of the normal value (intact muscle). In contrast, the amount of AChR of the laser irradiated denervated muscle remained near the normal value. Thirty days after denervation in the laser-treated group the amount of AChRs increased to 180% compared to 278% in the non-laser group. It is interesting that 4 months after denervation, in spite of progressive muscle atrophy, the amount of AChRs in the laser-treated group remained at 53% of the normal value compared to only 27% in the non-irradiated group. Statistical analysis showed borderline significance ($p = 0.056$) between denervated laser-treated and non-irradiated denervated muscles (Fig. 2).

Our findings suggest that in the early stage of muscle degeneration, laser treatment may temporarily preserve the denervated muscle close to its physiological status before injury and during progressive stages of muscle degeneration, partially maintain the amount of AChRs in the denervated muscle compared to the non-laser treated muscle.

C. Is Laser Phototherapy Damaging to the Muscle?

During 4 months of follow-up period we found no evidence of laser induced damage after irradiation. Moreover, in the laser-irradiated intact muscle group we found a significant increase in CK activity 60 days into the follow-up period ($p=0.008$) and an increasing amount of AChRs ($p=0.0008$) compared to the non-irradiated intact muscle. These findings suggest a possible positive therapeutic effect of laser phototherapy on the muscle.

**Conclusion:** Late denervation has been widely studied in animal models. In rats it has been shown that for the first 7 months after denervation myofibers exhibit a net loss of nuclear domains followed by nuclear groupings. If not re-innervated, the regenerating myofibers undergo atrophy and degeneration. To obtain a decrease in, or temporary prevention of this process, especially in cases of complete peripheral nerve injury where the affected nerve is reconstructed by grafts, tube or primary anastomosis, laser phototherapy can be an effective tool that preserves denervated muscle until nerve sprouting into the muscle occurs.

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**Fig. 2.** Graph illustrating the results of a study evaluating acetylcholine receptors (AChRs) synthesis in intact and denervated rat gastrocnemius muscle. The AChR synthesis levels (fmol/mg protein) are shown at 7, 30 and 120 days in intact and denervated muscles with and without laser treatment.

**Fig. 3.** Histological section of the crush area of the rat sciatic nerve showing the response of the nerve to laser phototherapy.

a. Nonirradiated nerve. Note of the scar in the fibrous tissue.
This experimental study suggests that the function of denervated muscles can be restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury. These findings could lead to direct therapeutic applications in the possible treatment of denervated muscles.

II. ANIMAL STUDIES: PHOTOTHERAPY IN PERIPHERAL NERVE REGENERATION

Posttraumatic nerve repair continues to be a major challenge in restorative medicine. Although enormous progress has been made in surgical techniques over the past three decades, functional recovery after severe lesion of a major nerve trunk is often incomplete and sometimes unsatisfactory.

A. Incomplete Peripheral Nerve Injury

1. Experimental Peripheral Nerve Crush Injury

Under general anesthesia the rat sciatic nerve was exposed and crushed at a pressure of 6.3±0.7 MPa for 30 seconds using an ordinary closed hemostat. Studies investigating the effects of low levels of laser irradiation on injured peripheral nerves of rats have found that it provides: 1) immediate protective effects which increase the functional activity of the injured peripheral

Fig. 4. Paraffin section from the anterior horn of the corresponding segments of a rat spinal cord 14 days after crush injury to the sciatic nerve, showing the spinal cord response to laser treatment of the injured peripheral nerve.

a: Section from a control animal shows extensive chromatolysis and cytoplasmic atrophy found in 40% of the motor neurons (arrows).

b: Section from a laser-treated animal shows minimal degenerative changes found in 20% of the motor neurons (arrows). (Cresyl fast violet stain, original magnification x 800).


Fig. 5. Photomicrographs of semithin sections stained with toluidine blue showing the axonal response to laser treatment of the injured (crushed) peripheral nerve in the rat.

One group of rats was treated using laser phototherapy for 20 consecutive days after injury. Twenty-one days after injury the nerves were excised and stained.

a. Site of crush injury in an untreated nerve showing nerve fibers that appear to be smaller and mostly nonmyelinated. Numerous macrophages and phagocytes are observed.

b. Site of crush injury in a laser-treated nerve demonstrating that most axons are ensheathed with myelin and a very few infiltrating macrophages are observed.

(Neurosurgery 20: 843-847, 1987)
nerve; 38) 2) maintenance of functional activity of the injured nerve over time; 39) 3) decrease or prevention of scar tissue formation at the site of injury (Fig. 3); 40) 4) prevention or decreased degeneration in corresponding motor neurons of the spinal cord (Fig. 4) and 5) increase in the rate of axonal growth and myelination (Fig. 5). 39) Moreover, direct laser irradiation of the spinal cord at specific segments improves recovery of the corresponding injured peripheral nerve. 42) These results, like others, 43) 44) suggest that laser phototherapy accelerates and improves the regeneration of the incompletely injured peripheral nerve.

B. Complete Peripheral Nerve Injury

1. Regeneration of the Transected Sciatic Nerve in the Rat after Primary Anastomosis

In acute cases where a peripheral nerve is completely transected, the treatment of choice is direct anastomosis. Some means of enhancing regeneration is essential, since degeneration is always inevitable in severely damaged peripheral nerves.

The therapeutic effect of 780 nm laser phototherapy on peripheral nerve regeneration after complete transection and direct anastomosis of rat sciatic nerve was evaluated in a double-blind randomized study. 45) After surgery, 13 of 24 rats received post-operative laser treatment, applied transcutaneously for 30 minutes on a daily basis, for 21 consecutive days: 15 minutes to the injured sciatic nerve and 15 minutes to the corresponding segments of the spinal cord. Positive somatosensory evoked responses were found in 69.2% of the irradiated rats ($p=0.019$), compared to 18.2% of the non-irradiated rats. Immunohistochemical staining in the laser-treated group showed an increased total number of axons ($p=0.026$) and a better quality of the regeneration process, which became evident by an
increased number of large diameter axons ($p=0.021$), compared to the non-irradiated control group (Fig.6). The study suggests that postoperative laser phototherapy enhances the regenerative processes of peripheral nerves after complete transection and anastomosis.

2. Regeneration of the Sciatic Nerve in the Rat after Complete Segmental Loss and Neurotube Reconstruction

In cases where the peripheral nerve is injured and complete segmental loss exists, the treatment of choice is nerve reconstruction using an autogenous nerve graft. The use of a regenerating guiding tube for the reconstruction of segmental loss of a peripheral nerve has some advantages over the regular nerve grafting procedure.

We performed a double-blind randomized study to evaluate the efficacy of 780-nm laser phototherapy on the acceleration of axonal growth and regeneration after experimental peripheral nerve reconstruction using a guiding tube. A 5 mm segment of the right sciatic nerve was removed and the proximal and distal parts were inserted into an artificial neurotube (Fig. 7). The rats were divided into two groups: the laser treated and non-laser treated groups. Postoperative laser phototherapy was applied transcutaneously for 30 minutes: 15 minutes on the transplanted peripheral nerve area and 15 minutes on the corresponding segments of the spinal cord, during 14 consecutive days. Conductivity of the sciatic nerve was studied by stimulating the sciatic nerve and recording the somatosensory evoked potentials (SSEPs) from the scalp. Three months after surgery SSEPs were found in 70% of the rats in the laser-treated group in comparison with 40% of the rats in the non-irradiated group. Morphologically, the previously transected nerve had good reconnection in both groups and the neurotube had dissolved (Fig. 8). Growth of myelinated axons, which crossed through the composite neurotube, was observed and the continuation of axonal sprouting through the area of the tube to the distal part of the nerve was recognized. The laser treated group showed more intensive axonal growth compared to the non-irradiated control group.

III. CLINICAL STUDIES: PHOTOTHERAPY IN PERIPHERAL NERVE REPAIR

1. Intraoperative clinical use of laser phototherapy following microsurgical treatment of the severely injured peripheral nerve

The use of low incident levels of laser irradiation during peripheral nerve surgery as a photo-stimulator device is an innovative method. The following study presents our experience with applying intraoperative laser phototherapy during the microsurgical release and neurolysis of the injured peripheral nerve (Fig. 9). Twenty-three patients with peripheral nerve injury underwent microsurgical repair of the injured peripheral nerve.

SURGICAL METHODS: Neurolysis - Using high microscopic magnification, combined external and interfascicular neurolyses were performed under intraoperative...
electrophysiological control (Fig. 10).

**INTRAOPERATIVE ELECTROPHYSIOLOGICAL RECORDING:** During the external and interfascicular neurolysis, compound muscle action potentials (CMAPs) were recorded from corresponding muscles during stimulation of the peripheral nerve to identify functional fascicles. After microsurgical release and neurolysis of the peripheral nerve, stable evoked responses were recorded and the peripheral nerve was subjected to direct laser irradiation using fiber optics. Laser phototherapy for 15 min was applied directly to the peripheral nerve after completion of neurolysis, using a fiberoptic-based system. We found that intraoperative laser treatment significantly increased compound evoked responses after direct laser irradiation of the injured peripheral nerve. The effect was mainly determined by CMAP recording (Fig. 11). Intraoperative direct laser irradiation caused “preventive” therapeutic effects which averted additional disturbances to the nerve and improved functional activity of the surgically treated nerve. An experimental animal study and clinical investigation of direct laser irradiation of the nerve, revealed an increased electrophysiological activity immediately after laser treatment, thus promoting improvement of nerve function.

### 2. 780-nm Laser Phototherapy in a Clinical Trial

Based on the outcome of animal studies, a clinical double-blind, placebo-controlled randomized study was performed to measure the effectiveness of 780 nm laser phototherapy in patients who had been suffering from incomplete peripheral nerve and brachial plexus injuries for 6 months up to several years. Most of these patients had been discharged by orthopedic sur-

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**Fig. 10** (a) Injured peripheral nerve surrounded by scar tissue.  
(b) nerve after intraneural removal of scar tissue (interfascicular neurolysis)

**Fig. 11** Laser phototherapy is illustrated being applied directly via a fiberoptic waveguide to the surgically treated nerve. Intraoperative recording shows that direct laser application to the surgically treated nerve significantly increased the amplitude of CMAP responses.
geons, neurosurgeons and plastic surgeons without further treatment. In this study 18 patients with a history of traumatic peripheral nerve /brachial plexus injury (mean duration from injury to treatment: 7 months in laser-treated group and 11.5 months in the placebo group) with a stable neurological deficit and a significant weakness, were randomly divided to receive either 780 nm laser or placebo (non-active light) irradiation.

The laser or placebo (non-active light) treatment was applied transcutaneously, each day for 21 consecutive days, 5 hours daily (3 hours to the injured area of the peripheral nerve and 2 hours to the corresponding segments of the spinal cord). The laser or placebo device was set up approximately 40 cm from the target area of the skin to be treated, and focused on the injured area of the peripheral nerve or corresponding level of the spine (area of corresponding segments of the spinal cord).

**Laser dosage:**
Laser irradiation of the spinal cord area was performed transcutaneously directly above the projection of the corresponding segments of the spinal cord, which was divided into 2 intravertebral levels. Each level was irradiated for 60 minutes a day (150 J/mm²), totaling 120 minutes a day (300 J/mm²).

Laser irradiation of the peripheral nerve area was performed transcutaneously directly above the projection of the injured nerve, which was divided into 3 parts: proximal, injured area and distal. Each section was irradiated for 60 minutes a day (150 J/mm²), totaling 180 minutes a day (450 J/mm²). The irradiating spot size was 3x2 mm (6 mm²). Clinically useful penetration of the near-infrared 780 nm wavelength was assessed to be approximately 4 cm. Analysis of results of this trial in the laser-irradiated group showed statistically significant improvement in motor function in the previously partially paralyzed limbs, compared to the placebo group, where no statistical significance in neurological status was found (Fig. 12). Mean motor function of the most influential (functionally dominant) muscle for movement of the affected limb showed a statistically significant improvement in the laser-irradiated group compared to the placebo group. The function was improved mostly by increasing power of the

![Fig. 12](image1.png)  
**Fig. 12** Graph demonstrating the motor function follow-up in injured patients who underwent 780-nm laser phototherapy or placebo treatment. Mean motor function (±SD) of all affected muscles was examined in injured patients using the Medical Research Council (MRC) Grading System. The analysis of the results showed that at baseline the 780-nm laser-treated and placebo groups were in clinically similar conditions (p = 0.887). The analysis of motor function during the 6-month follow up period compared to baseline showed statistically significant improvement (p = 0.0001) in the laser-treated group compared with the placebo group.  

![Fig. 13](image2.png)  
**Fig. 13** Graph illustrating the motor unit recruitment in injured patients who underwent either 780-nm laser phototherapy or placebo treatment. Motor unit recruitment, the mean of all examined muscles (±SD), was monitored in injured patients. The 780-nm laser-treated and placebo groups were in similar conditions at baseline (p = 0.934). In the laser-treated group, statistically significant improvement (p = 0.0006) was found in motor unit recruitment during the 6-month follow up period, compared with the placebo group.  
dominant muscles and not the intrinsic muscles. Electrophysiological observation during the trial supplied us with important diagnostic information and helped to determine the degree of functional recovery in nerve-injured patients. The electrophysiological analysis also showed statistically significant improvement in the recruitment of voluntary muscle activity in the laser-irradiated group, compared to the placebo group (Fig. 13).

This study must not be regarded as not the ultimate word and recommendations regarding 780 nm laser phototherapy in peripheral nerve injured patients. The limitations of this study are the small amount of patients, different nerves and etiology of injury. Nevertheless, this pilot study suggests that in peripheral nerve injured patients 780 nm laser phototherapy could progressively improve peripheral nerve function. Further controlled studies with larger populations and a multicenter trial perspective are definitely warranted.

Conclusions

This experimental study on denervated muscles suggests that treatment with laser phototherapy was capable of restoring muscular and neural function to a substantial degree when initiated at the earliest possible post-injury stage. These findings could have direct therapeutic applications for preserving the function of denervated muscle after peripheral nerve injury.

Extensive review articles, which have been published in Muscle and Nerve and Neurosurgical Focus revealed that most of the experimental studies showed that phototherapy could promote the recovery of the severely injured peripheral nerve. This review makes it possible to suggest that a time for broader clinical trials has arrived. The significance of the experimental and clinical studies is the provision of new laser technology for the treatment of severe nerve injury.

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