

# **Laser Phototherapy: A New Modality for Nerve Cell Tissue Engineering Technology, Cell Therapy and Nerve Repair**

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## **Basic Sciences And Clinical Trial**

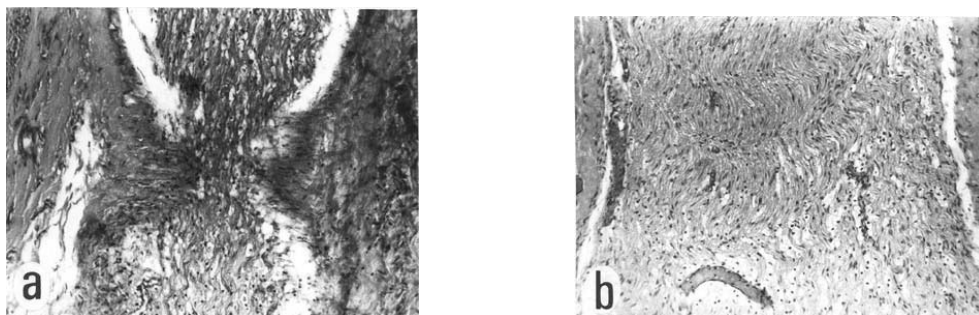
Studies, which evaluated the effects of 632.8nm and 780nm laser irradiation on Schwann<sup>1</sup> and nerve cell<sup>2</sup> cultures and injured peripheral nerves of animals<sup>3-7</sup> showed positive results. Laser phototherapy induces Schwann cell proliferation<sup>1</sup> and affects nerve cell metabolism and induces nerve processes sprouting<sup>2</sup>.

### *I - Laser phototherapy for treatment of experimental peripheral nerve injury*

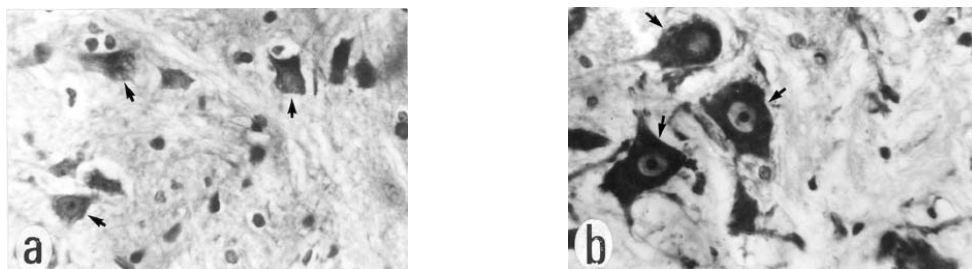
Laser phototherapy significantly improves recovery of the injured peripheral nerve<sup>3,4,6,7</sup> and, in addition, decreases posttraumatic retrograde degeneration of the neurons in the corresponding segments of the spinal cord.<sup>5</sup>

Our previous studies investigating the effects of low power laser irradiation 632.8 and 780nm on injured peripheral nerves of rats have found:

1. Protective immediate effects which increase the functional activity of the injured peripheral nerve.<sup>8</sup>
2. Maintenance of functional activity of the injured nerve over time.<sup>4</sup>
3. Influence of the LPLI on scar tissue formation at the injured site (Fig.1).<sup>6</sup>
4. Prevention or decreased degeneration in corresponding motor neurons of the spinal cord (Fig.2)<sup>5</sup>
5. Influence on axonal growth and myelination (Fig.3)<sup>4,7</sup>

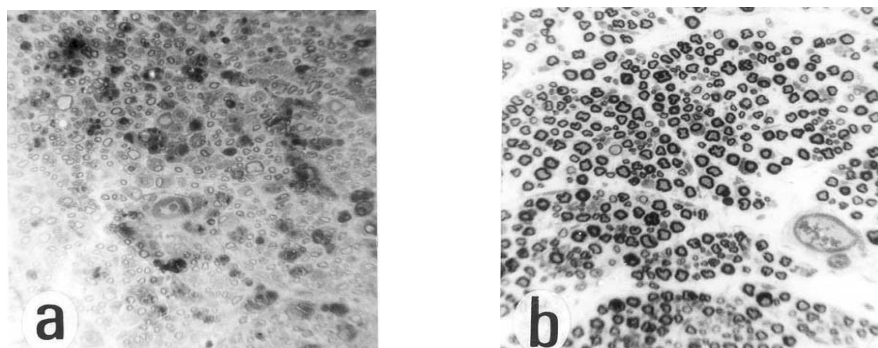


*Fig.1. Decrease or prevention of scar tissue formation at the site of injury. (Lasers in Surgery and Medicine 7:441-443, 1987) A- Scar in the place of the injury in the non-laser treated nerve. B- Prevention of scar formation after laser treatment.*



*Fig.2. (Spine 15: 6-10, 1990). Progressive degeneration changes in the corresponding neurons of the spinal cord after peripheral nerve injury in the control non-irradiated group (A). Decrease of degeneration process after laser treatment (B).*

Moreover, direct laser irradiation of the spinal cord improves recovery of the corresponding injured peripheral nerve.<sup>7,9</sup> Our results suggest that laser phototherapy accelerates and improves the regeneration of the injured peripheral nerve.



*Fig.3. (Neurosurgery 20: 843-847, 1987) Increase in rate of axonal growth and myelination: a- without treatment; b- laser treated nerve*

## 780nm Laser Phototherapy in Clinical Study

### II - Clinical double-blind, placebo-controlled randomized trial

Since our animal studies were positive, an evaluation of the response to 780nm laser phototherapy was in order. Therefore, a clinical double-blind, placebo-controlled randomized study was performed to measure the effectiveness of 780nm low power laser irradiation on patients who had been suffering from incomplete peripheral nerve and brachial plexus injuries for 6 months up to several years.<sup>10</sup> Most of these patients were discharged from initial orthopedics, neurosurgeons and plastic surgeons without further treatment.

In this study 18 patients with a history of traumatic peripheral nerve / brachial plexus injury (at least six months after the injury), with a stable neurological deficit and a significant weakness, were randomly divided to receive either 780nm laser or placebo (non-active light) irradiation. The analysis of the 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.4).

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 recruitment of voluntary muscle activity in the laser-irradiated group, compared to the placebo group (Fig.5)

This study shows that in long-term peripheral nerve injured patients 780nm low power laser irradiation can progressively improve peripheral nerve function, which leads to significant functional recovery.

### III - Further development in peripheral nerve reconstruction and role of 780nm laser phototherapy

This study was done to show the use of low power laser treatment enhances the regeneration and repair of a reconstructed injured peripheral nerve.<sup>11</sup>

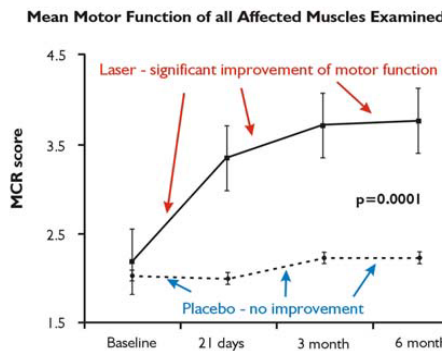


Fig.4

Photomedicine & Laser Surgery 25: 436-442, 2007)

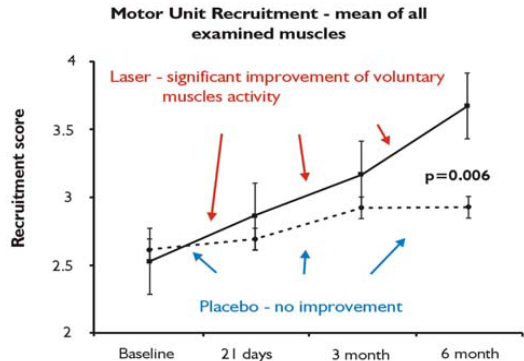
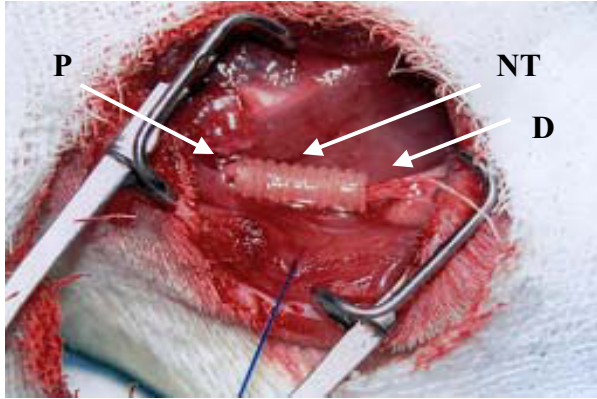


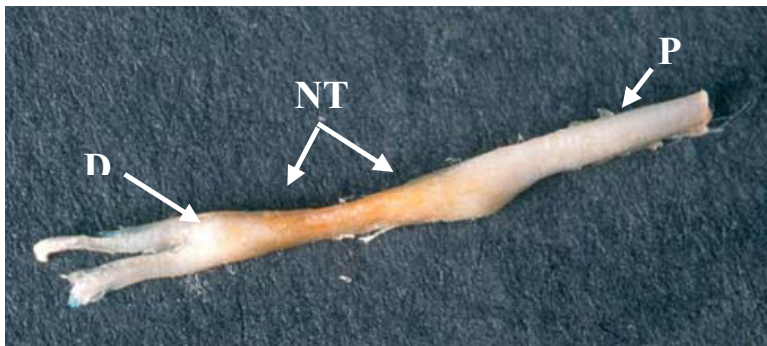
Fig.5



*Fig.6 (Photomedicine & Laser Surgery: 25: 137-143, 2007) A neurotube (NT) placed between the proximal (P) and the distal (D) parts of the nerve for the reconnection of 0.5 cm nerve defect (arrows).*

The 5mm segment of the right sciatic nerve was removed and proximal and distal parts were inserted into a bioabsorbable neurotube (Fig.6).

The rats were divided into two groups laser treated and non-laser treated. Postoperative low power laser irradiation was applied for 30 min. transcutaneously on the transplanted peripheral nerve area and 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 somato-sensory evoked potentials (SSEP) from the scalp. Three months after surgery SSEP somato-sensory evoked potentials were found in 70% of the rats in the laser-treated group in comparison with 40% of the rats in the non-irradiated Group.<sup>11</sup> Morphologically, the previously transected nerve had good reconnection four months after surgery in both groups and the neurotube had dissolved (Fig.7).



*Fig.7 (Photomedicine & Laser Surgery: 25: 137-143, 2007) Sciatic nerve of adult rat which was reconstructed by the neurotube (see arrows: NT-neurotube area, D-distal part, P-proximal part).*

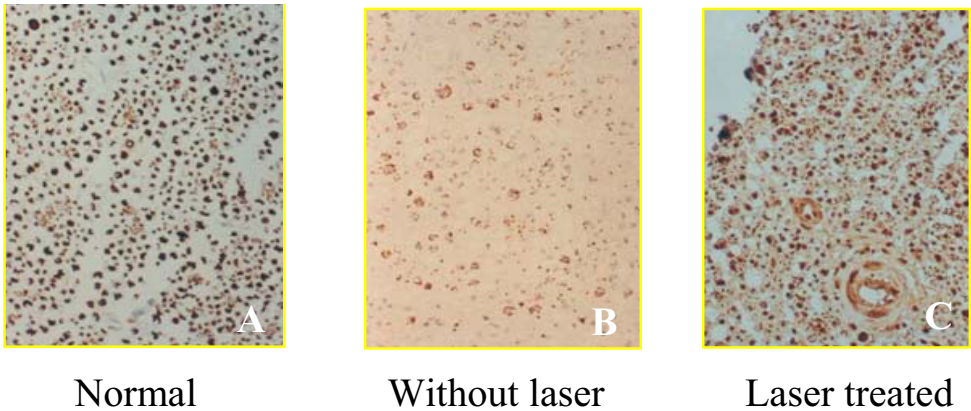


Fig.8.

The immuno-histochemical staining (Fig.8) using a monoclonal antibody-neurofilament showed more intensive axonal growth in neurotube-reconstructed and laser-treated rats (C) compared with the results of the non-laser treated group (B).

#### *IV- Influence of 780nm low power laser irradiation on nerve cell growth in vitro*

In this work the effect of 780nm laser phototherapy on sprouting and cell size of embryonic rat brain cells on microcarriers (MC) NVR-N-Gel in culture<sup>12</sup> was investigated. *Cell cultures:* Whole brains were dissected from 16-day old rat embryos (Sprague Dawley). After mechanical dissociation, cells were seeded directly in NVR-N-Gel, or suspended in positively charged cylindrical MC. Single cell-MC aggregates were either irradiated with LPLI within one hour after seeding, or cultured without irradiation. *NVR-N-Gel* (hyaluronic acid and laminin) was enriched with the following growth factors: BDNF and IGF-1.

*780nm Low Power Laser irradiation:* Laser powers were 10, 30, 50, 110, 160, 200 and 250 mw. Dissociated cells or cell-MC aggregates embedded in NVR-N-Gel, were irradiated for 1, 3, 4 or 7 min. A rapid sprouting of nerve processes from the irradiated cell-MC aggregates was detected already within 24h after seeding (Fig.9).

The extension of nerve fibers was followed by active neuronal migration. Differences between controls, and irradiated stationary dissociated brain cultures, became evident at about the end of the first week of cultivation - several neurons in the irradiated cultures exhibited large perikarya and thick elongated processes (Fig 10).

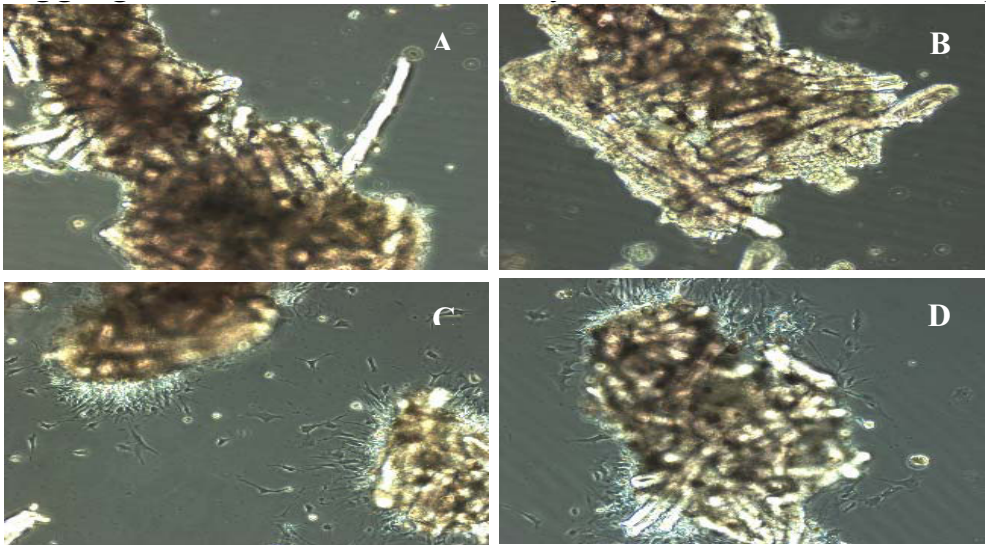


Fig 9. Effect of 780nm low power laser irradiation on initial sprouting and migration from DE-53 MCs in NVR-N-Gel. Initial sprouting and cellular migration is observed in irradiated cultures but not in non-irradiated control, already one day after the transfer to stationary cultures in NVR-N-Gel. A&B: Non-irradiated controls. C: Single irradiation of 250mW, for 1min. D: Single irradiation of 250mW for 3min. Original magnification: 200X.

### V - Further development in spinal cord reconstruction and role of 780nm laser phototherapy

The following treatment method was developed recently in our laboratories to enhance regeneration and to repair traumatic paraplegia in rats, resulting from spinal cord transection.<sup>13,14</sup> Embryonal spinal cord cells dissociated

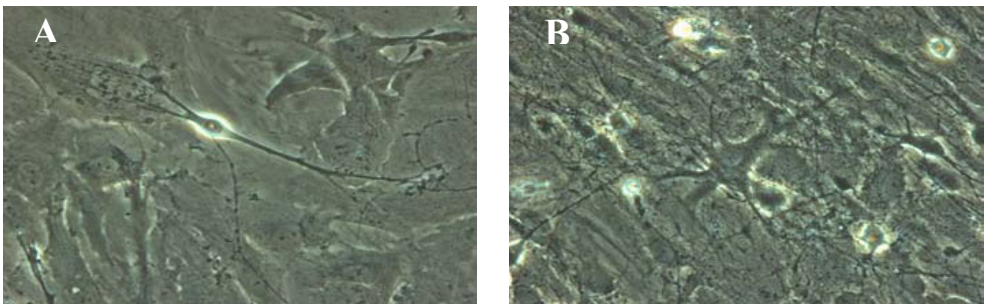


Fig 10. Effect of LPLI treatment on perikarya and fibers of nerve cells derived from rat embryonic brain. Dissociated brain cells were embedded in NVR-N-Gel and were either exposed to single irradiation of 160mW for 3min (B), or served as non-irradiated controls (A). Large neural cells exhibiting thick fibers were observed in 8 days in vitro (DIV) irradiated cultures. Original magnification: 200X.



Biodegradable microcarriers



Embryonal spinal cord cells

Fig.11A (*Neurological Research 24: 355-360, 2002*). *In vitro* reconstructed composite implants containing neuronal cells attached to gelatinous microcarriers (MCs).

Fig.11B Embryonal spinal cord cells (B).

from rat fetuses were cultured on biodegradable microcarriers (MCs) (Fig.11A) and embedded in hyaluronic acid (HA) (Fig.11B).

The cell-MCs aggregates were implanted into sites of the completely transected spinal cord of adult rats. These implants served as regenerative and repair sources for reconstructing neuronal tissue. During the following 14 post-operative days, the implanted area of the spinal cord was irradiated transcutaneously, 30 minutes daily to enhance the neuro-regenerative repair process.

The post-operative follow-up (from 3 to 6 months) showed that the rats which underwent embryonic nerve cell transplantation and laser treatment showed that most effective re-establishment of limb function, gait performance and intensive axonal sprouting occurred and after nerve cell implantation and laser irradiation (Fig. 12A,B), compared to rats without treatment (Fig. 13A,B).

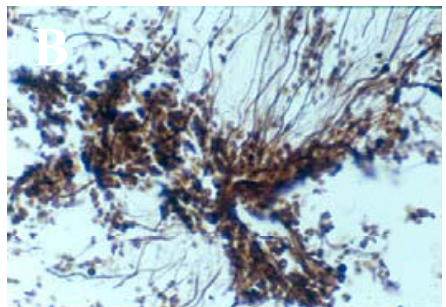


Fig. 12 (*Neurol Res 24: 355-360, 2002*) Active movement in both legs after embryonal nerve cell implantation in the transected spinal cord followed by low power laser treatment (A). Diffuse sprouting of axons at the site of nerve cell implantation followed by laser irradiation (modified Bodian's stain X 400) (B).

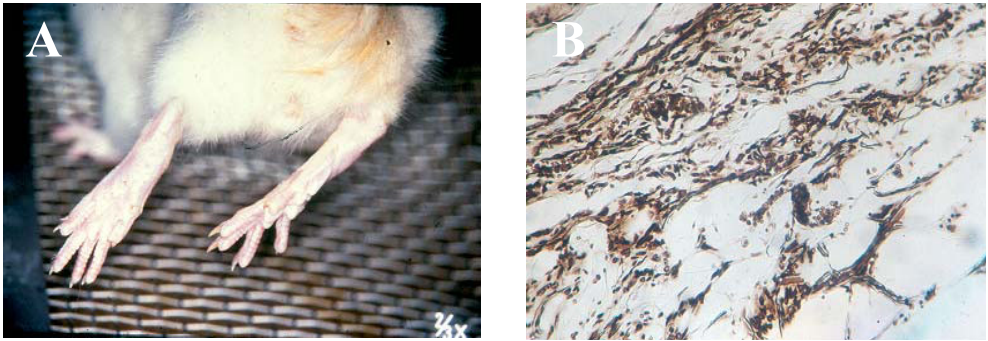


Fig. 13 (*Neurol Res* 24: 355-360, 2002) Complete paralysis of legs after spinal cord transection (A). Proliferating fibroblast and capillaries in a transected spinal cord of a rat without treatment (modified Bodian's stain X 400) (B).

The rats which underwent spinal cord transection only remained completely paralyzed in the lower extremities (Fig. 14A)

This study suggests that nerve cell implants which contain embryonal spinal cord cells attached to microcarriers and embedded in hyaluronic acid are a regenerative and reparative source for the reconstruction of the transected spinal cord. In addition, low power laser irradiation accelerates axonal growth and spinal cord regeneration.

*In conclusion:* The extensive review article, which was published in *Muscle and Nerve* in 2005<sup>15</sup> revealed that most of experimental studies showed phototherapy to promote the recovery of the severely injured peripheral nerve. This review makes possible to suggest that time for broader clinical trials has come.

The significance of our experimental and clinical studies is the provision of new nerve tissue engineering technology and 780nm laser phototherapy for treatment of severe nerve injury.

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