

# Photoengineering of Neural Tissue Repair Processes in Peripheral Nerves and the Spinal Cord: Research Development with Clinical Applications

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## ABSTRACT

The purpose of the present paper is to provide our data on the effects of phototherapy on peripheral nerve recovery. The aim is to call attention to an issue that still requires much research to elucidate the biological mechanisms through which phototherapy exerts its effects on nerve tissue, and to provide clinicians with the basis for planning clinical trials on the use of phototherapy for enhancing post-traumatic nerve regeneration.

## INTRODUCTION

STUDIES THAT EVALUATED the effects of 632.8-nm and 780-nm laser irradiation on Schwann<sup>1</sup> and nerve cell<sup>2</sup> cultures and injured peripheral nerves of animals<sup>3–9</sup> showed positive results. Laser phototherapy induces Schwann cell proliferation,<sup>1</sup> affects nerve cell metabolism, and induces nerve processes sprouting.<sup>2</sup> The number of experimental studies that have reported on the promoting action of phototherapy on peripheral nerve regeneration make it possible to suggest that the time for broader clinical trials has come.<sup>3</sup>

Injury of a peripheral nerve frequently results in considerable disability. In an extremity, such lesions may be associated with loss of sensory and motor functions, which leads to severe occupational and social consequences.

Surgical repair is the preferred modality of treatment for the complete or severe peripheral nerve injury.<sup>10–13</sup> In most cases, the results can be successful if the surgery is performed in the first 6 months after injury, in comparison to long-term cases where surgical management is less successful, although in related literature, there are several publications of surgical treatment of long-term injuries of the brachial plexus and peripheral nerve.<sup>14–17</sup> For most patients who suffered from long-term peripheral nerve injuries, the continuation of rehabilitation therapy was recommended, especially in those re-

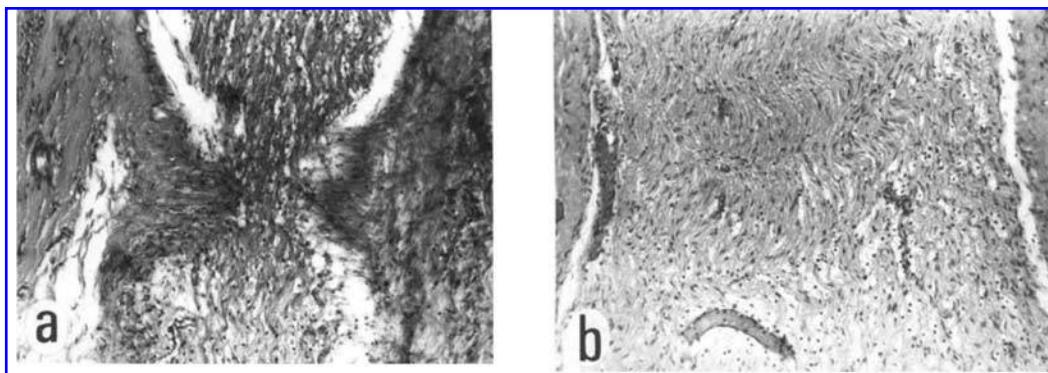
gions or countries that do not have specially dedicated peripheral nerve surgeons. Unfortunately, spontaneous recovery of long-term severe incomplete peripheral nerve injury is often unsatisfactory. The usual results after such an injury are degeneration of the axons and retrograde degeneration of the corresponding neurons of the spinal cord, followed by a very slow regeneration. Recovery may eventually occur, but it is slow and frequently incomplete. Understandably, therefore, numerous attempts have been made to enhance and/or accelerate the recovery of injured peripheral nerves. One of the methods studied is the use of different wavelengths of low-power laser irradiation to enhance the recovery of peripheral nerve injuries.

## 632.8- AND 780-NM LASER PHOTOTHERAPY FOR TREATMENT OF EXPERIMENTAL PERIPHERAL NERVE INJURY

Laser phototherapy significantly improves recovery of the injured peripheral nerve<sup>4,5,7,8</sup> and in addition decreases post-traumatic retrograde degeneration of the neurons in the corresponding segments of the spinal cord.<sup>6</sup>

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

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**FIG. 1.** Decrease or prevention of scar tissue formation at the site of injury. (a) Scar in the place of the injury in the non-laser-treated nerve. (b) Prevention of scar formation after laser treatment. (From Rochkind et al.<sup>7</sup>)

1. Protective immediate effects, which increase the functional activity of the injured peripheral nerve.<sup>18</sup>
2. Maintenance of functional activity of the injured nerve over time.<sup>5</sup>
3. Influence of the low-power laser irradiation on scar tissue formation at the injured site (Fig. 1).<sup>7</sup>
4. Prevention or decreased degeneration in corresponding motor neurons of the spinal cord (Fig. 2).<sup>6</sup>
5. Influence on axonal growth and myelinization (Fig. 3).<sup>5,8</sup>

Moreover, direct laser irradiation of the spinal cord improves recovery of the corresponding injured peripheral nerve.<sup>8,19</sup>

Most of the previously published results as well as our results suggest that laser phototherapy accelerates and improves the regeneration of the injured peripheral nerve.<sup>3</sup>

#### 780-NM LASER PHOTOTHERAPY IN CLINICAL STUDY

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

Since our animal studies were positive, an evaluation of the response to 780-nm laser phototherapy was in order. Therefore, a clinical double-blind, placebo-controlled randomized study was performed to measure the effectiveness of 780-nm

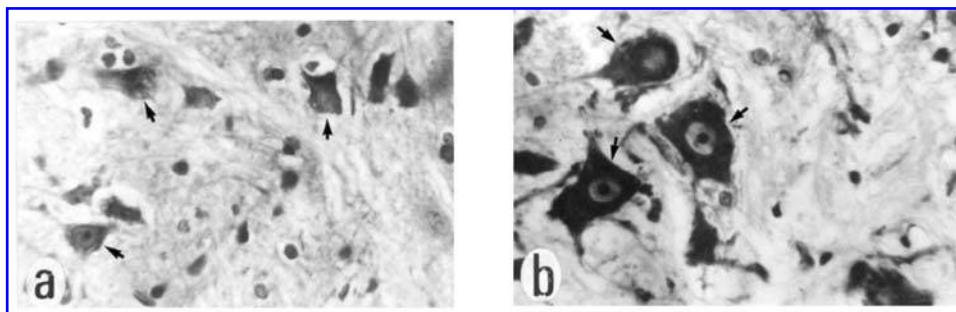
low-power laser irradiation on patients who had been suffering from incomplete peripheral nerve and brachial plexus injuries for 6 months up to several years.<sup>20</sup> Most of these patients were discharged from initial orthopedics, neurosurgeons, and plastic surgeons without further treatment.

In this study, 18 patients with a history of traumatic peripheral nerve/brachial plexus injury (at least 6 months after the injury), 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 analysis of the results of this trial in the laser-irradiated group showed statistically significant improvement in motor functions as well as in the recruitment of voluntary muscle activity in the previously partially paralyzed limbs, compared to the placebo group, where no statistical significance was found.

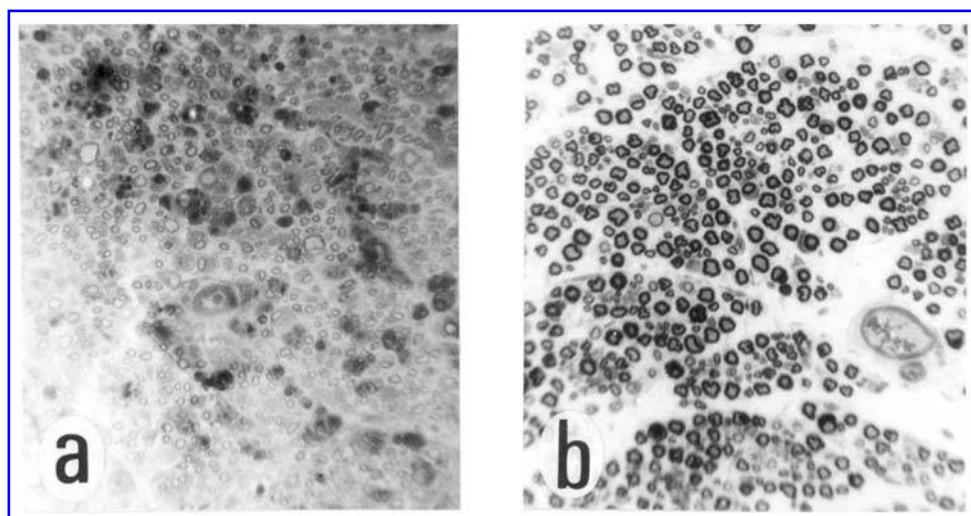
This study shows that, in long-term peripheral nerve injured patients 780-nm low power laser irradiation can progressively improve peripheral nerve function, which leads to significant functional recovery.

##### *Intraoperative clinical use of laser phototherapy after microsurgical treatment of the severely injured peripheral nerve*

The use of low-power laser irradiation during peripheral nerve surgery as a photo-stimulator device is an innovative



**FIG. 2.** 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). (From Rochkind et al.<sup>6</sup>)



**FIG. 3.** Increase in rate of axonal growth and myelinization. (a) Without treatment. (b) Laser-treated nerve. (From Rochkind et al.<sup>28</sup>)

method.<sup>21</sup> This study presents our experience in applying intraoperative low-power laser irradiation during the microsurgical release and neurolysis of the injured peripheral nerve. 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 neurolysis were performed under intraoperative electrophysiological control (Fig. 4).

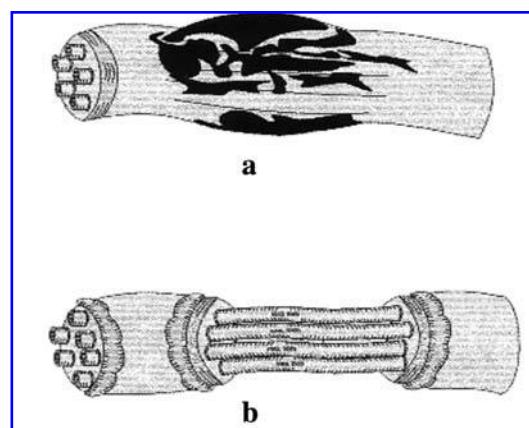
*Intraoperative Electrophysiological Recording.* During external and interfascicular neurolysis, compound muscle action potentials (CMAPs) were recorded from corresponding muscles during stimulation of the peripheral nerve to identify functional fascicles. After microsurgical release and neurolysis of the peripheral nerve, stable evoked responses were recorded, and the peripheral nerve was subjected to direct laser irradiation using fiber optics. Direct low-power laser irradiation was applied after completion of neurolysis, directly to the peripheral nerve, for 15 min, using fiber-optic instrument. We found that intraoperative laser treatment significantly increases compound evoked responses after direct laser irradiation of the injured peripheral nerve. The effect was mainly determined by CMAP recording. Figure 5 demonstrates a typical response of a patient whose injured nerve was microsurgically released followed by direct laser treatment of the nerve.

Intraoperative direct laser irradiation caused “preventive” therapeutic effects in order to prevent additional disturbances to the nerve and improve functional activity of the surgically treated nerve.<sup>21</sup> The experimental animal study<sup>18</sup> and clinical investigation of direct laser irradiation of the nerve reveal increased electrophysiological activity immediately after laser treatment, suggesting that low-power laser irradiation causes an immediate change in membrane permeability and thus promotes improved nerve function.

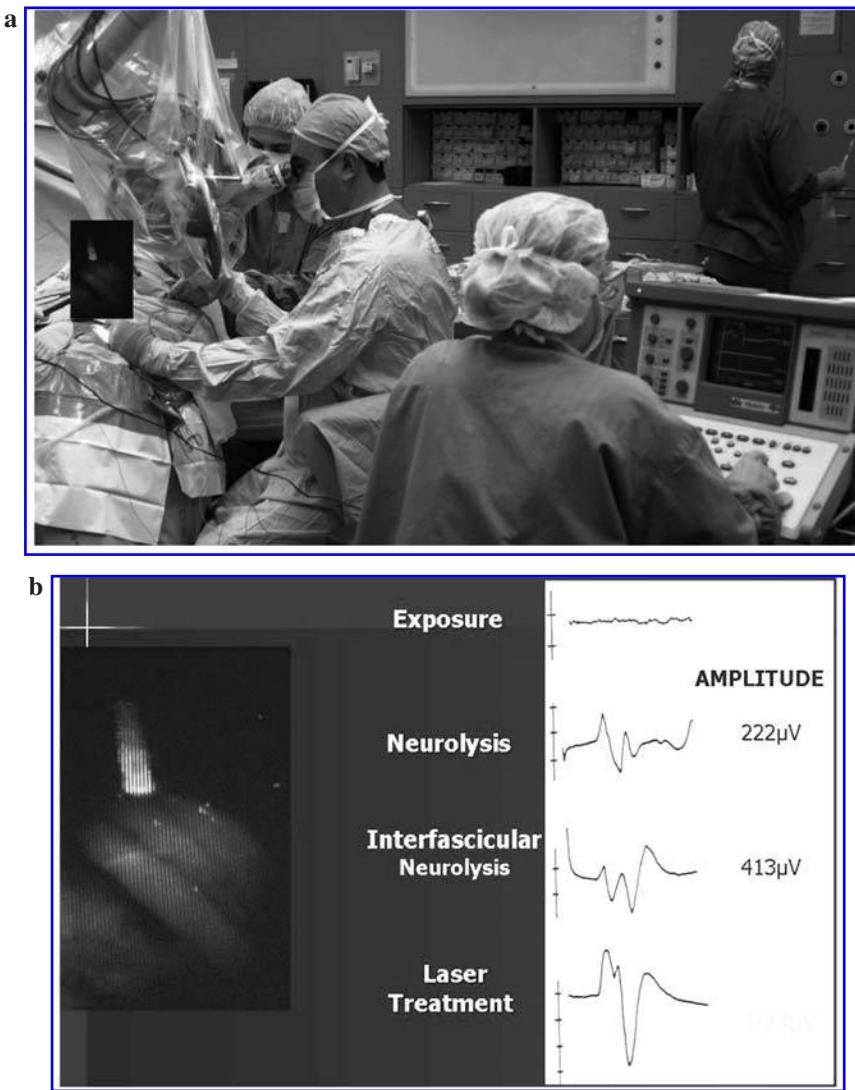
#### FURTHER DEVELOPMENT IN PERIPHERAL NERVE RECONSTRUCTION AND ROLE OF 780-NM LASER PHOTOTHERAPY

Artificial nerve guiding tube is one of the most challenging problems in peripheral nerve reconstruction. The use of both biodegradable artificial nerve tubes has been extensively investigated *in vivo*.<sup>22</sup> Recently, biodegradable composite transplant based on cell tissue-engineering technology was used for the treatment of complete peripheral nerve injury in rats.<sup>23</sup> The laser phototherapy was applied as a supportive factor for accelerating and enhancing axonal growth and regeneration after reconstructive peripheral nerve procedure.<sup>24</sup>

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



**FIG. 4.** (a) Injured nerve surrounded by scar tissue. (b) Nerve after intraneuronal removal of scar tissue (interfascicular neurolysis).



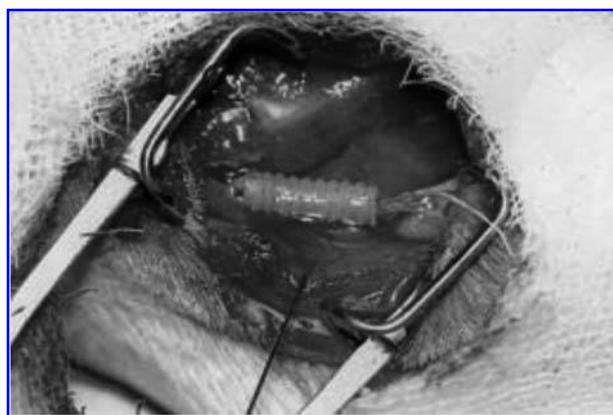
**FIG. 5.** (a) Low-power laser irradiation applied on the surgically treated nerve. (b) Intraoperative application of laser irradiation to the surgically treated nerve significantly increased the amplitude of compound muscle action potential (CMAP) responses.

The rats were divided into two groups: laser-treated and non-laser-treated.

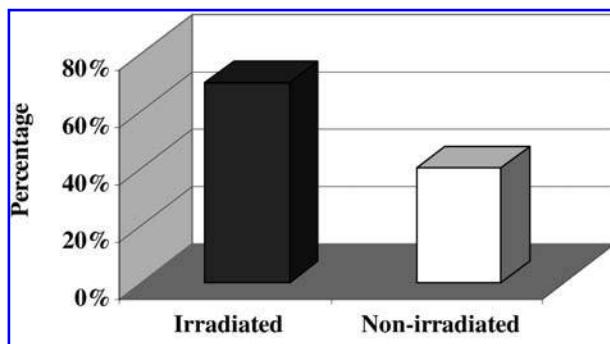
Postoperative low-power laser irradiation was applied for 30 min transcutaneously on the transplanted peripheral nerve area and corresponding segments of the spinal cord, during 14 consecutive days. Somatosensory evoked potentials (SSEP) were recorded 3 months after complete transection and tube reconstruction of the 0.5-cm nerve defect in the right sciatic nerve of the rats. Seven (70%) out of the 10 rats in the irradiated group had positive SSEP responses, and three (30%) had no response. In the non-irradiated group, four (40%) out of 10 rats had a positive response and six (60%) had no response (Fig. 7).

The biodegradable polyglycolic acid neurotube re-created the anatomical connection of the previously transected and divided nerve, and a distance of 0.5 cm was re-created. The neurotube had dissolved at this time (Fig. 8).

Three months after surgery, the growth of myelinated axons, which crossed through the composite neurotube, was found,



**FIG. 6.** 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).



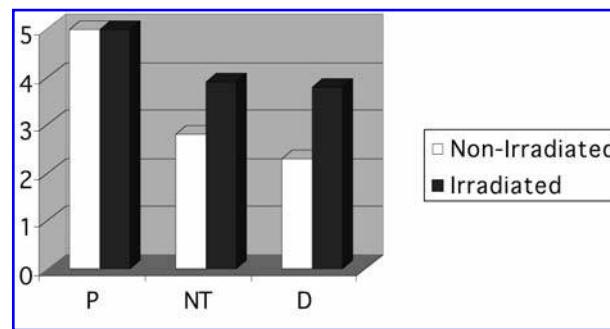
**FIG. 7.** Percentage of rats with positive somato-sensory evoked potentials at 3 months after neurotube reconstruction.

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 (Fig. 9). Presence and arrangement of axons from 10 rats in the laser-irradiated group were compared with 10 rats in the non-irradiated group. In the neurotube reconstructed areas, the amount of myelinated axons in the laser-treated group received a higher score of  $3.7 \pm 0.2$  in comparison to  $2.6 \pm 0.2$  in the non-laser-treated group. In the distal parts, the laser-treated group received a higher score of  $3.4 \pm 0.4$  in comparison to a  $2.0 \pm 0.3$  score in the non-laser-treated group. (Fig. 9).

#### FURTHER DEVELOPMENT IN SPINAL CORD RECONSTRUCTION AND ROLE OF 780-NM LASER PHOTOTHERAPY

Severe loss of sensory and motor function below the site of injury are typical characteristics of spinal cord injuries in mammals. Spinal cord reconstruction using implantation of cells from various sources has been gaining momentum in recent years.<sup>25,26</sup>

One feasible innovative way of repairing injured mammalian spinal cord is by creating a composite implant, which contains cultured cells from autologous or allogeneic source.



**FIG. 9.** Mean intensity of the axons into proximal (P), neurotube (NT), and distal (D) parts of the sciatic nerve.

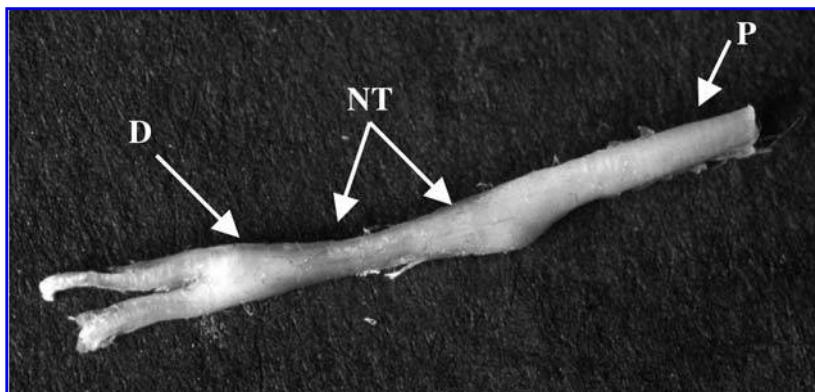
The implanted cells grow and serve as a vital bridge to connect the stumps of the severed spinal cord.<sup>26</sup>

The following photoengineering method was developed in our laboratories to enhance regeneration and to repair traumatic paraplegia in rats, resulting from spinal cord transection.<sup>27</sup> Embryonal spinal cord cells (Fig. 10a) dissociated from rat fetuses were cultured on biodegradable microcarriers (MCs) (Fig. 10b) and embedded in hyaluronic acid (HA).

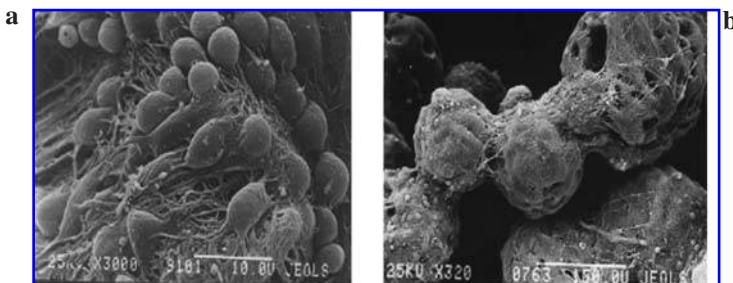
The cell-MC aggregates were implanted into sites of the completely transected spinal cord of adult rats. These implants served as regenerative and repair sources for reconstructing neuronal tissue. During the following 14 post-operative days, the implanted area of the spinal cord was irradiated transcutaneously,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

Three months after spinal cord transaction and implantation of embryonal nerve cells, SSEP were investigated in the non-laser-treated and laser-treated groups. The study showed the most effective re-establishment of electrophysiological signals occurred after embryonic nerve cell implantation and laser irradiation, compared to transection alone or nerve cell implantation without laser treatment. The transplant re-created the anatomical connection of the previously transected spinal cord (Fig. 11).

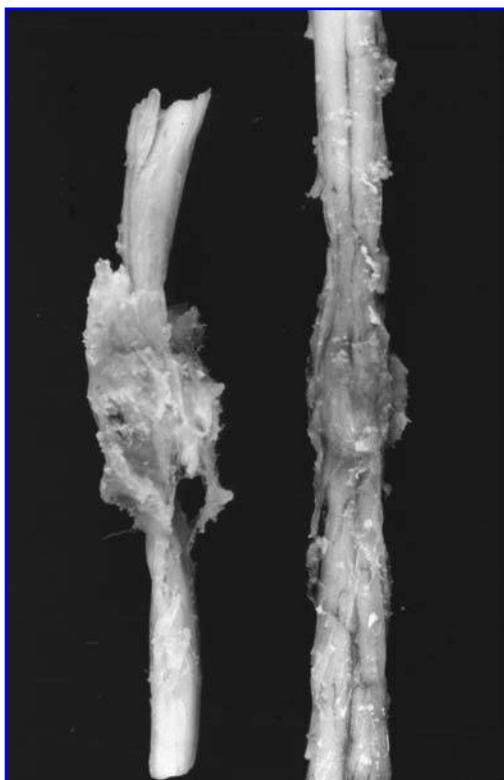
The post-operative follow-up (at 3–6 months) on operated rats which underwent embryonic nerve cell implantation in the



**FIG. 8.** Sciatic nerve of adult rat that was reconstructed by the neurotube (arrows: NT, neurotube area; D, distal part; P, proximal part).



**FIG. 10.** Components of the spinal cord transplant. (a) Embryonal spinal cord cells. (b) Biodegradable microcarriers.



**FIG. 11.** Transected spinal cord of adult rat reconstructed by the embryonic nerve cell microcarriers followed by laser treatment (3 months after surgery).

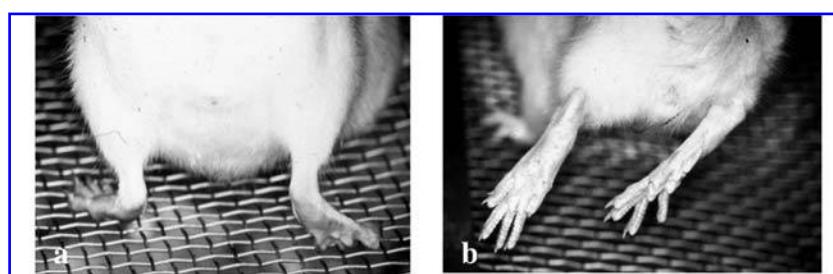
transected area of the spinal cord showed that the most effective re-establishment of active leg movements were found in the laser-treated group. The rats which underwent embryonic nerve cell transplantation and laser treatment showed that most effective re-establishment of limb function and gait performance occurred after nerve cell implantation and laser irradiation (Fig. 12a), compared to rats that were treated by nerve cell implantation alone. The rats which underwent spinal cord transection only remained completely paralyzed in the lower extremities (Fig. 12b).

Intensive axonal sprouting was observed in the group which was implanted with embryonic nerve cells–MC culture in HA and treated with low-power laser irradiation (Fig. 13a), in comparison with the untreated completely transected spinal cord area that contained only proliferating fibroblasts and blood capillaries (Fig. 13b).

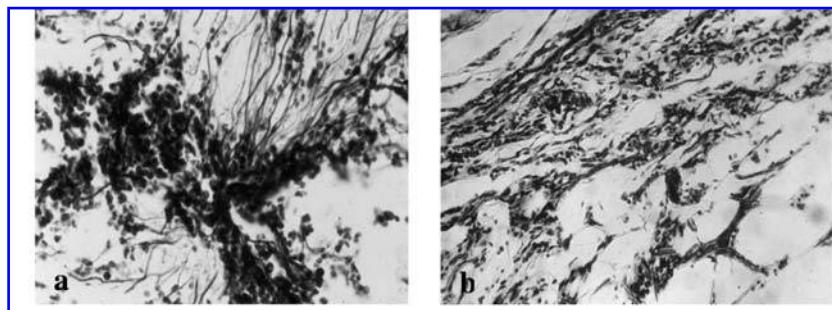
This study suggests that nerve cell implants that contain embryonal spinal cord cells attached to MCs and embedded in HA are a regenerative and reparative source for the reconstruction of the transected spinal cord. In addition, low-power laser irradiation accelerates axonal growth and spinal cord regeneration.

## CONCLUSION

The significance of these studies is the provision of new nerve tissue engineering technology and phototherapy for future treatment of patients suffering from complete peripheral nerve or spinal cord injury.



**FIG. 12.** (a) Active movement in both legs after embryonal nerve cell implantation in the transected spinal cord followed by laser treatment. (b) Complete paralysis of legs after spinal cord transection. (From Rochkind et al.<sup>27</sup>)



**FIG. 13.** (a) Diffuse sprouting of axons at the site of nerve cell implantation followed by laser irradiation. (b) Proliferating fibroblast and capillaries in a transected spinal cord of rat without treatment. (From Rochkind et al.<sup>27</sup>)

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