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# Large-area irradiated low-level laser effect in a biodegradable nerve guide conduit on neural regeneration of peripheral nerve injury in rats

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ABSTRACT

This study used a biodegradable composite containing genipin-cross-linked gelatin annexed with β-tricalcium phosphate ceramic particles (genipin-gelatin-tricalcium phosphate, GGT), developed in a previous study, as a nerve guide conduit. The aim of this study was to analyse the influence of a large-area irradiated aluminium–gallium–indium phosphide (AlGaInP) diode laser (660 nm) on the neural regeneration of the transected sciatic nerve after bridging the GGT nerve guide conduit in rats. The animals were divided into two groups: group 1 comprised sham-irradiated controls and group 2 rats underwent low-level laser (LLL) therapy. A compact multi-cluster laser system with 20 AlGaInP laser diodes (output power, 50 mW) was applied transcutaneously to the injured peripheral nerve immediately after closing the wound, which was repeated daily for 5 min for 21 consecutive days. Eight weeks after implantation, walking track analysis showed a significantly higher sciatic function index (SFI) score ($P < 0.05$) and better toe spreading development in the laser-treated group than in the sham-irradiated control group. For electrophysiological measurement, both the mean peak amplitude and nerve conduction velocity of compound muscle action potentials (CMAPs) were higher in the laser-treated group than in the sham-irradiated group. The two groups were found to be significantly different during the experimental period ($P < 0.005$). Histomorphometric assessments revealed that the qualitative observation and quantitative analysis of the regenerated nerve tissue in the laser-treated group were superior to those of the sham-irradiated group. Thus, the motor functional, electrophysiologic and histomorphometric assessments demonstrate that LLL therapy can accelerate neural repair of the corresponding transected peripheral nerve after bridging the GGT nerve guide conduit in rats.


## Introduction

Peripheral nerve injuries are common in clinical practice because of trauma such as crushing and sectioning. Lesions of the nerve structure result in loss of or diminished sensitivity and/or motor activity in the innervated territory. The degree of lesion depends on the specific nerve involved, the magnitude and type of pressure exerted and the duration of the compression.[11] The results of the injury commonly include axonal degeneration and retrograde degeneration of the corresponding neurons in the spinal medulla, followed by very slow regeneration.[18] Although there is a certain degree of recovery in most nerve injuries, the process is slow and often incomplete.[19] The adverse effect on the daily activities of patients with a peripheral nerve injury is a determinant factor in establishing the goals of early recovery.[20] Despite major advances in microsurgical techniques, the functional results of peripheral nerve repair remain largely unsatisfactory. In particular, the therapy of long gap peripheral nerve injuries is always a puzzle clinically. It usually requires a nerve graft, as promoting nerve regeneration is often necessary to bridge the proximal and distal nerve stumps.

Nerve autografting has been the first choice for repairing peripheral nerve defects. However, this recognised 'gold standard' technique for peripheral nerve repair has inevitable disadvantages, such as limited supply of available nerve grafts, permanent loss of the donor nerve function and potential differences in tissue structure and size. Although past studies indicated that xenografts and allografts are common alternatives to autografts, they have poor success rates and may be prone to immune rejection.[14,8] To avoid these impairments following such operations, researchers have put a considerable amount of effort into developing synthetic nerve conduits for repair of peripheral nerve defects. A nerve bridge approach involves the introduction of ends of injured nerve stumps into a tubular chamber, which promotes guidance of the

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growth of nerve fibres along suitable paths by mechanical orientation and confinement and increases the accuracy of the stump approximation.[5,24]

Clinicians have turned the focus of their attention to more effective methods of promoting nerve regeneration, target-organ reinnervation and functional restoration after peripheral nerve injury. Many physical factors also play a part in promoting nerve regeneration, in addition to neurotrophic factors and a number of pharmaceutical drugs. It is a common practice in physiotherapy to use therapeutic instruments for regenerative purposes.[9] Electrical,[15] ultrasound[17] and low-level laser (LLL) stimulation have been used to accelerate the regenerative processes, with the aim of an early recovery of functionality for the patient. LLL therapy began to be used in the regeneration and functional recuperation process of peripheral nerves in the 1970s, and the results obtained so far have been inconsistent.[3] Many animal experiments and clinical studies indicate that LLL irradiation can attenuate injury, promote repair and stimulate axonal sprouting and propagation, but its mechanism of action is not well understood.[1] A review of the literature on phototherapy for peripheral nerve repair found that the use of laser was based on several wavelengths (632–904 nm), lesion types (crushing, neurorrhaphy and tubulation), sample types, the duration and manner of the emission and the assessment types (such as functional, electrophysiological and morphometric).[9] In many studies, descriptions of the irradiation parameters, such as dose, average power, time and application methods, are expressly varied, which hampers methodological comprehension for the reproduction of results and hinders comparisons between studies.

A small number of studies have been conducted that used a laser with light-emitting diodes in nerve lesions by tubulation, and to the best of our knowledge, no studies have investigated the effects of LLL therapy on neural regeneration in a biodegradable nerve conduit. This study used a previously developed biodegradable composite containing genipin-cross-linked gelatin annexed with β-tricalcium phosphate (TCP) ceramic particles (genipin-gelatin-tricalcium phosphate, GGT) as a nerve guide conduit.[28] Furthermore, a large-area irradiated diode laser with 20 laser emitters was used. In the present study, a 10-mm gap in the rat sciatic nerve defect was bridged with a GGT nerve guide conduit. The 660-nm aluminium–gallium–indium phosphide (AlGaInP) LLL therapy was then applied transcutaneously to the transected nerve for 5 min daily for 21 consecutive days. Using gross and microscope observation, sciatic functional index (SFI) measurement, electrophysiology examination and axon image analysis, we investigated the effects of LLL therapy on peripheral nerve restoration and regeneration and collected data which may be of value for future clinical applicability in the treatment of nerve injuries.

## Materials and methods

### *Animals*

Twelve adult Sprague–Dawley rats, each weighing 250–300 g, were provided by the National Laboratory Animal Center, National Applied Research Laboratories (NARL). They were kept under controlled lighting and temperature, with standard food and water available *ad libitum*. Prior to the beginning of the study, the protocol was approved by the ethical committee for animal experiments of Central Taiwan University of Science and Technology, which determined that the experiment appropriately minimised the number of animals used and their suffering.

### *Preparation of GGT nerve conduits*

A homogeneous 18% gelatin solution (Bloom number 300, Sigma, Saint Louis, MO, USA) was prepared in a water bath at 60 °C. The gelatin solution was then mixed with weight ratios (gelatin/TCP = 1/2) of β-TCP (Fluka, Alzenau, Germany) ceramic particles to obtain the final gelatin–TCP (GT) mixtures. A silicone rubber tube (Helix Medical, Inc., Carpinteria, CA, USA) was used as an inner mandrel vertically dipped into the mixture at a constant speed where it remained for 1 min. The mandrel was then withdrawn slowly and allowed to stand for 5 min for air drying. The mandrel was rotated horizontally consistently to reduce variations in the wall thickness along the axis of the tube. Three coating steps were used to obtain a GT tube. The GT-coated mandrel was then immersed in a 1% (w/w) genipin (Challenge Bioproducts Co., Taichung, Taiwan, ROC) solution for 48 h to allow for sufficient cross-linking reactions within the gelatin. The genipin-cross-linked GT tubes were labelled GGT. The GGT-coated mandrel was rinsed twice with distilled water and further dried in a freeze dryer. The GGT nerve guide conduits were then slipped off the silicone rubber mandrel. The hollow GGT nerve guide conduits were subsequently sterilised by $^{60}$Co gamma ray irradiation (25 kGy) and stored in a desiccator at room temperature for further experiments.

### *Surgical procedure*

The animals were anaesthetised using an inhalational anaesthetic procedure (VMS, Matrix, New York, USA). Following the skin incision, fascia and muscle groups were separated using blunt dissection, and the left sciatic nerve was severed into proximal and distal segments. The proximal stump was then secured using a single 10/0 prolene suture (Johnson-Johnson, Edinburgh, UK) through the epineurium and the outer wall of the nerve guide conduits (12 mm in length). The distal stump was secured similarly into the other end of the chamber. Both the proximal and the distal stumps were secured to a depth of 1 mm into the chamber, leaving a 10-mm gap between the stumps. The muscle layer was re-approximated using 4/0 chromic gut sutures, and the skin was closed with 2/0 silk sutures. After surgery, the rats were housed separately under temperature- and humidity-controlled conditions with a 12 h light/12 h dark cycle and with free access to food and water.

### *Laser therapy*

After the injured nerves were bridged with the GGT nerve guide conduits, 12 adult Sprague–Dawley rats, each weighing approximately 250–350 g, were randomly divided into two groups. In the control group (GGT, $n = 6$), animals were sham-irradiated controls. In the laser-treated group (GGT/laser, $n = 6$), animals received a treatment of LLL irradiation. The diode laser (Megalas®-AM-800, Konftec Co., Taipei, Taiwan, ROC) is a compact multi-cluster laser system for area therapy. It has 20 AlGaInP laser diodes (output power, 50 mW; frequency, 50 Hz) that emit a continuous 660-nm AlGaInP laser beam and irradiate an area of about 314 cm$^2$. Laser irradiation was performed transcutaneously and focused on the area of the injured nerve. The energy density was 3.84 J cm$^{-2}$, the power density was 0.0032 W cm$^{-2}$ and the length of application was 5 min. The laser therapy was started on the first day after the operation and was continued for 21 consecutive days. The animals in the control group were subjected to the same procedure, but with the laser switched off. The specimens were removed upon sacrifice of the rats at 8 weeks.

### *Walking track analysis*

Analysis of a rat's walking pattern by recording its footprints and calculating the SFI is a well-established and commonly used method for the assessment of motor nerve recovery after sciatic