# Increase of Neuronal Sprouting and Migration Using 780 nm Laser Phototherapy as Procedure for Cell Therapy

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**Background and Objectives:** The present study focuses on the effect of 780 nm laser irradiation on the growth of embryonic rat brain cultures embedded in NVR-Gel (cross-linked hyaluronic acid with adhesive molecule laminin and several growth factors). Dissociated neuronal cells were first grown in suspension attached to cylindrical micro-carriers (MCs). The formed floating cell-MC aggregates were subsequently transferred into stationary cultures in gel and then laser treated. The response of neuronal growth following laser irradiation was investigated.
**Materials and Methods:** Whole brains were dissected from 16 days Sprague–Dawley rat embryos. Cells were mechanically dissociated, using narrow pipettes, and seeded on positively charged cylindrical MCs. After 4–14 days in suspension, the formed floating cell-MC aggregates were seeded as stationary cultures in NVR-Gel. Single cell-MC aggregates were either irradiated with near-infrared 780 nm laser beam for 1, 4, or 7 minutes, or cultured without irradiation. Laser powers were 10, 30, 50, 110, 160, 200, and 250 mW.
**Results:** 780 nm laser irradiation accelerated fiber sprouting and neuronal cell migration from the aggregates. Furthermore, unlike control cultures, the irradiated cultures (mainly after 1 minute irradiation of 50 mW) were already established after a short time of cultivation. They contained a much higher number of large size neurons ($P<0.01$), which formed dense branched interconnected networks of thick neuronal fibers.
**Conclusions:** 780 nm laser phototherapy of embryonic rat brain cultures embedded in hyaluronic acid—laminin gel and attached to positively charged cylindrical MCs, stimulated migration and fiber sprouting of neuronal cells aggregates, developed large size neurons with dense branched interconnected network of neuronal fibers and, therefore, can be considered as potential procedure for cell therapy of neuronal injury or disease. Lasers Surg. Med. 41:277–281, 2009. © 2009 Wiley-Liss, Inc.

**Key words:** axonal sprouting; cross-linked hyaluronic acid with laminin gel; embryonic nerve cells; low power laser irradiation; microcarriers

## INTRODUCTION

The therapeutic effect of low power laser irradiation (LPLI) was detected on peripheral and central nervous systems [1–6]. Previous studies, which evaluated the effects of LPLI on crushed injured peripheral nerves of rats, discovered protective immediate effects which increase the functional activity of the injured peripheral nerve [7]; maintenance of functional activity of the injured nerve over time [8]; decrease or prevention of scar tissue formation at the injured site [9]; prevention or decreased degeneration in corresponding motor neurons of the spinal cord [10]; increase in the rate of axon growth and myelinization, thus accelerating and improving the regeneration of the injured nerve. LPLI was found to increase migration and neurite sprouting of cultured embryonic nerve cells [11], as well as cultured adult brain microexplants [12], and to alter gene expression of olfactory ensheathing cells [13]. Our previous studies found that LPLI accelerated axonal growth into injured rat's spinal cord after an implantation of a composite implant, which was based on embryonic spinal cord nerve cells and cultured on biodegradable microcarriers (MCs) that were embedded in hyaluronic acid [6].

In this in vitro study we investigated the effect of 780 nm laser phototherapy on growth and development of embryonic brain neurons and their fibers in culture.

## MATERIALS AND METHODS

### Cell Culture

Sixteen-day-old rat embryos' (Sprague–Dawley) whole brains were dissected. After mechanical dissociation with narrow pipettes, $5\times10^6$ cells were suspended in medium attached to DE-53 positively charged cylindrical MCs in 60 mm bacteriological plastic dishes as previously described [14]. After 4–14 days in suspension, the formed floating cell-MC aggregates were collected and seeded in NVR-Gel (in 12 wells or 35 mm plastic dishes) as stationary cultures. Each single cell-MC aggregate was either treated with LPLI within 1 hour after seeding, or cultured without irradiation.

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Accepted 24 January 2009
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20757