



*Low level laser therapy for traumatic brain injury*

|                       |   |
|-----------------------|---|
| <b>Citation</b>       | Wu, Qiuhe et al. "Low level laser therapy for traumatic brain injury." Mechanisms for Low-Light Therapy V. Ed. Michael R. Hamblin, Ronald W. Waynant, & Juanita Anders. San Francisco, California, USA: SPIE, 2010. 755206-8. ©2010 SPIE. |
| <b>As Published</b>   | <a href="http://dx.doi.org/10.1117/12.841014">http://dx.doi.org/10.1117/12.841014</a>   |
| <b>Publisher</b>      | SPIE  |
| <b>Version</b>        | Final published version   |
| <b>Accessed</b>       | Sat Feb 04 11:13:16 EST 2012  |
| <b>Citable Link</b>   | <a href="http://hdl.handle.net/1721.1/58575">http://hdl.handle.net/1721.1/58575</a>   |
| <b>Terms of Use</b>   | Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.  |
| <b>Detailed Terms</b> |   |

# Low level laser therapy for traumatic brain injury

Qiuhe Wu<sup>1,2,3</sup>, Ying-Ying Huang<sup>1,2,4</sup>, Saphala Dhital<sup>1,5</sup>, Sulbha K Sharma<sup>1</sup>, Aaron C-H Chen<sup>1,6</sup>, Michael J Whalen<sup>7</sup>, Michael R Hamblin<sup>1,2,8,\*</sup>

<sup>1</sup> Wellman Center for Photomedicine, Massachusetts General Hospital, Boston MA

<sup>2</sup> Department of Dermatology, Harvard Medical School, Boston MA

<sup>3</sup> Department of Burns and Plastic Surgery, Jinan Central Hospital Affiliated to Shandong University, Jinan, P.R.China.

<sup>4</sup> Aesthetic and Plastic Center of Guangxi Medical University, Nanning, P.R China

<sup>5</sup> Department of Microbiology, University of Tokyo, Japan

<sup>6</sup> Boston University School of Medicine, Graduate Medical Sciences, Boston MA

<sup>7</sup> Department of Pediatrics, Massachusetts General Hospital, Boston MA

<sup>8</sup> Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

\* corresponding author: BAR414, 40 Blossom Street, Massachusetts General Hospital, Boston, MA02114. email: [Hamblin@helix.mgh.harvard.edu](mailto:Hamblin@helix.mgh.harvard.edu). Fax :617-726-8566

## ABSTRACT

Low level laser (or light) therapy (LLLT) has been clinically applied for many indications in medicine that require the following processes: protection from cell and tissue death, stimulation of healing and repair of injuries, and reduction of pain, swelling and inflammation. One area that is attracting growing interest is the use of transcranial LLLT to treat stroke and traumatic brain injury (TBI). The fact that near-infrared light can penetrate into the brain would allow non-invasive treatment to be carried out with a low likelihood of treatment-related adverse events. LLLT may have beneficial effects in the acute treatment of brain damage injury by increasing respiration in the mitochondria, causing activation of transcription factors, reducing key inflammatory mediators, and inhibiting apoptosis. We tested LLLT in a mouse model of TBI produced by a controlled weight drop onto the skull. Mice received a single treatment with 660-nm, 810-nm or 980-nm laser (36 J/cm<sup>2</sup>) four hours post-injury and were followed up by neurological performance testing for 4 weeks. Mice with moderate to severe TBI treated with 660-nm and 810-nm laser had a significant improvement in neurological score over the course of the follow-up and histological examination of the brains at sacrifice revealed less lesion area compared to untreated controls. Further studies are underway.

**Keywords:** photobiomodulation, low level laser therapy, traumatic brain injury, mouse model, neurological testing

## 1. Introduction

Severe and moderate traumatic brain injury (TBI), accidental or inflicted, is a major health and socio-economic problem throughout the world. In the United States alone, approximately 2 million injuries occur each year resulting in 56,000 deaths and 18,000 survivors suffering from permanent neurological impairment [1, 2, 3]. The consequent direct and indirect annual costs in the United States are estimated at \$56 billion [4]. The World Health Organization (WHO) has projected that by 2020, road traffic accidents, a major cause of TBI, will rank third as a cause of the

global burden of disease and disablement, behind only ischemic heart disease and unipolar major depression [1]. Although this has led the WHO to set a priority for prevention of head injuries, the incidence of head injuries continues to increase across the world. Efforts to improve treatment and outcome must therefore remain the priority for clinicians and researchers.

The pathophysiology of TBI is very complex and still poorly understood. Immediately following the primary impact, activation of several different pathways begins, resulting in secondary brain injury. These include inflammation, oxidative stress, ionic imbalance, increased vascular permeability, mitochondrial dysfunction, and excitotoxic damage [6, 7]. These processes result in brain edema, increased intracranial pressure (ICP), and impaired cerebral perfusion [2]. This combination of cellular and physiologic disturbances causes increased neuronal cell death; enlargement of infarct size; and neurological, motor, and cognitive impairment. Since there are no approved specific pharmacological agents that block the progression of the secondary injury, the current management of TBI is mainly supportive and aims at treating brain edema, reducing ICP, and combating complications such as hypoxia and shock [3]. Despite promising preclinical data, most of the trials that have been performed in recent years have failed to demonstrate any significant improvement in outcome [10].

Low level laser (or light) therapy (LLLT) has been clinically applied for many indications in medicine that require the following processes: protection from cell and tissue death, stimulation of healing and repair of injuries, and reduction of pain, swelling and inflammation [4, 5]. One area that is attracting growing interest is the use of LLLT to treat stroke, traumatic brain injury, neurodegenerative diseases and spinal cord injuries [6]. The notable lack of any effective drug-based therapies for most of these diseases has motivated researchers to consider the use of light as a real approach to mitigating what is considered to be a group of serious diseases. The fact that near-infrared light can penetrate into the brain and spinal cord could allow non-invasive treatment to be carried out with a low likelihood of treatment-related adverse events. Although in the past it was generally accepted that the central nervous system could not repair itself, recent discoveries in the area of neuronal stem cells have brought this dogma into question [7]. LLLT may have beneficial effects in the acute treatment of brain damage by improving mitochondrial function, by reducing the excitotoxicity consequent upon glutamate release, inhibiting neuronal apoptosis and upregulating several protective factors (Figure 1). In this study the effects LLLT mediated by different wavelengths of light were tested in a mouse model of closed head injury and by measuring the neurobehavioral and histological outcome of the traumatized mice.

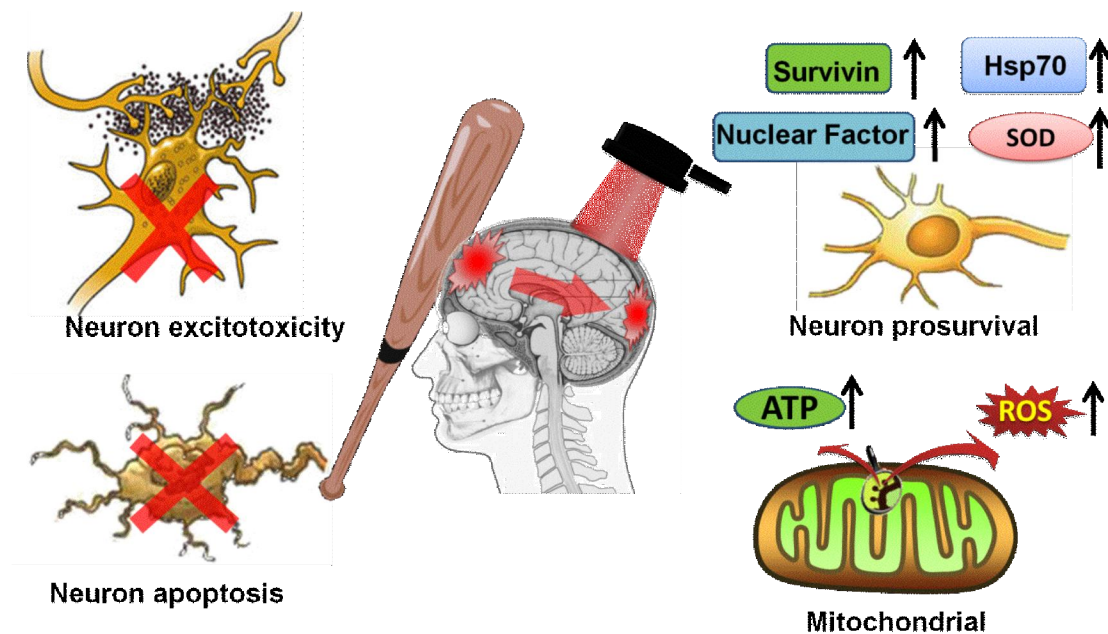


Fig 1. Schematic representation of the use of LLLT to treat TBI

## 2. Materials and methods

### *Animal*

Adult male BALB/c mice (weight 20 to 25 g; Charles River Laboratories, Wilmington, MA) were used in the study. The animals were housed at one mouse per cage and were maintained on a 12-h light–12-h dark cycle with access to food and water ad libitem. All animal procedures were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital and met the guidelines of the National Institutes of Health.

### *Traumatic Brain Injury Model*

Closed head injury (CHI) was induced under isoflurane anesthesia using a weight drop device. The weight drop apparatus includes a vertical 15 cm long plastic tube (diameter 32 mm) with a 69 g weight (diameter 25 mm) dropped through the upper end of the tube. Briefly, following anesthesia, a midline longitudinal incision was performed and the skull was exposed. A Teflon-tipped cone (3 mm diameter) was placed 1 mm lateral to the midline in the mid-coronal plane. The head was manually held in place and a 69 g weight was dropped on the cone from a height that was adjusted to yield a trauma that could be categorized as moderate to severe [Neurological Severity Score (NSS) 6–8] to the left hemisphere. After brain trauma, the skin was sutured. Mice with depressed skull fracture or visible hemorrhage were excluded from the study. The severity of the trauma was confirmed 1 h later by assessing the NSS scores. After recovery from anesthesia, the mice were returned to their cages with postoperative care and ad libitem access to food and water.

### *Neurobehavioral Evaluation*

The neurological status of the traumatized mice was evaluated at different time intervals after CHI according to a neurological severity score (NSS). The neurological tests are based on the ability of the mice to perform 10 different tasks (Table 1) that evaluate the motor ability, balancing, and alertness of the mouse. One point is given for failing to perform each of the tasks; thus, a normal, uninjured mouse scores 0. The severity of injury is defined by the initial NSS, evaluated 1 h post-CHI, and is a reliable predictor of the late outcome. Thus, fatal or near-fatal injury is defined in mice having an NSS of 9–10; severe injury in mice with an NSS of 7–8; moderate injury with NSS of 5–6, and mild injury in mice with an NSS of <5.

**Table 1. Neurological Severity Score (NSS) for Head-Injured Mice**

| <b>Task</b>  | <b>NSS</b> |
|--|------------|
| <b>Presence of mono- or hemiparesis</b>                    | <b>1</b>   |
| <b>Inability to walk on a 3-cm-wide beam</b>               | <b>1</b>   |
| <b>Inability to walk on a 2-cm-wide beam</b>               | <b>1</b>   |
| <b>Inability to walk on a 1-cm-wide beam</b>               | <b>1</b>   |
| <b>Inability to balance on a 1-cm-wide beam</b>            | <b>1</b>   |
| <b>Inability to balance on a round stick (0.5 cm wide)</b> | <b>1</b>   |
| <b>Failure to exit a 30-cm-diameter circle (for 2 min)</b> | <b>1</b>   |
| <b>Inability to walk straight</b>                          | <b>1</b>   |
| <b>Loss of startle behavior</b>                            | <b>1</b>   |
| <b>Loss of seeking behavior</b>                            | <b>1</b>   |
| <b>Maximum total</b>                                       | <b>10</b>  |

### ***Laser Treatment Design***

All mice were subjected to CHI and assessment of neurological score (NSS) was performed 1 h post-injury. The NSS scores of the mice ranged from 6 to 8, indicating a moderate-severe trauma.

The mice were then divided into three groups of eight mice per group, so that the means NSS in each group were similar, to ensure similar average severity of injury in all groups. One group of mice received a single treatment with 670-nm laser ( $36 \text{ J/cm}^2$ ) four hours post-injury, and the second group received a single treatment with 810-nm laser ( $36 \text{ J/cm}^2$ ) four hours post-injury while the third group, serving as a sham-operated control, underwent the same procedures as the laser-treated group, but did not receive actual laser irradiation.

In the laser-treatment group, after the mice were fixed on a plastic plate, the distal tip of the fiber optic of the laser was set to deliver a power density of  $150 \text{ mW/cm}^2$  to the whole brain and the duration of laser irradiation was 4 min (energy density of  $36 \text{ J/cm}^2$ ).

### ***Histology and Lesion size***

At the end of the 4-week follow-up period, mice were anesthetized and perfused transcardially with 10% phosphate-buffered formalin. And then the brains were removed and fixed in the same formaldehyde solution for 3 days. 2-mm-thick cross-sections were taken from the trauma region of the brain. The 2-mm-thick brain slices were processed in an automated tissue processor and embedded in paraffin. Five-micron-thick coronal sections were prepared from each 2-mm-thick brain slice block. Three random sections from each brain slice were stained with hematoxylin and eosin, and the lesion area in each section was calculated by Image-Pro Plus 6.0 software. The whole brain area and the area occupied by brain tissue were calculated separately in each histological section. Lesion size (in percentage) was expressed as whole brain area minus area occupied by brain tissue divided by the whole brain area.

### ***Statistical analysis***

Data are presented as mean  $\pm$  SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by Student *t*-test. Significance was defined as  $p < 0.05$ . SPSS statistics V17.0 software was used for statistical analyses.

## **3. Results**

### ***Neurobehavioral evaluation after 665-nm laser***

The neurobehavior evaluated by neurological severity score in control and the 670-nm laser ( $36 \text{ J/cm}^2$ ) 4h post injury treatment group are shown in (Fig. 1). The results show an overall decrease in the neurological score in control as well as the 670-nm laser treatment group after 24h. At all the time point after 24h post injury the neurological severity score showed a significant ( $p < 0.05$ ) difference in the treatment group and the control group. The neurological severity score decreased to 21% in the 670-nm laser ( $36 \text{ J/cm}^2$ ) treatment group on the 9 days after treatment with 52% retention of the score in the control group. After 28 days the neurological severity score in the treatment group decreased to 11% as compared to 28% in the control group. Thus the decrease in % neurological severity score was significantly low in 666 nm laser treated group at all time point as compared to the control group.

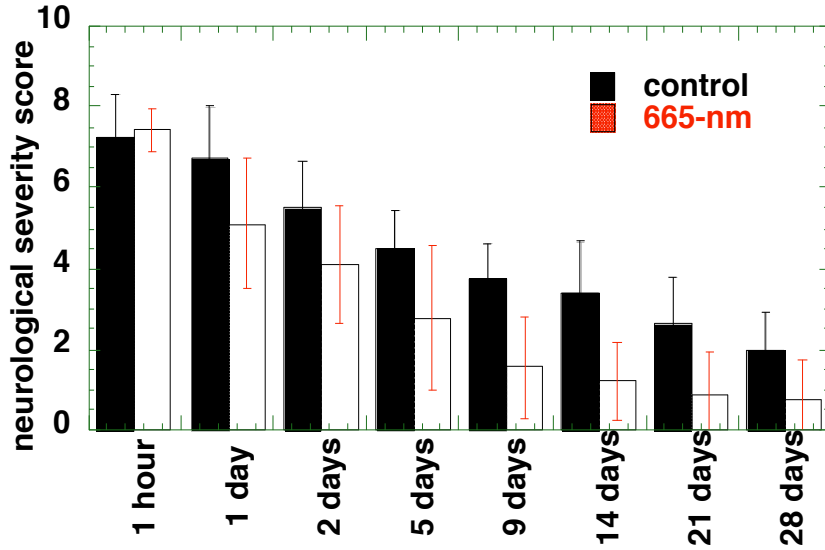


Fig 2. Neurological score (NSS) of control non-laser-treated and 665-nm laser-irradiated mice at different time intervals after induction of brain trauma. Results are expressed as mean ± SD.

**Neurobehavioral evaluation after 810-nm laser**

The neurological severity score in control and the 810-nm laser (36 J/cm<sup>2</sup>) 4h post injury treatment group are shown in (Fig. 2). The graph shows an overall decrease in the neurological score in control as well as the 810-nm laser treatment group after 24h. At all the time point after 24h post injury neurological severity score showed a significant difference ( $p < .05$ ) in the treatment group and the control group . This neurological severity score was 32% decreased in the 810-nm laser (36 J/cm<sup>2</sup>) treatment group on the 9 days after treatment with only 52% decrease in the control group. After 28 days the neurological severity score in the treatment group decreased to 5% as compared to 28% in the control group. Thus the decrease in the % neurological severity score in the 810 nm laser treatment group was lower as with the control group at all time point.

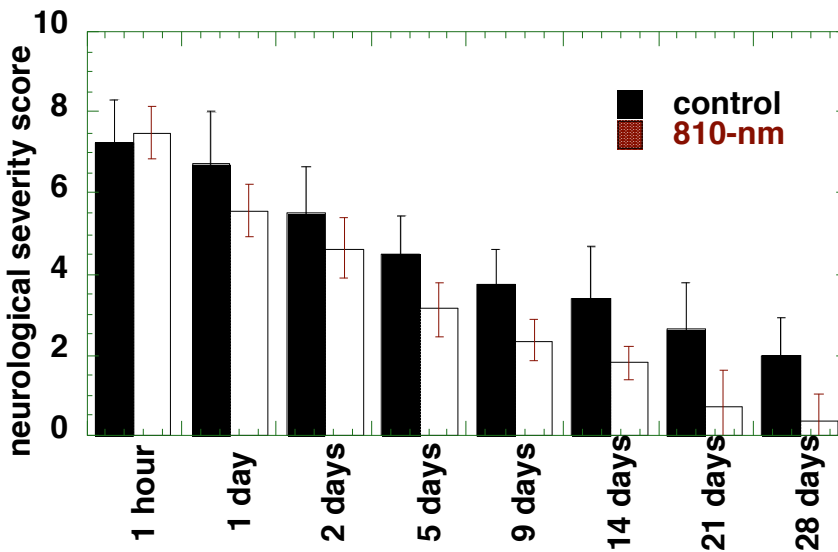
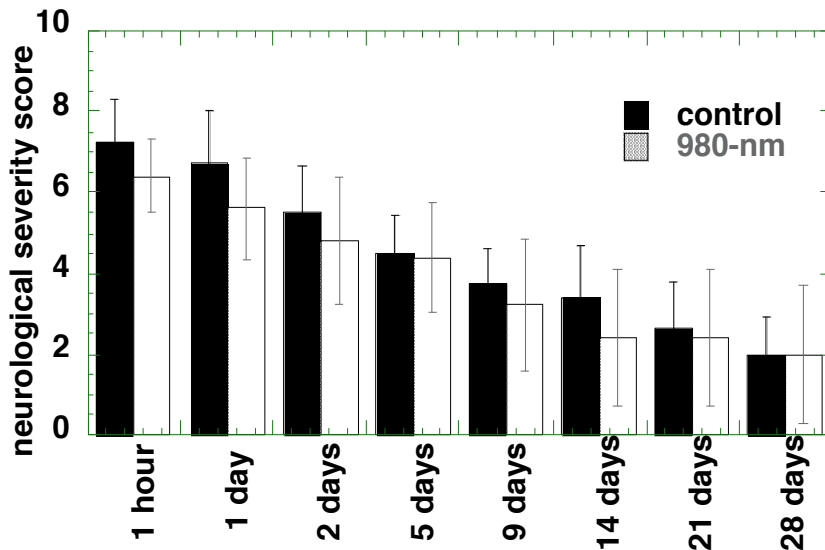


Fig 3. Neurological score (NSS) of control non-laser-treated and 810-nm laser-irradiated mice at different time intervals after induction of brain trauma. Results are expressed as mean ± SD.

### Neurobehavioral evaluation after 980 nm laser

The neurological severity score in control and the 980-nm laser (36 J/cm<sup>2</sup>) 4h post injury treatment group are shown in (Fig. 2). The graph shows an overall decrease in the neurological score in control as well as the 980-nm laser treatment group after 24h. At all the time point after 24h post injury neurological severity score showed a no significant ( $p > 0.05$ ) difference in of the treatment and the control group. The % decrease in the neurological severity score in the treatment group and the control group was 50% and 52 % respectively 9 days post after induction of brain trauma. After 28 days the score reached to 31% and 28% in the treated and the control groups respectively. Thus there was no significant difference in the neurological severity score between the two groups at all time points.



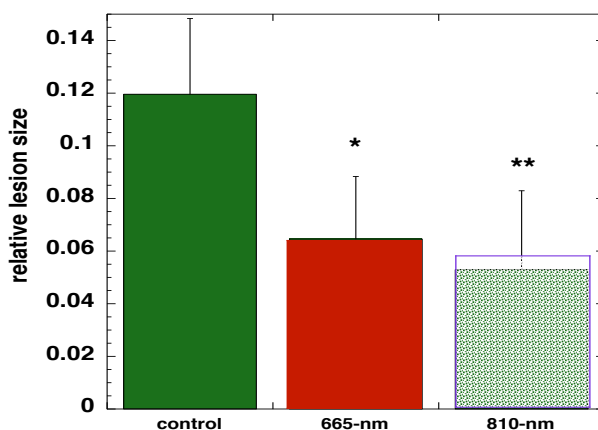
**Fig 5.** Neurological score (NSS) of control non-laser-treated and 980-nm laser-irradiated mice at different time intervals after induction of brain trauma. Results are expressed as mean  $\pm$  SD.

### Histology and lesion size

The histological studies with the brain cross-sections removed 28 days post TBI of the control and the treated groups are shown in Figures 6 and 7. There was a significant difference in the lesion size between the control group and both of the two laser-treated groups ( $p < 0.05$ ) and no significant difference between the two (670nm, 810nm) laser-treated groups ( $p > 0.05$ ). The lesion size in the histological sections measured with ImageJ software shows (Fig.7) that the lesion size in the control group was 12 area units, while the laser treated group 670-nm and 810nm showed a reduced lesion size which was 6 and 5 area units respectively.



**Fig 6.** Representative Micrographs of cross-sections of (a) control non-laser-treated mice brains. (b) 670 nm laser-treated mice brains (c) 810 nm laser-treated mice brains.



**Fig 7.** Quantification of lesion areas from micrographs of brain slices of mice in different treatment groups. \*  $p < 0.05$ ; \*\*  $P < 0.01$

#### 4. Discussion

This study has shown that both red laser (665-nm) and NIR laser (810-nm) can significantly and dramatically improve the neurobehavioral performance of mice after CCI TBI. NIR laser at 980-nm however did not produce the same positive effects. The principal tissue chromophore that is proposed to be responsible for photobiomodulation effects is cytochrome c oxidase (CCO). CCO has distinct absorption bands in the red (around 665-nm) and in the NIR (around 810-nm) [8]. At 980-nm however there is a notable absorption band of water (more than 10 times higher than absorption of water at 810-nm) and therefore 980-nm photons are more likely to produce tissue heating rather than the photochemical effects consequent upon absorption by CCO. Studies from Wong-Riley et al [9] indicated that 670-nm and 810-nm light has positive effects in protecting visual cortical neurons from cytotoxicity mediated by potassium cyanide.

LLLT is increasingly being used for tissue preservation and functional stimulation at the cellular level, after various forms of injury. Stimulated wound healing, reduction of edema and inflammation, pain relief, and prevention of tissue loss are the main applications. During LLLT, absorption of red or near-infrared photons by cytochrome c oxidase in the mitochondrial respiratory chain causes an increase in cellular respiration that continues for much longer than the light is present when delivered at appropriate fluence and exposure durations [10]. Primary cellular effects include increases in mitochondrial activity and ATP levels, production of low levels of reactive oxygen species, induction of transcription factors (including the pro-survival NF- $\kappa$ B), and inhibition of apoptosis [11].

Secondary injuries after TBI are attributable to spreading cellular damage that can grow to encompass a much greater area of the brain than was originally damaged and results from excitotoxic cell death after glutamate release and sequelae of inflammation. Secondary injury cascades can affect neurological function unrelated to specific tissue damage. Glutamate depolarizes neurons that suffer a huge influx of sodium and calcium but could survive if they had sufficient ATP to power the Na<sup>+</sup>/K<sup>+</sup>-ATPase pumps. ATP is increased several-fold by LLLT and can prevent excitotoxicity. LLLT modulates many biological and disease processes increasing fibroblast migration, proliferation and differentiation, angiogenesis and healing in ischemic heart and skeletal muscles, tendons, bones. In methanol-induced retinal toxicity, LLLT prevented death of retinal neurons. LLLT can affect both nitric oxide synthase activity and upregulation of TGF- $\beta$ 1 simultaneously. There are reports of beneficial effects of LLLT on functional recovery of injured peripheral nerves and also of central neurons in spinal cord.



LLLT has been used to significantly improve recovery in human stroke patients, following only one transcranial near infrared (NIR, 808 nm) laser treatment at ~18 hours after stroke [12]. A recent study in mice indicates that LLLT can reduce histopathology after closed head contusion TBI [13], and another study suggests that LLLT can improve learning and memory in middle-aged mice [14], suggesting that LLLT might also ameliorate learning and memory deficits associated with TBI even if applied long after the injury.

Many further questions are raised by our results. Are the laser parameters used in our study optimal? What is the optimum time after injury for laser application? Does repeated laser treatment give any additional benefit over the single treatment used in our study? Nevertheless, given the total lack of adverse effects of LLLT, transcranial laser is an extremely promising therapy for brain injury in humans.

### **Acknowledgements**

This work was supported by NIH grant R01AI050875, Center for Integration of Medicine and Innovative Technology (DAMD17-02-2-0006), CDMRP Program in TBI (W81XWH-09-1-0514) and Air Force Office of Scientific Research (FA9950-04-1-0079)

### **Reference**

- [1] Finfer, S. R., and Cohen, J., "Severe traumatic brain injury," *Resuscitation*. 48, 77-90 (2001)
- [2] Nortje, J., and Menon, D. K., "Traumatic brain injury: physiology, mechanisms, and outcome," *Curr Opin Neurol*. 17, 711-718 (2004)
- [3] Bullock, M. R., and Povlishock, J. T., "Guidelines for the management of severe traumatic brain injury. Editor's Commentary," *J Neurotrauma*. 24 Suppl 1, 2 p preceding S1 (2007)
- [4] Hamblin, M. R., and Demidova, T. N. "Mechanisms of low level light therapy – an introduction." *In: [Mechanisms for low-light therapy]*, San Jose, 2006, pp. 61001-61012.
- [5] Huang, Y.-Y., Chen, A. C.-H., and Hamblin, M. R. "Advances in Low-Intensity Laser and Phototherapy." *In: V. V. Tuchin (ed.), [Handbook of Photonics for Medical Science.]* London, UK: Taylor and Francis, 2010.
- [6] Huang, Y.-Y., Hamblin, M. R., and De Taboada, L. "Low-level Laser Therapy in Stroke and Central Nervous System". *In: V. V. Tuchin (ed.), [Handbook of Photonics for Medical Science.]* London, UK: Taylor and Francis, 2010.
- [7] Demir, O., Singh, S., Klimaschewski, L., and Kurnaz, I. A., "From birth till death: neurogenesis, cell cycle, and neurodegeneration," *Anat Rec (Hoboken)*. 292, 1953-1961 (2009)
- [8] Karu, T. I., Pyatibrat, L. V., Kolyakov, S. F., and Afanasyeva, N. I., "Absorption measurements of a cell monolayer relevant to phototherapy: Reduction of cytochrome c oxidase under near IR radiation," *J Photochem Photobiol B*. 81, 98-106 (2005)
- [9] Wong-Riley, M. T., Liang, H. L., Eells, J. T., Chance, B., Henry, M. M., Buchmann, E., Kane, M., and Whelan, H. T., "Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase," *J Biol Chem*. 280, 4761-4771 (2005)
- [10] Lane, N., "Cell biology: power games," *Nature*. 443, 901-903 (2006)
- [11] Karu, T. I., "Mitochondrial Signaling in Mammalian Cells Activated by Red and Near-IR Radiation," *Photochem Photobiol*. 84, 1091-1099 (2008)
- [12] Lampl, Y., Zivin, J. A., Fisher, M., Lew, R., Welin, L., Dahlof, B., Borenstein, P., Andersson, B., Perez, J., Caparo, C., Ilic, S., and Oron, U., "Infrared Laser Therapy for Ischemic Stroke: A New Treatment Strategy. Results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1)," *Stroke*. (2007)
- [13] Oron, A., Oron, U., Streeter, J., de Taboada, L., Alexandrovich, A., Trembovler, V., and Shohami, E., "low-level laser therapy applied transcranially to mice following traumatic brain injury significantly reduces long-term neurological deficits," *J Neurotrauma*. 24, 651-656 (2007)
- [14] Michalikova, S., Ennaceur, A., van Rensburg, R., and Chazot, P. L., "Emotional responses and memory performance of middle-aged CD1 mice in a 3D maze: effects of low infrared light," *Neurobiol Learn Mem*. 89, 480-488 (2008)