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UP TO 90% MORE PHOTONS
REACH THE TARGET TISSUE
ALL LASERS WITH 15 W OF OUTPUT ARE NOT EQUAL...

"WHENEVER POSSIBLE, THE CONTACT MODE OF TREATMENT IS PREFERRED FOR THE SIMPLE REASON THAT THE LOSS OF ENERGY IS MINIMAL—MANY TIMES EVERY PHOTON EMANATING FROM THE APPLICATOR ENTERS THE PATIENT'S SKIN OR TISSUE. THIS IS NOT THE CASE WITH THE NONCONTACT MODE OF TREATMENT, IN WHICH SOME OF THE PHOTONS ARE REFLECTED OR REFRACTED FROM THE SURFACE OF THE SKIN RESULTING IN LOSS OF ENERGY AND DIMINISHING THE INTENDED AMOUNT OF TREATMENT ENERGY."


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2015 begins as an exciting year for Laser Therapy. As Laser Therapy continues to grow in acceptance in the medical community, it is important to keep the focus on the underlying science. The biggest threat to the effective use of this powerful treatment modality is confusion and misinformation.

The fundamental principles that underpin laser therapy treatments as currently understood in the scientific literature are relatively straightforward. There is consensus that the application of a therapeutic dose of light to impaired or dysfunctional tissue leads to a cellular response mediated by mitochondrial mechanisms that reduce pain and inflammation and speed healing. However, as the technology has become more popular, manufacturers have gone beyond these bounds with elaborate claims about specific pulsing protocols and application-specific wavelengths to gain advantage and create confusion in the market. In reality these product-specific bells and whistles are more marketing hype than science and supported only by cherry-picking outdated literature or the manufacturer’s internal testing if at all.

The purpose of this Scientific Report is to make clear how LiteCure® has developed devices and dosing protocols for both Companion® and Pegasus® Therapy Lasers. Well-characterized cellular responses inform treatment guidelines and complexity is only added when dictated by reproducible science. Reprints provided in the piece demonstrate the fundamental principles that guide protocol development at LiteCure:

1. Protocols are developed through an understanding of cellular response and the penetration of light to the target tissue.
2. Protocols are validated by independent academic labs in blinded randomized trials with long-term follow-up.
3. Rigorous design of studies is focused on sham-controlled hard science and evaluated using quantifiable measures.

These simple principles form the powerful backbone of protocol development at LiteCure. The studies provided in this piece are only 4 of the 9 publications in 2013 using LiteCure therapy lasers with many more underway.

As you gain understanding of this powerful technology we encourage you to ask tough questions, understand the reasons for all the bells and whistles, and ask for recent publications to support claims. The clinical team at LiteCure will be there to help you every step of the way.

Sincerely,

Brian A. Pryor, PhD
CEO, LiteCure
In Vitro and In Vivo Optimization of Infrared Laser Treatment for Injured Peripheral Nerves

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ABSTRACT

BACKGROUND AND OBJECTIVE: Repair of peripheral nerve injuries remains a major challenge in restorative medicine. Effective therapies that can be used in conjunction with surgical nerve repair to improve nerve regeneration and functional recovery are being actively investigated. It has been demonstrated by a number of peer reviewed publications that photobiomodulation (PBM) supports nerve regeneration, reinnervation of the denervated muscle, and functional recovery after peripheral nerve injury. However, a key issue in the use of PBM as a treatment for peripheral nerve injury is the lack of parameter optimization for any given wavelength.

The objective of this study was to demonstrate that for a selected wavelength effective in vitro dosing parameters could be translated to effective in vivo parameters.

MATERIALS AND METHODS: Comparison of infra-red (810 and 980 nm wavelengths) laser treatment parameters for injured peripheral nerves was done beginning with a series of in vitro experiments using primary human fibroblasts and primary rat cortical neurons. The primary rat cortical neurons were used for further optimization of energy density for 980 nm wavelength light using measurement of total neurite length as the bioassay. For these experiments, the parameters included a 1 W output power, power density of 10 mW/cm², and energy densities of 0.01, 0.1, 0.5, 2, 10, 50, 200, 1,000, and 5,000 mJ/cm². For translation of the in vitro data for use in vivo it was necessary to determine the transtunaneous penetration of 980 nm wavelength light to the level of the peroneal nerve. Two anesthetized, male White New Zealand rabbits were used for these experiments. The output power of the laser was set at 1.0 or 4.0 W. Power density measurements were taken at the surface of the skin, sub-dermally, and at the level of the nerve. Laser parameters used in the in vitro studies were calculated based on data from the in vitro studies and the light penetration measurements. For the in vivo experiments, a total of 22 White New Zealand rabbits (2.34–2.89 kg) were used. Translated dosing parameters were refined in a pilot study using a transection model of the peroneal nerve in rabbits. Output powers of 2 and 4 W were tested. For the final set of in vivo experiments, the same transection nerve injury model was used. An energy density of 10 mW/cm² at the level of the peroneal nerve was selected and the laser parameters were further refined. The dosing parameters used were: 1.5 W output power, 43 seconds exposure, 8 cm² area and a total energy of 65 J.

RESULTS: In vitro, 980 nm wavelength light at 10 mW/cm² significantly improved neurite elongation at energy densities between 2 and 200 mJ/cm². In vivo penetration of the infrared light measured in anesthetized rabbits showed that on average, 2.45% of the light applied to the skin reached the depth of the peroneal nerve. The in vivo pilot study data revealed that the 4 W parameters inhibited nerve regeneration while the 2 W parameters significantly improved axonal regrowth. For the final set of experiments, the irradiated group performed significantly better in the toe spread reflex test compared to the control group from week 7 post-injury, and the average length of motor endplates returned to uninjured levels.

CONCLUSION: The results of this study demonstrate that treatment parameters can be determined initially using in vitro models and then translated to in vivo research and clinical practice. Furthermore, this study establishes that infrared light with optimized parameters promotes accelerated nerve regeneration and improved functional recovery in a surgically repaired peripheral nerve. Lasers Surg. Med. ©2013 Wiley Periodicals, Inc.

KEY WORDS: immunolabeling; light therapy; motor end plates; peripheral nerve injury; photobiomodulation; regeneration; re-innervation; toe spread reflex
INTRODUCTION
Post-traumatic repair of peripheral nerve injuries remains a major challenge in restorative medicine. In the United States, 50,000 peripheral nerve repair procedures are performed annually [1]. Although major advances have been made in the microsurgical repair of injured peripheral nerves [2], clinical results and functional recovery have been disappointing [3,4]. The current challenge is to identify effective therapies that can be used in conjunction with surgical nerve repair to improve nerve regeneration and functional recovery.

There is an impressive number of peer reviewed publications on the treatment of peripheral nerve injury with photobiomodulation (PBM). Based on reviews of this literature, PBM supports peripheral nerve regeneration, reinnervation of the denervated muscle, and functional recovery after peripheral nerve injury [5–7]. However, a key issue in the use of PBM as a treatment for peripheral nerve injury is the lack of parameter optimization for any given wavelength. Selection of treatment parameters was often based on published reports or transfer of parameters from other studies in the laboratory or clinic. Preliminary studies to optimize the treatment parameters were rarely done.

Review of this literature also underscores that many wavelengths can support peripheral nerve injury repair. The wavelengths primarily used in these studies ranged in the 600–904 nm range [6,7]. Wavelength selection was also often based on devices available to the laboratory or clinic, published reports, or transfer of wavelength selection to peripheral nerve injury studies from in vitro or in vitro experiments on other types of injuries.

Successful therapeutic use of any particular wavelength requires penetration to the target tissue and treatment parameters that can deliver a therapeutic dose at the tissue. Currently many available laser devices used for PBM emit at least a percentage of 980 nm wavelength light (personal communication with Dr. Smith). However, there are no published reports on optimization of parameters for this wavelength. Therefore, 980 nm wavelength light was chosen for this study. The objective of this study was to demonstrate that for a selected wavelength effective in vitro dosing parameters could be translated to effective in vivo use. A systematic approach was taken for optimization of treatment parameters beginning with a series of in vitro cellular models including primary human fibroblasts and rat cortical neurons. For the primary human fibroblasts, laser parameter optimization was initially done with 810 nm wavelength light. We previously reported that 810 nm wavelength light at several combinations of power density and treatment time supported differentiation of normal human neural progenitor cells in vitro [8]. We were interested in determining if these same optimized combinations of power density and time for 810 nm wavelength light could have a photobiomodulatory effect on a different cell type, the primary human fibroblast, and measured by a different bioassay, mitochondrial metabolism. Once the effective combinations of power density and time for the 810 nm wavelength light were identified for the primary human fibroblasts, these same combinations were tested using 980 nm wavelength light. The in vitro studies were then expanded to include further optimization of the 980 nm wavelength light using rat cortical neurons. In vivo penetration measurements of 980 nm wavelength light to the depth of the peroneal nerve were done to aid in the translation of the in vitro optimized parameters for use in vivo. An in vivo pilot study was then conducted to refine the dosing parameters followed by a definitive test of the optimized parameters based on behavioral and immunohistological analyses.

The results of this study demonstrate that treatment parameters can be determined initially using in vitro models and then translated to animal model research and clinical practice. Furthermore, this study establishes that infrared light with optimized parameters promotes accelerated nerve regeneration and improved functional recovery in a surgically repaired peripheral nerve.
MATERIALS AND METHODS

In Vitro Experiments: Initial Optimization of Laser Parameters using In Vitro Human Fibroblasts and Rat Cortical Neurons

Culture of primary human fibroblasts. Primary human fibroblasts (ATCC, Manassas, VA) were cultured in DMEM with 10% fetal bovine serum (FBS) and 4.5 g/L D-glucose. After trypsinization, 5 x 10^3 cells were seeded per well in a 24-well plate with DMEM containing 2% FBS and 1 g/L D-glucose. The fibroblasts were allowed to attach by incubating for 1 hour at 37°C, 5% CO2, before irradiation.

Laser irradiation of primary human fibroblasts. After attachment to the substrate, fibroblasts were exposed to light with a wavelength of 810 or 980 nm. A continuous wave (CW) 810 nm diode laser (Thor International, Chesham, UK; 200 mW output, modified and homogenized with a delivery optical fiber resulting in an output power of 150 mW) was used. A 980 nm CW laser (LiteCure, LLC, Newark, DE; Model No. PLT-980-10; output power: 1 to 10 W) was used at 1 W output power. A power density of 10 mW/cm^2 and energy densities of 200, 1,000, or 5,000 mJ/cm^2 were used for both lasers. Six out of 24 wells in each culture plate were seeded in a group of two adjacent wells. The height of laser above the plate was adjusted to attain an energy density of 10 mW/cm^2 and to irradiate two adjacent wells simultaneously. Light irradiation was performed from above with the lid off. The three groups were on the edges of the plate and separated from each other to eliminate the effects of light scatter during treatment. Also a black mask was used to cover the wells that were not treated. The 980 nm wavelength laser had an aiming beam (650 nm wavelength, 3.5 mW output power on the high setting) that remained on during laser treatment. Therefore, an experimental group was included (aiming beam group) that was irradiated with the aiming beam only. These cells were treated the same as the 980 nm wavelength group except only the aiming beam was on (irradiation time: 8 minutes and 20 seconds, power density: 0.01 mW/cm^2 and energy density: 5 mJ/cm^2).

MTS assay. At 40 minutes after irradiation, the MTS assay (Promega, Madison, WI) was performed to measure the metabolic activity of the cells. The test was performed according to the manufacturer’s instruction and was duplicated for each combination of parameters. A series of tests were done to determine the optimal incubation time in the MTS solution. One hour and 30 minutes resulted in the best absorption reading. After incubation with MTS solution, the supernatant was removed for absorption reading at a wavelength of 492 nm.

Culture of primary rat cortical neurons. One well in a 2-well chamber slide was coated with 30 μg/ml Poly-D-Lysine and 2 μg/ml Laminin according to manufacture protocol. Rat cortical neurons (Lonza, Inc., Walkersville, MD) were thawed from liquid N_2 and seeded 8 x 10^3 cells/well in primary neuron growth medium (Lonza, Inc.) with glucose concentration of either 25 mM as control or 180 mM as high glucose level. Neurons were subjected to high glucose concentrations to cause impairment of their metabolism and neurite extension. The high glucose concentration was established in a preliminary study which found a 28% decrease of total neurite length (P<0.001) when rat cortical neurons were cultured in 180 mM glucose compared to control (25 mM glucose) (Table 1).

Laser irradiation of rat cortical neurons. For comparison of the effects between 980 and 810 nm wavelength light, cells were irradiated with either a 980 nm CW laser (LiteCure, LLC; 1 W) or an 810 nm CW laser (Thor International, UK; 150 mW). The parameters for both lasers were power density of 10 mW/cm^2 and energy densities of 10, 50, 200, 1,000, and 5,000 mJ/cm^2. Cells were treated immediately after seeding and once again 24 hours later. Cells were fixed 24 hours after the second irradiation.

For further optimization of energy density for 980 nm wavelength light, a broader range of energy densities was studied. The parameters were 1 W output power, power density of 10 mW/cm^2, and energy densities of 0.01, 0.1,
0.5, 2, 10, 50, 200, 1,000, and 5,000 mJ/cm². A shutter controller (UNIBLITZ Corp., Rochester, NY) was placed in the path of light between the laser and chamber slide to control treatment time. The heights of the laser and shutter controller were adjusted so that the beam size was larger than the culture chamber (2 cm x 2 cm) and the power density on the cells was 10 mW/cm². Cells were treated immediately after seeding and again 24 hours later. Cells were fixed 24 hours after the second irradiation for further assessment.

Neurite assessment. The cells were fixed with 4% paraformaldehyde at 24 hours after the second irradiation. Each condition was duplicated. Images of all neurons (between 60 and 150 neurons in each group) were digitally photographed using an Olympus DP72 microscope digital camera (Olympus Imaging America, Inc., Center Valley, PA). The images of the neurons were uploaded to ImageJ with NeuronJ plugin (Version 1.45s). Neurites were counted for each neuron and neurite length was measured. Total neurite length was calculated as the sum of the length of all neurites from each neuron.

Temperature measurement. An infrared imaging system (FLIR Systems ThermoVision A40, moviMED, Irvine, CA) was used to detect temperature change in culture medium of the rat cortical neurons during irradiation. The computerized camera was focused on the culture dish without direct contact and temperature was recorded throughout the duration of the irradiation.

IN VIVO EXPERIMENTS

Animals. A total of 22 male White New Zealand rabbits (2.34–2.89 kg) were used in this study. The animal use protocol was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Use Committee. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council publication, 1996 edition.

Surgery. The rabbits were anesthetized with Ketamine/Xylazine (25 mg/kg; 5 mg/kg) and endo-tracheal intubation was performed. Anesthesia was maintained with isoflurane (3.0% with 1.5 L/minute O₂). Povidone iodine surgical scrub was used to clean the skin in the surgical area after shaving the hair. The skin was incised along the posterior aspect of left thigh and the superficial fascia along the sciatic vein was sharply divided. The biceps muscle was bluntly dissected away from the semitendinous muscle and retracted laterally to fully expose the peroneal nerve. The left peroneal nerve was completely transected and sutured back together by end-to-end epineural suture using 8–0 Ethilon suture. Skin was closed using a buried running subcutaneous suture technique with 4–0 Monocryl suture and cleaned with sterile saline. The rabbits recovered in heated post-surgical recovery chambers before they were returned to their cages.

Light penetration measurements. Penetration of 980 nm wavelength light through the skin and femoral biceps muscle overlying the peroneal nerve was measured in two anesthetized, male White New Zealand rabbits. A near infrared power meter was designed and built by B&W Tek, Inc. (Newark, DE) to measure the power density (Fig. 3 A,B) below the skin and at the level of the peroneal nerve. A small photo sensor (2.0 mm X 2.5 mm) was sealed in glass tube. The output voltage of the sensor was calibrated such that a reading of 1mV represented a power density of 1 mW/cm². Surgery was done as described above to expose the peroneal nerve. The output power of the laser was set at 1.0 or 4.0 W. Power density measurements were taken at the surface of the skin, sub-dermally, and at the level of the peroneal nerve. Each measurement was done in triplicate and the average number was used for calculation of penetration.
In Vitro and In Vivo Optimization of Infrared Laser Treatment for Injured Peripheral Nerves

In Vitro Cortical Neuron Neurite Length and Number

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control neurons 25 mM glucose</th>
<th>Impaired neurons 180 mM glucose</th>
<th>Decrease ratio (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length (micron)</td>
<td>163.10 ±5.89</td>
<td>118.00 ± 5.48</td>
<td>28% (P&lt;0.001)</td>
</tr>
<tr>
<td>Average length (micron)</td>
<td>45.94 ±1.47</td>
<td>39.63 ± 1.67</td>
<td>14% (P&lt;0.05)</td>
</tr>
<tr>
<td>Neurite number</td>
<td>3.55 ±0.11</td>
<td>2.98 ± 0.11</td>
<td>17% (P&lt;0.01)</td>
</tr>
</tbody>
</table>

Neurite extension was measured using ImageJ with NeuronJ Plugin. Measurements were analyzed by one-way ANOVA with Tukey’s multiple comparison test. There was a 28% decrease in total neurite length, a 14% decrease in average neuron length, a 17% decrease in neurite number when rat cortical neurons were cultured in 180 mM glucose compared to control (25 mM glucose). Based on this data, total neurite length was used as the assessment measure for optimization of the laser treatment parameters for 980 nm wavelength light.

Calculation of laser parameters used in pilot and long term studies. Based on our data from the in vitro experiments on rat cortical neurons, the optimal parameters for laser irradiation with 980 nm wavelength light were energy densities between 2 and 200 mJ/cm² at a power density of 10 mW/cm². Penetration measurements of 980 nm wavelength light to the level of the peroneal nerve in the living anesthetized rabbit revealed that approximately 2.45% (average of the 4.0 and 1.0 W measurements) of 980 nm wavelength light penetrated to the level of the nerve. To assure adequate treatments time for the in vivo experiments, calculations were based on the high end of the range of effective in vitro energy densities (200 mJ/cm²). Therefore, the energy density needed at the surface of the skin would be (200 mJ/cm²)/0.0245 = 8.16 J/cm². The treatment area necessary to cover the length of the injured peroneal nerve in the thigh was 8 cm² and the total energy delivered to this area based on an energy density of 8.16 J/cm² was 65 J. Therefore the 980 nm wavelength laser parameters used in the pilot study were: 2 W group (output power: 2.0 W, irradiation time: 32 seconds, area: 8 cm², 65 J delivered over the area) and 4 W group (output power: 4.0 W, irradiation time: 16 seconds, area: 8 cm², 65 J delivered over the area).

Pilot study. Eight White New Zealand rabbits underwent peroneal nerve transaction followed by end-to-end epineural suture as described above. The rabbits were randomly placed into three groups (four rabbits for control and two rabbits per laser treatment group): Control (received no laser treatment), 2 W group (980 nm wavelength, CW, output power: 2.0 W, treatment time: 32 seconds, 8 cm² area, 65 J delivered over the area) and 4 W group (output power: 4.0 W, treatment time: 16 seconds, 8 cm² area, 65 J). Laser treatment was done once daily for 10 consecutive days starting immediately after surgery. The control group rabbits were treated exactly the same but the laser was off. The rabbits had been gentled and were held during the treatments. Two control rabbits were euthanized at 14 days post-surgery with sodium pentobarbital (150 mg/kg, IP) for silver stain analysis and six rabbits were euthanized at 21 days post-surgery.
days post-surgery for axonal immunolabeling.

**Long term study.** Twelve White New Zealand rabbits were randomized into 2 groups: control (no laser treatment) and LT (laser treatment group, laser treatment parameters: 980 nm wavelength, CW, output power: 1.5 W, treatment time: 43 seconds, and 8 cm² area, 65 J). Surgery was performed as in the pilot study. The left side of peroneal nerve was completely transected and then repaired using epineural suture. Light was applied immediately after closure of the skin. Daily irradiation was performed for 10 consecutive days. The control group was handled the same way as the light-treated group except the laser was off during treatment time. Rabbits were euthanized at 9 weeks post-surgery with sodium pentobarbital (150 mg/kg, IP).

**Toe spread reflex test.** The toe spread reflex test has been shown to be an effective measure of function for the peroneal dependent muscles of rabbit [9]. Briefly, rabbits were lifted in the air and suddenly lowered without letting them touch a surface. The injured animals lose the native reflex to spread the toes. The behavioral test was video recorded from the front of the animal so that the full width of both hind feet could be captured. The toe-spreading reflex test was performed at baseline and weekly starting from 4 weeks post-surgery. Images of the toe spread were extracted from the video and analyzed by ImageJ. The width of the feet were measured pre- and post-injury. The toe spread was calculated as a ratio of post-injury to pre-injury width on the left foot.

**Silver stain.** The peroneal nerves were harvested 14 days post-injury and fixed in 4% paraformaldehyde overnight. The nerves were then cryoprotected in 30% sucrose for 48 hours. Transverse sections (10 μm thickness) of the nerve samples from 1 cm proximal and 2, 3, and 4 cm distal to injury site were cut using a cryostat. Immunohistochemistry was performed using antibody against protein gene product 9.5 (PGP9.5). Expression of PGP9.5 is highly specific to neurons and to cells of the diffuse neuroendocrine systems and stains regenerating neurons. Heat induced antigen retrieval was first performed by soaking the slides in heated citrate buffer (pH 6.0) for 30 minutes. The sections were blocked with 10% normal goat serum in PBS for 15 minutes at room temperature. After incubating with primary antibody (Mouse anti-PGP9.5, AbD Serotec, Raleigh, NC) for 1 hour, secondary antibody (Alexa-Fluor488 Goat anti-mouse IgG, Life Technologies, Grand Island, NY) was used to visualize the antigen. The nerve sections were photographed and images digitally collected using an Olympus DP72 microscope digital camera (Olympus Imaging America, Inc.). The intensity of positive labeling was analyzed using ImageJ software.

**Labeling of motor end plates in long term study.** For the long-term study, the peroneal nerve related muscles from both left side (injured) and right side (uninjured) were collected and fixed in 4% paraformaldehyde overnight. The collected muscles included the Peroneus Brevis and Peroneus Tertius. The muscles were transferred to 30% sucrose for 48 hours. Serial 20 μm thick longitudinal sections were cut. Neurofilaments were immunolabeled to visualize the peroneal nerve axons. Sections were washed in PBS and blocked in 0.2% Triton, 10% goat serum in PBS for 30 minutes at room temperature, followed by overnight incubation with Mouse anti-Neurofilament 160/200 (NF160/200, Life Technologies, Corp., 1:100 diluted in 1% goat serum, 0.2% Triton in PBS) at 4°C. After washing with 1% goat serum, 0.2% Triton in PBS, the sections were incubated for 1 hour with Goat anti-Mouse secondary antibody conjugated to Alexa Fluor 488 (Life Technologies,
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Corp. 1:300 diluted in 1% Goat serum, 0.2% Triton in PBS), and α-bungarotoxin (α-BTX) conjugated to Alexa Fluor 594 (Life Technologies, Corp., 2 μl/ml final concentration) at room temperature. α-BTX was used because it binds to acetylcholine receptors on muscle fiber. The main location of this receptor is on muscle end plates. After washing with PBS, slides were coverslipped with Vectashield mounting medium (Vectors Laboratories, Inc. Burlingame, CA) and sealed with nail polish. The images of motor endplates collected from the sections were uploaded to ImageJ (Version 1.45s) and the length of each motor endplate was measured. Motor end plates lengths are presented as mean ± SEM.

Statistical analyses. Statistical analyses were performed using GraphPad Prism (version 3.02, GraphPad Software, Inc., La Jolla, CA). MTS assay, neurite assessment and PGP9.5 immunolabeling were analyzed by one-way ANOVA with Tukey’s multiple comparison test. The toe spread test were analyzed with repeated measures ANOVA with time as a within-subjects factor, groups (LT and control) as a between-subjects factor, and ratio to baseline as the dependent variable. Dunnett’s multiple comparisons test was used to compare each time point to week 4 separately for each group, and Sidak’s multiple comparisons test was used to compare groups at each time point. Familywise error rate was set at alpha=0.05. The motor end plate data was analyzed by one-way ANOVA with Tukey’s multiple comparison test. A statistically significant difference was defined as P value less than 0.05.

RESULTS

Primary Human Fibroblasts – MTS Assay

The effects of altering time of irradiation at a power density of 10 mW/cm² for two different wavelengths of light on in vitro human fibroblast mitochondrial metabolism are summarized in Figure 1. The laser had an aiming beam that remained on during laser treatment. Therefore, an experimental group was included (aiming beam group) which was irradiated with the aiming beam only. These cells were treated the same as the 980 nm wavelength group. There was no statistical difference in the mitochondrial metabolism of the non-treated controls and the fibroblasts treated with the aiming beam as measured by mitochondrial dehydrogenase activity using the MTS assay.

![In Vitro Human Fibroblasts](https://via.placeholder.com/150)

For the range of energy densities investigated, 810 nm wavelength light at an energy density of 5,000 mJ/cm² caused a statistically significant increase in mitochondrial metabolism. Over the same range of energy densities, 980 nm wavelength light caused a statistically significant decrease in mitochondrial dehydrogenase activity for all energy densities tested. ***P<0.001. Bars represent the mean and the error bars represent the standard error of the mean.

This finding is important and...
illustrates that it cannot be assumed that laser parameters optimized for one wavelength can be used for other wavelengths.

Primary Rat Cortical Neurons—Total Neurite Length
To determine effective energy densities for 980 nm wavelength light, a series of experiments was done using in vitro rat cortical neurons. The neurons were subjected to high glucose concentrations to cause impairment of their metabolism and neurite extension. The glucose concentration used in these experiments was established in a preliminary study in which it was found that a 28% decrease of total neurite length ($P<0.001$), a 14% decrease in average neuron length ($P<0.05$), and a 17% decrease in neurite number ($P<0.01$) occur when rat cortical neurons were cultured in 180 mM glucose compared to control (25 mM glucose) (Table 1).

Our experiments using in vitro human fibroblasts indicated that lower energy densities of 980 nm wavelength light may be required compared to 810 nm wavelength light for a specific power density. Therefore, for the first experiment determining effects of 810 or 980 nm wavelength light on total neurite extension of neurons cultured in 180 mM glucose compared to the control neurons grown in 180 mM glucose or 25 mM glucose, a lower range of energy densities (10, 50, 200, 1,000, and 5,000 mJ/cm$^2$) was examined at a power density of 10 mW/cm$^2$. For cortical neurons subjected to high glucose concentration, 980 nm wavelength light significantly promoted neurite extension at energy densities of 10 and 50 mJ/cm$^2$ compared to the control neurons cultured in 180 mM glucose (Fig. 2A). These data led us to examine a broader range of low energy densities of 980 nm wavelength light at a power density of 10 mW/cm$^2$ (Fig. 2B). The 980 nm wavelength light significantly promoted total neurite extension at energy densities of 10 and 50 mJ/cm$^2$ compared to the control neurons cultured in 180 mM glucose (Fig. 2B).

An infrared imaging camera was used to detect temperature change in the rat cortical neuron cultures during irradiation (Fig. 2B) to determine if there was a thermal component to the effect. A temperature rise of approximately 0.1°C was found beginning with an energy density of 1,000 mJ/cm$^2$ and increased to approximately 0.7°C at an energy density of 5,000 mJ/cm$^2$. It is important to note that these fluences did not support neurite elongation. Therefore the
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significant increases in total neurite at fluences of 2, 10, 50, and 200 mJ/cm² could not be attributed to a thermal effect but to a photobiological effect. These data indicate that lower energy densities of 980 nm wavelength light were required compared to 810 nm wavelength light for a specific power density.

Penetration of 980 nm Wavelength Laser
Penetration of the 980 nm wavelength light through the skin and femoral biceps muscle overlying the peroneal nerve was measured in an anesthetized rabbit. Surgery was performed to expose the peroneal nerve by retracting the skin and femoral biceps muscle. The power meter was placed sub-dermally; or under skin and muscle (Fig. 3A), with the sensor positioned on the surface of the nerve (Fig. 3B). In this region, the skin and muscle had a thickness of 1 and 11 mm, respectively. The light penetrated to the depth of the peroneal nerve. At an output power of 1.0 W and a power density on the skin surface of 1.8 W/cm², the power density was 0.6 W/cm² sub-dermally, and 34 mW/cm² at the level of the nerve (Fig. 3C). At an output power of 4.0 W and a power density on the skin surface of 7.0 W/cm², the power density sub-dermally was 2.1 W/cm², and 200 mW/cm² at the level of the nerve (Fig. 3C). Therefore 30% of the power density delivered to the skin surface penetrated through the skin for both output powers settings (1.0 and 4.0 W). With a 1.0 W output power, 1.9% of the power density delivered to the skin penetrated to the level of the nerve while with a 4.0 W output power, 2.9% penetrated to the level of the nerve.

Pilot Study
Gross observation of the injury site at 14 days post injury revealed that there was reattachment of the ends of the transected peroneal nerve and an enlargement of the area (Fig. 4A). Silver staining of the transected nerve revealed significant degenerative changes (Fig. 4B and C). A comparison of sections 1 cm proximal to the lesion site (Fig. 4B) and 1 cm distal to the lesion site (Fig. 4C), showed an almost complete degeneration of the axons and their associated myelin at 1 cm distal to the lesion site. Fine granular and/or brown stained material was present in the areas bounded by the epineurium indicating the last stages of Wallerian degeneration (Fig. 4C). The majority of the circular epineurial profiles were completely empty. Also a few of the epineural profiles contained a normal staining axon indicating that a few neurons are beginning the regenerative process. These axons are not myelinated since that occurs at a later stage of regeneration (Fig. 4C). Analysis of the density of immunolabeling for PGP9.5, revealed that at 21 days post-injury there was significantly less labeling in the 4 W group than control and 2 W groups ($P<0.001$) at 3 and 4 cms distal to the transaction site (Fig. 4D–F). These data show that 4 W laser parameters inhibited nerve regeneration. Too much light energy has been shown to be inhibitory to a wide variety of cellular processes. At 2 cm post-lesion there was no significant difference between the control and the 2 laser treatments. At 3 and 4 cm distal to the injury site, the 2 W laser treatment had significantly better axonal regeneration than the control. It is important to note that in our rabbit model, the distance from the lesion site on the peroneal nerve to the muscles in the foot innervated by the peroneal nerve was 240–280 mm. It has been reported that the average rate of regeneration of motor axons of the peroneal nerve after transection and suturing was approximately 2 mm a day [10]. Since the survival time was only 3 weeks in this pilot study, improvement in function was not expected for the control and light treated nerves because at 3 weeks the nerves would have regenerated only in the 3 to 6 cm range. Schmitz and Beer [9] reported that the earliest the onset of peroneal nerve motor recovery as detected by the toe spread reflex was 9 weeks post injury.
Fig. 3. In vivo measurement of light penetration in an anesthetized rabbit. **A**: The photograph shows the experimental set up. The power meter is inserted into the incision and the laser probe is placed in contact with the shaved skin. **B**: In this photograph, the peroneal nerve can be seen coursing below femoral biceps muscle. The small photo sensor (2 mm x 2.5 mm) in the glass tube is placed on top of peroneal nerve. **C**: Table listing the power densities measured at the skin surface, sub-dermally and at the level of the nerve at output powers of 1 and 4 W.

<table>
<thead>
<tr>
<th>Output power</th>
<th>1 W</th>
<th>4 W</th>
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<td>Sub-dermal</td>
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Fig. 4. Pilot study results. A: Injury site at 14 days post-injury (indicated by arrows). B, C: Silver stained peroneal nerve samples 14 days after transection injury. B: Photomicrographs of the nerve 1 cm proximal to the injury site and C 1 cm distal to the injury site. The density of PGP9.5 immunolabelling was analyzed at 2 cm (D), 3 cm (E), and 4 cm (F) distal to the transection site at 21 days post-injury. There was significantly less labeling in the 4 W group than control and 2 W groups (P<0.001) at 3 and 4 cm distal to the transection site (E,F). Photomicrographs of PGP9.5 antigen visualized by the green fluorescence label in peroneal nerve at 14 days post-injury: (G) 1 cm proximal to the transection site, (H–J) 4 cm distal to the transection site for (H) Control, (I) 2 W, (J) 4 W. *P of 0.01 to 0.05, **P of 0.001 to 0.01, ***P<0.001. Bars represent the mean and the error bars represent the standard error of the mean.

Long Term Study: Toe Spread Reflex Test

Rabbits with a complete, unilateral, peroneal nerve transection were used for the long-term study. The LT group had irradiation for 10 consecutive days post-surgery. The toe spread reflex behavior test was performed before surgery as a baseline (Fig. 5A) and weekly beginning at 4 weeks post-surgery. At week 4, the mean toe spread decreased to 63.7 ± 2.6% and 68.2 ± 3.8% of baseline for control and LT groups respectively (Fig. 5B,D), which indicated functional loss in both groups. There was no significant functional recovery in either the LT or control groups at week 5 and 6 post-injury. At weeks 7, 8, and 9, the LT group showed statistically significant functional recovery compared to week 4 (P<0.001). Multiple comparisons of each time point to week 4 in the control group did not show any functional recovery (Fig. 5D). The LT group consistently
performed significantly better in the toe spread reflex test compared to the control group starting from week 7, with a return of function to 89.1 ± 4.0% in the LT group and 72.2 ± 3.6% in the control group (P<0.05) by week 9 (Fig. 5C,D). The significant improvement in the LT group demonstrated that light treatment promoted earlier and faster nerve regeneration and functional recovery.

Assessment of Motor Endplates
The length of motor endplates in sections of the Peroneal Tertius and Brevius muscles were measured (Fig. 6). The motor endplates were visualized by labeling with α-BTX conjugated to Alexa Fluor 594. Motor endplate length is directly related to whether it is innervated or deinnervated. The average length of the motor endplates in the muscles innervated by the peroneal nerve on the right side (uninjured) was 17.36 ± 0.26 μm. The average motor endplate length decreased to 11.20 ± 0.72 μm at 9 weeks post-injury while the average motor endplate length on the injured and light treated side (16.94 ± 0.52 μm) returned to uninjured levels. These data indicate that light irradiation supported peroneal nerve regeneration and reinnervation of the involved muscles by 9 weeks post-injury.

DISCUSSION
Numerous peer reviewed studies have established the efficacy of PBM for treatment of peripheral nerve injury. Reviews of the relevant literature demonstrate that different wavelengths can support peripheral nerve injury repair. The majority of the wavelengths used in these studies were in the 600–904 nm range [6,7]. Wavelengths longer than 904 nm typically have not been used for peripheral nerve repair and their effects in promoting peripheral nerve regeneration have not been adequately researched. Recently, 940 nm wavelength irradiation was used to treat a crush injury of the rat sciatic nerve [11]. The results were positive with a reported reduction in edema and inflammation and increased functional recovery on post-injury days 7, 14, and 21 based on the sciatic function index (SFI).

A key issue in the field of PBM is the lack of parameter optimization. Preliminary studies to optimize the treatment parameters are rarely done. A few studies have attempted to compare the efficacy of two or more wavelengths for peripheral nerve injury repair. In these experiments, the same parameters were used for both wavelengths without regard for differences in wavelength penetration to the target tissue or optimization of the parameters for each wavelength [12,13]. Barbosa et al. [10] examined the comparative effects of 660 and 830 nm wavelength light on crush injury of the rat sciatic nerve. The parameters used for both wavelengths included an output power of 30 mW.
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and 10 J/cm² on the skin surface. The 660 nm wavelength light resulted in improved functional recovery on day 14. No statistical difference was seen between the sham, 660 nm wavelength group and 830 nm wavelength group at 7 days or 21 days. The authors concluded that the 660 nm wavelength used with these parameters was more effective than 830 nm wavelength light. However, a significant difference at only one time point examined suggests that the parameters used for both wavelengths were not optimal. The effects of 660 and 780 nm wavelength light on neuromuscular and functional recovery were investigated using a crush injury model of the rat sciatic nerve [13]. The same laser parameters were used for both wavelengths. Neither wavelength improved function and suggests that the laser parameters for both wavelengths were not optimal.

There is one report in the literature on PBM treatment of peripheral nerve injury in which laser parameters were used in an animal model and then translated to the human [14,15]. Light, 780 nm wavelength, was used to treat severe sciatic nerve injury in a rat model [14] and in a human randomized double-blind placebo-controlled study of long term incomplete peripheral nerve injury [15]. The laser parameters used in the rodent study were 780 nm wavelength light, output power 200 mW, 15 minutes transcutaneous treatments over the reconstructed sciatic nerve and the corresponding spinal cord segments, daily for 14 days [14]. Spot size was not reported so neither energy density nor power density could be calculated and compared to the non-effective parameters used for 780 nm wavelength light in the Gigo-Benato et al. study [13].

These treatment parameters resulted in an increased total number of myelinated axons and improved motor function based on the SFI behavior test [14]. The laser parameters used in the human trial were 780 nm wavelength light, output power 250 mW, 3-hour transcutaneous treatments over the injured peripheral nerve (450 J/mm²) and 2 hours to the corresponding spinal cord segments (300 J/mm², spot size 6 mm²) daily for 21 days [15]. The authors reported that there was accelerated nerve regeneration and a progressive improvement in motor nerve function over a 6-month period. No explanation was given for the selection of the parameters used in either the animal study or the clinical trial or how the clinical trial parameters related to those used in the rodent study. Furthermore, the nerves treated in the patients in the laser treated group included the median, ulnar, radial peroneal, axillary nerves, and the upper trunk of the brachial plexus. All these nerves were transcutaneously treated with the same laser parameters regardless of the depth of the nerve. Even though a positive outcome was reported it is unlikely that optimization of the laser parameters was achieved.

There is no clear consensus on what wavelength may be best to use for a specific nerve and what parameters should be used for that wavelength. This lack of parameter optimization is responsible for the variability in results across studies and which is a criticism of PBM. As recently suggested [16], it is unreasonable to expect that a single light dose will have a universal application. However, many wavelengths may be efficacious for treatment of nerve injury if the wavelength can penetrate to the target tissue and the parameters used are optimized so that a therapeutic dose of light is applied.

For the majority of PBM studies, dose parameters reported were values delivered to the skin surface and not what was actually delivered to the target tissue. Our laboratory was the first to measure light penetration in an anesthetized rat from the dermis to the depth of the spinal cord using a smart, tissue-activated optical fiber probe attached to a spectrophotometer [17]. We also investigated the effect of PBM on peripheral nerve regeneration and function after severe median nerve injury and microsurgical autologous nerve graft repair using fibrin glue [18]. For these experiments, the percentage of output power of an 810 nm wavelength laser transcutaneously transmitted to the depth of the median nerve was measured in an anesthetized rat. Power transmitted through the skin to the depth of the nerve was 47% of the output power. Based on
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Fig. 6. Motor endplate measurement and labeling. A: Graph showing means of motor endplate length (μm) for the uninjured (right side), injured, and injured and LT (left side) groups. Measurement of motor endplate length tested by one way ANOVA with Tukey’s multiple comparison test showed significant difference between uninjured and injured (**P<0.001) and injured LT and injured (**P<0.001) groups. (B1–B2) Motor endplates from the Peroneal Tertius and Brevius muscles of rabbits at 9 weeks after nerve transection and LT (red, labeled with α-BTX conjugated to Alexa Fluor 594) innervated by immunolabeled peroneal nerve (green). C,D,E: Labeled motor endplates: (C) from the uninjured side (right) of the rabbit, (D) from injured left side of the rabbit with peroneal nerve transection, (E) from a rabbit that had nerve transection and light therapy. Bars represent the mean and the error bars represent the standard error of the mean. Scale bar = 10 μm.

In these measurements, laser parameters were chosen so that a power density of 10 mW/cm² was achieved at the depth of the nerve. Laser treatment with these parameters resulted in faster functional recovery of grip strength (P<0.05), shorter compound muscle action potential latency (P<0.05), and higher S-100 immunoreactivity (P = 0.0213).

Based on our data from the in vitro experiments on rat cortical neurons, the optimal parameters for infrared laser irradiation were energy densities between 2 and 200 mJ/cm² at a power density of 10 mW/cm². To assure adequate treatment time for the in vivo experiments, calculations were based on the high end of the range of effective in vitro energy densities (200 mJ/cm²). The data from the penetration experiments of 980 nm wavelength light revealed that approximately 2.45% (average of 4.0 W and 1.0 W measurement) of the light penetrated to the level of the peroneal nerve. The total energy delivered on the skin surface was calculated to be 65 J. Two different
output powers (2 and 4 W) were used for the pilot study. Although the total energy was kept at 65 J for both groups, nerve regeneration was significantly better with the 2 W laser treatment compared to the control while regeneration of the injured nerves was inhibited when treated with 4 W. Therefore, both energy density (200 mJ/cm²) and power density (10 mW/cm²) were considered for refining the calculation for the long-term study. Based on the toe spread reflex, these laser treatment parameters promoted an earlier and faster nerve regeneration and functional recovery compared to the non-treated controls and supported nerve regeneration and reinnervation of the involved muscles by 9 weeks post-injury.

Comparison between 980 and 810 nm wavelength light was done with two in vitro models. The experiments using human fibroblasts demonstrated that inhibition of mitochondrial metabolic activity was caused by 980 nm wavelength light, at 10 mW/cm² and energy densities that were stimulatory to mitochondrial metabolic activity with 810 nm wavelength light. In the cortical neuron experiments, 980 nm wavelength light at 10 mW/cm² and lower energy densities supported neurite elongation while at 10 mW/cm² and comparable energy densities 810 nm wavelength light had no effect on neurite elongation. These data demonstrate that for a given power density 980 nm wavelength light altered cellular activity at lower energy densities compared to 810 nm wavelength light. Thus, for different wavelengths of light at a given power density, the energy density necessary to achieve a desired effect may be very different.

This study demonstrates that treatment parameters for a specific wavelength of light can be determined initially using in vitro models and that these parameters can be translated to animal model research and clinical practice. The translation of dose from in vitro to in vivo was accomplished by first determining the effective laser parameters at the cellular level. The in vitro neuronal model used in this study involved compromised neurite elongation and not transection injury which was used in the in vivo animal model. Although the in vitro and in vivo models differed, the results demonstrate that optimizing the in vitro neuronal response to light at the cellular level provided a valid approach for the initial step in parameter optimization. The in vitro dose was then adjusted based on light penetration measurements. These results also validate that infrared light with optimized parameters is effective for treatment of nervous system injuries.

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The Effectiveness of Therapeutic Class IV (10 W) Laser Treatment for Epicondylitis

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ABSTRACT

BACKGROUND AND OBJECTIVE: Photobiomodulation has been shown to modulate cellular protein production and stimulate tendon healing in a dose-dependent manner. Previous studies have used class IIIb lasers with power outputs of less than 0.5 W. Here we evaluate a dual wavelength (980/810 nm) class IV laser with a power output of 10 W for the purpose of determining the efficacy of class IV laser therapy in alleviating the pain and dysfunction associated with chronic epicondylitis.

METHODS: Sixteen subjects volunteered for laser therapy, or an identically appearing sham instrument in a randomized, placebo-controlled, double-blinded clinical trial. Subjects underwent clinical examination (pain, function, strength, and ultrasonic imaging) to confirm chronic tendinopathy of the extensor carpi radialis brevis tendon, followed by eight treatments of 6.6 ± 1.3 J/cm² (laser), or sham over 18 days. Safety precautions to protect against retinal exposure to the laser were followed. The exam protocol was repeated at 0, 3, 6 and 12 months post-treatment.

RESULTS: No initial differences were seen between the two groups. In the laser treated group handgrip strength improved by 17 ± 3%, 52 ± 7%, and 66 ± 6% at 3, 6, and 12 months respectively; function improved by 44 ± 1%, 71 ± 3%, and 82 ± 2%, and pain with resistance to extension of the middle finger was reduced by 50 ± 6%, 93 ± 4%, and 100 ± 1% at 3, 6, and 12 months, respectively. In contrast, no changes were seen until 12 months following sham treatment (12 months: strength improved by 13 ± 2%, function improved by 52 ± 3%, pain with resistance to extension of the middle finger reduced by 76 ± 2%). No adverse effects were reported at any time.

CONCLUSIONS: These findings suggest that laser therapy using the 10 W class IV instrument is efficacious for the long-term relief of the symptoms associated with chronic epicondylitis. The potential for a rapidly administered, safe and effective treatment warrants further investigation.


KEY WORDS: epicondylalgia; photobiomodulation; tendinopathy; tendinosis; tenosynovitis
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INTRODUCTION

Tendinopathy is a common and painful condition that occurs following damage to a tendon [1–3]. The onset of symptoms is associated with overuse, increased load, vibration and/or repetitive movements and while tendon injuries are sometimes acute, they are most often chronic in nature resulting in significant restriction of activity and lost work-time [3,4]. Characteristic findings include necrosis [3], abnormal neovascularization [5], edema, crepitus, and impaired function [4,6]; however, the etiology remains incompletely understood. Furthermore, while most cases resolve themselves within 12 months of rest, approximately 15–20% are persistent, with reoccurrence of symptoms when activity is resumed [6,7].

There is little consensus regarding effective treatments for tendinopathy [4,8]. Rest, ice, and analgesics are general guidelines used to provide pain relief. Orthotic devices [9], ultrasonography [10] and deep transverse friction massage [11] are often recommended, although there is no conclusive evidence as to the effectiveness of these treatments. Similarly, while eccentric exercises have been shown to be more effective than no treatment in relieving symptoms for some tendinopathies, compliance can be problematic and there is a great deal of heterogeneity in protocols [12]. Randomized controlled studies of epicondylitis have determined that oral non-steroidal anti-inflammatory treatment was not significantly better than placebo [13] and although early corticosteroid injection did provide symptom relief in some patients, studies that were extended to 3 [14] and 12 [13] months post-injection indicated that corticosteroid injection could even produce a detrimental outcome. Extracorporeal shock therapy for treatment of tendinopathy is also not supported by systematic reviews of the literature [15], except perhaps for cases resistant to conventional treatments [16]. Another drawback is that there is a significant amount of pain associated with this therapy [17].

In contrast, low level laser therapy (LLLT), also known as photobiomodulation (PBM) has been shown to be effective at the cellular level increasing cytochrome C oxidase production and reversing the effects of cellular inhibitors of respiration [18]. Accelerated tissue healing has been reported, including an increase in collagen fibril size [19] and a decrease in prostaglandin E2 levels [20,21] in a dose dependent manner [22]. Samoilova et al. [23] reported activation of nitric oxide synthase, and recently increased blood flow in the treated limb has been demonstrated [24]. Given that tendinopathies have been shown to be associated with matrix degeneration, these combined effects would be likely to have an influence in improved healing of damaged tendon. While one meta-analysis of LLLT for lateral epicondylitis suggested that LLLT was not more effective than placebo [25], two more recent examinations of the literature based upon treatment protocol concluded a positive effect. In studies where the tendon was directly irradiated using wavelengths between 630 and 1,064 nm, doses of 0.5–8 J were effective in achieving improvements in decreased pain and increased strength both acutely and up to 8 weeks following treatment [26,27].

Previous clinical studies on LLLT have used lasers with a typical output of less than 0.5 W. However, a dual wavelength (980 and 810 nm) laser with an output power of up to 10 W has recently been developed for use in laser therapy. At full power (10 J/seconds) these instruments can deliver 8–9 J/cm² at the skin surface, achieving a distributed photochemical biomodulatory dose in only minutes. The body of evidence indicating that LLLT is efficacious suggests that the using a higher power laser would allow for an effective treatment to be delivered in a shorter time over a larger area, and with a more uniform dose than the point administration of low power lasers. However, to date, no randomized placebo controlled trials have been undertaken utilizing this instrumentation. Hence, the purpose of this investigation was to investigate the efficacy of a laser with a higher power output for the treatment of tendinopathy of the extensor carpi radialis brevis tendon in a clinical setting.
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METHODS
The study design was a single center randomized (1:1), placebo-controlled, double-blinded, parallel group clinical trial, conducted in the United States. Level of Evidence: Therapy, 2 [28]. Independent ethical review was conducted by the IRB Promedica Health Systems, Toledo, OH. Volunteers were recruited by advertisement for 4 months. Of the 28 subjects who volunteered for this randomized controlled trial, 16 subjects were accepted into the study. Exclusion criteria included factors which may have affected treatment administration such as photosensitivity or pigment around the cubital area which could have resulted in different levels of absorption of the administered light. The use of corticosteroids or, injection of the cubital area within the past 3 months also precluded participation. Informed consent was obtained and the rights and privileges of the patients were observed at all times. Clinical evaluation of lateral epicondylitis was made by blinded Sport Medicine Fellows on the basis of standard clinical tests including: 

- Handgrip strength from three maximal trials using the Smedley III Digital Grip Strength Tester (Creative Health Products, Plymouth, MI) according to standard protocol of Ashford et al. [29];
- Ratings of pain using the Visual Analog Sale (VAS) of 1–10 (where 10 is intolerable pain) with maximal handgrip using the Ashford et al. protocol [29], with moderate palpation of the common extensor tendon, and with resistance to extension of the middle finger (affected elbow stabilized at 90°, forearm pronated, wrist in neutral); And functional impairment (scale of 1–5 with 5 being no impairment and 1 being unable to use hand during daily tasks).

Participants also underwent ultrasonic imaging of the extensor carpi radialis brevis tendon to confirm the diagnosis based upon the presence of tendon thickening relative to the adjacent osseous structures, discriminate focal areas of hypoechomonicity or anechomonicity, focal tears, and the presence of calcifications. Ultrasonographs were evaluated by an experienced radiologist with specialization in musculoskeletal radiology who was also blinded to the treatment groups.

Subjects were then randomly assigned to placebo (sham) or laser treatment (LT) groups by drawing sealed envelopes from a box. Two identically appearing 10 W lasers were used for treatments (LiteCure LLC, Newark, DE), however the sham laser light was disabled and only the aiming beam (identical to that utilized on the true device) remained, subjects and clinicians were unable to discriminate between which instrument was which. The true laser was a solid-state diode dual wavelength (980/810 nm fixed ratio 80:20) laser. The aiming beam was a single wavelength class 3a laser (650 nm), with a power output 4 mW. All treatments were administered in a sport medicine clinic (Toledo, OH) by a trained technician according to the following schedule: three treatments on consecutive days, four additional treatments over the next 10 days and one final treatment during the third week. The testing protocol was repeated following the final treatment, and again at 3, 6, and 12 months to generate the primary outcome measures. The study flow diagram is shown in Figure 1.

Subjects abstained from all other forms of treatment including non-steroidal anti-inflammatory or topical medications, braces, physical therapy, ultrasound, acupuncture and shock wave therapy until completion of the study. If a patient chose to institute an alternative treatment they were withdrawn from the study at that point, their data was only included in analyses performed when the original laser or sham was the only treatment. Activities causing pain or irritation of the tendon were restricted until the laser treatment was complete (the first 3 weeks), after which subjects were encouraged to resume normal activity. Due to ethical considerations subjects randomized into the sham group were offered the true treatment after the 3-month follow up (Fig. 1), since any sham subjects choosing the true treatment were no longer blinded to the treatment their data was not crossed over and was not included in the treatment data analysis after the 3-month assessment.
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Procedure For Administration of the Laser Treatment

The area to be treated was demarcated at 1/2 the distance from the lateral epicondyle to the ulnar styloid and 1/3 of the distance from the lateral epicondyle to the acromion process. The non-contact laser probe was kept perpendicular to, and approximately 2.5 cm above the surface of the dermis, creating a spot size 5.7–9.6 cm². The laser was set at full power output of 10 W with a continuous wave form generating a total dose per treatment of 3000 J in 5 minutes or 6.6 ± 1.3 J/cm², within the recommended therapeutic guidelines [26,30]. The first 2.5 minutes of the laser treatment were administered with the arm in full extension, and the second 2.5 minutes were administered while passively moving the joint through its range of motion in order to better allow full illumination of the tissue. The laser probe was moved in a “painting” fashion with half of the treatment delivered along the long axis to the tendon, with the other half delivered transverse to the tendon while covering the anterior, lateral and posterior aspects of the lateral epicondyle.

Fig. 1. CONSORT flow diagram for the laser study.
Safety precautions were placed in effect to minimize the risk of exposure of the retina of the eye to laser light. Both clinician and subject wore specifically designed safety goggles provided by the manufacturer to shield against reflected laser light. Jewelry and other reflective surfaces were removed from the treatment area which was designated by MSDS signage, and was restricted access. At no time was the laser probe directed upwards towards the head of the clinician or subject.

Statistical Analysis
The data analysis was generated using the MIXED procedure for repeated measures with unbalanced design [31] (SAS/STAT software, Version 9.2 of the SAS System for Windows, Cary, NC) with the level of significance taken at $P < 0.05$. Power analyses were performed for each parameter using G*Power3 [32].

RESULTS
Demographic information is presented in Table 1. No differences were observed between the two groups prior to treatment. Clinical exams and ultrasonography confirmed a diagnosis of chronic tendinopathy of the extensor carpi radialis brevis tendon in all subjects. The ultrasonography revealed tendons to be thickened, with heterogeneous areas. Regions of hypochoogenicity and anechoogenicity were noted, and in some cases, proximal calcifications were also visible.

All subjects tolerated the treatments well; there were no reports of discomfort during the laser therapy or adverse reports made at any time, and all LT subjects completed the full treatment course as well as returning for the full 12 months of follow-up testing. One SG subject withdrew prior to the beginning of the treatment course because they did not want to risk being randomized into the sham treatment group, two SG subjects chose to obtain the true treatment when it was offered following the 3-month exam (their data were included prior to and including the 3-month assessment, but not afterwards as they were no longer blinded to the treatment), two SG subjects opted for corticosteroid injections following the 3-month assessment (their data were included prior to and including the 3-month assessment, but not following the injection), and the remaining three control subjects continued with no alternate treatment until completion of the 12-month follow up (Fig. 1). No significant differences were observed in any of the primary outcome measures of strength or pain in the sham group until 12 months (Figs. 2–6). In contrast, all pain measures as well as perceived functional impairment were significantly improved by the end of the treatment protocol in the laser group (Figs. 3–6). Handgrip strength was slower to recover and was not significantly improved over pre-treatment levels until 6 months post-treatment (Fig. 2).

DISCUSSION
This study is the first report of a clinical trial of a 10 W laser for treatment of tendinopathy. Our findings confirm the positive effects of PBM in enhancing tendon healing that

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<th>TABLE 1. Demographic Information for Sham and Laser Treatment Groups</th>
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<td>Sham ($n = 7$)</td>
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Data presented as mean ± SD.
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have previously been reported with LLLT trials [19,21,27]. In the current study, reductions in pain and return of strength and function were observed in the treatment group with greater speed and magnitude than in the sham group (Figs. 3–6), with improvement continuing up to and including the final time point at 12 months post-treatment. While it is possible that these effects were due at least in part to neurological changes [33], we propose that the improvements in the primary outcome measures may have occurred due to tendon repair over time as patients reported continued reduction of pain and increased strength even though they increased their use of the affected tendon over the same time period. In contrast, pain, strength, and functional impairment in the sham group remained undiminished until 12 months (Figs. 2–6). This outcome is as expected, as tendinopathy has been shown to resolve itself in approximately 12 months without treatment other than reduced use [6]. That the laser group showed

![Handgrip strength (kg)](image)

**Fig. 2.** Handgrip strength (kg) in the affected arm, pre and post laser and sham treatment. Shown $P$ values are for the difference in strength between laser and sham treatment ($df = 48$). Results for the unaffected arm are also shown for comparison. *Significantly different from pre-treatment. $P < 0.0001$. Power = 0.74.

![Functional Impairment (1–5, 1 = Useless)](image)

**Fig. 3.** Functional Impairment (1–5, 1 = Useless). Shown $P$ values are for the difference in perceived functional impairment between laser and sham treatments ($df = 48$). Significantly different from pre-treatment. *$P < 0.0001$ for the laser group, †$P < 0.002$ for the sham group at 12 months. Power = 0.77.
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continued decline of tendinopathy symptoms in spite of increased function immediately post-treatment through to 12 months (Fig. 3) indicates that PBM treatment provides a superior outcome to no treatment.

One weakness in the current study is the small sample size, particularly in the sham group. With continued pain and dysfunction at 3 and 6 months, the majority of patients in the sham group switched to the true treatment or sought other modalities (Fig. 1). However, our statistical power analysis of 0.62–0.89 supports the validity of our observations in spite of the small sample size [32].

The higher power output of the 10 W laser allowed for the delivery of an effective PBM dose in minutes. Here, a total of 3000 J were delivered over the entire area of the extensor carpi radialis brevis tendon from 1/2 the distance from the lateral epicondyle to the lateral stylus of the...

Fig. 4. Lateral Pain With Palpation (VAS 1-10). Shown P values are for the difference in perceived pain between laser and sham treatments (df = 48).
*Significantly different from pre-treatment P < 0.001; †Significantly different from pre-treatment P < 0.05. Power = 0.74.

Fig. 5. Pain with resistance extension middle finger (VAS 1-10). Shown P values are for the difference in perceived pain between laser and sham treatments (df = 48). *Significantly different from pre-treatment, P < 0.001; †P < 0.02. Power = 0.89.
The Effectiveness of Therapeutic Class IV (10 W) Laser Treatment for Epicondylitis

Fig. 6. Pain With Maximum handgrip contraction (VAS 1-10). Shown P values are for the difference in perceived pain between laser and sham treatments (df = 48). Significantly different from pre-treatment; *P < 0.001, †P < 0.02. Power = 0.62.

carpus and 1/3 of the distance from the lateral epicondyle to the acromion process in 5 minutes. This fluence (6.6 ± 1.3 J/cm²) is within the published guidelines for LLLT [30] which are designed for much lower power lasers using point treatment as opposed to the current beam diameter of 5.7–9.6 cm². The wider beam diameter and higher power allow for a much more even distribution of energy over a larger area such as a whole muscle or large tendon which is likely to be advantageous due to the dose response effect of PBM. Furthermore, because the point delivery of lower power lasers is so narrow, some of the inconsistency in outcomes following laser treatment has been attributed to the heterogeneity of delivery of the laser to the affected tissue [34].

Studies of LLLT treatment with 0.5 W lasers and point treatment have been shown to have beneficial outcomes in the treatment of tendinopathy [26,27], thus we cannot exclude that the 4 mW laser light in the aiming beam (650 nm) used in both the 10 W laser and the sham device contributed to the PBM dose applied in the current investigation. However, due to the short time period of exposure the total energy delivered by the aiming beam would have only amounted to 1.2 J, well under the level that has been identified as being an effective dose [30], making it unlikely that the aiming beam had any therapeutic effect. Furthermore, in the current study there was a clear difference in the effectiveness of the two devices in ameliorating the pain, weakness and dysfunction associated with lateral epicondylitis. There is also a possibility that the higher power output of the 10 W laser resulted in some heating of the tissue exposed to the laser and that the kinetic energy rather than PBM may have had an effect on the tendon repair. While this cannot be entirely discounted it is highly unlikely as the increase in skin temperature after a 5 minutes exposure to the laser was only 8°C (unpublished results) and there are no conclusive reports of heat being an effective treatment for epicondylitis in the literature.

Numerous cellular findings support the suggestion that the mechanism of the positive effect of laser treatment on tendinopathy in increased function, strength, and reduced pain may be due to enhanced repair of tendonous tissues. Reports of reduced levels of pro-inflammatory mediators TNF-α, IL-6, TGF-β cytokines, and COX-2 enzyme [35], PG₃ [21], and increased activation of NO [23] in damaged tendon have been made following LLLT treatment. Fibroblast metabolism appears to be enhanced with increased fibroblast proliferation [35] reduced fibroblast apoptosis [36] and an increase in collagen fibril size [19] and biomechanical strength [37] in response to PBM. Given
that tendinopathy tends to be characterized by tendon degeneration that is persistent to existing treatment modalities, these findings are very promising to the many individuals who suffer from chronic tendon dysfunction.

CONCLUSION

Laser therapy using a 10 W class IV solid state diode dual wave-length (980/810 nm) laser with eight treatments of 3,000 J each over 18 days was found to be a safe and efficacious treatment for the reduction of pain and loss of strength seen with chronic tendinopathy of the extensor carpi radialis brevis tendon. The potential for a quickly administered, safe and effective treatment of tendinopathy warrants further investigation.

ACKNOWLEDGEMENTS

The authors wish to recognize Dr. M. Petznick, Dr. J. Kiefer, Dr. P. Alasky, F. Hurley, and K. Zicarelli for their assistance with the data collection as well as Dr. D. Sachs for his assistance with the statistical analysis and A. Roberts and H. Gerelle for their assistance with data handling and manuscript preparation.

REFERENCES


High Powered Laser Therapy Enhances Muscle Healing

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1Department of Physical Therapy, Duquesne University, Pittsburgh PA; 2Bucaramanga; 3Penn State University School of Medicine

ABSTRACT
High powered laser therapy is an emerging modality used in rehabilitation; however little is known of its effectiveness in enhancing skeletal muscle repair.

PURPOSE
The purpose of this study was to determine the effect of high powered laser treatment on markers of muscle damage and repair in humans and to determine if these effects were muscle cell autonomous by treating muscle cells (C2C12) in culture.

METHODS
Male and female subjects (n=12) were recruited and underwent a standardized muscle damage protocol (20 sets of 10 repetitions of eccentric contractions of the vastus lateralis) using an isokinetic dynamometer. Forty-eight hours after damage, laser was administered to one leg while the contra-lateral leg served as the control. Six hours after laser treatment, bilateral muscle biopsies were collected from each participant. C2C12 cells were grown in standard culture conditions and received daily laser treatment.

RESULTS
Damage was confirmed by creatine kinase assays (320% increase, P<0.01) and loss of force production (~30%, P<0.05). Biopsies were examined for markers of muscle repair (e.g. IGF1 and VEGF). Laser treatment increased markers of muscle repair (IGF1, 2.3 ± 0.6 fold, p < 0.05) and enhanced muscle cell proliferation in culture.

CONCLUSIONS
Additional markers (in biopsies) and myotube formation (in C2C12s) are currently being examined in our laboratory. High powered laser treatment is effective in increasing molecular markers of muscle repair after damage and these effects are likely muscle cell autonomous.

INTRODUCTION
• After skeletal muscle injury full functional recovery often does not occur and there are few modalities beyond traditional exercise training that have a proven physiologic effect.
• Currently very little is known about the effect of high-powered laser treatment other than it may increase ATP production in human and animal cells.
• High-powered laser treatment affects neuron outgrowth in cells in culture.
• Low powered laser has been reported to have beneficial effects in healing tissue including in muscle.

METHODS
Cell Culture
The murine myoblast cell line (C2C12) was obtained from American Type Culture Collection (ATCC) (Rockville, MD). Cells were maintained in DMEM (ATCC) with 10% fetal bovine serum (FBS) at 37°C and 5% CO2 during proliferation. Cells were treated with laser every day for 5 days and collected 24 hours after the final treatment. N=4 for each experimental group. Proliferation experiments were repeated twice.

12 healthy men and women, moderately physically active (classified in category 2 IPAQ), aged 18 to 29 years, were randomly assigned to either Control (CON) or experimental group (EXP); lower limbs were also randomized to receive or not laser. All subjects were informed of the procedures and potential risks associated with the study and gave their written informed consent to participate.

Muscle damage protocol
200 Maximal isotonic unilateral muscle-lengthening contractions of the quadriceps femoris were performed in each leg (20 sets of 10 reps) using the Biodex dynamometer (Biodex-System3, Biodex Medical Systems, Inc., USA). This protocol was conducted 48 h before the UST for the EXP group. Muscle damage was confirmed by Creatine Kinase activity assay and MVC (Isometric peak torque).
Laser Treatment
Both groups received laser in one leg, the contralateral leg was assigned as their control. Continuous High-powered (10 W) laser was delivered for 10 min on a standardized area of the vastus lateralis. Subjects refrained from physical activity during 6 h after the laser until the biopsy procedure.

Dietary control
Each subject will consume a 350-kcal defined formula diet (67% carbohydrates, 18% protein, 15% fat; by mass) immediately before laser treatment and 1 hour before the exercise bout.

Muscle Biopsies
Bilateral percutaneous needle biopsies were obtained from the midportion of the vastus lateralis 6 hours after laser treatment, under local anaesthetic (1% lidocaine).

Figure 1. Experimental design of human study
High Powered Laser Therapy Enhances Muscle Healing

Figure 2. Muscle cells were treated in 6-well plates within the laminar flow hood.

<table>
<thead>
<tr>
<th>Time (seconds)</th>
<th>Control</th>
<th>10 mW/cm</th>
<th>10 mW/cm</th>
<th>50 mW/cm</th>
<th>50 mW/cm</th>
<th>50 mW/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 s</td>
<td>10 s</td>
<td>180 s</td>
<td>2 s</td>
<td>4 s</td>
<td>60 s</td>
<td></td>
</tr>
<tr>
<td>Energy density</td>
<td>0</td>
<td>0.1 J/cm²</td>
<td>2.0 J/cm²</td>
<td>0.1 J/cm²</td>
<td>2.0 J/cm²</td>
<td>2.0 J/cm²</td>
</tr>
<tr>
<td>Total energy delivered</td>
<td>0</td>
<td>100 J</td>
<td>1800 J</td>
<td>30 J</td>
<td>60 J</td>
<td>600 J</td>
</tr>
</tbody>
</table>
Regeneration is dependent upon muscle satellite cells

Figure 3. Model of muscle repair. Muscle repair after damage or injury is dependent upon satellite cell proliferation and IGF1 expression.

RESULTS

Figure 4. C2C12 cells in culture after 5 days or proliferation.

Control cells

4 treatments of 50 mW/cm² (100 J)
**CONCLUSION**

Laser treatment increased IGF1 gene expression in healing muscle and affected muscle cell proliferation in a dose-dependent manner. HPLT is a modality that enhances the muscle healing process.

---

**Figure 5. C2C12 proliferation counts after 5 days of laser treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proliferation Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl 6 min</td>
<td>20000</td>
</tr>
<tr>
<td>Ctrl 12 min</td>
<td>15000</td>
</tr>
<tr>
<td>20 s</td>
<td>10000</td>
</tr>
<tr>
<td>2 min</td>
<td>5000</td>
</tr>
<tr>
<td>6 min</td>
<td>3500</td>
</tr>
<tr>
<td>12 min</td>
<td>3000</td>
</tr>
</tbody>
</table>

**Fold difference in IGF1 mRNA**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>15</td>
<td>3.0</td>
</tr>
<tr>
<td>20</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Insulin-like Growth Factor 1 Expression**

- Untreated leg (after damage)
- Laser treated leg (after damage)
Over the last five years, there has been a dramatic increase in the use of laser as a therapeutic modality in small animal veterinary clinics. The popularity of the technology has correlated directly with the availability of higher-powered Class IV therapy lasers; there are, however, a wide variety of laser devices currently on the market with an extensive array of features and a deafening amount of marketing hype to back up their claims.

Unfortunately, to date there has been a shortage of good clinical research to establish clear and effective dosing strategies to produce consistent clinical results. The goal of the researchers participating in this study is to report the first large series of data using consistent dosing parameters to help establish an informed dosing strategy for the clinician.

Laser therapy uses photobiomodulation to stimulate cellular metabolism, reduce inflammation and speed the natural healing process. A number of biochemical mechanisms have been described previously that facilitate this effect. The key to effective laser therapy treatment is therefore the delivery of an adequate amount of therapeutic light to the necessary anatomical structures.

Initially, this would seem to only require a simple calculation of the amount of light delivered and depth of tissue penetration. But in the current environment with little data to substantiate claims, the dosing conversation quickly deteriorates into claims about laser pulsing parameters and different wavelengths of invisible light. This can be tedious for a population of potential users trained in veterinary medicine rather than laser physics.

Although a recent review of the literature concluded that continuous emission of light (CW) remains the gold standard for clinical efficacy, and a wide therapeutic window of laser wavelengths have been demonstrated to be efficacious, a cloud of confusion remains. The goal here is to provide the first large series of cases using a consistent dosing strategy to establish the efficacy of laser therapy in the small animal clinic.

**METHODS**

Case data were collected over four months from Pierrefonds Animal Hospital, Ste. Genevieve, Quebec, Canada; Stoney Creek Veterinary Hospital, Morton, PA, United States; and Dyrlægehuset, Odense, Denmark. All cases were treated with Companion CTS or CTC laser therapy devices using pre-set protocols and SmartCoat dosing technology. Patient assessments were scored on a four-point scale (excellent, good, average, poor) by the clinician and correlated to appropriate quantitative measures for the condition when possible.

For example, rehabilitation outcomes after stifle repair or hip dysplasia were tallied by improvements in lameness scoring. Similarly, most wound-healing outcomes were graded by measures observed in the clinic or reported by the owner. All cases with sufficient information to draw a clinical conclusion by the end of the collection period were included in the study.

In addition to the treatment protocols provided with the laser therapy system, condition-specific treatment heads were used to optimize delivery. Conditions such as wounds or lick granulomas were treated off-contact with the large, compression of superficial tissues with laser firing, at bottom.
A multi-center case series on laser therapy

Deep muscular injuries including hip dysplasia and osteoarthritis were treated with the large convex contact treatment head that can be used to compress superficial tissues, displacing excess fluid and enhancing laser penetration to deep structures.

Conditions such as otitis and post surgical wounds, which require delivery of laser to tight spaces or of smaller laser spot sizes, were treated with small contact or non-contact treatment heads.

A summary of the conditions that were treated and average treatment parameters is presented in Table 1.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dogs</th>
<th>Cats</th>
<th>Average Joules</th>
<th>Dosing Range (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td>0</td>
<td>5</td>
<td>810</td>
<td>6-8</td>
</tr>
<tr>
<td>Anal Gland</td>
<td>5</td>
<td>0</td>
<td>721</td>
<td>6-8</td>
</tr>
<tr>
<td>Bruise/Trauma</td>
<td>1</td>
<td>0</td>
<td>900</td>
<td>8-10</td>
</tr>
<tr>
<td>Claudication</td>
<td>1</td>
<td>0</td>
<td>8-10</td>
<td></td>
</tr>
<tr>
<td>Ear Infection</td>
<td>8</td>
<td>1</td>
<td>1395</td>
<td>3-4 Superficial</td>
</tr>
<tr>
<td>8-10 Deep Fracture</td>
<td>2</td>
<td>3</td>
<td>1486</td>
<td>8-10</td>
</tr>
<tr>
<td>Hip Dysplasia</td>
<td>3</td>
<td>1</td>
<td>1162</td>
<td>8-10</td>
</tr>
<tr>
<td>Hot Spot</td>
<td>7</td>
<td>1</td>
<td>1080</td>
<td>3-4</td>
</tr>
<tr>
<td>Infected Wound</td>
<td>2</td>
<td>0</td>
<td>623</td>
<td>3-4</td>
</tr>
<tr>
<td>IVDD</td>
<td>8</td>
<td>2</td>
<td>927</td>
<td>8-10</td>
</tr>
<tr>
<td>Kidney Stone</td>
<td>0</td>
<td>3</td>
<td>912</td>
<td>8-10</td>
</tr>
<tr>
<td>Lick Granuloma</td>
<td>9</td>
<td>0</td>
<td>1430</td>
<td>30</td>
</tr>
<tr>
<td>Ligament</td>
<td>34</td>
<td>0</td>
<td>1870</td>
<td>3-4</td>
</tr>
<tr>
<td>Operative Wound</td>
<td>10</td>
<td>19</td>
<td>459</td>
<td>3-4</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>5</td>
<td>1</td>
<td>1716</td>
<td>6-10</td>
</tr>
<tr>
<td>Pain</td>
<td>7</td>
<td>3</td>
<td>3450</td>
<td>8-10</td>
</tr>
<tr>
<td>Wound</td>
<td>18</td>
<td>6</td>
<td>1440</td>
<td>3-4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120</strong></td>
<td><strong>45</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS & DISCUSSION

In all, 165 cases were contributed from the three sites. The reported cases are indicative of a random sampling of the standard caseload in a small animal veterinary practice.

A summary of the conditions and average treatment parameters is provided in Table 1. Across all sites, tissue treatment areas from 100 cm² to 700 cm² were treated with dosing of 3 to 4 Joules/cm² for superficial conditions and higher dosing of 6 to 10 J/cm² for deeper pathologies. This is a dosing strategy that can only be achieved clinically with the higher output power provided by a Class IV therapy laser.
These dosing parameters were observed to be overwhelmingly effective by the patient assessments summarized in Table 2. Of the 120 canine cases, 95.8 percent were judged to have outcomes defined as good or excellent. A similar trend was observed in the results for the feline population of 45 cases with 93.3 percent of the outcomes scored as good or excellent.

This large series provides the first multi-center insight into the effectiveness of laser therapy in a standard clinical practice setting. The dosing strategy of treating superficial wounds, operative wounds, hot spots and superficial tendon injuries with 3 to 4 J/cm² over a large area with good margins was shown to be effective. Similarly, the dosing strategy of treating deep wounds, arthritis, contusions, hip dysplasia, and disc disease with higher doses of 6 to 10 J/cm² over a large area with good margins was also shown to be effective.

These dosing regimes appeared to be complemented by protocol adjustments for coat and skin color and animal size by the SmartCoat technology in the Companion Therapy lasers, as well as the versatility of the condition-specific treatment heads.

As laser therapy becomes ubiquitous in veterinary practice, a rigorous assessment of its efficacy is critical. In the complete absence of large, multicenter studies, this case series stands out as a significant step towards establishing uniform dosing and technique in the use of this modality in common veterinary practice.

Table 2

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>52 (43.3%)</td>
<td>22 (48.9%)</td>
</tr>
<tr>
<td>Very Good</td>
<td>15 (12.5%)</td>
<td>4 (8.9%)</td>
</tr>
<tr>
<td>Good</td>
<td>48 (40.0%)</td>
<td>20 (44.4%)</td>
</tr>
<tr>
<td>Fair</td>
<td>3 (2.5%)</td>
<td>3 (6.7%)</td>
</tr>
<tr>
<td>Poor</td>
<td>2 (1.7%)</td>
<td>3 (6.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>45</td>
</tr>
</tbody>
</table>

* Pierrefonds Animal Hospital used a 5-point scale.

The effectiveness of Class IV power and dosing strategy were consistent at all study sites. These data provide a foundation for dosing strategy that will inform future work to further establish the efficacy of laser therapy in the small animal veterinary clinic of the future.

Special thanks to Jennifer Johnson, VMD, CVVP of Stoney Creek Veterinary Hospital; Maria Cecere, RVT, CCRP, and Laurie Dunbar, DVM, CCRP, of Pierrefonds Animal Hospital; and Dr. Kim Qvist of Dyrlægehuset for providing the data for this series.

CONCLUSIONS

This is the first published uniform multicenter, international case series to assess the effectiveness of any laser therapy technique in veterinary practice. Class IV Companion Therapy Lasers using SmartCoat technology have been clearly demonstrated to empower small animal practices to achieve consistent positive outcomes over a wide range of common clinical conditions.
Jason Smith is the Director of Clinical Affairs at LiteCure LLC. He is focused on the coordination and support of research and clinical activity to better understand the fundamentals and applications of laser therapy.

His research has focused on wound healing and the modulation of the body’s response to medical devices. He serves as an ad-hoc reviewer for scientific journals including Langmuir and Experimental Cell Research and has published many peer-reviewed articles. Clinically, he has designed IDE and post market randomized multicenter clinical trials for a wide range of medical devices.

He holds bachelor’s and master’s degrees in Materials Science and Engineering from North Carolina State University and a doctorate in biomedical engineering from Duke University.

REFERENCES


INTRODUCTION
Therapeutic lasers have been used around the world for over twenty years, yet only recently has this technology been widely integrated into mainstream medical practice. Technology and manufacturing advancements now allow laser units to be affordable and to have adequate output power to perform comprehensive treatments in a reasonable time. As more studies are being completed, we are finding that therapeutic lasers are effective for treating many common disorders. It is important to understand the basic mechanisms of laser therapy in order to use this versatile tool properly for appropriate applications. This understanding will allow the clinician to use lasers as a stand-alone therapy, or as an adjunct to other treatments.

Photochemical effects occur when laser light is absorbed by chromophores (the light absorbing part of a molecule) within a target cell, and biochemical change is inspired. Photobiomodulation, which is the term science and industry agree is most descriptive, is an example of a photochemical process in which photons from a laser source interact with target cells and cause stimulatory or inhibitory biochemical change.

There are over 3,000 published studies on non-ablative laser therapy. Many of these studies have been done on cells in vitro and have shown compelling results concerning laser light’s effect on various types of cells. These studies have shown increases of angiogenesis, neurite extension, normalization of ion channels, stabilization of the cellular membrane and many other cellular changes.

The exact mechanism of action for photobiomodulation is still being debated in the scientific community. It is likely that several mechanisms are involved, depending on the type of cell being stimulated. The most supported mechanism to date is that cytochrome c, which is found within the intercellular membrane of the mitochondria, acts as a photoreceptor. Cytochrome c absorbs light from 500 nm to 1100 nm due to specific properties of this large molecule. Once light is absorbed, cytochrome c is excited and can more readily bond with oxygen and become cytochrome c oxidase, a compound critical to the formation of ATP. ATP is the activated carrier of energy in the cell, and facilitates a host of biologic responses or secondary mechanisms. This cellular mechanism initiates the reduction of pain, the reduction of inflammation and the healing of tissue.

LIGHT-TISSUE INTERACTION
Cells and biological tissue respond to light in a wide range of wavelengths, from ultraviolet to the near-infrared. Selecting the correct wavelength ensures the light will penetrate through skin, fat and muscle to reach the target cells to be treated. Biological tissue either reflects, absorbs, scatters, or transmits light. The primary chromophores in tissue that are relevant for laser therapy applications are hemoglobin, oxyhemoglobin, water, and melanin. Figure 1 shows the absorption coefficient for these various components as a function of wavelength.

The ideal window for the delivery of light into tissue ranges from approximately 650 nm to 1.3 microns. As the melanin content increases, the window is narrowed to about 860 nm to 1.3 microns. Melanin is a critical component when determining how to treat specific tissue. To minimize absorption by melanin in darker skin and/or hair, longer wavelengths are required. Many studies fail to point this out when addressing depth of penetration. As can be seen from the values on the chart on the next page, it is a critical component to absorption. Water is another component which is often misunderstood. The adjacent chart is plotted on a logarithmic scale. This format allows the presentation of data that varies by many orders of magnitude on the same graph. Water absorption is not a substantial concern until above 1 micron. (For example, the absorption coefficient of water at 970 nm is 0.45 while the absorption coefficient of melanin is over 10, which is an order of magnitude greater.)

Figure 2 shows a spectrum for water absorption from the UV (ultraviolet) to the IR (infrared). Also illustrated is a section...
Figure 1. Optical absorption spectra of various tissue components in the ultraviolet to infrared frequency range. From The Warren Research Group at Duke University.

of the spectrum magnified (b.) to show a slight variance of absorption in this optical window. Although there are some peaks, the values are minimal when compared to the much higher peaks for water absorption seen at higher wavelengths.

Figure 3, reproduced from a published study, demonstrates the transmission of light through adipose breast tissue. This clearly shows the optimal region in the spectrum for transmission is from around 740 nm to 1.1 microns. It also shows that absorption in this region is fairly flat. The important component missing from this study is melanin.

This point is also illustrated in Figure 4, which shows the absorption spectrum of liver tissue. As expected, this spectrum correlates well with the breast tissue spectra. Studying liver tissue is a great example of light absorption in water and hemoglobin. However, the lack of melanin in the tissue makes it very different than treating through skin and hair. When treating patients with higher melanin concentration in their skin and/or hair, the penetration of shorter wavelengths is reduced significantly. Hair removal lasers, for example, are designed to target melanin utilizing the principle of selective photothermolysis (the preferential heating of the hair follicle with light). The higher relative absorption by the melanin kills the follicle, while not being absorbed to any great degree by the skin. The most popular wavelengths for this application are 755 and 810 nm, since the melanin absorption is high, and hemoglobin absorption is low.

Target cells of interest for laser therapy have such a generous absorption curve (UV through to IR), that choice of wavelength is as much determined by what the light is not absorbed by: water, melanin, hemoglobin etc.
Figure 3. Optical Transmittance through 3 mm of adipose breast biopsy and 500 microns of fibroglandular breast biopsy (green line). The absorption peaks marked F and A are due to fibroglandular and adipose tissue respectively. The absorption resonances marked 'OxyHb' are hemoglobin resonances in the tissue. Frontiers in Bioscience 3, a1-10, January 1, 1998 by Fay A. Marks General Electric Corporate Research and Development.

In order to deliver a therapeutic dosage to deep tissue, it is very important to choose the correct wavelength and use the correct amount of light. The textbook definition of "depth of penetration" is the depth at which the initial intensity of light drops to 1/e or ~37%. It does not consider the actual measurable amount of light. By this definition, the wavelength is the only important factor.

When determining practical dosages in a clinical setting, it is critical to consider wavelength as well as the initial intensity of light. For example, assume the depth of penetration of 800 nm light into muscle tissue is 5 mm. With 100 mW of power, 37 mW would be delivered at 5 mm. Raising the initial power to 10 W, 3.7 W would be delivered at a depth of 5 mm. The more power delivered to the surface of the tissue, the larger the dosage delivered to deeper tissue.

PROTOCOLS – WHERE DO THEY COME FROM?
As described previously, depth of a therapeutic dosage is dependent on the wavelength and the amount of light delivered to the surface. The goal of a therapy protocol is to optimize dosage at the cellular level. An example of proper protocol development is demonstrated by a recent series of laboratory studies on nerve repair.

An initial study was performed in vivo on rat dorsal root ganglion nerves. These nerve cells were split into 2 groups; one group was placed into a glucose medium, the other served as the control and put in a standard medium. The glucose group experienced severe impairment in axonal sprouting, while the control group remained unchanged. The impaired cells were then exposed to various wavelengths and dosages of laser light. The optimum axonal sprouting, which was 90% of the control, occurred when using a 980 nm laser with a dosage of 100 mJ/cm².

The second study was divided into two parts. The first part of the study was used to determine the correct dosage to deliver on the surface in order to achieve this dosage at the impaired nerve. In this first portion of the study, white New Zealand rabbits were used to determine how to get a dosage to the peroneal nerve. Small electro-optic detectors were introduced into the tissue and placed directly over the peroneal nerve. Since both hemoglobin and oxyhemoglobin have different absorption, the rabbits were anesthetized during these experiments, rather than euthanized. It was found that only 2.45% of the initial dosage (applied to the surface), penetrates to the peroneal nerve. This is only a depth of ~ 2.5 cm of tissue. Working backwards from the data gleaned in the Petri dish study, i.e. a dosage of 100 mJ/cm² at the nerve for optimal repair, it was calculated that the dose at the skin surface needed to be 4 J/cm².

The second part of the study then applied the given dosage to determine its therapeutic effects. In this second portion of the study, the peroneal nerve was transected and then immediately repaired surgically. The peroneal nerve is critical for the toe spread reflex, so each rabbit was videotaped in order to precisely measure the amount of toe spread. This was then compared to the unimpaired leg. Laser therapy, plus standard of care, was administered to half of the rabbits each day, for 8 days. The control group was treated with standard of care, plus a sham laser treatment for the same period of time. As early as six weeks, the laser-treated group showed a statistically significant amount of functional recovery indicating nerve repair when compared to the control. The laser group showed 90% repair after just 9 weeks post-transection.

This study is one example of how dosages and protocols are determined for specific laser treatments. In many cases, specific cells or tissues are tested in vivo, in order to determine a range of optimal parameters. Depth of penetration studies have been done on cadavers and on anesthetized animals. There have been many studies done on how light penetrates tissues. All of these parameters have been studied for years for medical laser applications including cosmetic / aesthetic procedures, laser surgery, and diagnostic applications.
APPLYING PROTOCOL DEVELOPMENT TO THE PATIENT

When performing laser therapy, it is critical to choose the correct dosage and wavelength, but equally important to consider the size of the area to be treated. (This is because the total energy required for a particular treatment protocol is a product of dosage in J/cm² and treatment area in cm²: Energy Required = Dosage x Treatment Area.) Using the 4 J/cm² dosage example from above and applying this to a 500 cm² treatment area, 2000 Joules of energy would be required for an effective treatment to be administered. The output power of the laser will have a dramatic impact on the time required to deliver the energy. 1 watt is equal to 1 J/sec and therefore the following treatment times would result at different laser power output levels:

<table>
<thead>
<tr>
<th>OUTPUT POWER</th>
<th>DOSAGE</th>
<th>SIZE OF TREATMENT AREA</th>
<th>TREATMENT TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mW</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>66 hours 40 minutes</td>
</tr>
<tr>
<td>100 mW</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>3 hours 20 minutes</td>
</tr>
<tr>
<td>250 mW</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>1 hour 20 minutes</td>
</tr>
<tr>
<td>500 mW</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>40 minutes</td>
</tr>
<tr>
<td>5 W</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>4 minutes</td>
</tr>
<tr>
<td>10 W</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>2 minutes</td>
</tr>
<tr>
<td>15 W</td>
<td>4 J/cm²</td>
<td>300 cm²</td>
<td>1 minute 20 seconds</td>
</tr>
</tbody>
</table>

This chart shows that treatment times are dramatically decreased as power is increased. Treatment at the higher power levels has been demonstrated to be both safe and efficacious in numerous studies and in clinical practice.

THE ROLE OF WAVELENGTH

The wavelength will be determined by the types of conditions you wish to treat. Superficial treatments such as wounds can be treated with shorter wavelengths in the red region of the spectrum. If deeper conditions are going to be treated, a longer wavelength in the range of 800 nm to 1 micron will be optimal. When treating darker skinned, or hirsute patients, the longer wavelengths will have optimal penetration. The dosage will be determined by the condition, the color of the patient’s skin and hair, and the depth, and type, of tissue to be treated. For additional information about the role of wavelength please reference LiteCure’s annotated bibliography.

CONCLUSION

The understanding of the fundamentals of laser therapy, the clinical research and science, as well as the commercial product technology have advanced significantly over the last 30 years. The medical industry is just now beginning to use and experience the benefits of these technology, scientific, and research advances.
REFERENCES


*LiteCure Therapy Lasers are indicated for the treatment of pain, inflammation, and the temporary increase of microcirculation. Research related to off-label indications is provided at the request of a licensed physician.
BRIAN PRYOR, PH.D.

Brian Pryor is co-founder and the Chief Executive Officer at LiteCure®, LLC in Newark, Delaware. He has developed and taken to market several lasers and light based technologies. Dr. Pryor is well published (having contributed to 35 papers and several book chapters) in the areas of chemistry, physics, laser development and applications, including lasers in medicine. He has recently published “Clinical Overview and Applications of Class IV Therapy Lasers.” He has also recently written chapters on the subject of laser therapy in Current Perspectives in Clinical Treatment and Management in Workers’ Compensation Cases and in Your Dog’s Golden Years: A Manual for Senior Dog Care.

Dr. Pryor holds bachelor degrees in Mathematics and Chemistry from Salve Regina University and a PhD in Physical Chemistry from the University of Pennsylvania.

LUIS DE TABOADA, MSEE

Luis De Taboada is Vice President of Research and Development at LiteCure®, LLC. He was previously Vice President of Research and Development at PhotoThera Inc., Director of Engineering at Laser Mechanisms, Engineering Manager at Medical Optics, a division of Kaiser Aerospace and Electronics where he led the development of novel Carbon Dioxide lasers, laser systems, fiber beam delivery systems, and research in head mounted displays for minimally invasive surgery.

With over twenty years as a manager and active contributor to the design, fabrication, and testing of devices and systems for industrial, scientific, medical, and dental applications, Mr. De Taboada has extensive design and management experience on multi-disciplinary product development projects and systems engineering encompassing diffractive and refractive optics, optical fibers, electro-optics, laser, RF, energy conversion, and software technologies; including designing test methods for system acceptance and compliance with FDA and EN requirements, and active management of large DoD contracts and grants. Mr. De Taboada holds a MS in Electrical Engineering and a BS in Applied Mathematics from the University of California, San Diego.
BRIAN W. LITTLE, M.D., PH.D.

Brian Little has research interests in biomedical imaging, neuromuscular disease and slow virus diseases such as Creutzfeldt-Jakob disease. He has served on several regional and national committees in medical education, and is on the editorial board of the Journal of Continuing Education in the Health Professions. Other interests include the history of medicine and health care economics.

Dr. Little was Vice President of Academic Affairs and Research for Christiana Care Health Services from October, 2000, to May 2011. Prior to that, he was at the MCP-Hahnemann University School of Medicine, where he was Senior Associate Dean for Graduate and Continuing Medical Education and Affiliate Affairs. In these positions, Dr. Little was responsible for the allied health, undergraduate medical and graduate medical education programs, the clinical research enterprise which involves clinical trials, health services research, the Institutional Research Board and the support infrastructure. Since retirement, Dr. Little has consulted with Universities, Hospitals, Medical Schools and Industry on medical education and research administration.

Dr. Little received his MD and PhD (Biochemistry and Enzymology) degrees from the University of Vermont. His undergraduate degree is a BA in Physics from Cornell University. His residency and fellowship training in anatomic and clinical pathology and neuropathology were all at the Medical Center Hospital of Vermont.

JUANITA J. ANDERS, PH.D.

Juanita J. Anders is a Professor of Anatomy, Physiology and Genetics and Professor of Neuroscience at Uniformed Services University of the Health Sciences. She specializes in peripheral and central nervous system injury and repair mechanisms, and light tissue interactions. She is recognized as an expert in photobiomodulation and has been invited to speak and chair sessions at numerous international laser conferences.

Dr. Anders received her PhD in Anatomy from the University of Maryland Medical School then joined the National Institutes of Health in the Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS. Dr. Anders serves on the Executive Councils and Scientific Advisory Boards of several laser societies. She is the past president of the North American Association of Laser Therapy, a founding member of the International Academy of Laser Medicine and Surgery, and currently is the President of the American Society of Lasers in Medicine and Surgery. She has recently been appointed as a board member of the International Society of Laser in Medicine and Surgery. She serves on the Editorial Boards of Photomedicine and Laser Surgery, Lasers in Surgery and Medicine, Lasers in Medical Science, Physiotherapy Practice and Research and has published over 60 peer reviewed articles.
Veterinary Advisory Board

ROBIN DOWNING, DVM, CVPP, CVA, CCRP, CPE, DAAPM, DACVSMR

Robin Downing is Hospital Director of The Downing Center for Animal Pain Management, LLC, the first comprehensive pain prevention and management practice for pets in Northern Colorado. In 2000 she was named the Hill’s Animal Welfare and Humane Ethics Award winner, and in 2001 the World Small Animal Association presented Dr. Downing the Excellence in Veterinary Healthcare Award (Small Animal Veterinarian of the Year).

Dr. Downing is a founder of the International Veterinary Academy of Pain Management, a Certified Veterinary Pain Practitioner, a certified veterinary acupuncturist, a Certified Canine Rehabilitation Practitioner (University of Tennessee), a certified Tui Na practitioner (Chi Institute), is certified in canine medical massage (CSU), and is certified in animal chiropractic (IVCA). She is one of a handful of veterinarians to hold the Diplomate credential in the American Academy of Pain Management, the US’s largest interdisciplinary human pain management organization. In 2009 Dr. Downing became the first veterinarian to earn the designation Certified Pain Educator from the American Society of Pain Educators, a second human pain management credential.

As an affiliate faculty at the Colorado State University College of Veterinary Medicine, Dr. Downing mentors post-graduate veterinarians, veterinary students, veterinary technician students, and veterinary technician assistant students from various college and graduate programs.

Dr. Downing has been sharing her passion for facilitating, enhancing, lengthening, and strengthening the Family-Pet-Veterinary Bond with audiences around the world since 1996.

FELIX DUERR, DVM, MS, DACVS, DECVS

Felix Duerr earned his veterinary degree in Germany. He completed his surgical residency/master’s program at Colorado State University. Dr. Duerr is the only practicing veterinarian in Colorado that is double boarded by both the American College of Veterinary Surgeons (ACVS) and the American College of Veterinary Sports Medicine and Rehabilitation (ACVSMR). He worked in private practice for four years prior to joining Colorado State University in 2011. Dr. Duerr’s research focus is clinical studies aimed at improving animal health and quality of life related to orthopedic problems. His clinical interests include sports medicine and rehabilitation, cranial cruciate ligament injury, hip dysplasia, elbow dysplasia, minimally invasive surgery (arthroscopy) and arthritis. Current research projects include the development of novel gait analysis techniques, evaluation of new treatment options for arthritis (surgical and non-surgical), and investigation of techniques to enhance bone healing.

Dr. Duerr’s specific interest is to improve the quality of life for dogs suffering from orthopedic disease. His research is not limited to surgical treatment of these diseases but also focuses on preventative, minimally-invasive and non-surgical treatment options.
JOHN C. GODBOLD JR., DVM

John Godbold graduated from Centre College of Kentucky in 1974 with a BS in Biochemistry and Molecular Biology and received his DVM from Auburn University School of Veterinary Medicine in 1978. In 1980 he established Stonehaven Park Veterinary Hospital, a general small animal practice in Jackson, Tennessee, where he was a solo small animal practitioner for 33 years. Dr. Godbold currently works full time with Stonehaven Veterinary Consulting, teaching, and assisting colleagues as a consultant for laser surgery and laser therapy.


Dr. Godbold is in high demand as a laser trainer and has spoken at over 450 workshops, wet-labs and continuing education courses throughout the world.

DEBBIE GROSS, DPT, MSPT, OCS, CCRP

Debbie Gross has been involved in the field of canine physical rehabilitation and conditioning for over twenty years. She began her career in human sports medicine and quickly made the transformation over to canine physical rehabilitation and sports medicine. She began with a BS at Boston University, an advanced MS from Quinnipiac College, and a doctorate at the University of Tennessee. She is also a certified canine rehabilitation practitioner, and is one of the founding persons involved in the University of Tennessee Canine Rehabilitation Practitioner program. She continues to play an integral role in the continued growth and advancement of the program. In addition, she believes that each day should be a learning experience and continuously seeks continuing education opportunities in the classroom and in real life experiences.

Dr. Gross has been teaching throughout the world on many topics and is widely published on the topic of canine physical rehabilitation, laser therapy in a rehabilitation setting, as well as canine performance. She has many DVDs, articles, and additional information published for the dog lover on topics ranging from conditioning, structure, injury prevention, stretching, strengthening, performance, and rehabilitation. She has been very involved with professional research in the area of canine performance and treatment of canine performance issues. In addition, a new passion has included the treatment of degenerative myelopathy with laser therapy. She absolutely loves spending time in her clinics, Wizard of Paws Physical Rehabilitation for Animals, LLC (www.wizardofpaws.net). Her love of animals and rehabilitation is demonstrated through her practice and her teaching. She is often sought out for her advice throughout the world and is thrilled to be able to offer her advice to others to help animals.
JÖRG MAYER, DVM, MS, DABVP (ECM), DECZM (SMALL MAMMAL), DACZM

Jörg Mayer grew up in Germany where he received his primary education. He went to Budapest, Hungary to study veterinary medicine shortly after the “iron curtain” fell. During his studies he was fortunate to be able to work with veterinarians in South Africa and Namibia for 6 months. He always had a strong interest in exotic animal medicine. After he received his doctoral degree from the University of Budapest/Hungary, he went to the USA for an internship in ‘Zoological Medicine and Surgery’ at the Roger Williams Park Zoo in Providence, RI. At the end of the internship he was part of a research team to study wild tree kangaroos in the rainforest of Papua New Guinea for 3 months. After this exciting experience, he went to the Royal Veterinary College in London, England to study for his MS degree in wild animal health. The masters project which focused on lead toxicity in the common loon, brought him to the Tufts Wildlife Clinic in the USA. There he was hired to serve as a clinical associate professor and as the head of the clinical service for exotic animals. In 2010 he moved to Athens, Georgia, to take the job of Associate Professor in Zoological Medicine at the University of Georgia.

Dr. Mayer lectures regularly at large national and international conferences on all aspects of exotic animal medicine. He qualified with the first batch of specialists in Exotic Companion Mammals as a Diplomate of the American Board of Veterinary Practitioners; he is also a Diplomate of the European College of Zoological Medicine. He served as the president of the Association of Exotic Mammal Veterinarians from 2010-2012. Since 2012 he has been an International Fulbright Specialist in Zoological Medicine.

Dr. Mayer has published many scientific articles and book chapters some of which have been translated into French, Spanish and Portuguese. His latest book, Veterinary Clinical Advisor: Birds and Exotic Pets, was published in early 2013 and contains over 750 pages.

EVELYN ORENBUCH, DVM, CAVCA, CCRT

Evelyn Orenbuch leads the veterinary staff at GA Veterinary Rehabilitation, Fitness & Pain Management. She has been practicing veterinary medicine since 1994 after graduating from The Ohio State College of Veterinary Medicine. For over a decade, Evelyn has dedicated herself to veterinary rehabilitation and pain management, prescribing, overseeing and implementing comprehensive treatment plans for dogs, cats, horses, and small animals. She has treated patients ranging from world class performance athletes to senior pets. Dr. Orenbuch takes great satisfaction in working intensively with each client and patient and seeing the patient improve under her care. In addition to physical rehabilitative and fitness medicine, she is experienced in veterinary acupuncture, chiropractic, and Chinese herbs.

Dr. Orenbuch earned her rehab certification (Certified Canine Rehabilitation Therapist) from the Canine Rehabilitation Institute, studied acupuncture with the International Veterinary Acupuncture Society, and is certified in veterinary chiropractic through the American Veterinary Chiropractic Association. She is a past president of the American Association of Rehabilitation Veterinarians, and member of the International Veterinary Academy of Pain Management and the American Canine Sports Medicine Association.

In her spare time, Dr. Orenbuch enjoys the outdoors, has earned world championship medals in the sport of dragon boat racing, and has volunteered as a veterinarian in Guatemala, Israel, Mexico and Thailand. She shares her life with her husband, Stuart, a professor at Kennesaw State University; Pia, their mixed breed rescue dog; and Chuck, an orange tabby rescue cat.
RAY ARZA, DVM

Ray Arza earned his DVM at the University of Tennessee in 1979. He was a small animal general practitioner for 23 years with a special interest in surgery and dentistry. Dr. Arza started using a surgical laser in 1998, and soon thereafter became a frequent lecturer at conferences, universities, and seminars on laser technologies. In 2002, he left private practice to join the industry as an educator, trainer, consultant, and lecturer. He is the co-author of both volumes of Class IV Laser Therapy Treatment of Common Conditions and contributor to the veterinary protocols programmed in LiteCure’s veterinary lasers.

RONALD J. RIEGEL, DVM

Ronald Riegel is cofounder of the American Institute for Medical Laser Applications (AIMLA). His background in laser technology encompasses human, companion animal, and equine disciplines. He has spent the last two decades lecturing nationally and internationally to human and veterinary healthcare professionals. He has worked with therapeutic lasers since 1979.

Dr. Riegel has authored or co-authored more than a dozen papers and books including: Clinical Overview and Applications of Class IV Therapy Lasers, Laser Therapy for the Equine Athlete, and Laser Therapy in the Companion Animal Practice. He also founded and managed Equistar Publications and Illustrated Animal Books LLC.

Dr. Ron Riegel earned his undergraduate degree in Chemistry from the University of Delaware. His doctoral degree in Veterinary Medicine came from the University of Illinois. He served as a clinical instructor with the Ohio State University and is a member of the Academy of Human Neuromuscular Physiology.
HEIDI WARD, DVM, DACVIM

Heidi Ward earned her DVM Degree from The Ohio State University in 1989. She performed an internship in small animal medicine and surgery at Veterinary Specialists of Connecticut in 1989-1990, a residency in Small Animal Medicine at the University of Florida College of Veterinary Medicine in 1990-1992 and completed a second residency in Medical Oncology at The Ohio State University College of Veterinary Medicine in 1994. She was a clinical assistant professor in the small animal internal medicine service at The Ohio State University College of Veterinary Medicine for three years and worked within the internal medicine department of Med Vet in Columbus, Ohio, for 1 year prior to moving to Florida in 1998. She currently works at Gulfcoast Veterinary Oncology and Internal Medicine in Sarasota, Florida, and sees patients in Naples and Fort Myers on Wednesdays.

LISA MILLER, DVM, DACVIM, CCRT

Lisa Miller is the Veterinary Medical Director for Companion® Animal Health. Dr. Miller is a graduate of the University of Tennessee, College of Veterinary Medicine. After graduation in 2003, Dr. Miller completed an internship in internal medicine and then became certified in canine rehabilitation therapy at the Canine Rehabilitation Institute. Working in a large referral practice, she practiced canine rehabilitation, sports medicine, neurological rehabilitation and acupuncture for several years before returning to general small animal practice. Always maintaining an interest in the canine athlete/performance dog, Dr. Miller is also active on the health committees for two purebred dog breed clubs. Dr. Miller is a horse owner and enthusiastic equestrian.
ERICA SHOULTS, DVM

Erica Shoults is the Product Manager for Companion® Therapy Products. Dr. Shoults graduated from Kansas State University's veterinary college in 2005 and has been helping veterinary clinics all over the world incorporate alternative therapies into their practices for the past 7 years. She has worked with several organizations in product development and improvement including the creation of the Stance Analyzer for lameness diagnosis. Her extensive knowledge of hydrotherapy has helped place dozens of canine underwater treadmills throughout North America and she has worked on several new hospital builds in designing physical rehabilitation suites. She resides in her hometown of Kansas City with her dog Splash and two cats, Missy and Sully.

Cell culture experiments on rat cortical and dorsal root ganglion neurite extension were used to determine optimal laser dosing at 980 nm and 810 nm. After measuring real penetration to the target tissue, these dosing criteria were then tested successfully in functional models of rabbit peroneal nerve injury and a rat spared nerve injury. The authors conclude that infrared light with optimized parameters promotes accelerated nerve regeneration and improved functional recovery in a surgically repaired peripheral nerve.


The effect of laser therapy on muscle tissue was evaluated using both cell culture and clinical trial using an established muscle injury protocol. Cell culture experiments demonstrated that laser therapy increases cell proliferation in a dose dependent manner. Analysis of human tissue biopsies taken 6 hours after laser therapy treatment (10 W CW 980/810 nm for 10 minutes) revealed that 10 J/cm² increased IGF-1 signaling and decreased the presence of inflammatory markers.


 Cultures of C2C12 murine skeletal muscle cells were treated with different laser doses and mitochondrial biogenesis signaling molecules (AMPK, p38) were measured to determine optimal dosage. Then the optimal treatment dose was applied over 4 consecutive days and a significant increase in mitochondrial biogenesis markers was observed. The authors conclude that laser treatment is capable of altering mitochondrial signaling and regulatory proteins in skeletal muscle tissue without cardiovascular and/or inflammatory influence.


15 patients with documented chronic tendinosis of the elbow were randomized into sham or laser therapy treatment. The laser group received eight 5.5 minute treatments of 10 J/cm² every-other day for 18 days. Follow-up evaluations were made at 3, 6 and 12 months. Multiple pain measures, handgrip strength and functional impairment all improved with respect to sham treatment. Statistical significance was maintained to 1 year follow-up.


39 women with fibromyalgia syndrome were randomized to receive laser therapy treatment or sham heat gun treatment. Treatments were administered twice a week for 4 weeks at eight tender points across the neck, shoulders and back. Laser therapy treatment significantly improved upper body flexibility, fibromyalgia impact score and a composite measure of pain compared to the sham treatment.

Knapp, Daniel J. "Postherpetic neuralgia: Case study of class 4 laser therapy intervention." The Clinical Journal of Pain (April 17, 2013) Published online ahead of print. (LiteCure® Laser used in study)

Case study of laser treatment on a woman diagnosed with PHN (postherpetic neuralgia). The 73-year-old woman had pain in her upper back, shoulder and arm that had lasted for 15 years after a case of shingles. Pain decreased after 8 weekly class 4 laser treatments (2 to 4 W, 3.5 to 7.1 J/cm²).


Pilot study consisting of ten patients with at least one month of myofascial pain. Patients underwent two weeks of class IV laser treatment. A majority of the patients who underwent laser therapy showed improvement in pain assessment at 25 and 30 days post treatment. This study warrants a larger, controlled clinical study.

Study examined the effects of laser treatment on rats with nerve injury. Rats were injured and either treated with a 980 nm wavelength laser (LT) or in a control group (CTRL). Laser treatment significantly decreased mechanical allodynia. In the LT group there was also regeneration of the intra-epidermal nerve fibers, re-innervation of the LC and a decrease in expression of PGP9.5.


Review of published laser therapy studies. Phototherapy before resistance exercise may enhance contractile function, reduce exercise-induced muscle damage, and facilitate post-exercise recovery.


42 right-hand dominant subjects, equally divided in age groups 18-35 yrs or 65-90 yrs, were randomized to treatment and control groups. The treatment group received 10 J/cm² pre-treatment to the first dorsal interosseous before exercise to failure. Results indicate that laser therapy shows promise to enhance time to task failure, prevent loss of muscular strength and delay the onset of musculoskeletal fatigue in older adults.


Dr. Anders presented a series of studies on the effect of laser therapy on nerves. Cell culture experiments on rat cortical and dorsal root ganglion neurite extension were used to determine optimal laser dosing at 980 nm and 810 nm. After measuring real penetration to the target tissue, these dosing criteria were then tested successfully in functional models of rabbit peroneal nerve injury and a rat spared nerve injury. Preliminary histology results were presented to support the functional result.


27 patient prospective case series on patients with clinically verified TMJ disorder. Subjects received 5 weekly treatments using a 830 nm laser on 3 points in contact mode at an energy density of 15.4 J/cm². After treatment population VAS was reduced by 85% and jaw range of motion increased by 24%. “The laser therapy was effective in the improvement of the range of temporomandibular disorders (TMD) and promoted a significant reduction of pain symptoms.”


30 consecutive patients with clinically verified sub-acute or chronic low back pain. Patients were randomized to receive 15 treatments (5 days a week for 3 weeks) of either ultrasound or laser therapy. Ultrasound was administered at 1 MHz for 10 minutes. 2600 J of laser therapy was administered to the same treatment area over 10 minutes. The laser therapy group “showed a statistically significant reduction of Visual Analog Scale pain and Oswestry Low Back Pain Disability Questionnaire scores with respect to Ultrasound at the end of treatment (P<0.005).”


2 case studies are presented that demonstrate the potential effectiveness of laser therapy applied transcranially to the forehead and scalp for the treatment of traumatic brain injury (TBI).

70 patients with clinically verified subacromial impingement were randomized to receive 10 treatments (5 days a week for 2 weeks) of either ultrasound or laser therapy. Ultrasound was administered at 1 MHz for 10 minutes. 2050 J of laser therapy was administered to the same treatment area over 10 minutes. After the treatment period the laser therapy group had reduced pain, improved articular movement, functionality and muscle strength in the affected shoulder compared to the ultrasound group (P<0.005).


A rat model of induced tendonitis was used to evaluate the effect of laser therapy on inflammatory signaling. 42 rats were randomized evenly into laser and control populations. Rats in the laser group were treated at 780 nm with a fluence of 7.7 J/cm² every-other day starting at 12 h or day 7 through the end of the study. The laser group had significantly lower IL-6, COX-2 and TGF-β than control animals in both acute and chronic phases. Laser therapy significantly reduced TNF-α only at the chronic phase. Laser therapy is effective for the reduction of mRNA expression for pro-inflammatory mediators.


44 studies of laser therapy were reviewed for inhibitory effects on peripheral nerve pain. In 13 of 18 human studies laser therapy was shown to slow conduction velocity and/or reduce the amplitude of compound action potentials. Several mechanisms for the analgesic effects of laser therapy are also discussed.


The Orthopaedic Section of the APTA recommends that clinicians should consider the use of laser therapy to decrease pain and stiffness in patients with Achilles tendinopathy.


In cell culture, primary fibroblasts treated with laser at 660 nm were shown to increase proliferation and decrease cell death at an irradiance of 2.5 W/cm² and a fluence of 150 J/cm².


33 studies comparing continuous wave (CW) and pulsed laser treatments were reviewed. A common criticism of these studies is the lack of direct, like-for-like comparison of pulsed treatment to CW. The article concludes “CW is the gold standard and has been used for all LLLT applications” and “there is no consensus on the effects of different frequencies and pulse parameters on the physiology and therapeutic response of various disease states that are often treated with laser therapy. This has allowed manufacturers to claim advantages of pulsing without hard evidence to back up their claims.”


55 patients with low back pain were randomized to receive either manual adjustment or adjustment followed by laser therapy. After 4 weeks the laser therapy group had a 71% reduction in pain score (VAS) and was significantly better than manipulation alone.
Annotated Bibliography


Primary cortical and dorsal root ganglion neurons were pre-treated in high glucose media to induce die-back in a cell culture model of diabetic cell damage. In cortical neurons 980 nm irradiation at 0.01, 0.05 and 0.2 J/cm² significantly increased neurite extension. For DRGs subjected to high glucose media, 810 nm light at 0.01 J/cm² significantly increased neurite extension.


A review of 25 clinical trials of laser therapy for the treatment of tendinopathy. 12 trials had positive results and 13 were inconclusive or showed no significant effect. Dosing in the 12 positive trials supports the existence of an effective dosing window from 1-9 J/cm² depending on the depth of the tendon.


Human fibroblasts were exposed to 980 nm and 810 nm irradiation at a range of irradiance and fluence parameters in cell culture. Response was measured by MTS assay for mitochondrial activity. A 980 nm dose of 5 J/cm² at 10 mW/cm² caused an 11% increase in mitochondrial activity. 810 nm doses of 1 and 5 J/cm² at 50 mW/cm² caused a 40% increase. Results indicate that different wavelengths require different dosing to increase mitochondrial activity.


Position paper from the International Association for the Study of Pain that recommends the use of laser therapy in the treatment of Myofascial Pain: “Laser Therapy shows strong evidence of effectiveness for pain relief.”


Seminal review of Laser Therapy for Neck Pain published in a top-tier medical journal. 16 RCTs including 820 individual patients were included in the review. Laser therapy was found to immediately reduce neck pain and maintained results up to 22 weeks after completion of treatment. Also, most statistical heterogeneity disappeared when Chow excluded studies with small doses or flaws in treatment procedure.


RCT of laser therapy vs. therapeutic ultrasound in 70 patients with clinically verified subacromial impingement syndrome. Laser therapy was shown to have significantly greater benefit than ultrasound in reducing pain and improving the articular movement, functionality and muscle strength of the affected shoulder.


This review paper covers the current theory on basic mechanisms of laser therapy and discusses the effects of different dosing strategies. Cytochrome c is identified as the primary absorber of light in the near infrared leading to cellular changes related to ATP, NO and ROS levels. Different doses of laser therapy are shown to either stimulate or inhibit multiple metabolic and cell signaling pathways.


World Health Organization (WHO) taskforce concludes that laser therapy is beneficial for the treatment of neck pain.

Review of 7 studies using laser therapy to treat carpal tunnel syndrome. 5 of the 7 studies (226 total subjects) had positive findings for laser therapy treatment. These studies had an average success rate of 84% in cases of mild/moderate carpal tunnel syndrome. In general higher dosages applied to the median nerve at multiple locations were used in the successful studies. “Photoradiation is a promising new, conservative treatment for mild/moderate CTS. It is cost effective compared to current treatments.”


This review paper covers the current theory on basic mechanisms of laser therapy. Cytochrome c oxidase is identified as the primary absorber of light in the near infrared leading to cellular changes related to ATP, NO and ROS levels. The “optical window” for tissue penetration is identified from 600 nm to 1.4 μm. A review of animal and clinical studies is also presented for applications including wound healing, nerve regeneration and numerous musculoskeletal conditions.


Bashkatov investigates the optical properties of human tissue in the visible and near infrared. Tissue penetration depth, defined as the depth of 37% of surface exposure, is found to be between 2.25 mm and 2.50 mm into skin and between 4.5 mm and 6.0 mm in mucous tissue for the wavelengths used in laser therapy devices.


This review presents several studies that evaluate the efficacy of laser therapy to promote regeneration and recovery of injured peripheral nerve. Studies include a crush injury model of the rat facial nerve, injured rat sciatic nerve and regeneration after surgical repair. All studies demonstrated the efficacy of laser therapy for treating nerves and the technique was identified as “one of the most promising therapies to date” for these difficult pathologies.


This study evaluated both continuous wave (CW) and pulsed laser therapy in an elliptic wound model in rats. This study is one of the few published reports that evaluates both pulsed and CW therapy compared to a sham control. All laser parameters improved wound healing. Maximal benefit was achieved using CW laser therapy. Optimal dosing for wound healing was 5 J/cm².


35 patients with TMJ verified by MRI were randomized to laser therapy treatment or control. Patients in the treatment group received 15 treatments of 3 J/cm² at 904 nm. Both groups improved in all measures over the study period. The laser group had significantly better improvement in the number of tender points and all range of motion measures.


23 consecutive pediatric patients that received chemotherapy in preparation for bone marrow transplant were given 4 J/cm² phototherapy daily for 14 days at 670 nm. Ulcerative oral mucositis rates in this case series were reduced by 20-40% compared to historical controls.

Absorption changes in HeLa cells were evaluated from 530-890 nm. Irradiation causes changes in the spectrum of the cells in the ranges from 754-795 nm and 812-873 nm. Cytochrome c oxidase becomes more oxidized (implying increased cellular oxidative metabolism) at all wavelengths used. This study supports the hypothesis that the mechanism of photobiomodulation is related to the chromophore cytochrome c.


40 patients with fibromyalgia syndrome were randomized to receive laser therapy or placebo treatment 5 days a week for 2 weeks. Laser therapy consisted of treating all active trigger points for 3 minutes each. Both the sham and treatment groups improved in all measures except sleep disturbance and tender point sensitivity. The treatment group improved significantly compared to sham in pain, muscle spasm, morning stiffness and number of tender points.


Several effects of light on living cells are discussed. Proliferation, migration, metabolism, DNA synthesis, cell signaling and antimicrobial effects are identified. Future areas for research are identified.


Early study of laser therapy mechanisms that evaluated the structure of cytochrome c and other potential chromophores in the respiratory chain. Mechanisms for stimulation and inhibition are discussed as well as effects of laser therapy at the systemic level and possible limitations of the technology.

*LiteCure Therapy Lasers are indicated for the treatment of pain, inflammation, and the temporary increase of microcirculation. Research related to off-label indications is provided at the request of a licensed physician.