The Pillars Paper

Validating Multi Radiance Laser Technology

A Scientific Monograph

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The Pillars Paper:
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Introduction:

With over 5,200 studies on the use of therapeutic light, it is clear that light has a very powerful influence on the body, stimulating some biological processes while inhibiting others. This is called photobiomodulation (PBM) and it can be utilized to repair damaged tissue, accelerate recovery from injuries, modulate pain and alter disease states. However, several factors still remain unclear such as optimal doses, ideal treatment parameters, and the combined effects of different light sources (laser and LEDs) to achieve these desired effects. In this white paper, we will discuss the "pillars" of research that specifically support and validate the unique and patented (Multi Radiance Medical) synergistic combination of light, manufactured by Multi Radiance Medical.

Our proof of concept (POC) process began with basic mechanistic studies that involved both in-vivo and in-vitro trials that explored the interaction of combined multi-light sources – a novel innovation in the field of photobiomodulation. The In-vitro experiments explored the very basic interactions of photons and chromophores (molecules that serve to capture or detect light energy) to successfully detect that photobiological actions are occurring. In-vivo studies were carried out in both animal and human subjects to optimize the parameters of the device, such as power, dose and treatment frequency to maximize the previously identified in-vitro effects. The search for the optimal parameters were then refined in lab-controlled trials, where confounding factors are limited and direct relationships were validated and discussed. Finally the outcome data from previous studies was translated into clinical practice by performing human clinical trials to validate protocols, doses and parameters. Outcomes were closely documented not only to show statistical difference but clinical significance as well.

All experiments, trials and studies were supervised by the Laboratory of Phototherapy in Sports and Exercise (Sao Paulo, Brazil) and conducted at clinics and labs located both domestically and around the world. The following POC validates the combined synergistic effects of the different light sources (laser and LEDs) found in the Multi Radiance Medical devices and identify the optimal doses, treatment parameters for the safe delivery of consistent, clinically relevant patient outcomes.

About Multi Radiance Technology:

Multi Radiance Medical has combined the efforts of over 20 years of technical achievements and thousands of clinical sites around the world to create superior devices that reliably deliver the most reproducible and clinically significant results. The unique and patented core technology is the synergistic synchronization of Super Pulsed Laser (GaAs 905 nm), infrared and red LEDs (875 nm and 640 nm). Contained in a cluster probe, these specific wavelengths and light sources were selected to optimize the biological effects of the entire phototherapeutic window, provide a greater depth of penetration and eliminate the thermal barrier.

Scientific Foundations:

In-Vitro Experiments and The Mechanism of Action Pillar

Validating the Photobiological Response

One of the basic mechanisms of photobiomodulation (PBM) is the acceleration of electron transfer by electromagnetic radiation in the visible and near infra-red region of the spectrum. [1,2] Modulation of cytochrome c-oxidase activity (CCO) has been suggested as a possible key mechanism for PBM. To produce a phototherapeutic effect, photons must be absorbed by a chromophore. Albuquerque-Pontes et al[3] investigated the effects of phototherapy with the combination of different light sources to establish if an optimal dose or wavelength was needed to stimulate the enhancement of adenosine triphosphate (ATP) production. Three different low level laser therapy (LLLT) wavelengths (660, 830 and 905 nm) were used to analyze CCO, an enzyme in muscle, brain, and other tissues that catalyzes the transfer of a phosphate group from ATP to creatine, producing adenosine diphosphate (ADP) and phosphocreatine (PCr) expression by immunohistochemistry. Data was analyzed at 5, 10, 30 minutes, and at 1, 2, 12 and 24 hours. The results demonstrated that LLLT increased ($p<0.05$) CCO expression mainly with the following wavelengths and doses: 660nm with 1 J,

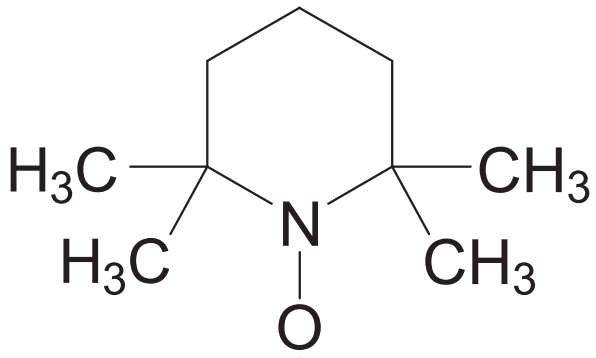

Figure 2
2,2,6,6-tetramethyl piperidine-N-oxyl

830nm with 3 J and 905nm with 1 J at each time-point. Using a stable free radical (2,2,6,6-tetramethyl piperidine-N-oxyl or TEMPO) which can readily accept an electron or react with an unstable free radical, is a very sensitive method for measuring electron transfer and free radical production by the observation of the decay of the EPR (electron paramagnetic resonance) signal of TEMPO. Eichler et al[4] found that red-light illumination of cell samples during 5 minutes with an energy dose of 150 J/cm^2 and an intensity of 500 mW/cm^2 produced a reduction of about 20% in the TEMPO EPR signal. Friedmann et al[5] repeated this experiment with the Multi Radiance Medical TQ Solo to study the combined effects of different light sources on the electron transfer chain. Friedmann found that the combination of laser, and light emitting diodes enhanced ATP production, cell proliferation, and reduced pain via neutralization of Reactive Oxygen Species (ROS). It accomplished this but only required 1/10 the average energy dose from the Eichler et al experiment while producing the same TEMPO EPR signal reduction. The acceleration of the electron transport chain by light plays an antioxidant role, which neutralizes the ROS.[6]

Depth of Penetration and Penetration of Light through Skin:

Researchers have recently been studying the effects of depth of penetration by testing various wavelengths to determine which are better suited for deeper or superficial applications. A review of the available literature has demonstrated that depth of penetration is directly related to the wavelength. Actual measurements of the skin penetration of light over time are necessary to understand how light enters the body.

Depth of penetration is wavelength specific as demonstrated by Sangkwan and Jong-In[7]. The 830 nm wavelength showed that the highest diffuse reflectance and the deepest penetration when compared with other wavelengths like 655 nm, 980 nm and 1064 nm. While increasing the available power will deliver a net greater energy to the tissue, it does not improve the penetration characteristics of the wavelength. Hudson et al[8] found 808 nm, 1 mW/cm^2 was achieved at 3.4 cm, but for 980 nm, 1 mW/cm^2 was achieved at only 2.2 cm depth of tissue. It was determined that 808 nm of light penetrates as much as 54% deeper than 980 nm light in bovine tissue.

The prior studies only focused on continuous wave (CW) laser operation. It has been suggested in the literature that other modes, such as super pulsing, may have different skin penetration time profiles. Brondon, et al[9] found the super pulsed mode of operation better able to penetrate through melanin filters, indicating that pulsing may be beneficial in reaching deep target tissue in dark-skinned patients. Joensen et al[10] evaluated a super pulsed 904 nm LLLT and found the light energy penetrated 2-3 easier through the rat skin barrier than 810 nm continuous. There was an interesting linear increase in penetrating energy from 38% (SEM±1.4) to 58% (SEM±3.5) during 150 sec of exposure during

the study. Therefore, the greatest depth of penetration occurs at the 904/905 nm wavelengths and that the absorption occurs not just at the superficial layers of the skin, but in deeper layers as well.

Leal-Junior et al studied the effects of depth of penetration with multiple wavelengths of 640nm Red LED, 875nm IRED and 905nm SPL to establish how various wavelengths and light sources interact when applied concurrently through the skin. Using the Multi Radiance MR4 SE25 and a ThorLabs Power Meter, Leal-Junior reproduced the protocol established by Joensen et al in an effort to understand if the combined wavelengths of the MR4 SE25 emitter impacted overall penetration of the light through the skin. One other major difference was in the thickness of the skin flaps used. In the original study (Joensen et al.), skin was taken from the gastrocnemius while in the latest study, it was taken from the dorsum which can be nearly double the thickness. The data suggests and demonstrates a similar pattern of linearly increasing penetration of the light sources with time as the previous study, but interestingly when the combined wavelengths were compared to the predicted measurement (summated total of each individual measured light source) the actual penetration of the combined sources was nearly 100% greater at all of the time points.

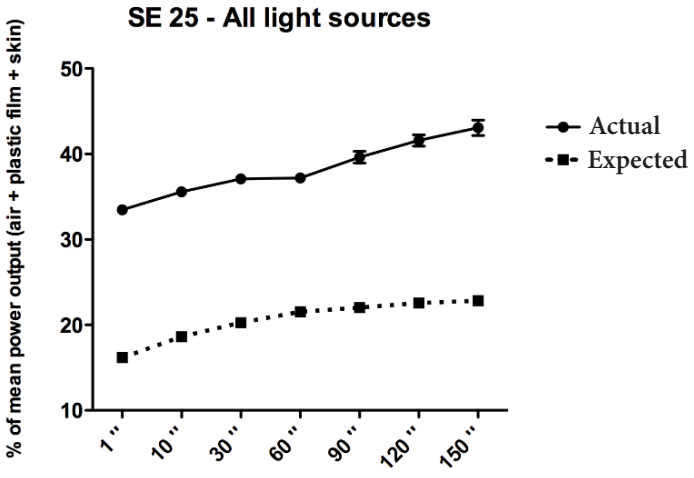

Figure 3
Comparison of Actual versus Expected Percentage of Energy Penetrating through the Skin.

The study concludes that a combination of multiple wavelengths creates a "synergism" that enhances each individual wavelength's ability to penetrate the skin. The increase in the skin penetration time profile created by multiple wavelengths allows greater amounts of light energy to penetrate sub dermal with lower average mean power outputs of power. This reduces the amount of energy being transformed into heat, where heat can lead to a reduction in photobiomodulation effects and a dangerous rise in tissue temperature.

Key Note: When multiple wavelengths are combined, there is a 100% increase in the available light below the skin and confirms the presence of synchronicity between wavelengths or the "Triple Cascade Effect".

Photobiomodulation and the Thermal Effect

The primary effects of photobiomodulation are based on photochemical changes and not the result of thermal changes in tissue.[11 12] However, most continuous wave lasers/LEDs and all high powered Class 4 lasers produce a considerable amount of heat as a byproduct. Super pulsing has been suggested as a means to prevent the absorption of photons from being converted to heat or transferred to the surrounding tissue in an effort to maximize the photobiomodulation effect.[13] Joensen et al[14] studied the effects of a 200 mW, 810 nm laser and a 60 mW mean output of power (20 W peak power) 904nm to demonstrate the thermal effects on healthy participants of differing skin color, age, and gender. Their results demonstrated that when higher doses are used, a super pulsed 904nm laser with a constant pulse train produced significantly higher temperatures in dark than in light skin and the continuous wave 200 mW 810nm laser induced three to six times more heat in dark skin than in the other skin color groups. Eight of thirteen participants with dark skin asked for LLLT to be stopped because of uncomfortable heating.

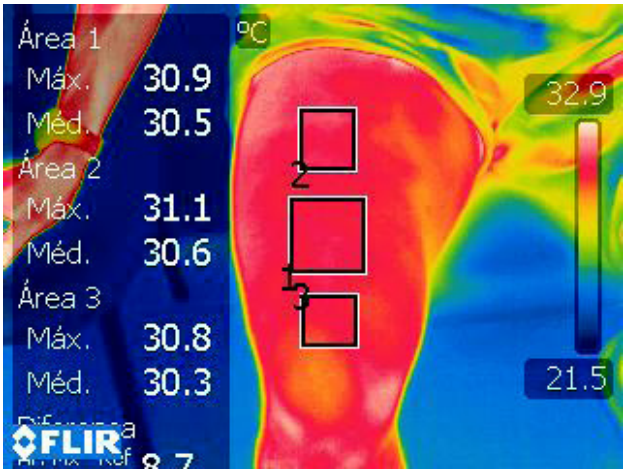

Figure 4
Thermal Image of Quadriceps during testing of the LS50

Leal-Junior et al replicated this study utilizing the Multi Radiance MR4 LaserShower (LS50: 640 nm, 875nm and 905nm) emitter with a set frequency of 250 Hz to

deliver a placebo, 10 J, 30 J and 50 J to sixty healthy adult volunteers divided by gender, age, and skin color stratified according to Von Luschan's chromatic scale. The previous study (Joensen et al.) found that at the higher doses, temperature increased significantly, as much as 22.3°C and the photothermal effects may exceed the thermal pain threshold for humans with dark skin color.

The MR4 LS50, at all doses (10, 30 and 50 J), did not increase temperature by 22.3°C in any of the test subjects. Therefore, the group did not find excessive photothermal effects that may affect patient safety and comfort. The study concluded that the stimulatory effects from the Multi Radiance devices are not created or influenced by photothermal reactions and therefore must be photochemical in nature. This may be attributed to the ultra-short pulse structure related to the frequency of the super pulsed laser and pulsing of the LEDs and IREDs as compared to the devices in the previous study (Joensen et al.) which utilized a continuous wave laser and a super pulsed laser that utilizes "constant" pulse trains.

Key Note: No significant temperature increases were found in any skin pigmentation types, therefore, the application of the LS50 at all doses was safe and no concerns about excessive heating are warranted. Additionally, the biological effects of the MR4 are related to photochemical effects and not due to excessive photothermal tissue changes that may be dangerous to patients.

Discovering the Optimal Parameters: *The Controlled Laboratory Studies Pillar*

A previous trials by Santos et al[15] evaluated the effects of multiple wavelengths and doses for the effects on muscle performance, fatigue, and skeletal muscle damage induced by tetanic contractions. Three different laser doses (1, 3, and 10 J) were applied with three different wavelengths (660, 830, and 905 nm) before six tetanic contractions induced by electrical stimulation. Blood and muscle samples were taken immediately after the sixth contraction. Qualitative assessment of morphology revealed lesser tissue damage in most LLLT-treated groups, with doses of 1–3 J/660 nm and 1, 3, and 10 J/905 nm providing the best results. Optimal doses of LLLT significantly delayed the development of skeletal muscle fatigue and protected skeletal muscle tissue against damage. All doses with wavelengths of 905 nm but only the dose of 1 J with 660 nm wavelength decreased creatine kinase (CK) activity ($p < 0.05$). The findings also lead the researchers to conclude that the combined use of different wavelengths at the same time can represent a therapeutic advantage in clinical settings.

A controlled study by Leal-Junior et al[16] was conducted to evaluate the effects of phototherapy on skeletal muscle performance and post-exercise recovery to biphasic dose response activity and measure the effects of multi light sources (laser and LEDs of the MR4 LS50). Forty healthy untrained male volunteers were recruited and randomly assigned to one of the four groups (placebo, 10, 30 or 50 J) and immediately after baseline testing had one of four doses applied prior to six sites of the quadriceps muscle prior to executing an isokinetic eccentric exercise fatigue protocol. Measurements of creatine kinase (CK) activity, visual analogue scale and strength of maximum voluntary contraction measurements were recorded.

Biphasic Dose Response and Validation

The biphasic dose-response or Arndt-Schulz curve in LLLT has been shown both in-vitro studies and in animal experiments.[17] Since laser, like ultrasound, at low levels can stimulate and extreme levels become destructive,[18] it is necessary to understand the values of this biphasic dose curve to stimulate biological processes or inhibit them. Due to selection of inappropriate dosimetric parameters that led to negative studies[19], this information becomes imperative when looking to regulate the photobiological response.

To measure the biphasic dose response, CK levels were drawn as well as Visual Analogue Scale (VAS) measurements taken on each subject at several time points (before, 1 minute, 1, 24, 48, 72 and 96 hours).

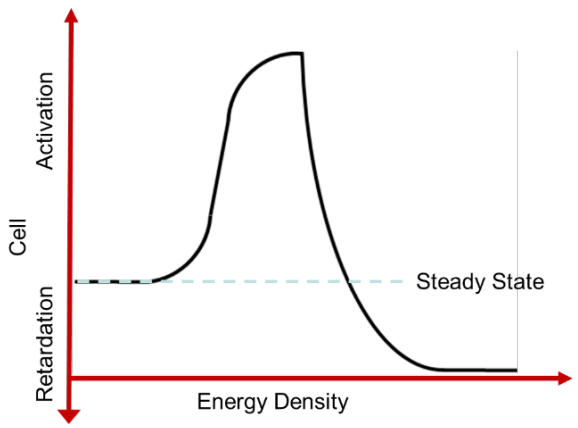

Figure 5
Depiction of the biphasic dose-response or Arndt-Schulz curve

Stimulation: Dose Validation of Inflammatory Marker Reduction via CK Activity

A stimulatory dose is regarded as one that typically will reduce inflammation and stimulate tissue repair. A CK test is used to detect inflammation of muscles (myositis) or serious muscle damage[20]. The placebo group results indicated that considerable muscle damage occurred as a result of the study protocol. Typically CK is greatest at 48 hours post exercise and the results showed all active groups experienced a delay in muscle damage. The active groups significantly decreased ($p<0.05$) CK activity compared to placebo. This minimized the delayed onset muscle soreness (DOMS) and damage from the fatigue protocol within the first 24 hours.

While all doses were beneficial, it was the 30 J group that represented the most stable measurement across all time points. The lower (10J) and higher (50J) doses did provide some protection, however at the peak of CK of 48 hours these doses did not perform as well as the 30 J dose. This is an excellent example of stimulatory portion of the biphasic dose-response or Arndt-Schulz curve. The smallest of the tested doses (10 J) stimulated the biological processes and as the dose was increased (30 J dose) even more favorable stimulatory results were noted. However once the larger dose (50 J) was applied the biological effects began to diminish and photobioinhibition began to take effect.

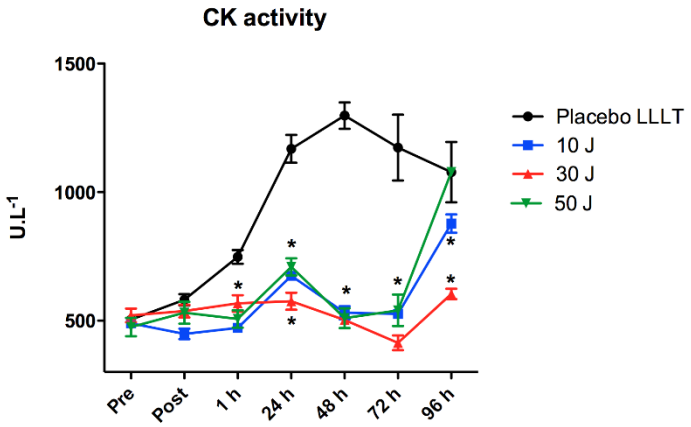

Figure 6
Creatine kinase (CK) activity based upon delivered dose over time. The 30 J dose was the most consistent across all time points.

Inhibition: Dose Validation for the Modulation of Pain and VAS Values

Pain relief is typically achieved when larger doses of energy are applied to the tissue creating a photobioinhibitive effect. This can effectively relieve pain of various etiologies.[21] Of the three doses, the 50 J dose significantly reduced short term pain in the first 24 hours – exhibiting the inhibitory aspect of the biphasic response. After 24 hours both the 50 and 30 J doses were effective, however, it was not until 72 hours the 10 J dose was able to return to baseline. As time progressed all doses were able to reduce VAS, however the higher doses (50 J) are most effective immediately and during the first 24 hours.

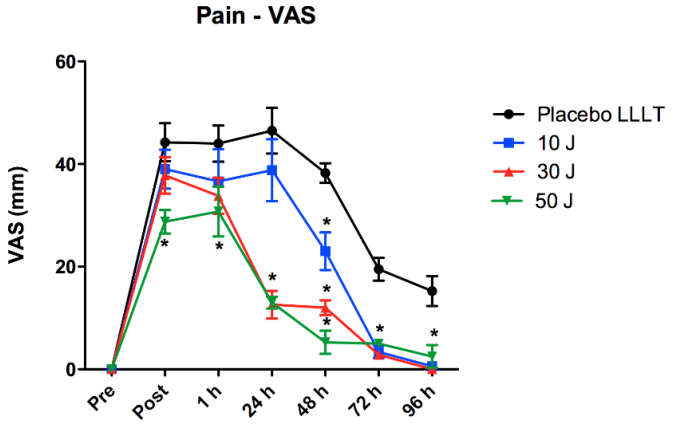

Figure 7
Visual Analogue Scale (VAS) measurements based upon delivered dose over time. The 50 J dose was significantly better than placebo at all time points.

Powering Athletic Performance and Optimizing Recovery

Fatigue has been found to play a big role in limiting performance in just about every individual and in every sport. Muscle fatigue, or physical fatigue, is the decline in ability of a muscle to generate force as a result of vigorous exercise. There are two main causes of muscle fatigue. The limitations of a nerve's ability to generate a sustained signal (neural fatigue) and the reduced ability of the muscle fiber to contract (metabolic fatigue). Leal Junior measured a maximum voluntary contraction (MVC) of the quadriceps of each subject in the four groups during the eccentric exercise protocol to utilize as a measure of performance levels. The observed effect was an expected decrease in performance (placebo group), however each of the active groups had a positive and immediate impact on the performance data. A single dose (any) was able to maintain MVC ($p<0.05$) or diminish fatigue as com-

pared to placebo immediately following the application of the Multi Radiance MR4 LS50. Power was not only maintained during the fatigue session, but it improved above baseline at 48 hours and continued to improve at 96 hours post exercise. This demonstrates an increase in performance of the muscle. This reduction of fatigue can be crucial to maximizing field performance but can possibly impact injury rates.

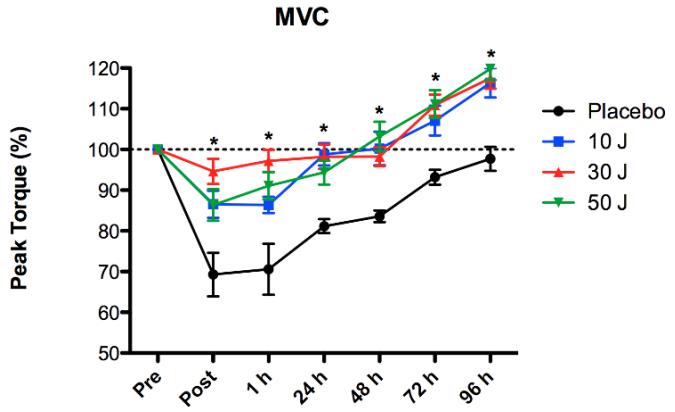

Figure 9
Maximum voluntary contraction (MVC) measurements based upon delivered dose over time. The 30 J dose was significantly better than placebo at all time points and during the exercise.

It is no secret, in order to improve performance, athletes must train intensely. Exercise is a stressor and exhibits catabolic effects on the body. Recovery is the body's reaction to adapt and replenish tissues at a higher level than that existing before exercising. Realizing full recovery is a function of time and failure to adequately rest can have many negative effects. A recent systematic review with meta-analyses performed by Leal-Junior et al has shown positive results of phototherapy applied before exercise to not only enhance athletic performance but also accelerate the recovery process.[22] All subjects in the active group demonstrated an accelerated full recovery to baseline at 48 hours compared to the placebo group that required a full 96 hours to return to original baseline readings. This represents a 300% increase in recovery rate!

The study authors concluded that the phototherapy treatment had a "protective" effect on the exercising muscles which minimized damage to exercising muscles. The current phototherapy paradigm only focuses on the utilization post injury to accelerate healing, however this study suggests two additional benefits: the use of phototherapy for the prevention of injuries/diseases and the performance enhancing drug free improvement of athletic performance.

Key Note: Pre-exercise phototherapy with combination of low-level laser and LEDs, mainly with 30 J dose, significantly increases performance, accelerates recovery and improves biochemical markers related to skeletal muscle damage.

Validation of the Outcomes: *The Clinical Trials Pillar*

Reducing Knee Pain in Clinical Practice with the Combined Use of Super Pulsed Laser and LEDs

Phototherapy has arisen as an interesting alternative to drugs in treatment of musculoskeletal disorders. However, there is a lack of studies investigating the effects of combined use of different wavelengths for musculoskeletal disorders. Leal-Junior and Johnson, et al[23] conducted a randomized, placebo-controlled, double-blinded clinical trial of eighty-six patients to evaluate the short and long term effects of a MR4 LaserShower (LS 50) + MR4 SE25 (SE 25)for non-specific knee pain. Patients of the active group received 12 treatments with active phototherapy (with 905nm super pulsed laser, 640nm and 875nm LEDs) and conventional treatment (physical therapy or chiropractic care), patients of the placebo group were treated the same way but with a placebo phototherapy device. Pain assessments (VAS) and quality of life assessments (SF-36®) were performed at baseline, 4th, 7th and 10th treatments, after the completion of treatments and at one month follow-up visit to determine success.

Testing a Treatment Methodology, the Priority Principle™

The emitters used in the trial were selected after early feasibility work was performed. Selection was based upon several criterion; including surface area, how best to mechanically remove blood from the area via compression, and how to ensure the aperture was in direct contact with the skin to minimize photon loss due to reflection or scattering. The MR4 LS50 was used where the treatment area was larger, since the emitter covered 20 cm^2. This was in the back, the groin and the back of the knee. The SE25 was utilized when a smaller aperture was required to make sure that direct contact with the knee was necessary, especially surrounding the

patella. The treatment protocol included both primary targets (SE25) and systemic targets (LS50) highlighting the methods of the Priority Principle. Approximately 40% of the total energy was delivered directly to the knee while the remaining 60% was divided between 3 other selected systemic "targets."

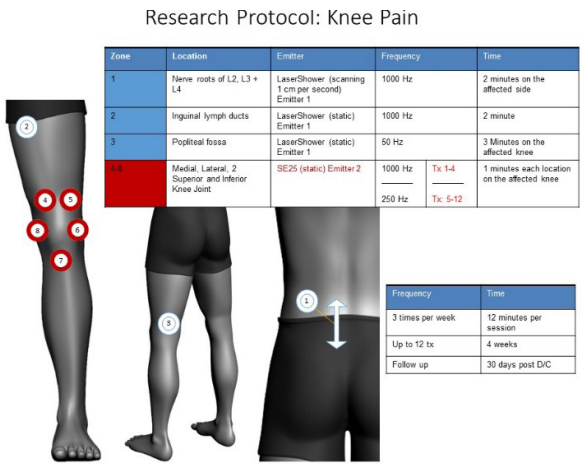

Figure 10
Protocol for the study, highlighting the Priority Principle. Methods included were local pain and tissue repair, systemic pain relief and tissue repair and reduction of swelling. Note the change in protocol frequency selection at the local site of pain after treatment four.

The Importance of Clinical Significance for Consistent Outcomes

It has been suggested that a 33% decrease in pain represents a reasonable standard for determining that a change in pain is meaningful from the patient's perspective.[24] The results demonstrated a decreasing trend in reported VAS scores in the active treatment group after treatment 4, while the control leveled off and increased slightly. The active group reached statistically significant improvements ($p<0.05$) at treatments 10, 12 and the thirty day follow-up increasing outcomes by 30% compared to placebo. The control group observed a drop in VAS pain of 35%, however statistical significance was not reached. Overall the active group resulted in a 50% improvement (15% greater than the placebo group) or one standard deviation improvement over the placebo group which is clinically significant. The analyses of the SF-36® data demonstrated an increasing trend in physical component score (PCS), and demonstrated a statistically significant improvement in physical functioning at the conclusion of both the treatment and follow-up phases. No adverse events or reactions were reported in the clinical trial. The study suggests that although other therapies (i.e. physical therapy or chiropractic therapy) are effective in treating knee pain, the addition of phototherapy enhances clinical outcomes in regards to pain and physical functioning.

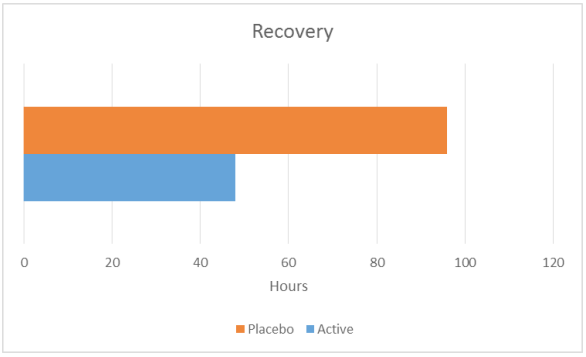

Figure 11
All active groups demonstrated an accelerated recovery to baseline at 48 hrs compared to the placebo group that required a full 96 hrs.

Key Note: The MR4 Laser Therapy System decreases acute and chronic pain (statistically and clinically) while improving the quality of life in patients who suffer with different kinds of knee pain.

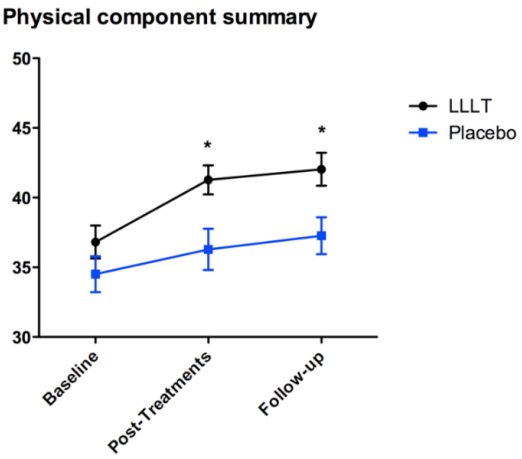

Figure 12
SF-36 data physical component score (PCS) demonstrated statistically significant improvement in physical functioning at treatments twelve and at the one month follow-up visits.

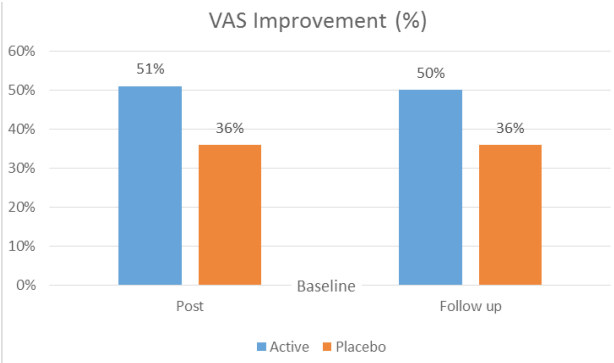

Figure 13
Percentage of improvement from baseline at treatments twelve and at the one month follow-up visits. Both represented statistical and clinical significance, however there was statistical significance noted between groups in favor of the Active Laser group.

Conclusion:

The unique and patented combination of wavelengths and light sources of the MR4 Laser Therapy System by Multi Radiance Medical was evaluated in a variety of in-vitro, human and animal in-vivo laboratory controlled studies and human clinical trials to clearly demonstrate proof of concept and overall efficacy utilizing only the gold standard scientific methods, not just VAS, but objective, biochemical measures. A biphasic dose response pattern was reported at 10 J (stimulatory response) and 50 J (inhibitory response). There were no adverse events noted in any of the trials; and since skin temperatures did not exceed the thermal threshold for skin tolerance, MR4 devices can be provided with a high degree of safety to both patient and clinician. These studies have confirmed that a synergistic relationship between the three different light sources exists and was able increase ATP production by optimizing the absorption spectrum of CCO stimulation, provide greater depth of penetration than when working independently, creates a minimal thermal heating all while decreasing pain, reducing inflammatory markers and improving athletic performance and accelerating recovery from fatigue. This monograph should give clinicians confidence that the Multi Radiance Medical products are supported by science tested and clinically proven to produce consistent, positive patient outcomes.

The Future is on the Horizon.

Multi Radiance Medical has committed to on-going clinical and scientific studies of its technology to push new industrial product designs, to become an innovative leader in discovering new clinical applications and move light-based medicine forward into the future and toward mainstream acceptance.

Multi Radiance Medical is currently investing into Research and Development at a faster pace than anyone else in the industry.

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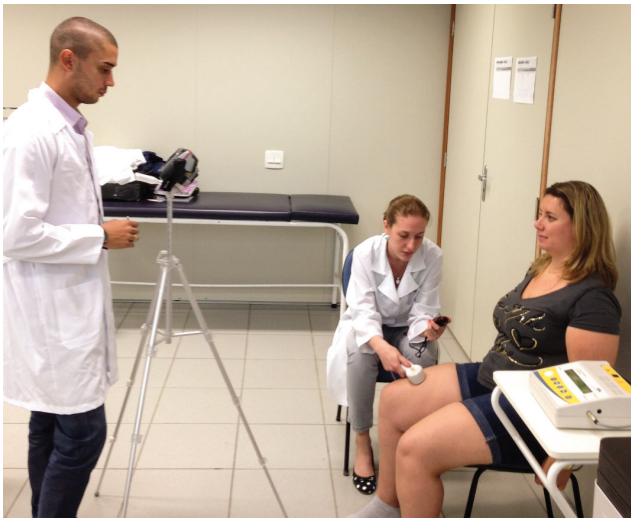
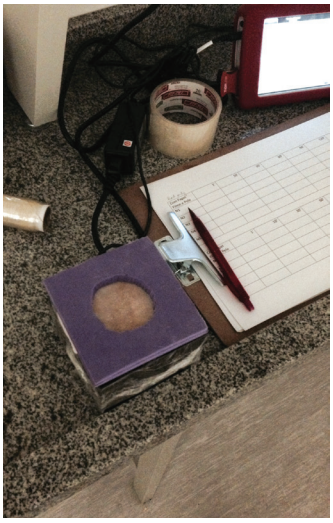


The thermal effects of simultaneous applied super-pulsed laser and light emitting diodes phototherapy with 905, 875 and 640 nm wavelengths on human skin – Laboratory setup.



Skin Penetration Time-Profiles for simultaneous applied super-pulsed laser and light emitting diodes phototherapy with 905, 875 and 640 nm wavelengths in a Rat Model – Laboratory setup.

Here are the names...

From left to right: Henrique Dantas Pinto (M.Sc. student), Kadma Monteiro (Ph.D. candidate), Eduardo Foschini Miranda (Post-Doctoral fellow), Fernanda Colella Antonialli (M.Sc. student), Ernesto Leal-Junior (Professor, Head of Laboratory), Vanessa Grandinetti (M.Sc. student), Ivo Aleixo Junior (M.Sc. student), Adriane Vanin (Ph.D. candidate), Gianna Pontes (Ph.D. candidate), Paulo Paiva (M.Sc. student).

About the Authors:

Douglas Johnson, ATC, EES, CLS, is a certified athletic trainer with over 20 years of clinical/industrial experience. He attended Wayne State University and The University of Detroit-Mercy where he earned a Summa Cum Laude Bachelors of Science degree in Sports Medicine in 1994.

He is the Senior Vice President, Clinical and Scientific Affairs at Multi Radiance Medical and is involved in numerous research studies involving super pulsed laser. Recently he was named as a clinical advisor to Laser Therapy U, invited to speak at the Annual 2014 NATA Symposium about Laser Therapy and is a member of the NAALT/WALT Scientific Education Committee for the 2014 Program. He studied the effect of super-pulsed laser and light emitting diodes phototherapy on non-specific knee pain which was published in Lasers in Medical Science, May 2014 and is a reviewer for the Journal of Athletic Training.

Currently he is updating his text, Phototherapy 101, to Laser Therapy 101 due to be published 2014, completing a Laser Methods Post Graduate Course, and has been invited to speak at Euroscience 2015 in London, England.

Ernesto Cesar Pinto Leal Junior, PT, PhD has a bachelor degree in Physiotherapy from 2002 in Brazil. In 2004 he got his Master´s degree in Biomedical Engineering at University of Vale do Paraiba (Univap) in Brazil, and he defended his PhD thesis in 2010 at University of Bergen - Norway (Section of Physiotherapy Science, Department of Public Health and Primary Health Care, Faculty of Medicine and Dentistry). In 2012 he finished his Post-Doctoral at Department of Pharmacology of University of Sao Paulo.

Dr. Leal Junior has been a lecturer at 2 Universities in Brazil (Unilasalle University and University of Caxias do Sul) between February 2005 and July 2009, and is a reviewer of several international peer-review journals, specifically in the Phototherapy and Sports Science fields (Photomedicine and Laser Surgery, Lasers in Medical Science, Physiotherapy Research International, Journal of Sports Sciences, Journal of Photochemistry and Photobiology, Biology, Photochemistry and Photobiology). Since April 2014 he has been a member of the editorial board of Photomedicine and Laser Surgery. His current position is as Full Professor at Nove Julho University in Sao Paulo, Brazil.

His expertise in research is phototherapy in skeletal muscle disorders. A special interest has been developed in phototherapy research (Low-Level Laser Therapy and Light Emitting Diode Therapy) for skeletal muscle fatigue delaying, performance enhancement, injury prevention and recovery after strenuous physical activity.

Currently Dr. Leal Junior has had 70 scientific papers published, 48 of them in international peer-reviewed journals (indexed by Pubmed/Medline). This includes 2 papers recently accepted in international journals. He has presented more than 40 scientific papers at National and International Congresses and in September 2011, Dr. Leal Junior was awarded by NAALT with the Young Clinical Research Award in Phototherapy.

The Pillars

1) Scientific Foundations: In-Vitro Experiments and The Mechanism of Action Pillar
   a) In-Vitro Experiments
      i) Friedmann H, Lipovsky A, Nitzan Y, Lubart R. Combined magnetic and pulsed laser fields produce synergistic acceleration of cellular electron transfer. Laser Therapy, 2009, 18(3): 137-141

   b) In-Vivo (Animal)
      i) Albuquerque-Pontes GM, Leal-Junior EC, et al. Effect of different doses, wavelengths and application intervals of low-level laser therapy on cytochrome c-oxidase activity in intact skeletal muscle in rats. Lasers Med Sci June, 2014 Epub ahead of print]
      ii) Santos LA, Leal-Junior EC, et al. Effects of pre-irradiation of low-level laser therapy with different doses and wavelengths in skeletal muscle performance, fatigue, and skeletal muscle damage induced by tetanic contractions
      iii) Leal-Junior EC, et al. Skin Penetration Time-Profiles for simultaneous applied super-pulsed laser and light emitting diodes phototherapy with 905, 875 and 640 nm wavelengths in a rat model.

   c) In-Vivo (Human)
      i) Leal-Junior EC, et al. The thermal effects of simultaneous applied super-pulsed laser and light emitting diodes phototherapy with 905, 875 and 640 nm wavelengths on human skin.

2) Discovering the Optimal Parameters: The Controlled Laboratory Studies Pillar
   a) In-Vivo (Human)
      i) Leal-Junior EC, Antonialli F, Grandinetti V, Vanin A, Tomazoni S, Miranda. Phototherapy in skeletal muscle performance and recovery after exercise: Effect of combination of super-pulsed laser and light emitting diodes. Lasers Med Sci June, 2014 Epub ahead of print]

3) Validation of the Outcomes: The Clinical Trials Pillar
   a) In-Vivo (Human)
      i) Leal-Junior EC and Johnson D et al. Adjunctive use of combination of super-pulsed laser and light emitting diodes phototherapy on non-specific knee pain: double-blinded randomized placebo-controlled trial. Lasers Med Sci May, 2014 Epub ahead of print]

Glossary for Pillars Paper

Adenosine Triphosphate (ATP)
Adenosine triphosphate (ATP) is a molecule that transports chemical energy within cells for metabolism.

Constant Pulse Train
Mode of super pulsed laser operation where the mean output of power is NOT affected by the set frequency and the mean output of power is constant

Constant Pulse Width
Mode of super pulsed laser operation where the mean output of power is related to the frequency or number of impulses per second the laser fires.

Continuous Wave (CW)
An uninterrupted beam of laser light (as opposed to a pulsed beam)

Creatine Kinase (CK)
Elevation of CK in the blood is an indication of damage to muscle.

Cytochrome C Oxidase (CCO)
The enzyme cytochrome c oxidase or Complex IV is a large transmembrane protein complex found in mitochondrion that synthesize ATP. CCO proteins are chromophores which mean they absorb light, which can increase the production of ATP.

Delayed Onset Muscle Soreness (DOMS)
Delayed onset muscle soreness (DOMS) is the pain and stiffness felt in muscles several hours to days after exercise.

Electron Paramagnetic Resonance (EPR)
Electron paramagnetic resonance (EPR) is a technique for studying materials with unpaired electrons for the detection and identification of free radicals

Gallium Arsenide (GaAs)
A light emitting semiconductor diode that contains gallium and arsenide. GaAs has been used to produce (near-infrared) laser diodes since 1962

In Vitro
Studies that are in vitro are performed with cells or biological molecules studied outside their normal biological context; for example proteins are examined in solution, or cells in artificial culture medium.

In Vivo
Studies that are in vivo are those in which the effects of various biological entities are tested on whole, living organisms usually animals including humans, and plants as opposed to a partial or dead organism, or those done in vitro.

Light Emitting Diodes (LED)
Optoelectronic semiconductor devices which are emitting broadband optical radiation. They are similar to laser diodes, containing an electrically driven p-n junction and an optical waveguide, but lack optical feedback, so that no laser action can occur.

Low Level Laser Therapy (LLLT)
Photobiomodulation with low energy lasers to achieve therapeutic effects.

Maximum Voluntary Contraction (MVS)
The greatest amount of tension a muscle can generate and hold, however briefly, as in muscle testing.

Nanometer (nm)
Unit of measurement of energy delivered referring to wavelength

Performance Enhancing Drugs (PED)
Performance-enhancing drugs (also known as PED) are substances used by athletes to improve their performances.

Photobiomodulation (PBM)
Photostimulation or photobiomodulation, is the process where a chain of chemical reactions is triggered by exposure to light. Photonic energy is absorbed by the cellular mitochondria, generating ATP, NO and ROS. This stimulates or inhibits many physiological responses, resulting in the restoration of normal cell function

Physical Component Score (PCS)
A composite score and measurement of physical ability as seen from the patient's perspective from the SF36. This score includes physical functioning, role-physical, bodily pain, and general health.

Priority Principle
A dynamic methodology used for integrating photobiomodulation methods (dose/rate and techniques) into clinical practice by prioritizing the current physiological and functional needs of the patient and then applying the appropriate sequence of treatment methods to maximize the phototherapeutic responses and patient outcomes

Proof of Concept (POC)
A proof of concept (POC) or a proof of principle to verify that a concept or theory has the potential of being used.

Quality of Life Assessment (SF-36)
The SF-36 is a multi-purpose, short-form health survey with only 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index.

Reactive Oxygen Species (ROS)
Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis.

Super Pulsed Laser (SPL)
Super Pulsed Laser produces a high peak impulse of intense light for a fraction of a second. Thus, there are no damaging thermal effects in the tissue because the pulses are of very short duration.

2,2,6,6,Tetramethyl piperidine-N-Oxyl (TEMPO)
TEMPO is widely used as a radical trap, as a structural probe for biological systems in conjunction with electron spin resonance spectroscopy.

Visual Analogue Scale (VAS)
The visual analogue scale (VAS) is a psychometric response scale and a measurement instrument for subjective characteristics or attitudes that cannot be directly measured

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