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PODIATRIC | SCIENTIFIC REPORT

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Review of Laser Phototherapy in Podiatry

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July 19, 2010

The Well-Oiled Human Machine

By far the most obvious and fortunate conclusion we have been able to extract from *in vivo* studies (not only with respect to laser phototherapy) is that our immune system is capable of handling an extraordinary range of pathologies. The time scale and degree to which our cells can react and combat these contaminants is the subject of much study, but it is clear both that lasers do stimulate the immune system and that the restoration of healthy function continues well after the initial irradiation. The amount of healing done during the minutes of laser irradiation is minuscule compared to the time it takes to relieve the body of disease or infection. This leads to one very important piece of information: the body does most of the work itself and so the target for an effective laser treatment is *NOT* the pathology itself, but rather to stimulate the appropriate cell compartments that lead to the body's natural repair mechanisms. Basically, we want to stimulate the cell's metabolism (i.e. its ability to use oxygen to create energy).

Bacteria, on the Other Hand

There are about 1000 different types of bacteria commonly present in the human body most of which reside either on the skin, or in the digestive tract. Of these, only about 10% are maintainable in cell culture and able to be studied. Some are beneficial (e.g. those that aid in digestion of food) others pathological. With this wide variety of species, never-mind their different functions and chemical signatures, it is prohibitively difficult to target any individual candidate or even to make the generalization that these candidates are more abundant than any other with respect to a particular pathology. Instead we can capitalize on one common feature in most bacteria: they do not like oxygen. Most bacteria are anaerobes that proliferate and metabolize much better in the absence of oxygen. Fortunately, this is in direct contradiction with the way our cells flourish and so stimulating the oxygen intake and conversion process will simultaneously help our healthy cells and inhibit bacteria.

Mechanisms

The principle absorbers of mammalian tissue by light in the near infrared (NIR) range of the electromagnetic spectrum (other than melanin in the skin) are hemoglobin at the core of blood cells and cytochrome c oxidase in the mitochondria. As such, and before any attention to their function, the characterization of absorption of these complexes was of paramount importance, and the subject of much study. Action spectra (i.e. the dependence of wavelength on absorption) have been generated for these (and other) targets *in vitro* and the peaks have been isolated and correlated with the biologically state of these complexes (see section "Understanding K-Laser's Success").

Metabolic Action

The action spectra tells us where in the spectrum and at what rate laser radiation is absorbed by these chromophores, but we must address the biology of the cell to understand the subsequent chain of events that lead to a beneficial, curative result. As discussed earlier, the central goal is to stimulate the cell (and ultimately, the body) to perform its natural functions, but at an enhanced rate. These natural functions are not only extremely numerous (ranging from protein synthesis to enzyme secretion, from cell signaling to physical movement) but also highly cell-type dependent. Any attempt to directly target one of the multitude and variety of these specific enzymes is difficult, and fundamentally unnecessary. If instead, the metabolism, specifically the respiratory chain, can be stimulated, the cell will enhance the functionality of *all* of its natural processes.

Fortunately, both hemoglobin and cytochrome c oxidase are involved in cell metabolism and their roles in the respiration chain are linked. Hemoglobin is the molecule, at the core of red blood cells, that transports oxygen through the body to the cells. When it reaches the cell it has to be de-oxygenated or "reduced". The oxygen is then passed through the cell membranes and into the mitochondria where it is processed by a series of enzymes, the last of which is cytochrome c oxidase. Here the oxygen is again "reduced" as it is converted into water; this reaction is the stimulus for the enzyme ATP synthase to create ATP, the source of chemical energy in cells. This is the reason we need oxygen, slightly more in depth than "to breathe". Think of the hemoglobin as the faucet that governs the rate at which oxygen flows *into* the cell and cytochrome c oxidase as the drain that determines the rate at which oxygen can *exit* the cell in the form of ATP (energy). To optimize efficiency of the flow of oxygen through the respiratory process, the most appropriate course of action would be to open both the faucet and drain as wide as possible (opening one without

the other would not increase the overall throughput); that is, stimulate the amount of hemoglobin that reaches the cell, the rate at which it reduces its oxygen, and then the rate at which the cell can process that oxygen and output energy. The goal then is to increase local blood circulation, stimulate the reduction of hemoglobin, then stimulate both the reduction and immediate re-oxygenation of cytochrome c oxidase so the process can start again.

Clinical Functionality

Circulation

Recall the first goal of an effective therapy was to increase the amount of oxygen available for the cell to process. This means increasing blood circulation since the hemoglobin in red blood cells are the transporters of oxygen from the lungs to the cells. On the macroscopic scale, this relies on increasing the heart rate, which in turn slightly increases body (and blood) temperature. This is why exercise is good therapy for almost any ailment; increasing blood flow increases metabolism and stimulates the immune system. Locally around a wound, however, topographical heating does very little, resulting in neither an increase in circulation nor metabolism. This type of thermal effect is *not* the mechanism for laser stimulation of circulation. Laser irradiation instead creates local temperature gradients; that is, temperature differences on the molecular level that create potentials along which blood cells are more likely to flow. The stronger and more numerous the gradients, the more local circulation of oxygen can be stimulated. Recall that the cell is more than 80% water. If you can target the absorption of water by a particular wavelength of radiation, you can cause local resonances that reinforce themselves. In the entire NIR region (i.e. from 700-1000 nm) the strongest and most distinct peak in absorption is at 965 nm; the right side of Figure 1 shows the absorption spectrum of brain tissue in the NIR.

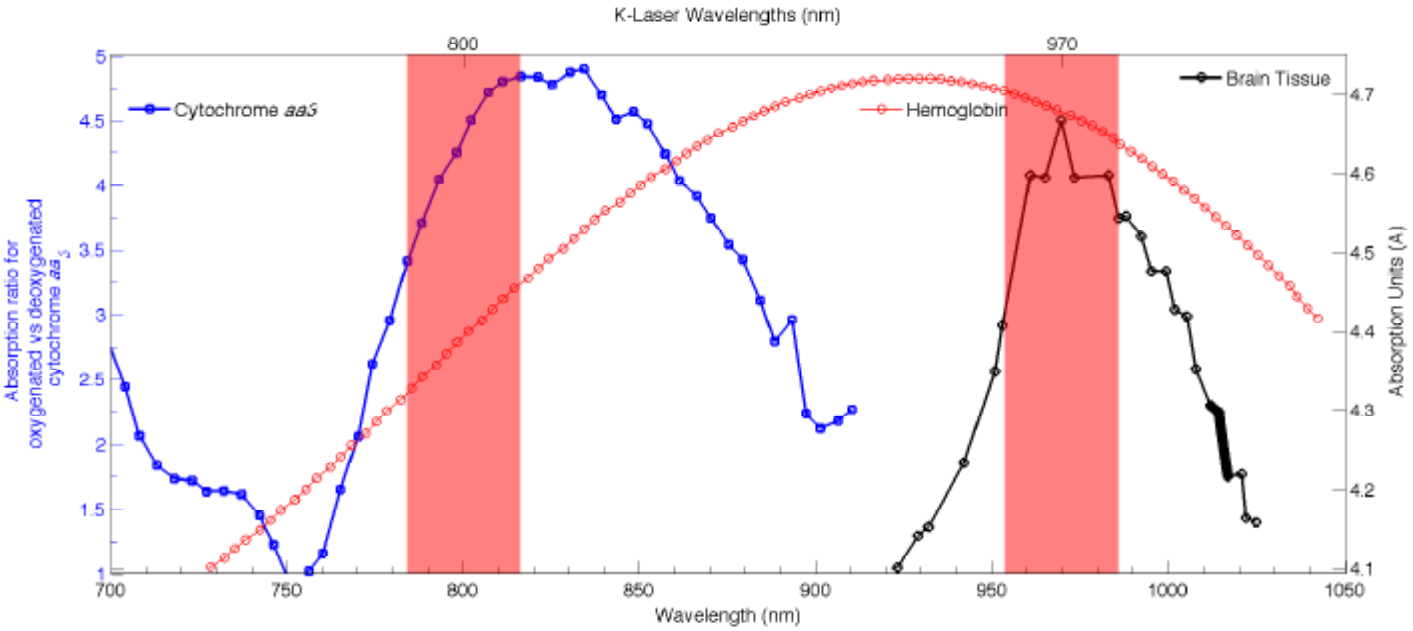

Figure 1: K-Laser's wavelengths coincide with the peaks of stimulated cytochrome reduction (left axis) and tissue absorption (right axis). The width of the bands corresponds to the ± 15 nm Gaussian spread in energy of our beam. Data re-digitized from [5].

Hemoglobin Deoxygenation

Once the increased circulation gets the blood to the cell, the hemoglobin that carry the oxygen in the blood have to drop off their oxygen supply. Oxygenated and deoxygenated hemoglobin have very distinct signatures in the NIR. We are not concerned with the process of re-oxygenating the hemoglobin, because this occurs in the lungs. Instead we are interested in the absorption spectrum of oxygenated hemoglobin (HbO_2) whose deoxygenation can be stimulated by the absorption of a photon of radiation. Figure 1 shows this rather broad peak that covers the higher end of the NIR.

Cytochrome c Oxidase Redox

As discussed earlier, the terminal enzyme in the respiratory chain of a cell, cytochrome c oxidase, is the principle absorber of radiation in the entire cell and governs the rate at which oxygen is processed into ATP. Unlike the one-way deoxygenation of hemoglobin, cytochrome receives and delivers its oxygen in cycles within the cell and so we need to stimulate both processes in order to maximize efficiency. It turns out that laser irradiation does both, depending on the oxidation state of the enzyme. When deoxygenated, laser

irradiation will stimulate oxygenation, and vice versa [3]. This effect has resounding implications and is thought to be the universal validation of laser therapy. The different oxygenation states of this enzyme have peaks throughout the visible-NIR spectrum, which is why virtually all wavelengths used have shown to be useful.

Laser phototherapy with wavelengths throughout the NIR spectrum enhances cellular metabolism, but there exists a peak in the absorption spectrum that can maximize this effect. Figure 1 shows the difference spectrum in the absorption of oxygenated vs. deoxygenated cytochrome. Remember, when the enzyme is either fully oxygenated or fully deoxygenated, irradiation will push the cycle along in the right direction, so we want to stimulate the process at both endpoints. The peak in the difference spectrum reflects the wavelength at which laser irradiation will have the greatest effect to change the oxygenation state, which will subsequently turn the wheels on the cellular metabolism most efficiently. This is analogous to firing the spark plugs at the exact time in the engine cycle to get the maximum effect.

Literature Review

A study by Bornstein et al [1] found that laser light at 870nm and 930nm significantly potentiated the effects of erythromycin, tetracycline and ciprofloxacin on the bacterial pathogens MRSA and *E. coli*. Another study by Bornstein et al [2] reported inactivation of *Staphlococcus aureus*, *E. coli*, *Candida albicans*, and *Trichophyton rubric*. They postulate that they achieved this by causing an optically mediated mechano-transduction of cellular redox pathways essentially stopping the organisms energy production without causing thermal damage to porcine tissue, nares, or to nail matrix tissue. A clinical study led by Adam Landsman et al [4] found that 85% of the treated toenails were improved by clear, linear nail growth at 180 days. For the more severe infection and dystrophy, the success rate dropped to approximately 64%; 30% of cultures were found to be unchanged at 180 days.

In an unpublished *in vitro* study by Larry Jensen at Midwestern University, 6 minute exposure to K-Laser's dual 800 and 970 nm beam at 8 Watts (for a total of 2880 Joules) caused complete toxicity in cultures of C.Albicans, T Rubrum and attenuation of P. Aeruginosa.

The clinical protocol of Bruce Weber (InMotion Foot and Ankle Specialists) consisted of weekly visits (3 treatments per visit) of 10 Watt, continuous wave (CW) exposure for 4 minute on great toes and 2 minutes for each of the lesser toes per treatment. They treated 35 patients with an average of 4 infected toenails per patient; these patients had moderate disease consisting of moderate thickening of nail plate in excess of 2 mm but not exceeding 5mm, discolored to the midportion of the nail with lifting of the nail from the nail bed to approximately the same level. At 180 days (6 months) 69% of the nail plates were totally cleared (i.e. the nail plate was clear, had adhered to the nail bed, and returned to normal thickness); 15% had marginal improvement of the nail appearance but it was noted that there was a decrease in subungual debris; and the remaining 16% of nails treated had varying degrees of improvement.

In a second group of 10 patients who received weekly treatment with a minimum 2800 Joules to the nail surface, we found full clearance of 77% of nails treated after 180 days; the classification of disease severity and induced clearance was the same. Additionally we had the patients treat their shoes with Tineacide shoe spray and topical application of Tineacide.

***In vivo* Penetration and Dose**

Power density is the only necessary intensity parameter for *in vitro* experimentation because there is no attenuation due to a monolayer of cells. From power density measurements, calculating the energy density (i.e. dose) is straightforward: power density in units of Watts/cm^2 multiplied by treatment time in seconds yields dose in units of Joules/cm^2. This is the energy deposited per area of irradiated tissue. *In vivo*, however, this parameter does not tell the whole story. Tissue is a highly scattering medium and there is non-trivial attenuation at depths in the human body. The power density simply refers to the intensity (number of photons) at the output of the laser. This intensity decays exponentially with depth in tissue, and the decay constant (related to the penetration depth) is determined by the wavelength of the laser and the optical properties of the tissue. Furthermore, radiation will scatter laterally (radially, since the beam is cylindrical) and so there will be dose deposited beyond the spot size of the laser.

Again, these are complicated phenomena that have to be modelled and measured to give an accurate description of the dose deposition of any laser beam to be used *in vivo*. Comparing lasers to each other must therefore include more than just power density analysis. Figure 2 is an example of such analysis. From these profiles and a detailed analysis of the optical properties of the different types of tissue, we can calculate the necessary treatment distances and times for therapeutic regimens.

Sample Calculation

If you wish to deliver 2000 Joules to an achilles tendonitis ailment at a depth of 1 inch (~2.5 cm), for example, you would think to take a 500 milliWatt Class III laser and treat for 4000 seconds (the most powerful Class III laser is only 500 milliWatt = 0.5 Joule/sec x 4000 sec = 2000 Joule). This is still a long time (over an hour) to deliver the necessary dose even to the surface of a toenail, for example. Since there is beam attenuation in tissue, though, you would only actually be delivering 1016 Joules to the affected area (0.5 Watt at surface x 50.8% intensity at 2.5 cm [from Figure 2 along the central beam axis] x 4000 seconds). In fact, you would have to irradiate for 7874 seconds (over 2 hours!!) to build up enough dose. If instead you used a 12 Watt laser, you could achieve 2000 Joules at 2.5 cm in 328 seconds (only about 5 minutes).

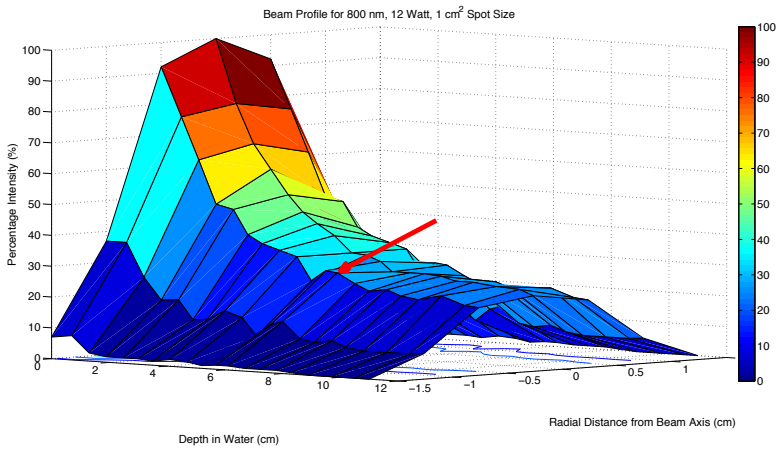

Figure 2: Example of an actual *measured* 3-D dosimetric beam profile. For instance, a point (red arrow) that is 6.1 cm deep in water and 0.5 cm from the central beam axis will be exposed to radiation whose intensity is 29% of the full intensity at the surface of the skin. This type of information is crucial to determining the dose delivered to tissue at a distance inside the body and is exclusive to K-Laser.

There is much more to consider when comparing lasers or predicting success of treatments than either a simple power output or surface energy value since all contributing factors lead to severe attenuation of the beam. If only superficial dermatology concerns you, than less intricate and less expensive Class II or III lasers may be suitable for you. But for any subcutaneous, and especially deep muscle or joint ailments, if you wish to achieve any analgesic or biostimulatory effects whatsoever, these lower power lasers simply cannot deliver sufficient dose at depths in the body in reasonable treatment times.

Understanding K-Laser's Success

The K-Laser has one wavelength (970 nm) that coincides with a peak in water absorption; again the cell is 80% water and so this will have the effect of most efficiently creating temperature gradients that will increase local blood flow and therefore oxygen flow. This wavelength, along with the other at 800 nm, lies within the broad peak in oxygenated hemoglobin absorption; this means once the blood gets to the cells, K-Laser irradiation will most efficiently stimulate the passing of oxygen from the hemoglobin into the cells for use in metabolism. Finally, the 800 nm beam lies at the peak in the cytochrome c oxidase redox cycle; once the oxygen is in the cell, K-Laser irradiation will most efficiently stimulate the cyclic process of using and replenishing oxygen, thereby maximizing the ATP (energy) throughput of the cell. Remember, the name of the game is oxygen: getting into the cell, getting the cell to use it faster to make more energy, and then letting the cell's natural processes boost the body's immune system. This will result in curative and analgesic effects upon every administration of treatment as well as continued relief in the future.

The power output ranging from 0.1-12 Watts and the beam size tunable from $1-5\,\mathrm{cm}^2$ provides fully adjustable power density output through the range of $20-12{,}000\,\mathrm{mW/cm}^2$. This combined with a frequency modulation potential of 1 - 20,000 Hz (along with the continuous wave (CW) capability) provides complete coverage of the therapeutic region. This fundamental optimization of absorption mechanisms combined with the power density and frequency modulation capabilities explains the clinical success that has been found with K-Laser irradiation.

Take Home Message

FACT: Laser phototherapy, if administered by someone trained in the art, is beneficial in almost all of its forms and has no adverse side effects.

The differences between commercially available laser units lie solely in the wavelength, power density, pulse modulation, and aesthetics. From these parameters, you can derive the penetration depth, dose distribution, treatment time, and the estimated biological effect. There is *NOT* a "magic" wavelength or setting that is the cure for a disease, and to claim otherwise (as many distributors or salesmen do) is irresponsible. There are, however, certain operating regimes that give better results than others and are more effective for particular symptoms. The select few modalities that have been specifically designed to isolate and capitalize on a fundamental therapeutic mechanism, have continually proved successful in the clinic. And since the primary mechanism of action is the stimulation of the body's natural anti-pathological immune system, the range of symptoms for which this treatment modality is useful knows no bound.

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Mechanisms of Low Level Light Therapy.

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ABSTRACT

The use of low levels of visible or near infrared light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing tissue damage has been known for almost forty years since the invention of lasers. Originally thought to be a peculiar property of laser light (soft or cold lasers), the subject has now broadened to include photobiomodulation and photobiostimulation using non-coherent light. Despite many reports of positive findings from experiments conducted in vitro, in animal models and in randomized controlled clinical trials, LLLT remains controversial. This likely is due to two main reasons; firstly the biochemical mechanisms underlying the positive effects are incompletely understood, and secondly the complexity of rationally choosing amongst a large number of illumination parameters such as wavelength, fluence, power density, pulse structure and treatment timing has led to the publication of a number of negative studies as well as many positive ones. In particular a biphasic dose response has been frequently observed where low levels of light have a much better effect than higher levels. This introductory review will cover some of the proposed cellular chromophores responsible for the effect of visible light on mammalian cells, including cytochrome c oxidase (with absorption peaks in the near infrared) and photoactive porphyrins. Mitochondria are thought to be a likely site for the initial effects of light, leading to increased ATP production, modulation of reactive oxygen species and induction of transcription factors. These effects in turn lead to increased cell proliferation and migration (particularly by fibroblasts), modulation in levels of cytokines, growth factors and inflammatory mediators, and increased tissue oxygenation. The results of these biochemical and cellular changes in animals and patients include such benefits as increased healing in chronic wounds, improvements in sports injuries and carpal tunnel syndrome, pain reduction in arthritis and neuropathies, and amelioration of damage after heart attacks, stroke, nerve injury and retinal toxicity.

Keywords: biostimulation, low level laser therapy, wound healing, biomodulation, cold laser, action spectra

1. HISTORY

In 1967 a few years after the first working laser was invented, Endre Mester in Semmelweis University, Budapest, Hungary wanted to test if laser radiation might cause cancer in mice [1]. He shaved the dorsal hair, divided them into two groups and gave a laser treatment with a low powered ruby laser (694-nm) to one group. They did not get cancer and to his surprise the hair on the treated group grew back more quickly than the untreated group. This was the first demonstration of "laser biostimulation". Since then, medical treatment with coherent-light sources (lasers) or noncoherent light (light-emitting diodes, LEDs) has passed through its childhood and adolescence. Currently, low-level laser (or light) therapy (LLLT), also known as “cold laser”, “soft laser”, “biostimulation” or “photobiomodulation” is practiced as part of physical therapy in many parts of the world. In fact, light therapy is one of the oldest therapeutic methods used by humans (historically as solar therapy by Egyptians, later as UV therapy for which Nils Finsen won the Nobel prize in 1904 [2]). The use of lasers and LEDs as light sources was the next step in the technological development of light therapy, which is now applied to many thousands of people worldwide each day. In LLLT the question is no longer whether light has biological effects but rather how energy from therapeutic lasers and LEDs works at the cellular and organism levels and what are the optimal light parameters for different uses of these light sources.

Mechanisms for Low-Light Therapy, edited by Michael R. Hamblin, Ronald W. Waynant, Juanita Anders,
Proc. of SPIE Vol. 6140, 614001, (2006) · 1605-7422/06/$15 · doi: 10.1117/12.646294

One important point that has been demonstrated by multiple studies in cell culture [3], animal models [4] and in clinical studies is the concept of a biphasic dose response when the outcome is compared with the total delivered light energy density (fluence). The reason why the technique is termed **LOW**-level is that there exists an optimal dose of light for any particular application, and doses lower than this optimum value, or more significantly, **larger** than the optimum value will have a diminished therapeutic outcome, or for high doses of light a negative outcome may even result.

There are perhaps three main areas of medicine and veterinary practice where LLT has a major role to play (Figure 1). These are (i) wound healing, tissue repair and prevention of tissue death; (ii) relief of inflammation in chronic diseases and injuries with its associated pain and edema; (iii) relief of neurogenic pain and some neurological problems. The proposed pathways to explain the mechanisms of LLLT should ideally be applicable to all these conditions.

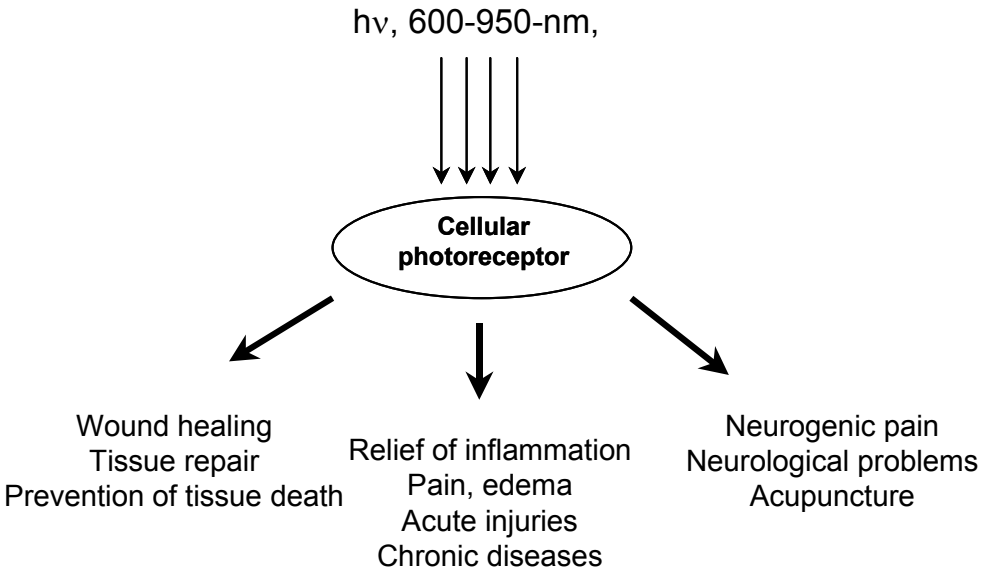

Figure 1. Schematic representation of the main areas of application of LLLT

2. BIOCHEMICAL MECHANISMS

2.1. Tissue photobiology

The first law of photobiology states that for low power visible light to have any effect on a living biological system, the photons must be absorbed by electronic absorption bands belonging to some molecular chromophore or photoacceptor [5]. One approach to finding the identity of this chromophore is to carry out action spectra. This is a graph representing biological photoresponse as a function of wavelength, wave number, frequency, or photon energy and should resemble the absorption spectrum of the photoacceptor molecule. The fact that a structured action spectrum can be constructed supports the hypothesis of the existence of cellular photoacceptors and signaling pathways stimulated by light.

The second important consideration involves the optical properties of tissue. Both the absorption and scattering of light in tissue are wavelength dependent (both much higher in the blue region of the spectrum than the red) and the principle tissue chromophore (hemoglobin) has high absorption bands at wavelengths shorter than 600-nm. For these reasons there is a so-called “optical window” The second important consideration involves the optical properties of tissue. Both the absorption and scattering of light in tissue are wavelength dependent (both much higher in the blue region of the spectrum than the red) and the principle tissue chromophores (hemoglobin and

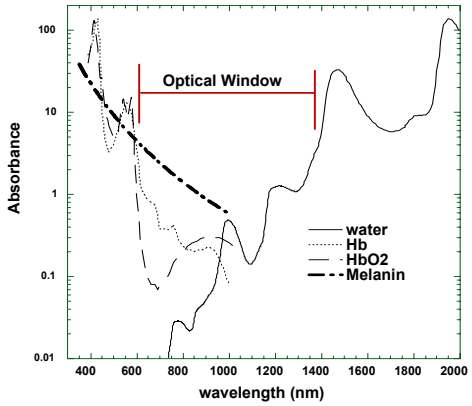

Figure 2. Optical window in tissue due to reduced absorption of red and near-infra-red wavelengths (600-1200 nm) by tissue chromophores

melanin) have high absorption bands at wavelengths shorter than 600-nm. Water begins to absorb significantly at wavelengths greater than 1150-nm. For these reasons there is a so-called "optical window" in tissue covering the red and near-infrared wavelengths, where the effective tissue penetration of light is maximized (Figure 2). Therefore although blue, green and yellow light may have significant effects on cells growing in optically transparent culture medium, the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-950-nm).

2.2 Action spectra

It was suggested in 1989 that the mechanism of LLLT at the cellular level was based on the absorption of monochromatic visible and NIR radiation by components of the cellular respiratory chain [6]. The inner mitochondrial membrane contains 5 complexes of integral membrane proteins: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome c oxidase (Complex IV), ATP synthase (Complex V) and two freely diffusible molecules ubiquinone and cytochrome c that shuttle electrons

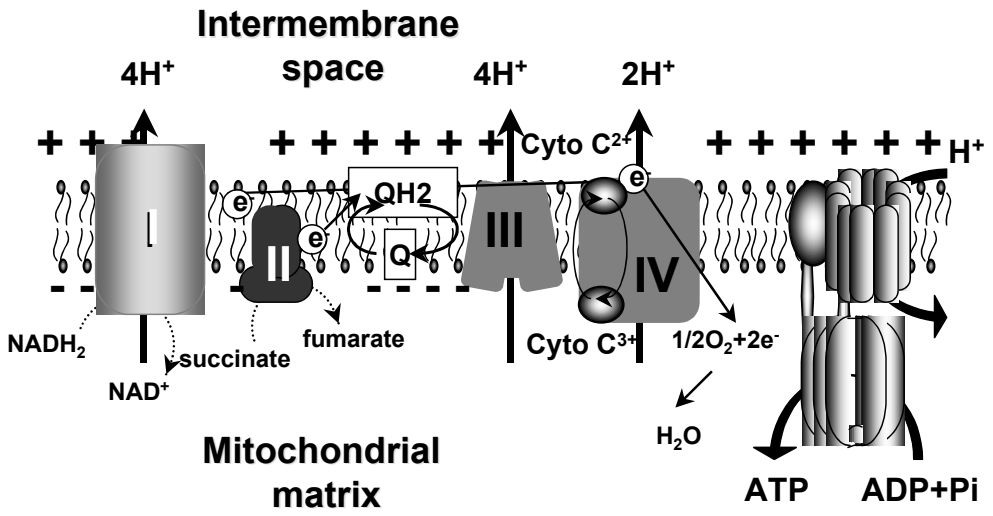

Figure 3. Structure of the mitochondrial respiratory chain

from one complex to the next (Figure 3). The respiratory chain accomplishes the stepwise transfer of electrons from NADH and $FADH_2$ (produced in the citric acid or Krebs cycle) to oxygen molecules to form (with the aid of

protons) water molecules harnessing the energy released by this transfer to the pumping of protons (H^+) from the matrix to the intermembrane space. The gradient of protons formed across the inner membrane by this process of active transport forms a miniature battery. The protons can flow back down this gradient, reentering the matrix, only through another complex of integral proteins in the inner membrane, the ATP synthase complex.

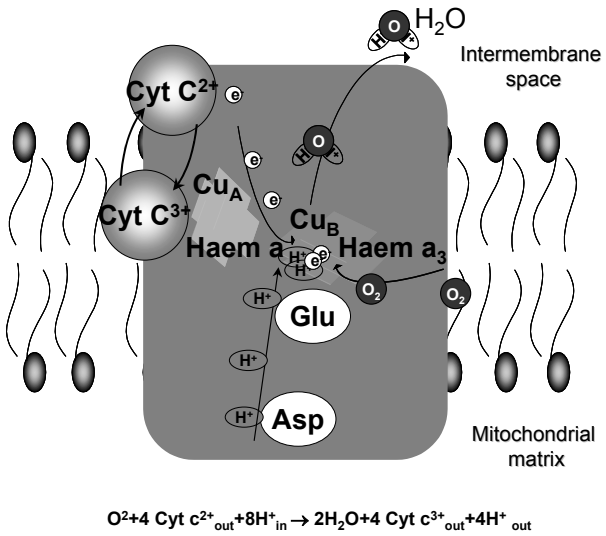

Figure 4. Structure and mode of action of cytochrome c oxidase

Absorption spectra obtained for cytochrome c oxidase in different oxidation states were recorded and found to be very similar to the action spectra for biological responses to light. Therefore it was proposed that cytochrome c oxidase is the primary photoacceptor for the red-NIR range in mammalian cells [7] (Figure 4). Cytochrome C oxidase contains two iron centers, haem *a* and haem a_3 (also referred to as cytochromes *a* and a_3), and two copper centers, Cu_A and Cu_B [8]. Fully oxidized cytochrome c oxidase has both iron atoms in the Fe(III) oxidation state and both copper atoms in the Cu(II) oxidation state, while fully reduced cytochrome c oxidase has the iron in Fe(II) and copper in Cu(I) oxidation states. There are many intermediate mixed-valence forms of the enzyme and other coordinate ligands such as CO, CN, and formate can be involved. All the many individual oxidation states of the enzyme have different absorption spectra [9], thus probably accounting for slight differences in action spectra of LLLT that have been reported. A recent paper from Karu's group [10] gave the following wavelength ranges for four peaks in the LLLT action spectrum: 1) 613.5 - 623.5 nm, 2) 667.5 - 683.7 nm, 3) 750.7 - 772.3 nm, 4) 812.5 - 846.0 nm.

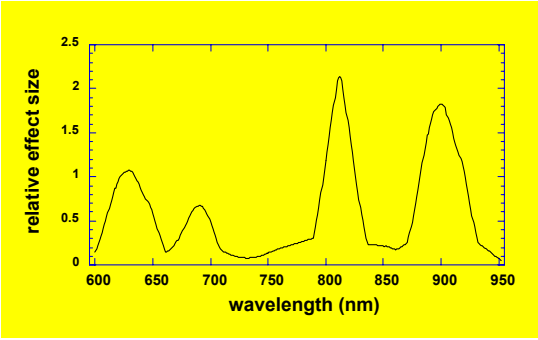

Figure 5. Generalized action spectrum for LLLT effects in cells, animals and patients. Data shown are an amalgamation of many literature reports from multiple laboratories.

A study from Pastore et al [11] examined the effect of He-Ne laser illumination on the purified cytochrome c oxidase enzyme and found increased oxidation of cytochrome c and increased electron transfer. Artyukhov and colleagues found [12] increased enzyme activity of catalase after He-Ne illumination.

Absorption of photons by molecules leads to electronically excited states and consequently can lead to acceleration of electron transfer reactions [13]. More electron transport necessarily leads to increased production of ATP [14]. Light induced increase in ATP synthesis and increased proton gradient leads to an increasing activity of the Na^+/H^+ and Ca^{2+}/Na^+ antiporters and of all the ATP driven carriers for ions, such as Na^+/K^+ ATPase and Ca^{2+} pumps. ATP is the substrate for adenyl cyclase, and therefore the ATP level controls the level of cAMP. Both Ca^{2+} and cAMP are very important second messengers. Ca^{2+} especially regulates almost every process in the human body (muscle contraction, blood coagulation, signal transfer in nerves, gene expression, etc.).

In addition to cytochrome c oxidase mediated increase in ATP production, other mechanisms may be operating in LLLT. The first of these we will consider is the "singlet-oxygen hypothesis." Certain molecules with visible absorption bands like porphyrins lacking transition metal coordination centers [15] and some flavoproteins [16] can be converted into a long-lived triplet state after photon absorption. This triplet state can interact with ground-state oxygen with energy transfer leading to production of a reactive species, singlet oxygen. This is the same molecule utilized in photodynamic therapy (PDT) to kill cancer cells, destroy blood vessels and kill microbes. Researchers in PDT have known for a long time that very low doses of PDT can cause cell proliferation and tissue stimulation instead of the killing observed at high doses [17].

The next mechanism proposed was the "redox properties alteration hypothesis" [18]. Alteration of mitochondrial metabolism and activation of the respiratory chain by illumination would also increase production of superoxide anions $O_2^{\bullet-}$. It has been shown that the total cellular production of $O_2^{\bullet-}$ depends primarily on the metabolic state of the mitochondria. Other redox chains in cells can also be activated by LLLT. NADPH-oxidase is an enzyme found on activated neutrophils and is capable of a non-mitochondrial respiratory burst and production of high amounts of ROS can be induced. [19]. These effects depend on the physiological status of the host organism as well as on radiation parameters.

The activity of cytochrome c oxidase is inhibited by nitric oxide (NO). This inhibition of mitochondrial respiration by NO can be explained by a direct competition between NO and O_2 for the reduced binuclear center CuB/a3 of cytochrome c oxidase and is reversible [20]. It was proposed that laser irradiation could reverse the inhibition of cytochrome c oxidase by NO and thus may increase the respiration rate ("NO hypothesis") [21]. Data published recently by Karu et al [21] indirectly support this hypothesis. Another piece of evidence for NO involvement in responses to LLLT is an increase in inducible nitric oxide synthase production after exposure to light (635 nm) [22]. While both observations support the hypothesis of NO dependent responses to LLLT, responses to different wavelengths of light in different models may be governed by distinct mechanisms.

2.3 Cell signaling

The combination of the products of the reduction potential and reducing capacity of the linked redox couples present in cells and tissues represent the redox environment (redox state) of the cell. Redox couples present in the cell include: nicotinamide adenine dinucleotide (oxidized/ reduced forms) NAD/NADH, nicotinamide adenine dinucleotide phosphate NADP/NADPH, glutathione/glutathione disulfide couple GSH/GSSG and thioredoxin/ thioredoxin disulfide couple Trx(SH)2/TrxSS [23]. Several important regulation pathways are mediated through the cellular redox state. Changes in redox state induce the activation of numerous intracellular signaling pathways, regulate nucleic acid synthesis, protein synthesis, enzyme activation and cell cycle progression [24]. These cytosolic responses in turn induce transcriptional changes. Several transcription factors are regulated by changes in cellular redox state. Among them redox factor –1 (Ref-1)- dependent activator protein-1 (AP-1) (Fos and Jun), nuclear factor κB (NF-κB), p53, activating transcription factor/cAMP-response element–binding protein (ATF/ CREB), hypoxia-inducible factor (HIF)-1α, and HIF-like factor. As a rule, the oxidized form of redox-dependent transcription factors have low DNA-binding activity. Ref-1 is an important factor for the specific reduction of these transcription factors. However it was also shown that low levels of oxidants appear to stimulate proliferation and differentiation of some type of cells [25-27]

It is proposed that LLLT produces a shift in overall cell redox potential in the direction of greater oxidation [28]. Different cells at a range of growth conditions have distinct redox states. Therefore, the effects of LLLT can

vary considerably. Cells being initially at a more reduced state (low intracellular pH) have high potential to respond to LLLT, while cells at the optimal redox state respond weakly or do not respond to treatment with light.

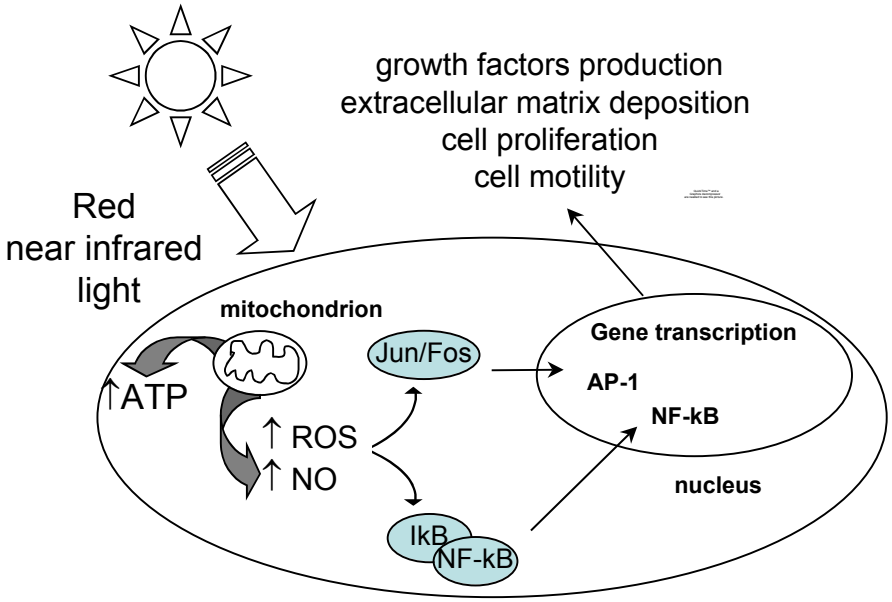

Figure 6. Cell signaling pathways induced by LLLT.

3. IN VITRO RESULTS

3.1 Cell types

There is evidence that multiple mammalian and microbial cell types can respond to LLLT. Much of Karu's work has used *Escherichia coli* (a Gram-negative aerobic bacterium) [29] and HeLa cells [30], a human cervical carcinoma cell line. However for the clinical applications of LLLT to be validated it is much more important to study the effects of LLLT on non-malignant cell types likely to be usefully stimulated in order to remedy some disease or injury. For wound healing type studies, these cells are likely to be endothelial cells [31], fibroblasts [32], keratinocytes [33] and possibly some classes of leukocytes such as macrophages [34] and neutrophils [35]. For pain relief and nerve regrowth studies these cells will be neurons [36-38] and glial cells [39]. For anti-inflammatory and anti-edema applications the cell types will be macrophages [34], mast-cells [40], neutrophils [41], lymphocytes [42] etc. There is literature evidence for in vitro LLLT effects for most of these cell types.

3.2. Isolated mitochondria

Since the respiratory chain and cytochrome c oxidase are located in mitochondria, several groups have tested the effect of LLLT on preparations of isolated mitochondria. The most popular system to study is the effects of HeNe laser illumination of mitochondria isolated from rat liver. Increased proton electrochemical potential and ATP synthesis was found [43]. Increased RNA and protein synthesis was demonstrated after 5 J/cm^2 [44]. Pastore et al [45] found increased activity of cytochrome c oxidase and an increase in polarographically measured oxygen uptake after 2 J/cm^2 of HeNe. A major stimulation in the proton pumping activity, about 55% increase of <--H+/e- ratio was found in illuminated mitochondria. Yu et al [13] used 660 nm laser at a power density of 10 mW/cm^2 and showed increased oxygen consumption (0.6 J/cm^2 and 1.2 J/cm^2), increased phosphate potential, and energy charge (1.8 J/cm^2 and 2.4 J/cm^2) and enhanced activities of NADH: ubiquinone oxidoreductase, ubiquinol: ferricytochrome C oxidoreductase and ferrocytochrome C: oxygen oxidoreductase (between 0.6 J/cm^2, and 4.8 J/cm^2).

3.3 LLLT cellular response

The cellular responses observed in vitro after LLLT can be broadly classed under increases in metabolism, migration, proliferation, and increases in synthesis and secretion of various proteins. Many studies report effects on more than one of these parameters. Yu et al reported [33] on cultured keratinocytes and fibroblasts that were irradiated with 0.5-1.5 J/cm^2 HeNe laser. They found a significant increase in basic fibroblast growth factor (bFGF) release from both keratinocytes and fibroblasts and a significant increase in nerve growth factor release from keratinocytes. Medium from HeNe laser irradiated keratinocytes stimulated [3H]thymidine uptake and proliferation of cultured melanocytes. Furthermore, melanocyte migration was enhanced either directly by HeNe laser or indirectly by the medium derived from HeNe laser treated keratinocytes.

The presence of cellular responses to LLLT at molecular level was also demonstrated [46]. Normal human fibroblasts were exposed for 3 days to 0.88J/cm^2 of 628 nm light from light emitting diode. Gene expression profiles upon irradiation were examined using a cDNA microarray containing 9982 human genes. 111 genes were found to be affected by light. All genes from antioxidant related category and genes related to energy metabolism and respiratory chain were upregulated. Most of the genes related to cell proliferation were upregulated too. Amongst genes related to apoptosis and stress response, some genes such as JAK binding protein were upregulated, others such as HSP701A, caspase 6 and stress-induced phosphoprotein were downregulated. It was suggested that LLLT stimulates cell growth directly by regulating the expression of specific genes, as well as indirectly by regulating the expression of the genes related to DNA synthesis and repair, and cell metabolism.

4. ANIMAL MODELS

There has been a large number of animal models that have been used to demonstrate LLLT effects on a variety of diseases, injuries, and both chronic and acute conditions, In this review we will therefore only discuss three particular applications for which there are good literature reports of efficacy.

4.1 Wound healing

The literature on LLLT applied to a stimulation of wound healing in a variety of animal models contains both positive and negative studies. The reasons for the conflicting reports, sometimes in very similar wound models, are probably diverse. It is probable that applications of LLLT in animal models will be more effective if carried out on models that have some intrinsic disease state. Although there have been several reports that processes such as wound healing are accelerated by LLLT in normal rodents [3, 34], an alternative approach is to inhibit healing by inducing some specific disease state. This has been done in the case of diabetes, a disease known to significantly depress wound healing in patients. LLLT significantly improves wound healing in both diabetic rats [35, 36] and diabetic mice [37, 38]. LLLT was also effective in X-radiation impaired wound healing in mice [39]. A study [47] in hairless mice found improvement in the tensile strength of the HeNe laser-irradiated wounds at 1 and 2 weeks. Furthermore, the total collagen content was significantly increased at 2 months when compared with control wounds. The beneficial effect of LLLT on wound healing can be explained by considering several basic biological mechanisms including the induction of expression cytokines and growth factors known to be responsible for the many phases of wound healing. Firstly there is a report [48] that HeNe laser increased both protein and mRNA levels of IL-1α and IL-8 in keratinocytes. These are cytokines responsible for the initial inflammatory phase of wound healing. Secondly there are reports [49] that LLLT can upregulate cytokines responsible for fibroblast proliferation and migration such as bFGF, HGF and SCF. Thirdly it has been reported [50] that LLLT can increase growth factors such as VEGF responsible for the neovascularization necessary for wound healing. Fourthly TGF-β is a growth factor responsible for inducing collagen synthesis from fibroblasts and has been reported to be upregulated by LLLT [51]. Fifthly there are reports [52, 53] that LLLT can induce fibroblasts to undergo the transformation into myofibloblasts, a cell type that expresses smooth muscle α-actin and desmin and has the phenotype of contractile cells that hasten wound contraction.

4.2 Neuronal toxicity

Studies from Whelan's group have explored the use of 670-nm LEDs in combating neuronal damage caused by neurotoxins. Methanol intoxication is caused by metabolic conversion to formic acid that produces injury to the retina and optic nerve, resulting in blindness. Using a rat model and the electroretinogram as a sensitive indicator of

retinal function, they demonstrated that three brief 670-nm LED treatments (4 J/cm(2)), delivered at 5, 25, and 50 h of methanol intoxication, attenuated the retinotoxic effects of methanol-derived formate. There was a significant recovery of rod- and cone-mediated function in LED-treated, methanol-intoxicated rats and histopathologic evidence of retinal protection [54]. A subsequent study [55] explored the effects of an irreversible inhibitor of cytochrome c oxidase, potassium cyanide in primary cultured neurons. LED treatment partially restored enzyme activity blocked by 10-100 microM KCN. It significantly reduced neuronal cell death induced by 300 μM KCN from 83.6 to 43.5%. LED significantly restored neuronal ATP content only at 10 microM KCN but not at higher concentrations of KCN tested. In contrast, LED was able to completely reverse the detrimental effect of tetrodotoxin, which only indirectly down-regulated enzyme levels. Among the wavelengths tested (670, 728, 770, 830, and 880 nm), the most effective ones (830 nm, 670 nm) paralleled the NIR absorption spectrum of oxidized cytochrome c oxidase.

4.3 Nerve regeneration

Animal models have been employed to study LLLT effects in nerve repair [56, 57]. Byrnes et al used 1,600 J/cm^2 of 810-nm diode laser to improve healing and functionality in a T9 dorsal hemisection of the spinal cord in rats [39]. Anders et al [58] studied LLLT for regenerating crushed rat facial nerves; by comparing 361, 457, 514, 633, 720, and 1064-nm and found best response with 162.4 J/cm^2 of 633-nm HeNe laser.

5. CLINICAL STUDIES

Low-power laser therapy is used by physical therapists to treat a wide variety of acute and chronic musculoskeletal aches and pains, by dentists to treat inflamed oral tissues and to heal diverse ulcerations, by dermatologists to treat edema, non-healing ulcers, burns, and dermatitis, by orthopedists to relieve pain and treat chronic inflammations and autoimmune diseases, and by other specialists, as well as general practitioners. Laser therapy is also widely used in veterinary medicine (especially in racehorse-training centers) and in sports-medicine and rehabilitation clinics (to reduce swelling and hematoma, relieve pain, improve mobility, and treat acute soft-tissue injuries). Lasers and LEDs are applied directly to the respective areas (e.g., wounds, sites of injuries) or to various points on the body (acupuncture points, muscle-trigger points). However one of the most important limitations to advancing the field into mainstream medical practice is the lack of appropriately controlled and blinded clinical trials. The trials should be prospective, placebo controlled and double blinded and contain sufficient subjects to allow statistically valid conclusions to be reached.

Clinical applications of low-power laser therapy are diverse. The field is characterized by a variety of methodologies and uses of various light sources (lasers, LEDs) with different parameters (wavelength, output power, continuous-wave or pulsed operation modes, pulse parameters). In recent years, longer wavelengths (~800 to 900 nm) and higher output powers (to 100 mW) have been preferred in therapeutic devices especially to allow deeper tissue penetration. In 2002 MicroLight Corp received 510K FDA clearance for the ML 830-nm diode laser for treatment of carpal tunnel syndrome. There were several controlled trials reporting significant improvement in pain and some improvement in objective outcome measures [59-61]. Since then several light sources have been approved as equivalent to an infrared heating lamp for treating a wide-range of musculoskeletal disorders with no supporting clinical studies.

6. UNRESOLVED QUESTIONS

6.1 Wavelength. This is probably the parameter where there is most agreement in the LLLT community. Wavelengths in the 600-700-nm range are chosen for treating superficial tissue, and wavelengths between 780 and 950 are chosen for deeper-seated tissues due to longer optical penetration distances through tissue. Wavelengths between 700 and 770-nm are not considered to have much activity.

6.2 Laser vs non-coherent light. One of the most topical and widely discussed issues in the LLLT clinical community is whether the coherence and monochromatic nature of laser radiation have additional benefits as compared with more broad band light from a conventional light source or LED with the same center wavelength and intensity. Two aspects of this problem must be distinguished: the coherence of light itself and the coherence of the interaction of light with matter (biomolecules, tissues).

6.3. Dose. Because of the possible existence of a biphasic dose response curve referred to above, choosing the correct dosage of light (in terms of energy density) for any specific medical condition is difficult. In addition there has been some confusion in the literature about the delivered fluence when the light spot is small. If 5J of light is

given to a spot of 5 mm^2 the fluence is 100 J/cm^2 which is nominally the same fluence as 100 J/cm^2 delivered to 10 cm^2, but the total energy delivered in the latter case is 200 time greater.

6.3 Pulsed or CW. There have been some reports that pulse structure is an important factor in LLLT; for instance Ueda et al [62, 63] found better effects using 1 or 2 Hz pulses than 8 Hz or CW 830-nm laser on rat bone cells, but the underlying mechanism for this effect is unclear..

6.4 Polarization status. There are some claims that polarized light has better effects in LLLT applications than otherwise identical non-polarized light (or even 90-degree rotated polarized light) [64]. However it is known that polarized light is rapidly scrambled in highly scattering media such as tissue (probably in the first few hundred μm), and it therefore seem highly unlikely that polarization could play a role except for superficial applications to the upper layers of the skin.

6.5. ***Systemic effects.*** Although LLLT is mostly applied to localized diseases and its effect is often considered to be restricted to irradiated area, there are reports of systemic effects of LLLT acting at a site distant from the illumination [65, 66].

ACKNOWLEDGEMENTS

M. R. Hamblin was supported by US National Institutes of Health (R01CA/AI838801 and R01 AI050875) T. N Demidova was supported by a Wellman Center Graduate Student Fellowship. We are grateful to R Rox Anderson for support.

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Lasers in Surgery and Medicine 41:271–276 (2009)

Effect of Low Level Laser Therapy (830 nm) With Different Therapy Regimes on the Process of Tissue Repair in Partial Lesion Calcaneous Tendon

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Background and Objective: Calcaneous tendon is one of the most damaged tendons, and its healing may last from weeks to months to be completed. In the search after speeding tendon repair, low intensity laser therapy has shown favorable effect. To assess the effect of low intensity laser therapy on the process of tissue repair in calcaneous tendon after undergoing a partial lesion.

Study Design/Materials and Methods: Experimentally controlled randomized single blind study. Sixty male rats were used randomly and were assigned to five groups containing 12 animals each one; 42 out of 60 underwent lesion caused by dropping a 186 g weight over their Achilles tendon from a 20 cm height. In Group 1 (standard control), animals did not suffer the lesion nor underwent laser therapy; in Group 2 (control), animals suffered the lesion but did not undergo laser therapy; in Groups 3, 4, and 5, animals suffered lesion and underwent laser therapy for 3, 5, and 7 days, respectively. Animals which suffered lesion were sacrificed on the 8th day after the lesion and assessed by polarization microscopy to analyze the degree of collagen fibers organization.

Results: Both experimental and standard control Groups presented significant values when compared with the control Groups, and there was no significant difference when Groups 1 and 4 were compared; the same occurred between Groups 3 and 5.

Conclusion: Low intensity laser therapy was effective in the improvement of collagen fibers organization of the calcaneous tendon after undergoing a partial lesion. Lasers Surg. Med. 41:271–276, 2009. © 2009 Wiley-Liss, Inc.

Key words: calcaneous tendon; diode laser; lesion tendon; low level laser therapy; physical therapy; repair tissue

INTRODUCTION

The calcaneous tendon is one of the most frequently injured tendons in human beings, followed by digital flexors, due to overuse, trauma caused by firearm wounds, and sharp objects [1].

Owing to the slow pace of healing, the rupture of the calcaneous tendon is considered a serious injury, and it has drawn the attention of several researchers [2].

Spontaneous rupture of the calcaneous tendon occurs between 2 and 6 cm of its insertion into the calcaneous bone. Histological examination has suggested that such tendons had already undergone primary degeneration [3] and showed important alterations in the type of collagen fibers [4].

In order to observe blood supply to the calcaneous tendon, CARR & NORRIS (1989) [5] verified that the number of blood vessels varies along the length of the tendon and their highest concentration occurs in the calcaneous insertion and up to 4 cm above it, considering that neoangiogenesis is a vital part of the healing process, as it restores normal circulation and carries more cells and nutrients to the injured location, thus limiting ischemic necrosis and allowing tissue repair [6].

Due to its low blood supply, the calcaneous tendon is a structure that can take weeks or even months to heal completely [2,7].

During the period of the lesion, it is customary for the patient to remain immobilized in order to prevent a new rupture, which could generate countless functional complications, including ultra-structural and biomechanical alterations in the tendon [8,9].

Such complications, caused by prolonged immobilization, can be minimized by shortening the duration of the tendon repair [3].

Trying to accelerate tendon repair, several physical agents such as ultrasound [10], electrical stimulation [11], and low level laser therapy [12] have shown beneficial effects.

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Accepted 10 February 2009
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20760

Lasers in Surgery and Medicine 42:559–565 (2010)

Collagen Changes and Realignment Induced by Low-Level Laser Therapy and Low-Intensity Ultrasound in the Calcaneal Tendon

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Background and Objective: The treatment of calcaneal tendon injuries requires long-term rehabilitation. Ultrasound (US) and low-level laser therapy (LLLT) are the most used and studied physical agents in the treatment of tendon injuries; however, only a few studies examined the effects of the combination of US and LLLT. Therefore, the purpose of this study was to investigate which treatment (the exclusive or combined use of US and LLLT) most effectively contribute to tendon healing.
Study Design/Materials and Methods: This was a controlled laboratory study with 50 rats whose Achilles tendon was injured by direct trauma. The rats were randomly divided into five groups and treated for 5 consecutive days, as follows: group 1 (control) received no treatment; group 2 was treated with US alone; group 3 was treated with LLLT alone; group 4 was treated first with US followed by LLLT; and group 5 was treated first with LLLT followed by US. On the sixth post-injury day, the tendons were removed and examined by polarized light microscopy. The organization of collagen fibers was assessed by birefringence measurements. Picrosirius-stained sections were examined for the presence of types I and III collagen.
Results: There was a significantly higher organization of collagen fibers in group 2 (US) than in the control group ($P = 0.03$). The amount of type I collagen found in groups 2 (US), 3 (LLLT), and 5 (LLLT+US) was significantly higher than that in the control group ($P \leq 0.01$), but no significant differences were found between treatment groups. There were no differences in the amount of type III collagen between groups.
Conclusion: Ultrasound, LLLT, and the combined use of LLLT and US resulted in greater synthesis of type I collagen; US was also effective in increasing collagen organization in the early stages of the healing process. Lasers Surg. Med. 42:559–565, 2010. © 2010 Wiley-Liss, Inc.

Key words: achilles tendon; birefringence; diode laser; physical therapy; picrosirius red; tendon injuries; ultrasonography

INTRODUCTION

The Achilles (calcaneal) tendon is one of the most commonly injured tendons in the human body. The primary healing of the tendon is believed to take at least 6 weeks [1–3].

The rupture of the Achilles tendon usually occurs 3–6 cm above its insertion into the calcaneus [4]. This may be explained by repetitive strain and poor blood supply to this region of the tendon [2,4]. Tendon healing requires long-term rehabilitation, but the use of immobilization cast may predispose the tendon to several complications [1,3,5,6].

Some studies suggest that the spontaneous rupture of the calcaneal tendon may occur due to previous degeneration [7,8]. The most common cellular changes prior to the spontaneous rupture of a tendon are: an increase in the amount of type III collagen (thin fibers), reduction of type I collagen (thick fibers), and decrease in collagen aggregation [2,7,8].

Recent studies have shown that physical agents, such as low-intensity ultrasound (US) [9,10] electrical stimulation [11], electromagnetic fields [12], and low-level laser therapy (LLLT) [13,14] my speed wound healing.

Therapeutic US has been shown to be beneficial in reducing edema, improving cellular metabolism, and increasing the tensile strength of the tendon. It also promotes synthesis of type I and type III collagen, and better alignment and organization of collagen fibers, accelerating tendon healing [6,9,15,16]. Histological and

Authorship Standards: Each person listed as an author has participated in the study to a significant extent. The authors accept full responsibility for the design and conduct of the study, had access to the data, and controlled the decision to publish.
The authors declare no conflict of interest or competing financial interests with regard to the manuscript entitled "Collagen changes and realignment induced by low-level laser therapy and low-intensity ultrasound in calcaneal tendon healing." We certify that we have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, and honoraria).

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Accepted 13 April 2010
Published online 15 July 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20932

Lasers in Surgery and Medicine 28:216–219 (2001)

Effects of Laser Irradiation on the Spinal Cord for the Regeneration of Crushed Peripheral Nerve in Rats

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Background and Objective: The purpose of the present study was to examine the recovery of the crushed sciatic nerve of rats after low-power laser irradiation applied to the corresponding segments of the spinal cord.

Study Design/Materials and Methods: After a crush injury to the sciatic nerve in rats, low-power laser irradiation was applied transcutaneously to corresponding segments of the spinal cord immediately after closing the wound by using 16 mW, 632 nm He-Ne laser. The laser treatment was repeated 30 minutes daily for 21 consecutive days.

Results: The electrophysiologic activity of the injured nerves (compound muscle action potentials—CMAPs) was found to be approximately 90% of the normal precrush value and remained so for up to a long period of time. In the control nonirradiated group, electrophysiologic activity dropped to 20% of the normal precrush value at day 21 and showed the first signs of slow recovery 30 days after surgery. The two groups were found to be significantly different during follow-up period ($P < 0.001$).

Conclusion: This study suggests that low-power laser irradiation applied directly to the spinal cord can improve recovery of the corresponding insured peripheral nerve. Lasers Surg. Med. 28:216–219, 2001.

Key words: peripheral nerve injury; compound muscle action potentials; low-power laser; spinal cord irradiation; rats

INTRODUCTION

Treatment of injuries to peripheral nerves has always constituted an important medical problem, and, although recovery does eventually occur in most cases, it is a very slow and frequently incomplete process [1]. Peripheral nerves are highly vulnerable to pressure. The amount of damage done depends on the specific nerve involved, the magnitude and type of pressure and the length of time the nerve is compressed. If the amount and duration of compression are slight, most nerves will recover either immediately or shortly after trauma. But if the pressure is intense and/or the duration is long, recovery is prolonged and often partial. One of the causes of nerve compression is the crush injury. The usual results after such an injury are degeneration of the axons and retrograde degeneration of the corresponding neurons of the spinal cord, followed by a very slow regeneration. Understandably, therefore, numerous attempts have been made to enhance and/or accelerate the recovery of injured peripheral nerves. One of the methods studied is the use of low-power laser irradiation to enhance the recovery of peripheral nerve injuries. The use of low-power laser irradiation in the treatment of experimental peripheral nerve injuries was reported by Rochkind in 1978 [2]. More recent publications describe the effect of low-power laser irradiation applied directly or transcutaneously to the crushed peripheral nerve alone [3–7] or to the crushed nerve and the corresponding segments of the spinal cord [8]. The results showed that low-power laser irradiation increases the recovery of the crushed sciatic nerve of rats [3,4,7] and decreases retrograde degeneration of the neurons in the corresponding segments of the spinal cord [6,7]. In this study, the recovery of the crushed sciatic nerve of rats after low-power laser irradiation applied to the corresponding segments in the spinal cord alone was studied.

MATERIALS AND METHODS

The present study was carried out on 17 Sprague-Dawley rats of uniform age (3 months) each weighing approximately 300 g. The rats were divided into two groups and were anesthetized intraperitoneally with diluted Nembutal 15 mg/kg weight. The right thigh along the sciatic nerve and the dorsolumbar region of the spine were shaved.

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Accepted 21 July 2000

Lasers Med Sci 2002, 17:146–153

Effects of Diode Laser Therapy on Blood Flow in Axial Pattern Flaps in the Rat Model

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Abstract. Axial pattern skin flaps are a very important reparative tool for the plastic and reconstructive surgeon in the reconstruction of tissue defects. From whatever unfortunate reason, part or all of such flaps occasionally suffers from irreversible ischaemia with loss of the flap. Infrared diode laser therapy has been shown to improve local and systemic circulation. The present study was designed to assess the effect of an 830 nm diode laser (power density, 18.5 W/cm^2, energy density 185 J/cm^2) on the blood flow of axial pattern flaps in the rat model and their survival, compared with unirradiated controls. The flaps were raised in all animals ($n=40$), and blood flow assessed with laser speckle flowmetry (LSF). In the experimental groups (3 groups, $n=10$ per group), the flaps were irradiated either directly over the dominant feeder vessel (iliolumbar artery), at the proximal end or at the distal end of the flap itself and blood flow assessed during irradiation. Flowmetry was performed again in all animals at 5 and 10 min postirradiation, and the flaps sutured back in position. The unirradiated controls were handled in exactly the same way, but the laser was not activated. The survival rate of the flaps was assessed on the fifth postoperative day. LSF demonstrated significant increased blood flow in the flaps at 5 and 10 min postirradiation in all experimental groups compared with the control animals. At five days postirradiation, there was significantly better survival of the flaps in all the experimental groups compared with the controls ($p<0.01$), but no significant difference was seen between any of the experimental groups. We conclude that laser therapy increases the blood flow and perfusion of transferred flaps, and that this has significant effects on the survival of the flaps. One possible mechanism of modulation of the autonomic nervous system is discussed.

Keywords: Autonomic nervous system; Diode laser; Failing skin flaps; Low level laser therapy; Photobioactivation; Tissue ischaemia

INTRODUCTION

In the field of plastic and reconstructive surgery, a variety of flaps have been developed to repair tissue defects, and failure of flaps is a major problem for the plastic and reconstructive surgeon. Despite good intraoperative care, for whatever reason, irreversible ischaemia sometimes occurs in the distal portion of random pattern skin flaps or in random portions of axial pattern skin flaps. If the peripheral blood flow is sufficient in the distal portion of the skin flap then flap necrosis would become much less of a problem. Low incident levels of laser irradiation have been shown to improve circulation [1,2]. If, for example, application of a diode laser can improve the circulation in the distal portion of the flap, then this should, in theory, help to ensure a greater survival area.

Since 1989, the author and others have been reporting on enhanced blood flow following the application of low incident levels of laser energy, often referred to as laser therapy, in research with animal models [3–5]. Of particular interest have been the reports on the effects of 830 nm GaAlAs diode laser irradiation on random pattern skin flap survival in the rat model. It has been demonstrated that GaAlAs diode laser therapy produced (1) higher vascular perfusion, (2) greater fluorescent areas under fluorescein angiography and (3) significantly larger flap survival areas than either non-coherent LED-irradiated or unirradiated control flaps. Laser therapy has been used in humans with significant success

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Lasers in Surgery and Medicine 12:441–449 (1992)

Therapeutic Effect of Ga-Al-As Diode Laser Irradiation on Experimentally Induced Inflammation in Rats

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We produced experimental inflammation models in rats by carrageenin and studied the effect of Ga-Al-As diode laser irradiation (780 nm, continuous wave, 31.8 j/sec/cm^2, spot size of 0.2 mm) on inflamed regions compared with those of indomethacin, a potent anti-inflammatory agent. We found that a low-power infrared laser has an anti-inflammatory effect on carrageenin inflammation. A low-power laser inhibits: (1) the increase of vascular permeability during the occurrence of an acute inflammation in the carrageenin-air-pouch model, (2) edema in the acute stage in the carrageenin-paw-edema model, and (3) the granuloma formation in the carrageenin-granuloma model after receiving laser irradiation once daily. In all cases, irradiation for less than 10 min was sufficient to inhibit the inflammation by 20–30%. The inhibitory effect of laser irradiation was not comparable to that of indomethacin (4 mg/kg, i.o.) in the air-pouch model and the paw-edema model, whereas laser irradiation was more potent than that of daily administration of indomethacin (1 mg/kg, i.o.) in the granuloma model.

In future studies of the mechanism of laser effect, it should be noted that irradiating a rat twice, before and after the provocation of inflammation, was essential in order to achieve an effective inhibition of paw-edema.

Key words: anti-inflammatory effect, carrageenin inflammation, granuloma, low-power laser, paw-edema, vascular permeability

INTRODUCTION

Various studies have tested the efficacy of low-power laser irradiation on a living body based on the nonthermal mechanism. However, there have been very few reports on the anti-inflammatory effect of laser in vivo, and most of them were reported in conjunction with the analgesic treatment of rheumatoid arthritis cases [1–4]. Studies on the anti-inflammatory effect of a low-power laser are still at the stage where more experimental cases should be demonstrated. As a part of that effort, we investigated the anti-inflammatory effect of a low-power laser on experimental inflammation models in rats. Carrageeni, a kind of polysuccaride extracted from Irish moss, was used as an inflammatory irritant to examine the anti-inflammatory effect of a Ga-Al-As semiconductor diode laser on vascular permeability, edema, and granuloma formation in comparison with the effect of indomethacin, a nonsteroidal anti-inflammatory drug.

Accepted for publication February 12, 1992.

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Irradiation at 830 nm Stimulates Nitric Oxide Production and Inhibits Pro-Inflammatory Cytokines in Diabetic Wounded Fibroblast Cells

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Background and Objective: Wound healing in diabetic patients remains a chief problem in the clinical setting and there is a strong need for the development of new, safe, reliable therapies. This study aimed to establish the effect of irradiating diabetic wounded fibroblast cells (WS1) in vitro on pro-inflammatory cytokines and the production of nitric oxide (NO).
Materials and Methods: Normal, wounded and diabetic wounded WS1 cells were exposed to an 830 nm laser with 5 J/cm^2 and incubated for a pre-determined amount of time. Changes in cellular viability, proliferation and apoptosis were evaluated by the Trypan blue assay, VisionBlue™ fluorescence assay and caspase 3/7 activity respectively. Changes in cytokines (interleukin—IL-6, IL-1β and tumour necrosis factor-alpha, TNF-α) were determined by ELISA. NO was determined spectrophotometrically and reactive oxygen species (ROS) was evaluated by immunofluorescent staining.
Results: Diabetic wounded WS1 cells showed no significant change in viability, a significant increase in proliferation at 24 and 48 hours ($P<0.001$ and $P<0.01$ respectively) and a decrease in apoptosis 24 hours post-irradiation ($P<0.01$). TNF-α levels were significantly decreased at both 1 and 24 hours ($P<0.05$), while IL-1β was only decreased at 24 hours ($P<0.05$). There was no significant change in IL-6. There was an increase in ROS and NO ($P<0.01$) 15 minutes post-irradiation.
Conclusion: Results show that irradiation of diabetic wounded fibroblast cells at 830 nm with 5 J/cm^2 has a positive effect on wound healing in vitro. There was a decrease in pro-inflammatory cytokines (IL-1β and TNF-α) and irradiation stimulated the release of ROS and NO due to what appears to be direct photochemical processes. Lasers Surg. Med. 42:494–502, 2010.

Key words: IL-1β; IL-6; lasers; NO; ROS; TNF-α

INTRODUCTION

The process of wound healing is a highly co-ordinated process that involves a series of overlapping events controlled by a variety of cells, growth factors, cytokines and metabolic enzymes released at the wound site. Dysregulation of this co-ordinated event leads to impaired wound healing; an abnormality which is frequently seen in conditions such as diabetes. There are many causes of chronic wounds, with diabetes, pressure ulcers and venous stasis as the three most common causes [1]. Impaired wound healing is an incapacitating complication of diabetes often necessitating amputation and poses a serious challenge in clinical practice.

Growth factors and cytokines such as interleukin-1-beta (IL-1β), IL-6 and tumour necrosis factor-alpha (TNF-α) have diverse modes of action and are released during wound repair [2]. IL-1β and TNF-α are both well-known pro-inflammatory cytokines and have similar functions or effects; however, they do not share chemical or structural resemblance and their effects are interceded by specific receptors. Together with IL-1, TNF-α is the first cytokine known to be upregulated during the inflammatory phase of wound healing and contributes to the oxidative stress within the wound by generating reactive oxygen species (ROS) [3]. IL-6 is induced during acute phase reactions and usually expressed in response to or together with IL-1 and TNF-α [4]. However, contradictory effects have been reported [5]; it suppresses TNF-α, IL-1 and IL-12. Its vital role in wound healing is its ability to cause cell differentiation and proliferation. TNF-α is the most critical accelerator of diabetes [6].

ROS and reactive nitrogen species (RNS) act as molecular messengers during cell signalling; however, they have a biphasic effect, being both beneficial and detrimental depending on their concentration. ROS and RNS are generated during wound healing and are important mediators in this carefully controlled process, however in chronic wounds there is an uncontrolled production of these molecules. Nitric oxide (NO) is significantly reduced in chronic ulcers and impaired healing of diabetic wounds is

Contract grant sponsor: University of Johannesburg (UJ); Contract grant sponsor: National Research Foundation (NRF) of South Africa; Contract grant sponsor: Medical Research Council (MRC) of South Africa.
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Accepted 5 August 2009
Published online 15 July 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20812

Journal of Athletic Training 2012:47(2):178–183

www.nata.org/jat

Limb Blood Flow After Class 4 Laser Therapy

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Context: Laser therapy is purported to improve blood flow in soft tissues. Modulating circulation would promote healing by controlling postinjury ischemia, hypoxia, edema, and secondary tissue damage. However, no studies have quantified these responses to laser therapy.

Objective: To determine a therapeutic dose range for laser therapy for increasing blood flow to the forearm.

Design: Crossover study.

Setting: Controlled laboratory setting.

Patients or Other Participants: Ten healthy, college-aged men (age = 20.80 ± 2.16 years, height = 177.93 ± 3.38 cm, weight = 73.64 ± 9.10 kg) with no current history of injury to the upper extremity or cardiovascular conditions.

Intervention(s): A class 4 laser device was used to treat the biceps brachii muscle. Each grid point was treated for 3 to 4 seconds, for a total of 4 minutes. Each participant received 4 doses of laser therapy: sham, 1 W, 3 W, and 6 W.

Main Outcome Measure(s): The dependent variables were changes in blood flow, measured using venous occlusion plethysmography. We used a repeated-measures analysis of variance to analyze changes in blood flow for each dose at 2, 3, and 4 minutes and at 1, 2, 3, 4, and 5 minutes after treatment. The Huynh-Feldt test was conducted to examine differences over time.

Results: Compared with baseline, blood flow increased over time with the 3-W treatment ($F_{3,9} = 3.468$, $P < .011$) at minute 4 of treatment (2.417 ± 0.342 versus 2.794 ± 0.351 mL/min per 100 mL tissue, $P = .032$), and at 1 minute (2.767 ± 0.358 mL/min per 100 mL tissue, $P < .01$) and 2 minutes (2.657 ± 0.369 mL/min per 100 mL tissue, $P = .022$) after treatment. The sham, 1-W, and 6-W treatment doses did not change blood flow from baseline at any time point.

Conclusions: Laser therapy at the 3-W (360-J) dose level was an effective treatment modality to increase blood flow in the soft tissues.

Key Words: therapeutic modalities, circulation, musculoskeletal injuries

Key Points

- Using a class 4 laser in a human clinical model, we found a protocol-response effect: a 3-W protocol at a 50% duty cycle applied to the biceps brachii muscle was the most effective for increasing blood flow to the distal forearm.
- Laser therapy is an effective, noninvasive treatment modality to improve blood flow and perhaps tissue healing in the clinical setting.

The use of laser as a clinical modality has increased greatly over the past decade. Positive effects of laser therapy for the treatment of acute and chronic musculoskeletal disorders include pain control[1,2] and improved tissue repair.[3,4] However, the underlying mechanisms and clinical effectiveness of laser therapy remain poorly understood.

Lasers are classified by power level and their ability to produce eye injury. These power and beam characteristic ratings are established by the American National Standards Institute and the International Electrotechnical Commission. Most therapeutic lasers available for use in clinical practice are classified as 3B or 4. Class 3B lasers emit power of 5 to 500 mW, whereas class 4 lasers emit power of more than 500 mW. A few therapeutic laser manufacturers offer divergent-beam power outputs greater than 10000 mW. Class 3B level emitting lasers are known as low-level, low-intensity, and cold lasers because they generate no significant thermal effect in the superficial tissue during irradiation. Class 4 lasers are known as high-power and hot lasers because they can produce rapid increases in superficial tissue temperatures when maximum permissible exposure limits are exceeded. Recent trends in laser therapy show a preference for class 4 lasers in patient care settings.[5] Class 4 lasers can emit greater photonic energy in a shorter period of time than class 3B lasers without producing an appreciable rise in tissue temperature under normal treatment protocols.[5] This higher power becomes important when treating injuries to deeper tissues such as ligaments, muscles, tendons, and cartilage.

Authors of most published clinical studies on laser therapy to treat musculoskeletal injuries have used class 3B low-power lasers. Several published reports[6,7] have questioned the ability of low-power lasers to effectively transmit energy beyond the skin into deep musculoskeletal tissues. Excessive beam scattering and attenuation within the skin limit the potential biostimulative effects of laser in the deeper target tissues because of several factors related to dosimetry, such as subthreshold optical power, insufficient treatment durations, and varied treatment frequencies.[8,9] Therefore, it is relevant and timely to study the dosimetric responses of specific infrared wavelengths of high-power class 4 lasers and their ability to modulate the physiologic effects that are conducive to healing.

Positive therapeutic effects of laser have been attributed to increased blood flow in soft tissues and, coincidentally, the

Low-Level Laser Therapy (808 nm) Reduces Inflammatory Response and Oxidative Stress in Rat Tibialis Anterior Muscle After Cryolesion

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Background and Objective: Muscle regeneration is a complex phenomenon, involving coordinated activation of several cellular responses. During this process, oxidative stress and consequent tissue damage occur with a severity that may depend on the intensity and duration of the inflammatory response. Among the therapeutic approaches to attenuate inflammation and increase tissue repair, low-level laser therapy (LLLT) may be a safe and effective clinical procedure. The aim of this study was to evaluate the effects of LLLT on oxidative/nitrative stress and inflammatory mediators produced during a cryolesion of the tibialis anterior (TA) muscle in rats.

Material and Methods: Sixty Wistar rats were randomly divided into three groups ($n = 20$): control (BC), injured TA muscle without LLLT (IC), injured TA muscle submitted to LLLT (IRI). The injured region was irradiated daily for 4 consecutive days, starting immediately after the lesion using a AlGaAs laser (continuous wave, 808 nm, tip area of 0.00785 cm^2, power 30 mW, application time 47 seconds, fluence 180 J/cm^2; 3.8 mW/cm^2; and total energy 1.4 J). The animals were sacrificed on the fourth day after injury.

Results: LLLT reduced oxidative and nitrative stress in injured muscle, decreased lipid peroxidation, nitrotyrosine formation and NO production, probably due to reduction in iNOS protein expression. Moreover, LLLT increased SOD gene expression, and decreased the inflammatory response as measured by gene expression of NF-kβ and COX-2 and by TNF-α and IL-1β concentration.

Conclusion: These results suggest that LLLT could be an effective therapeutic approach to modulate oxidative and nitrative stress and to reduce inflammation in injured muscle. Lasers Surg. Med. 44:726–735, 2012.

Key words: low-level laser therapy; photobiomodulation; muscle cryolesion; inflammatory mediators; nitrative stress; oxidative stress

INTRODUCTION

Skeletal muscle injuries are common consequences of sport and labor activities. Depending on the severity of the injury, they can affect muscle function, leading to atrophy, contracture, pain, and increased likelihood of re-injury [1–3].

Muscle repair is very complex and involves several highly organized molecular and cellular processes. Immediately following the disruption of the myofibers, neutrophils, and macrophages infiltrate to the lesion area, producing pro-inflammatory cytokines and proteases responsible for necrotic tissue removal and further propagation of the inflammatory response [4–6]. These processes

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Contract grant sponsor: NIH; Contract grant number: R01AI050875; Contract grant sponsor: Emergency Medicine Division; Contract grant number: LIM 51; Contract grant sponsor: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Contract grant number: 2006/01096-8, 2009/01990-9; Contract grant sponsor: Conselho Nacional de Desenvolvimento Científico (CNPQ); Contract grant number: 473537/2008-7, 151747/2007-5.

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Accepted 24 August 2012
Published online 21 September 2012 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/lsm.22077

Photomedicine and Laser Surgery
Volume 28, Number 1, 2010

Pp. 3–16
DOI: 10.1089/pho.2008.2470

Low Level Laser Treatment of Tendinopathy: A Systematic Review with Meta-analysis

Steve Tumilty, MPhty,[1] Joanne Munn, Ph.D.,[1] Suzanne McDonough, Ph.D.,[2] Deirdre A. Hurley, Ph.D.,[3] Jeffrey R Basford, Ph.D.,[4] and G. David Baxter, DPhil[1]

Abstract

Objectives: To assess the clinical effectiveness of Low Level Laser Therapy (LLLT) in the treatment of tendinopathy. Secondary objectives were to determine the relevance of irradiation parameters to outcomes, and the validity of current dosage recommendations for the treatment of tendinopathy. ***Background:*** LLLT is proposed as a possible treatment for tendon injuries. However, the clinical effectiveness of this modality remains controversial, with limited agreement on the most efficacious dosage and parameter choices. ***Method:*** The following databases were searched from inception to 1st August 2008: MEDLINE, PubMed, CINAHL, AMED, EMBASE, All EBM reviews, PEDro (Physiotherapy Evidence Database), SCOPUS. Controlled clinical trials evaluating LLLT as a primary intervention for any tendinopathy were included in the review. Methodological quality was classified as: high (≥6 out of 10 on the PEDro scale) or low (<6) to grade the strength of evidence. Accuracy and clinical appropriateness of treatment parameters were assessed using established recommendations and guidelines. ***Results:*** Twenty-five controlled clinical trials met the inclusion criteria. There were conflicting findings from multiple trials: 12 showed positive effects and 13 were inconclusive or showed no effect. Dosages used in the 12 positive studies would support the existence of an effective dosage window that closely resembled current recommended guidelines. In two instances where pooling of data was possible, LLLT showed a positive effect size; in studies of lateral epicondylitis that scored ≥6 on the PEDro scale, participants' grip strength was 9.59 kg higher than that of the control group; for participants with Achilles tendinopathy, the effect was 13.6 mm less pain on a 100 mm visual analogue scale. ***Conclusion:*** LLLT can potentially be effective in treating tendinopathy when recommended dosages are used. The 12 positive studies provide strong evidence that positive outcomes are associated with the use of current dosage recommendations for the treatment of tendinopathy.

Introduction

In recent times, the term "Tendinopathy" has been used as a general clinical descriptor to indicate pain in the region of the tendon without any indication of the underlying cause.[1] However, the prevalence of tendinopathies is apparently increasing. For example, in New Zealand the incidence of Achilles tendon ruptures more than doubled between the years 1998 to 2003, from 4.7/100,000 to 10.3/100,000, a phenomenon that follows international trends.[2] Patella tendinopathy accounted for 20% of all knee injuries reported over a six month period at a sports injury clinic,[3] while tennis elbow affects approximately 1%–2% of the population.[4] Other common sites of tendinopathy are golfer's elbow at the medial side of the elbow, and the rotator cuff tendons in the shoulder.

Perhaps because of the multifactorial nature of the pathogenesis of tendinopathy,[5,6] there is a plethora of treatment modalities available to reduce symptoms and to attempt to control or enhance the tendon healing response. These modalities, which include various electrotherapy modalities, eccentric exercise, a variety of injection techniques, and cross-fiber massage, provide mixed or uneven benefit across patient populations.[7–9]

Low level laser therapy (LLLT) or the use of laser sources at powers too low to cause measurable temperature increases, has been used to treat soft tissue injuries and inflammation since the 1960s, and studies from as early as the 1980s reported benefits in a variety of tendon and sports injuries.[10,11] More recently, the term LLLT has been used to describe not only the use of low power laser sources, but also

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INTERNAL DOSIMETRY: COMBINING SIMULATION WITH PHANTOM AND EX VIVO MEASUREMENT

Bryan J. Stephens, PhD, Wendy Baltzer, DVM, PhD, DACVS, Phil Harrington, DC, CMLSO

Introduction

Internal dosimetry of laser therapy is far too often overlooked or "guesstimated", but is crucial information for the design of treatment protocols and prediction of biological efficacy. In vitro studies have given us a general idea of the range of biostimulatory doses, but their results do not and should not be directly extrapolated to form conclusions in vivo.

The science of dosimetry has been extensively developed in other wavelength ranges of the electromagnetic spectrum to different degrees of precision based on the danger of exposure of each. Though we do not need the sub-millimeter accuracy of the radiation oncologist who delivers ionizing radiation that can destroy individual cells, the techniques they have developed offer a sensible guide to understanding the photon transport in biological tissue. Here we employ some of these tools as we aim to bridge this gap and understand exactly how dose is distributed at depth in the body.

Materials and Methods

Wavelengths investigated were 800 nm and 970 nm at powers ranging from 0.1 - 12 Watts using the K-1200 (K-LaserUSA, Franklin, TN). First-order predictions were made from power measurements on incremental depths in water and tissue phantoms. Second-order estimates were established by Monte Carlo photon transport simulation on actual MRI data with literature-referenced values of scatter, absorption, and reflection coefficients. Finally, the most robust data came from ex vivo Si photodiode detector measurement on six canine cadavers in a variety of anatomical geometries.

2.1 First-Order Approximation

Power meter employed was the PLUS (LaserPoint, Vimodrone, Milano, Italy) using the "LD" calibration setting (quoted by the manufacturer as appropriate for the 800-900 nm range; no significant differences in sensitivity were noted for the 970 nm wavelength).

The meter was placed face-up on a stand to maintain ambient airflow through the heat sink fins. On top was placed a 2 mm thick piece of aluminum with a 1 cm diameter hole punched through that served as an aperture so that spatial independence of the detector head could be verified and radial scattering could be measured. On top of this was placed a thin plastic beaker, whose attenuation was minimal (transmission loss of 2% was measured and all the data corrected accordingly). The laser's handpiece was fixed normal to and at a distance of 12 cm from the beaker/detector interface and irradiation was carried out. In the beaker, layers of water from 0.5 - 10 cm in 0.5 cm increments were added, and the power transmission measured. At each depth of water, the detector was moved relative to the central axis of the beam (laser handpiece position kept constant) to measure transmission values at distances of 0 - 1.5 cm in 0.5 cm increments from the central axis.

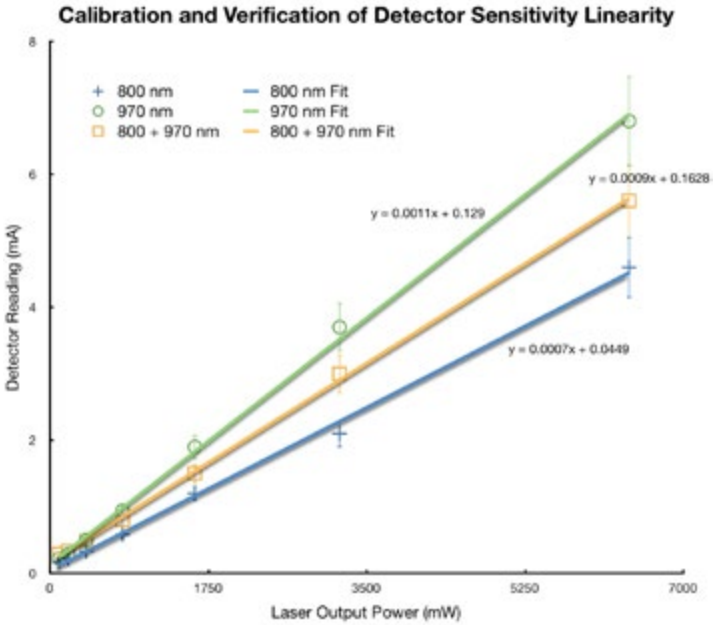

Figure 1: Measurement of Linearity of Response vs Exposed Power for the FDS-1010-CAL for each Wavelength

2.2 Second-Order Approximation

Combining techniques from radiation oncology and neurology, simulations can be performed to give the most detailed prediction of dose deposition. Radiation oncologists pre-plan their irradiations with full 3-dimensional simulations tracking accelerator head motion and collimator leaf manipulation and overlay these parameters on computed tomography images of the patient to ensure highly localized dose distributions. In fact, most linear accelerators on the market come equipped with software capable of performing such estimations, for quality assurance as well as by federal mandate. The interaction of ionizing radiation with biological matter is substantially different from infrared radiation, however, and so the core interactions can

not be modeled the same way. Neurologists started using radiation in the near-infrared (NIR) to map the oxygenation of brain tissue since gray- and white- brain matter have distinct signatures in the NIR. To this end, there have been several algorithms developed to track NIR photon transport; used here was the Monte Carlo eXtreme (MCX) [1].

Combining these resources has lead to the first Monte-Carlo simulation in laser therapy. The input parameters for the simulation are the absorption coefficient, scattering coefficient, refractive index, and anisotropy factor for each type of tissue. MRI images are used to delineate the exact location of each tissue type and a 7-dimensional matrix (three spatial and 4 parameters) can be formed. Then with enough processing time a computer can initiate a fixed number of photons, originating at any voxel in the matrix, initially traveling in any direction from that origin, and track their transport to every voxel at each time step. Run the simulation for long enough and you have a full laser therapy session modeled and the deposited dose at every voxel recorded.

2.3 Third-Order Approximation

Using a cadaver model and a sensitive photon detector system, a full ex vivo dosimetric profile can be established. Resecting various layers of dermis, fat, muscle, and connective tissue, the detector was placed at a variety of depths and the power density delivered to each depth was compared to the surface skin exposure. Normalizing these curves, an accurate model can be formulated to develop pre-planned treatment protocols and quantify the dose dependence of biological effects, post-irradiation. Used were two Si detectors (FDS-100-CAL and FDS-1010-CAL, ThorLabs, Inc., Newton, NJ) whose calibration is NIST traceable, but a power-linearity and wavelength-dependence calibration test was performed on each. Figure 1 shows the measured photocurrent vs exposed power for the full range of experimental values and their fit to a linear model.

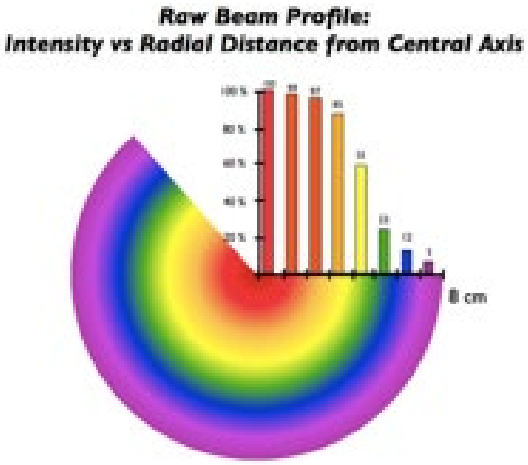

Figure 2: 2-Dimensional Beam Intensity Profile

The same aluminum aperture setup was used to test the spatial sensitivity differences on different parts of the detector wafer, with no significant differences found. This setup was also used to measure the 2-dimensional beam intensity profile, shown in Figure 2 which is clearly not uniform throughout the entire cross-section.

Results

3.1 First-Order Approximation

You can see from the full three-dimensional dose profile in Figure 3 that even in a simple water phantom at the most transparent wavelength (relative to the rest of the NIR) radiation intensity is strongly attenuated with depth. The anisotropy factor at this wavelength in water is about 0.8 which means that 80% of the scattering is directed in the forward hemisphere. This counteracts the absorption losses somewhat, but as you can see the attenuation is still quite steep.

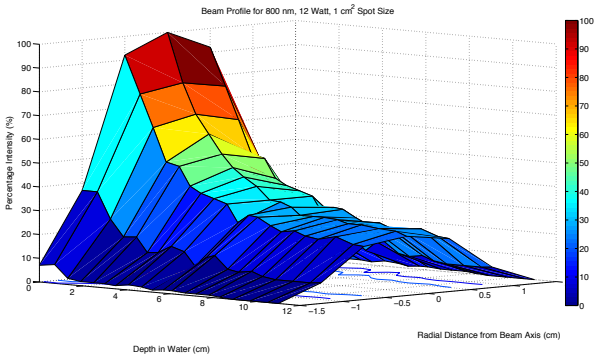

Figure 3: Measured 3-Dimensional Beam Profile in Water Phantom

3.2 Second-Order Approximation

Figure 4 shows the progression of stages in the simulation process. First, the anatomical positions of different tissue types need to be extrapolated from the MRI by a trained radiologist or surgeon. From there, the relevant literature was searched for optical properties of each tissue type at the given wavelength [2-4]. These parameters are overlaid on a contour map extracted from the MRI so that each voxel contains the absorption coefficient, scattering coefficient, anisotropy factor, and refractive index of the corresponding tissue type at the given wavelength. This particular simulation then initiated one billion photons each of which with the initial direction indicated by the red arrow and initial poistion distributed according to the measured 2-dimensional cross-sectional beam profile measured in Figure 2. The simulation then ran for fifty, 0.1 nanosecond times steps (remember radiation moves at the speed of light and so all the energy gets deposited very quickly) and recorded the absorbed dose in each voxel. Plotted are the values only in the plane of the MRI image, binned in 10% intervals, and normalized to 100% at the surface.

3.3 Third-Order Approximation

Ex vivo measurement is the most accurate, humane form of internal dosimetry estimation. Figure 5 shows the raw data taken on eight canine cadavers of a variety of breeds and in a variety of anatomical arrangements. They are plotted here for simplicity and to show the overall exponential trend in beam attenuation, but this plot does not take into account all of the different types of tissue through which the beam penetrated for each measurement.

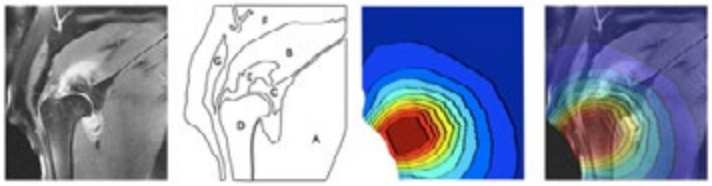

Figure 4: Stages of the Monte-Carlo Dosimetric Simulation. The different tissue types were identified as follows: A - muscle (subscapularis, teres major, latissimus dorsi, triceps) B - muscle (supraspinatus) C - bone (scapula) D - bone (humerus) E - tendon (of the supraspinatus) F - muscle and fat (omotransversarius) G - muscle (cleidobrachialis).

As you can see from Figure 4, these measurements included several combinations of skin/hair, fat, muscle, tendons/ligaments, and bone to compile a full dosimetric profile. Also, several beam paths were evaluated to acquire optimal penetration angles.

Example

The depth from the surface to the center of the joint where the detector was placed was measured (by digital caliper) to be 2.4 cm. From the curve in Figure 3, and assuming this dog to be a simple tank of water, we would predict the beam to transmit about 50% of its intensity to this depth. From an MRI-Monte Carlo simulation of this anatomical configuration, and including the estimated attenuation of skin, bone, fat, muscle, and joint tendons, we predict transmission of something more like only 5%. From the Si photodiode measurement, we find that only about 2% of the beam is transmitted to the center of the joint.

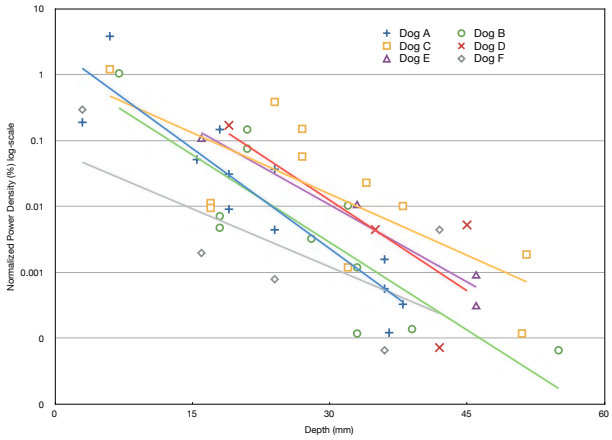

Figure 5: Generalized Penetration Data for Eight Canine Cadaver Legs

Discussion and Conclusions

As expected, the first-order experiments under-estimated the beam attenuation, but Monte Carlo results served as an accurate prediction of ex vivo observation. Dose delivered at therapeutic depths are up to 2 and 3 orders of magnitude less than those delivered to the surface. With enough data using a variety of skin, tissue, and bone thicknesses, this type of analysis will yield a full dosimetric profile.

Much more work remains to be done in quantitative internal dosimetry of laser therapy. This study, however, is a necessary step in the right direction on the path of understanding the orders of magnitude involved. Once further enlightened, we will be able to review both existing and future studies to better understand the biological effect of the delivered dose that came from the reported treatment prescriptions, and eventually converge on the optimal treatment parameters for clinical success.

Figure 6: Radiograph Examples of Anatomical Orientation of Detectors in Cadavers

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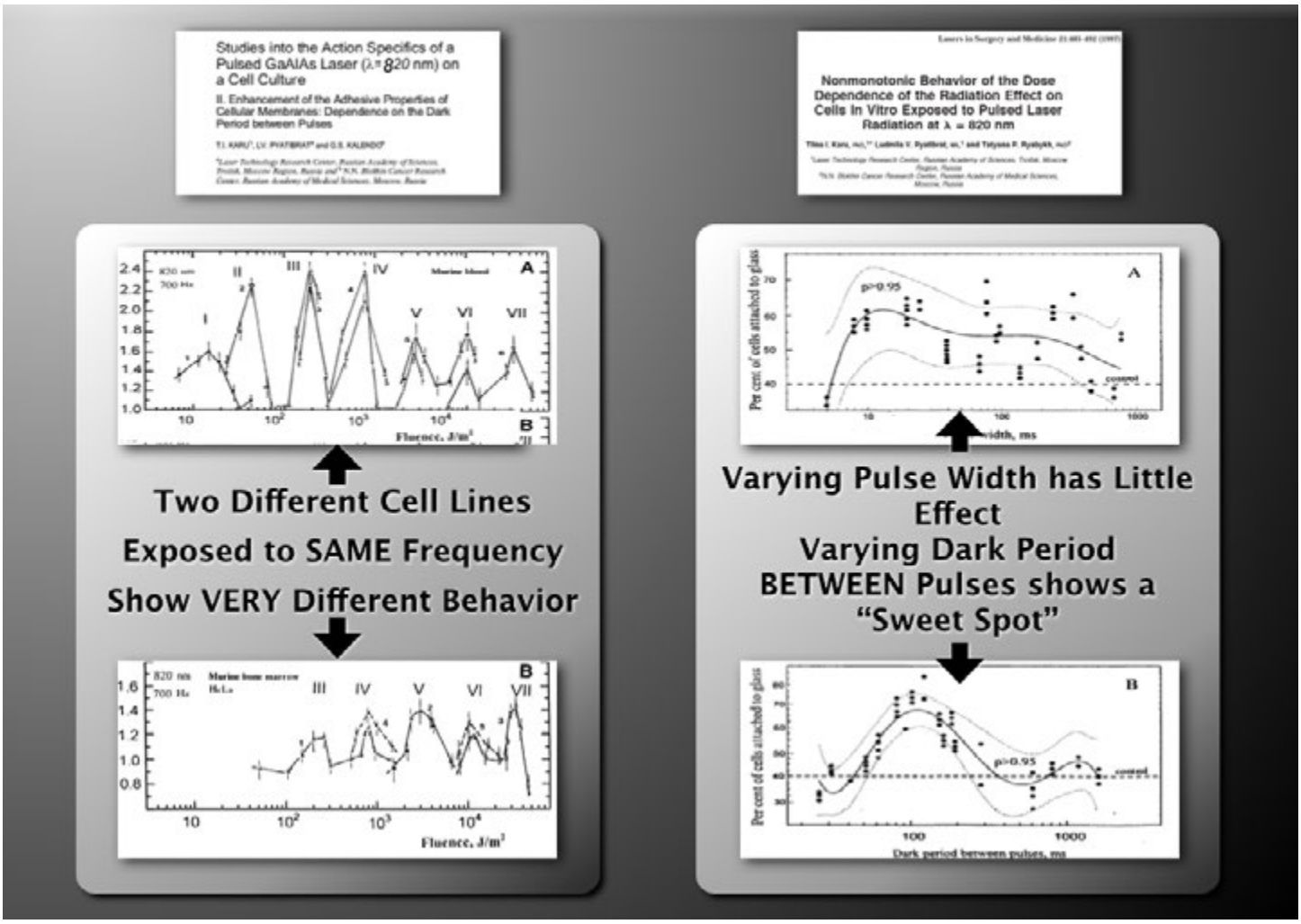
HOW DO WE KNOW



This slide is a graphical illustration of the idea that different cell types respond to different frequencies (and parameters, in general). Not every in vitro study measures "stimulation" by increased proliferation. Some measure adhesion to the glass, others spinning flagellum, others different biochemical secretions. And if you scour all the papers you'll find that the peak of stimulation occurs with different parameter sets. The closest thing to a truly side-by-side analysis is the Karu paper (attached).

Lasers in the Life Sciences, Vol. 9, pp. 211-217
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Studies into the Action Specifics of a Pulsed GaAIAs Laser (λ=*S20* nm) on a Cell Culture

II. Enhancement of the Adhesive Properties of Cellular Membranes: Dependence on the Dark Period between Pulses

T.I. KARU[*]', LV. PYATIBRAT[a] and G.S. KALENDO[b]

[a]*Laser Technology Research Center, Russian Academy of Sciences, Troitsk, Moscow Region, Russia and* [b] N.N. *Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia*

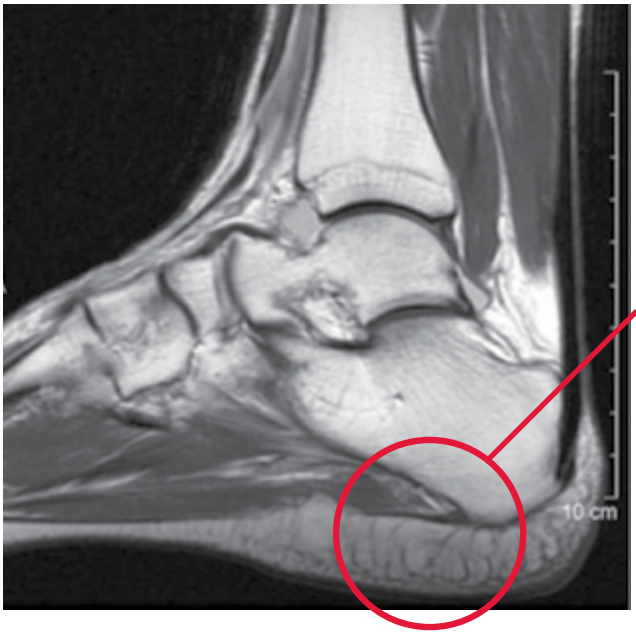
(Received January 02, 1999; In final form January 26, 1999)

Based on the number of cells attached to glass, changes are studied in the adhesive properties of cellular membranes 30 min. after irradiating a HeLa cell suspension with a pulsed GaAIAs laser (λ. = 820 nm. dose 60 J/m^2. pulse repetition frequency 0.1, 0.2, 0.5, 1.0, 2.5, 10, 50 or 100 Hz, duty factor 5, K), 20, 40, 70 or 95%). It is demonstrated that irradiation causes the number of the cells attached to the glass substrate to increase, but only when the duration of the dark period between pulses is in the range 50-200 ms (maximum increase at 100 ms).

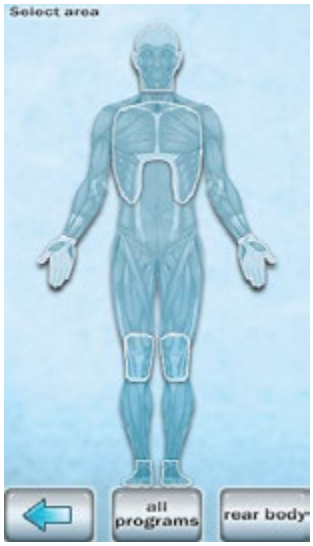
Keywords: Adhesion; dark period between pulses; GaAIAs laser; low-power laser therapy

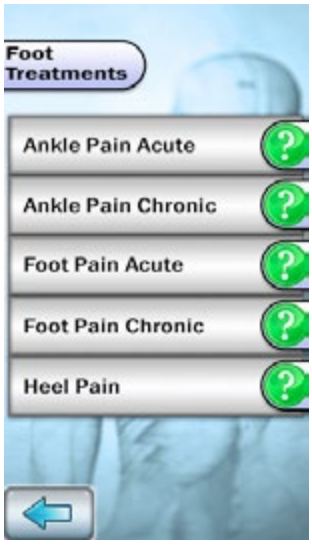
INTRODUCTION

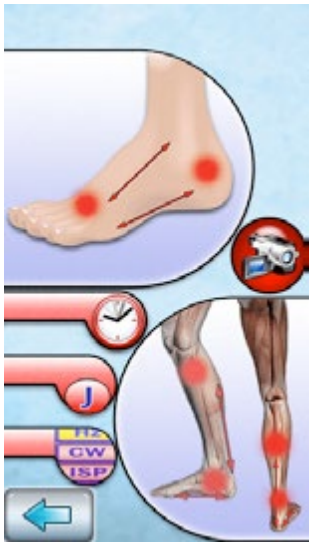
It is known that the sensitivity of eukaryotic cells to continuous-wave (CW) and pulsed laser radiation of one and the same wavelength and dose may be different (Karu *et al,,* 1996a and b, 1997; Karu 1998). In these

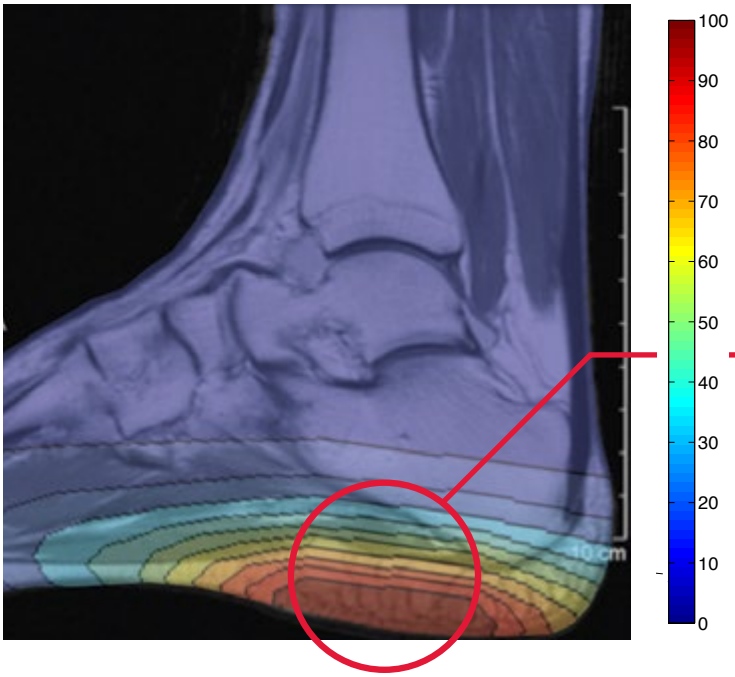


Where does the **PAIN** originate?





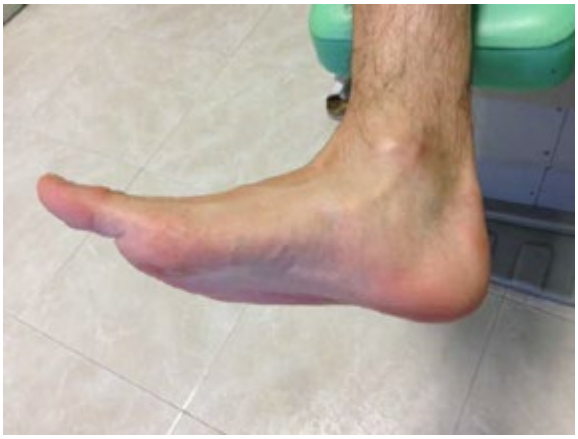




How much dose is actually **DELIVERED** there?

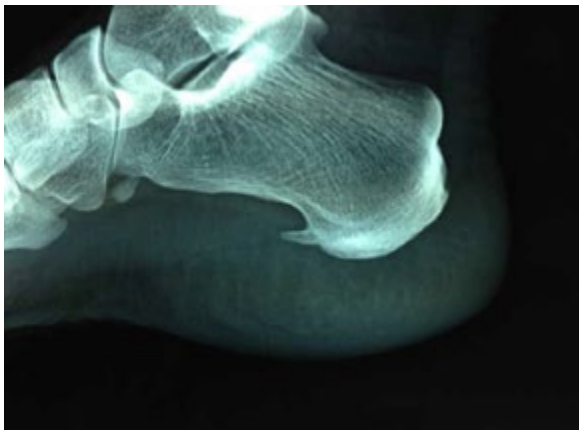
CASE REPORT:
HEEL SPUR TREATED WITH K-LASER CUBE

A.R., 35 years old male, checked in to the podiatric clinic of the Higher Learning Health Institute Claudiana in May 2014, complaining of an acute pain to the heel of the right foot when standing, and reporting an even more intense pain in the same area when putting his foot down first thing in the morning.



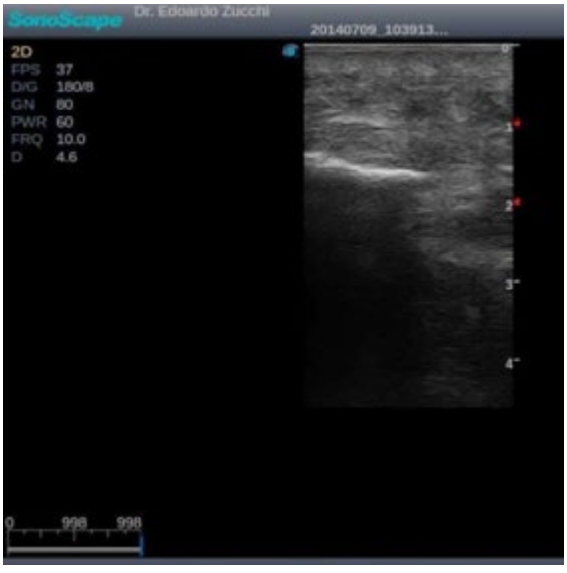
The patient had been suffering from chronic pain for 4 months now he decides to go to the clinic because of the pain intensification that causes a limitation in the usual movements and, consequently, a compensation through an antalgic gait but causing pain to the contralateral foot.

X-Ray and Ultrasound examinations confirmed the diagnosis of heel spurs.



The patient felt temporary relief by taking some NSAIDs (Nonsteroidal anti-inflammatory drugs) but he didn't resolve the problem in a definitive way.

Dr. Luca Rizzi, assisted by the third-year's students of the Master degree in Podiatry of the Higher Learning Health Institute Claudiana, knows very well the difficulties of the heel spur treatment and chooses the laser therapy's application using K-Laser Cube. First of all, the team wants to reduce the inflammation and the pain. The patient may also benefit from the biostimulating effects of laser therapy such as improved circulation and metabolic activity as well as the functioning of the nervous system, the immunoregulation and preventing the fibrotic tissue's formation.



The therapeutic program includes 3 lasertherapy's sessions in a week as well as a custom orthotic insole which relieves the painful area (Schwarz ring).

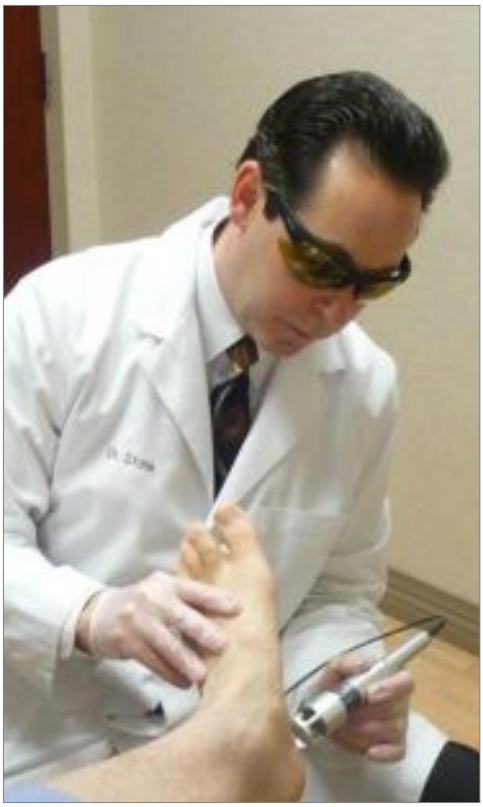
The program is set for acute pain and skin type II coloration. The treatment time per session is 4:35 minutes for two times, with a pause of ten minutes between the first and the second application. The total Joule is 1200 and the average power is 6 W. The operator uses the "ENT" handpiece directing the photons' beam to the insertion area of the plantar aponeurosis.

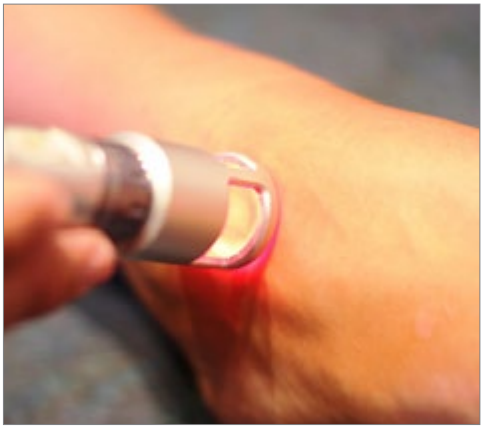
Just after the first application the patient refers an immediate improvement that increases in the two subsequent sessions. At the end of the therapy, the pain completely disappeared.

The use of the orthotic insole is important to safeguard the achieved mental and physical well-being and to prevent the relapse and any mechanical stress on the treated area.

Higher Learning Health Institute Claudiana
Via Lorenz-Böhler, 13 - 39100 Bolzano (Italy)

CASE REPORT: HEEL PAIN





54 year old white female with a one year history of chronic left heel pain. Conservative treatment consisting two steroid injections, custom made orthotic devices, and a course of physical therapy did not relieve her pain.

Examination revealed moderate pain to palpate the medial calcaneal tuberosity of the left foot. Due to failure of conservative treatment, soft tissue laser therapy was recommended.

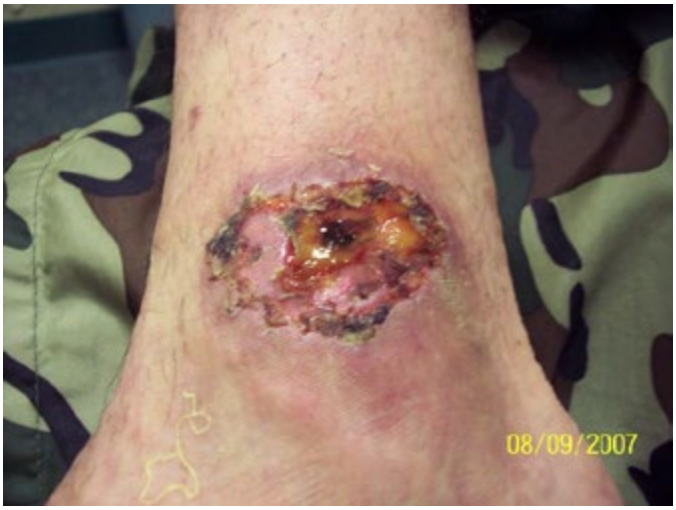
Laser therapy was performed twice weekly. The K-Laser Acute Ankle Pain setting was used at 10 Watts. After a total of six treatments, the patient's heel pain was resolved and she returned to full exercise activity without recurrence of her of pain.

DR. HOWARD STONE
NORTH SHORE PODIATRY GROUP

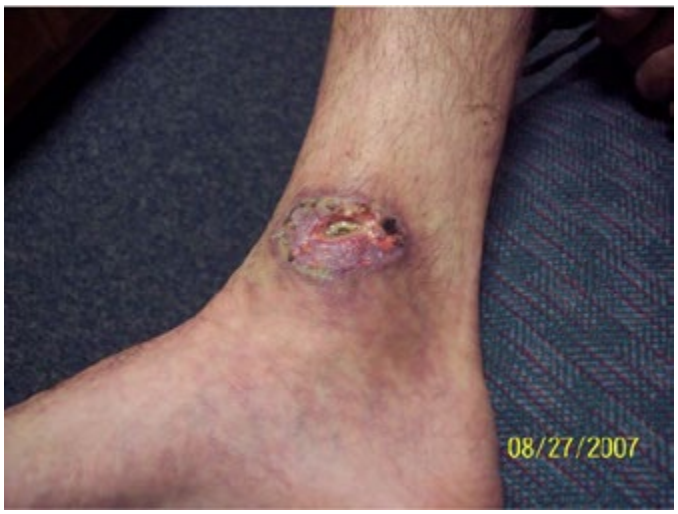
CASE REPORT: BURN

The injury occurred on July 4 ,2007 when a motorcycle fell on the patient's leg. His medial ankle was burned to the bone and he required a skin graft. On August 8, 2007 his medical doctor told him that he would require multiple skin grafts as the first one was not improving.

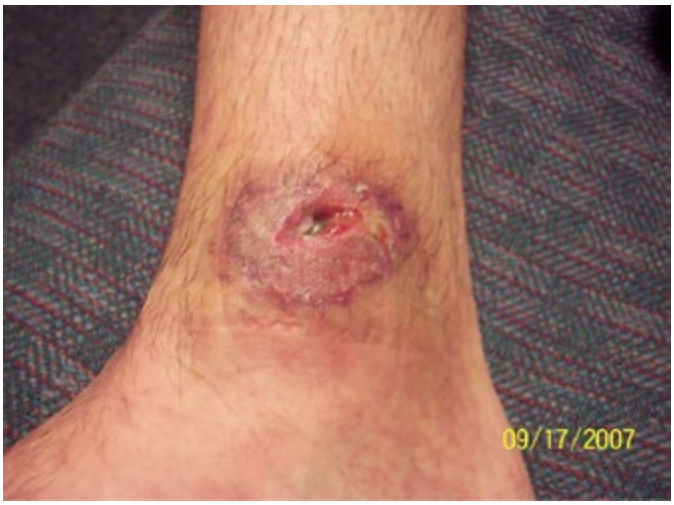
Scott chose to wait an additional 30 days after the first treatment. The K-Laser model 6D was used to relieve pain and promote healing. You'll see the results of the efficiency of the Klaser Model 6D in the following pictures.



Picture from one month and on skin graft after accident - August 9, 2007.



17 days and 4 K-Laser treatments later - August 27, 2007

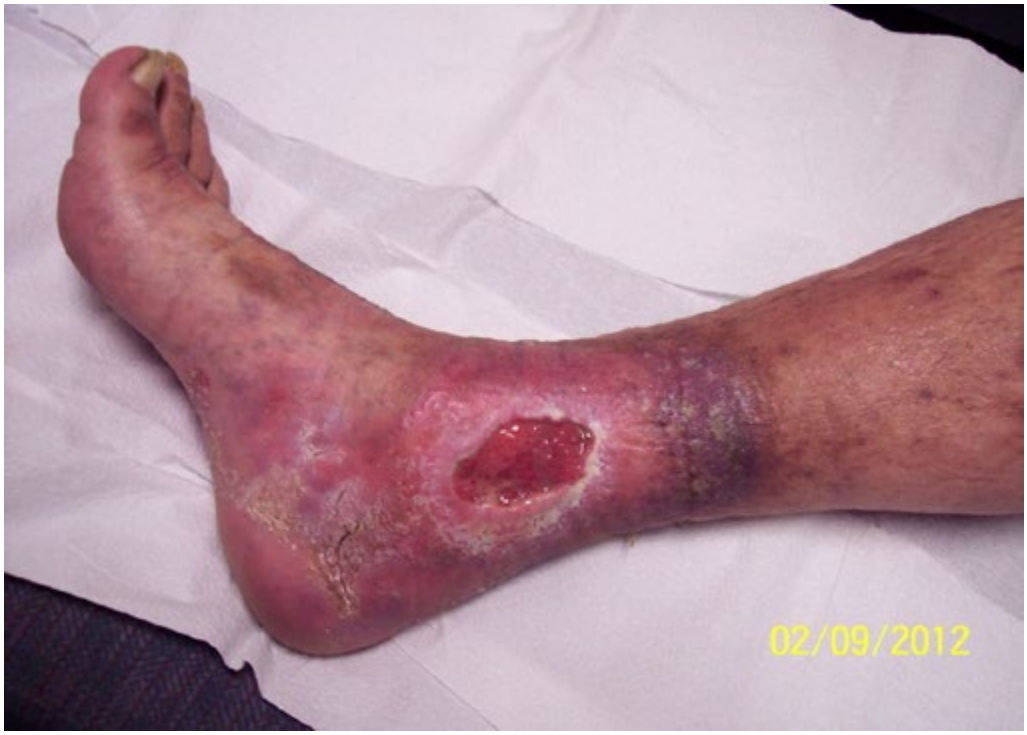


39 days and 8 K-Laser treaments - September 17, 2007

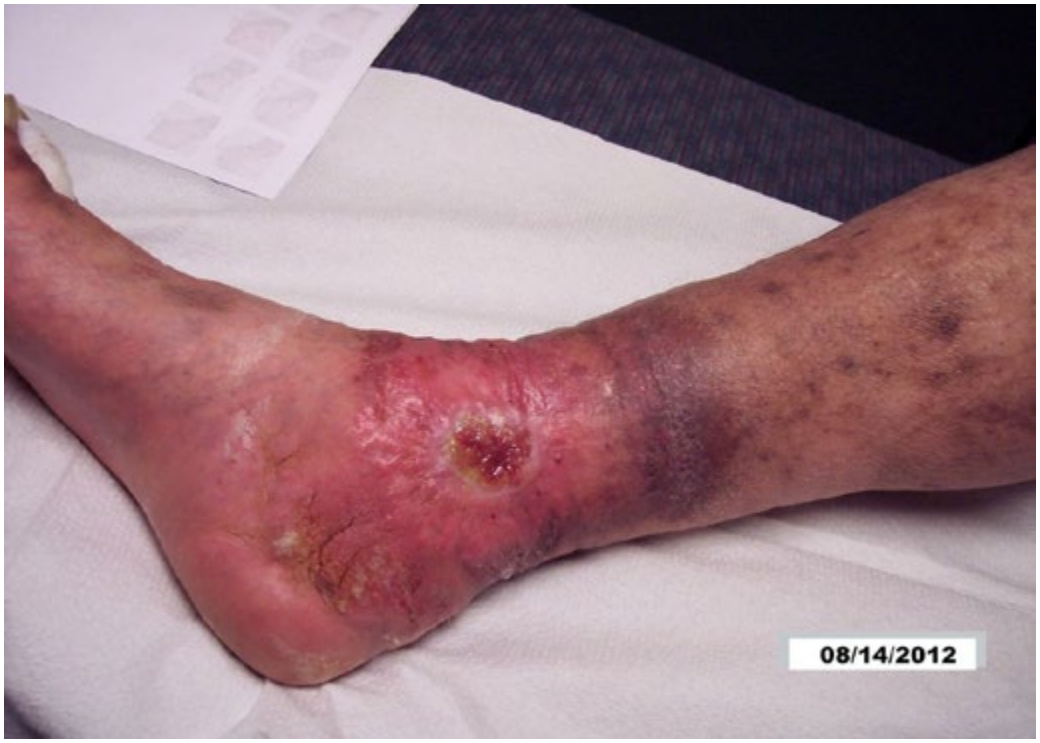
J. Rod McGinnis D.C., Sacramento, CA

CASE REPORT:
NON-HEALING WOUND

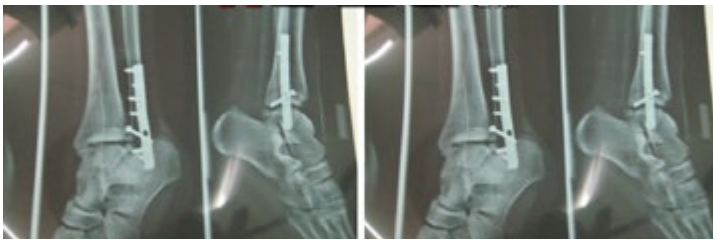
After four years of treatment with several methods



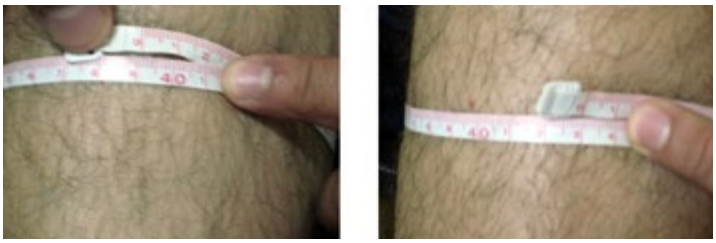
After 6 months using Klaser class IV laser

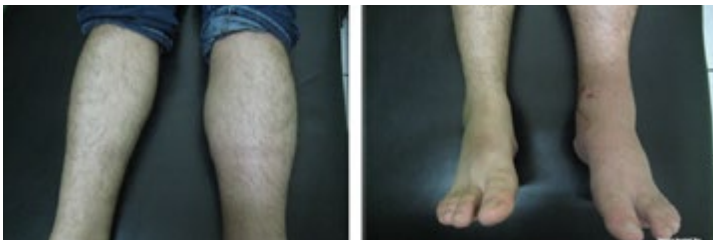


CASE REPORT: FRACTURED ANKLE

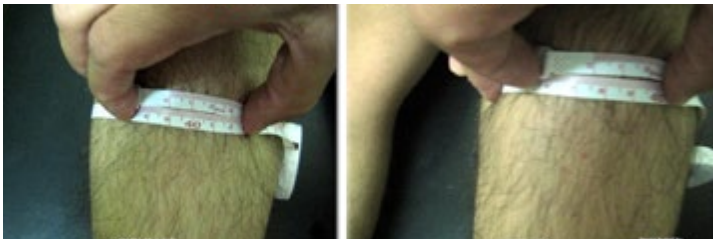


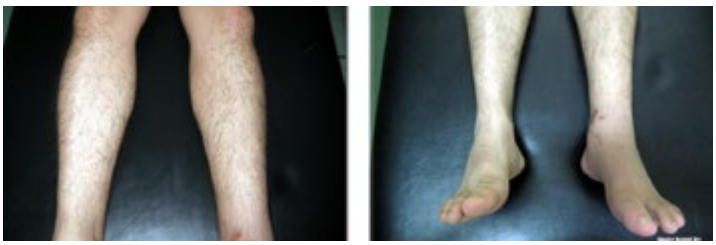
First day of physical therapy





After 8 K-Laser treatments





- Male patient, 35-years-old, suffers fractured ankle lateral malleolus of the fibula and ligament ruptures
- Deltoid on the inner face ankle and injured by abrasion medial malleolus on May 25, 2011
- The patient was surgically treated with reduction of fracture with osteosynthesis material, deltoid ligament plasty
- The patient has delayed of coagulation and therefore also slow the healing of the wound by abrasion before starting physiotherapy.

First day in physical therapy

- Patient walks with partial support up to 50% with axillary crutches
- Pain in ankle range of motion when forcing
- Range of motion; plantar flexion 40 degrees, -5 degrees dorsiflexion
- Important edema in leg, ankle and foot
- Leg metrics to evaluate edema: 39 cms diameter in right leg and 43 cms diameter in left leg - +4 cms

After 8 sessions of K-Laser therapy

- Patient with free march without crutches with light claudication
- No pain in the ankle, only a little tendinitis in the tendon of the peroneal, this caused friction on the plate osteosynthesis as the patient is performing daily activities already complete
- Range of motion: plantar flexion 45 degrees, 20 degrees dorsiflexion
- Edema is resolved
- Leg metrics to evaluate edema: 39 cms diameter in right leg; 37.5 cms diameter in left leg
- Now the left leg has a smaller diameter than the right, this is due to muscle atrophy of muscles of the leg itself with an injury of this nature

Unidad De Rehabilitacion & Terapia Laser, Mexico

DECREASING PAIN AND INCREASING RANGE OF MOTION IN DE QUERVAIN'S SYNDROME AND TRIGGER FINGERS WITH CLASS IV THERAPY LASER ST. THOMAS' HAND THERAPY UNIT

Stephen Barabas BSc, BVMS MRCVS* & Antonella Chierchia LTC*, Mr. Matthew James MB ChB FRCS (Plast) & Rachel Box BSc., OT$

INTRODUCTION

Laser therapy has shown clinical success in a wide variety of musculoskeletal and wound healing scenarios as both complementary and supplementary to the standard of care. This pilot study aims to quantify the clinical efficacy of K-Laser therapy on the treatment of carpal tunnel syndrome, trigger fingers, De Quervain's tenosynovitis, and other non-specific lower brachial injuries. The future trial shall quantify both the efficacy rate (expressed as percentage of symptom-free outcomes, based on finger-locking, pain, and function) and the duration of treatment (expressed both as number of hospital visits as well as total days from initial consults) in trigger finger and De-Quervain's cases. A reduction of either of these quantities would be of substantial benefit to the patient's quality and speed of healing and National Health Service standard of care and financial budgets. Twenty-two patients with severe pain and functional hand, wrist or finger disorders were treated with K-Laser for this pilot trial.

METHODOLOGY

Firstly, therapy lasers are an engine for microcirculation. Blood is the conduit for the transport of oxygen and nutrients to the cell and waste products like lactic acid and carbon dioxide away. In the capillaries, blood flow is regulated through microscopic pressure and thermal gradients. Targeting water with radiation (most efficiently in the Near Infrared (NIR) at 970 nm), is the best way to produce the temperature gradients that will increase localized blood flow.

Once more blood reaches the tissues, haemoglobin carrying the oxygen is reduced, releasing their oxygen supply to the surrounding cells, proportional to their metabolic demand. Simultaneously, these blood cells carry the waste metabolite products away from the cells. Laser therapy can speed up this process because the haemoglobin molecule absorbs light (most efficiently in the NIR at 905 nm), this "reduction" process increases blood oxygen release to the surrounding tissue cells to be processed into cellular energy. Free oxygen passes through the cell membrane and into the mitochondria where it is processed by a chain of respiratory enzymes whose end product is ATP.

Cytochrome oxidase is the transport enzyme between the end of the respiratory chain and ATP synthase, the enzyme that produces ATP. Each back-and-forth cycle produces a molecule of ATP, and without laser, this process happens at its normal metabolic rate. Cytochrome oxidase enzyme exists in "reduced" or "oxidized" state, if it absorbs laser light (most efficiently in the NIR at 800 nm), it will alter oxidation states more rapidly. More laser light energy means greater ATP production within the cells and quicker healing by the body's tissues.

Studies using laser light energy greater than the bio-stimulatory levels can affect trans-membrane proteins bridging cell membranes. Alterations in the flow of electrolytes across these cell signaling proteins can affect inflammation, oedema and pain perception.

THE LASER

Wavelengths (nm)	Frequencies (Hz)	Power Densities (W/cm^2)	Doses Delivered (J/cm^2)
660 nm 800 nm 905 nm 970 nm	Continuous Wave + 1- 20,000 Hz	Up to 1.6 W/cm^2	6-88J/cm^2

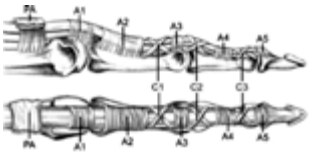
The effect of laser therapy is dose dependent. There is a broad consensus that from 2-10 J/cm2 is the optimal dose for bio-stimulation and increased healing, higher doses can have an analgesic effect. The central concept is that dose delivered at depth to the target tissue is significantly different to the dose to which the skin is exposed. To blindly extrapolate the bio-stimulatory results for a given dose delivered in vitro ,on a monolayer of cells 5 microns thick, to produce results from the same dose delivered to the skin of a patient or to treat subcutaneous soft

tissues or joints in vivo is naive and wrong by several orders of magnitude. The same rationale holds when using in vivo rat/mice studies to substantiate protocols developed for humans. Much research has been done by K-Laser and elsewhere to ascertain the optimal exposure levels that will deliver the optimal dosing of the hands and fingers in question, as well as how to modify the protocols to compensate for the variety in patient size. Research proves different tissues respond differently to different parameters, whether they be wavelengths or pulse frequencies. The laser therapy industry is relatively ignorant as to the "right" frequencies or wavelengths for any given condition, but we know that in the two conditions of this trial contain complex tissues, most notably bone, cartilage, smooth muscle, ligaments/ tendons, nerve cells and skin, each tissue type having different absorption to infra-red frequencies. K-Laser uses a variety of parameters within one treatment protocol to improve clinical efficacy. The laser does this automatically, and not randomly, but rather from a calculation of the average percentage breakdown of tissues involved and depth of treatment desired.

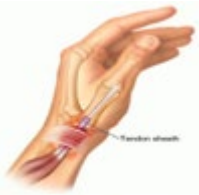
PATIENTS - INCLUSION CRITERIA

Trigger Finger - all adults aged 18 and over with single or multiple digit trigger finger disease, excluding those with a diagnosis of diabetes mellitus and all patients with a diagnosis of any form of cancer. Trigger finger in diabetics with bilateral disease- all adults aged 18 and over with symmetrical bilateral trigger finger(s) and a diagnosis of diabetes mellitus, excluding all patients with a diagnosis of any form of cancer.

De Quervains - all adults aged 18 and over with De Quervains tenosynovitis, excluding all patients with a diagnosis of any form of cancer.



Trigger Finger



De Quervains

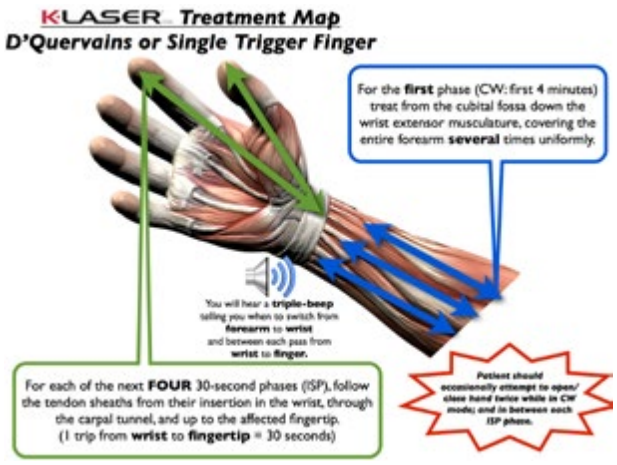
OUTCOME MEASURES

Pain - Pain was measured on a Visual Analog Scale (VAS) from 1 to 10 at every visit. Pain scores were recorded for several clinically relevant circumstances, including at night, during the day at rest, during normal daily activity, during exercise, and at full range of motion.

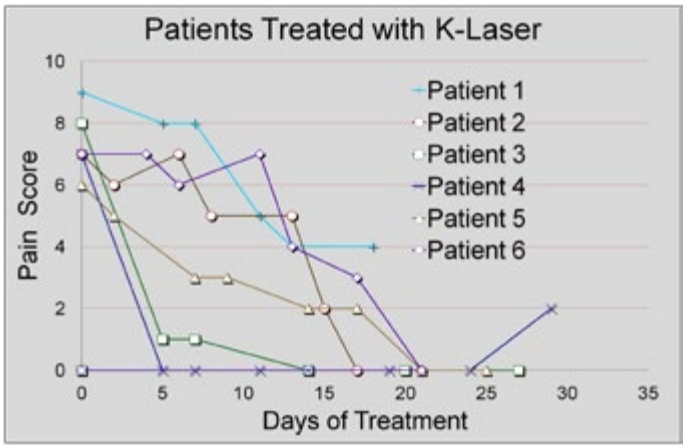
Range of Motion - Goniometers were used to measure full flexion and extension at every visit.

Grip Strength
Trigger Finger patients: Lateral pinch strength, Tripod pinch strength. Subjective: better / same / worse
DeQuervains patients: Pain on Finklestein test – Yes /No.
Pain on resisted MCPJ1 Extension – Yes /No.
Pain according to Visual Analogue Score

TREATMENT TECHNIQUE & FREQUENCY

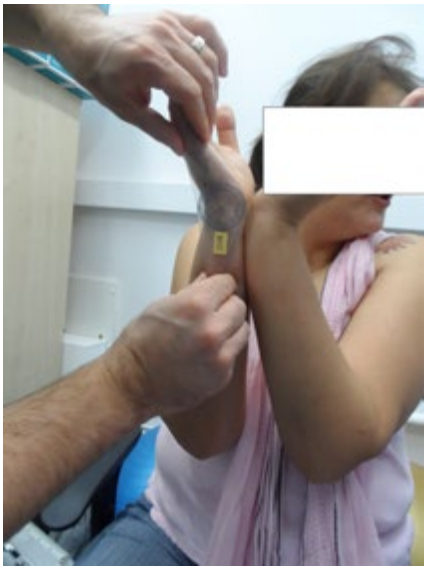
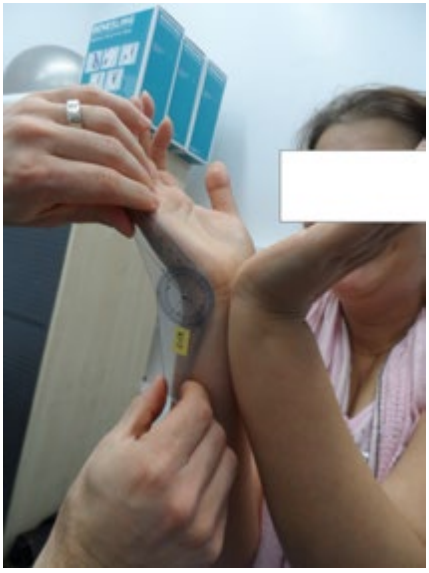


RESULTS



Patient 1 Light Blue: Multiple Tendinopathy and Cervical Pain ; Patient 2 Green: Scar Sensitivity following EPL Repair Patient 3 Dark Brown: Carpal Tunnel ; Patient 4 Purple: De Quervain's Tendinitis Patient 5 Dark Blue: Ligament tear, ulnar sided wrist pain; Patient 6 Light Brown: Tenosynovitis following trauma

Patient 2: Caucasian female, 72 years old. Has area of hypersensitivity over the dorsal surface of the left hand. Post EPL surgery and tendon transfer. Tender over the MCPJ's, no scar sensitivity. Objective/Treatment: Extension + 27oMCPJ and IPJ+27o Objective Post Treatment: Left thumb extension MCPJ:+12/IPJ: +14/. Still mildly sensitive over the MCPJs, but now able to touch hand without any difficulty. Analysis: Patient very happy with outcome and keen for discharge.


Measuring Changes in Range of Motion and Grip Strength Pre and Post K-Laser Class IV Therapy

Patient 4: Caucasian female, 65 years old. 18 years ago carpal tunnel syndrome, since returning to work De Quervain's R>L pain around the lateral aspects of the thumb and wrist area. Pain R 7/10 and L 2/10. Wears splint at night and has trouble sleeping due to the pain. Opted not to have steroid injection. Had pain on Finklestein assessment after 4/52 of splinting, initially. Subjective: Patient reported that her pain has gone.She has noticed that the sharp pain has decreased after manual labour. Objective Post Treatment: Right Finkelstein's -ve; left Finkelstein's test -ve. No pain on palpation at the 1st dorsal compartment.

Patient 7: Caucasian male, 74 years old. Two years ago given two steroid injections for wrist arthritis, returned for trapeziectomy six months later. Resulted in loss of radial and palmar cutaneous medial nerve sensation and lack of grip strength. Objective Pre Treatment: Grip strength 0.66 , MCP 32o and IPJ 55o Objective Post Treatment: Grip strength 4.33, MCPJ 46o and IPJ 68o improved hand and arm sensitivity except thumb

DISCUSSION

This is a pilot study looking at the effect of the Class IV laser therapy (K-Laser) on cases that had not shown a positive or had limited response to a combination of surgery, steroidal injections, splinting and/or hand therapy exercises in St. Thomas' Hand Unit Clinic. For the simplicity of the study K-Laser therapeutic protocols were provided to patients during the study for two sessions per week for on an average a four week period, with no other therapies being provided concurrently. Despite the variety, complexity and chronicity of cases treated there was a positive response to the analgesia and functional range of motion in all but three out twenty-two cases. In addition, several patients general skin sensitivity and capillary refill time improved during the short treatment period. Further studies are required to understand the benefits of K-Laser therapy when used in conjunction to surgery and hand therapy as part of a standardised rehabilitation program.

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