Energy FOR Health

International journal
of information and scientific culture

Volume [12]

Energy for Health
International journal
of information and scientific culture

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Contents

A predictive analysis of thermal effects in pigmented skin and underlying tissues during IR laser therapy.

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ABSTRACT

Infrared lasers are widely used in sport medicine and rehabilitation for their ability to induce a selective heating of localized portions of tissue. The desired effect is optimized by varying laser parameters (wavelength, emission modality, power). In this work we present a modelling study aimed at analyzing the thermal effects in the skin in dependence of irradiation conditions (treatment time and scanning mode of the laser probe) and skin pigmentation. The modelling study has been supported by a preliminary experimental study in albino and black mice. The results highlighted the dependence of the temperature values reached in different types of skin, on the concentration of epidermal melanosomes: the same laser induced thermal effects below the threshold of thermal damage in a light pigmented skin (45°C for a 5s arthrosis treatment) and might induce thermal damage in a dark pigmented skin (65°C in the same conditions). Moreover, it has been found out that the scanning mode of the laser light may be modulated in order to induce different thermal regimens in the skin (outer layers and deep layers). This predictive analysis may be used as an effective tool to draft guidelines for laser therapies as well as to design personalized clinical protocols.

INTRODUCTION

The therapeutic effects of tissue heating have been known since antiquity and new applications of instrumental therapies based on tissue heating continue to appear. Laser therapy is one of the most commonly used instrumental therapies that induce tissue heating and has the advantage of being able to selectively heat localized portions of tissue, even of very small volume. The rate of temperature rise in the exposed tissue volume, as well as the spatial and temporal evolution of thermal processes, depend on tissue optical properties (1), source features and treatment parameters, that is laser emission mode (continuous wave or pulsed and, in this case, on pulse width), wavelength, energy delivered to the tissue, dimensions of the directly irradiated area and treatment time duration (2).

Photothermal processes are an important consequence of laser-tissue interaction. They have direct effects on biomolecules, biochemical reactions, cell and tissue homeostasis and can be accompanied photomechanical effects. Obviously, based on the intensity and type of photothermal processes, the final effect can range from modifying a biological response to an irreversible tissue damage, and sometimes the threshold that separates the therapeutic effect from the damage is not clear and not easy to identify. Precise knowledge about the magnitude and spatial distribution of induced thermal effects in the tissue and their dependency on the treatment parameters is of decisive importance for the safe application of laser therapy and for taking advantage of thermal processes for therapeutic purposes. To predict the thermal effects induced by laser radiation in the tissue is quite difficult and implies also knowledge of the optical parameters of target tissues (3-6). Modelling is very important for the preparation of treatment protocols, since current laser systems could potentially provide personalized healthcare solutions, assuming that appropriate treatment parameters are chosen. The optical properties of a tissue are defined by its absorption coefficient (μa), scattering coefficient (μs) and anisotropy (g). μa is an index of the mean free path before absorption of radiation by tissue chromophores occurs and

depends on chromophore concentration; μs is an index of the mean free path of photons between scattering events; g indicates the angular deflection of a photon's trajectory caused by a scattering event (7,8). Absorption and scattering limit the penetration of laser light into the tissue (3,7,8). When laser thermal therapies are developed, the different biological effects that are induced into the tissue at different temperature ranges should be considered. When tissue temperature rises above 100°C, water evaporation and tissue desiccation occur. These effects are at the basis of laser surgery and laser thermotherapy for tumor removal (9-11). Temperatures over 60-70°C cause denaturation of structural proteins (coagulation) producing, even for short exposure times, immediately visible and irreversible tissue damage. Temperatures ranging from 40 to 50°C can affect biochemical reactions, enzyme structure and activity, cell morphology, extracellular matrix properties, leading to effects ranging from reversible changes to delayed cell death. In this range of temperatures, the biological effects strongly depend on the exposure time (12). From a therapeutic point of view, moderate heating may have significant effects.

Heat increases the rate of biochemical reactions, therefore stimulates tissue metabolism. Tissue heating also modifies physiological functions: blood flow increases, favouring the supply of nutrients and removing catabolites; inflammation increases and promotes phagocytosis and wound healing; muscle spasm decreases; fluid viscosity decreases, leading to the decrease of tissue stiffness and elongation of connective tissue due to the release of cross-linked collagen fibres. Moreover, heating promotes relaxation, therefore it has a general analgesic and sedative effect against soreness and aches. (13) Photomechanical effects can be considered secondary effects of the photothermal interaction: heating induces mechanical forces, which can act on both the extracellular matrix (ECM) and the cellular component of the tissue (2).

When irradiation is performed by very short laser pulses (pulse duration of the order of nanoseconds or less) dynamic compressive and tensile stresses inside a biological substrate can be generated even with small amounts of energy, due to the occurrence of stress and thermal confinement. These effects cause tissue damage and disruption, therefore they are useful in surgery and microdissection (14). Conversely, indirect mechanical effects can be induced by absorption of relatively long laser pulses (pulse duration longer than 1 μs), which allow the propagation of thermal energy out of the irradiated zone and through the tissue. In this case, predominant photothermal phenomena can generate mechanical deformations of the ECM components which may be conveyed at cellular level through the ECM integrins-cytoskeleton network (15-17). Mechanical stress plays a crucial role in maintaining the homeostasis of connective tissue, bone, muscle, cartilage and other tissues, and can also affect cell growth and differentiation, protein synthesis and ECM production.

With current pulsed infrared (IR) laser systems, including high power lasers, thermal processes and the induced biological effects may be appropriately modulated by choosing pulse energy, pulse width and duty cycle. Acting on these parameters, it has been possible to apply high power lasers in physical medicine, rehabilitation and sports medicine to stimulate tissue repair and recovery. These lasers allow to heat small volumes of tissue and to properly take advantage of the therapeutic effects of heat, but the treatment protocols must be carefully determined on the basis of the effect desired and the characteristics of the tissue to be irradiated. Worth noting are the characteristics of the skin (e.g. the phototype of the subject), since it is the first biological tissue with which laser radiation interacts in all treatments involving deeper tissues such as muscles or articular regions.

In this work we present a predictive analysis of the photothermal effects induced in skin by treatment with a MLS laser, equipped with a dual wavelength NIR source. MLS laser is currently applied in physical and sports medicine to treat musculo-skeletal diseases. In order to study the temperature dynamics in skins characterized by different phototypes, i.e. with different pigmentation, we set up in vivo experimental measurements on animal models (black and albino mice). The therapy was performed on the posterior legs by using a commercially available advanced laser system, characterized by multiwave and multimodal IR emission. The induced temperature dynamics on the superficial skin surface was monitored by the use of an infrared thermocamera. A modelling study was then developed in order to evaluate the laser induced thermal elevation in mice, not only on the skin surface, but also in the deep tissues. In fact, there is no experimental technique able to provide directly these data with a non-contact, non-invasive method. The thermographic data and the postprocessing results were compared. Then, the model analysis was used to study thermal effects in human skin, by considering skins with different concentrations of melanosomes (light skinned and darkly pigmented subjects). The results may be used to optimize clinical protocols.

MATERIALS AND METHODS
The laser system
The laser used in the experimental work is a commercial device (M1, ASA srl, 68x43x99 cm; 21 kg equipped with the handpiece #F9000166, having an external diameter of 20 mm). The light source is designed by combining the emission of two IR laser diodes.

The two modules have different wavelengths, peak power and emission mode. The first one is a pulsed laser diodes, emitting at 905 nm, with 25 W maximum optical power, the pulse width is 100 ns, while the frequency of pulses in a single pulse train may be varied till a maximum value of 90kHz, thus varying the total average power delivered to the tissue. Frequency of the pulse train may be varied in the range 1-2000 Hz. The second diode laser operates in continuous mode or in pulsed mode (maximum repetition rate 2000 Hz, pulse width 250 µs), emitting at 808 nm, with a maximum optical power output of 1.1W and a duty ratio of 50% independently of the pulse repetition rate. The light principal propagation axis of the two laser modules are coincident. In the pulse mode operation (MLSp), pulses from the two laser sources are synchronized in frequency (in the range 1- 2000 Hz), while in the continuous mode operation (MLSc) emission at 808nm is continuous (1.1W output power) and emission at 905nm is pulsed (2000Hz pulse train repetition frequency).

The animal model and the laser treatment
The animal models used in the experimental sessions were albino and black mice. Three black C57BL/6 mice (Harlan Laboratories,Inc., Indianapolis – IN, USA) and three albino ICR(CD-1®) mice (Harlan Laboratories, Inc., Indianapolis – IN, USA), were anesthetized with chloral hydrate (400 mg/kg, i.p.), then the external upper portion of the posterior legs was shaved. The eumelanin concentration in the albino and black mice skin is about 8 and 1/10 times than that of the human Caucasian race respectively (18,19).
All experiments involving laboratory animals were performed according to the Italian Guidelines for Animal Care (D.L. 116/92), in accordance with the European Communities Council Directives

(86/609/EEC). The laser treatment was performed by keeping the laser light handpiece close to the shaved skin and by slowly moving it, thus performing a scan on the treated area (in our case ~5 cm²). This procedure has been chosen because it is very frequently used in clinics, since it allows to treat large parts of the body (for example whole muscles) and to further limit the tissue heating. We considered two different treatment modalities: 1) continuous mode, 3 minutes treatment time, 2) pulsed mode (frequency: 700 Hz), 3 minutes treatment time. In the continuous wave irradiation mode, the power delivered to the tissue was 1.1 W emitted by the 808 nm diode module, while the 905 module emitted a mean power of 57.6 mW. In the frequency configuration (700 Hz), it has been delivered to the tissue a mean power of 550 mW for the 808 nm diode laser and 20.16 mW for the 905 diode laser.
These two treatment modalities correspond to the laser configurations used for the treatment of two important diseases, arthrosis and muscle contracture, respectively.

The temperature measurements
The temperature dynamics on the skin surface of the mice was monitored by the use of an infrared thermocamera (ThermoVision A20, FLIR Systems Inc., Wilsonville, OR, USA). The camera is equipped with a 17-mm-focal-length germanium lens, which allows a minimum working distance of 30 cm, resulting in a spatial resolution of 0.8 mm. The thermal sensitivity is 0.12°C at 30°C. The device is controlled via computer, by the use of the ThermaCam Researcher Software™, which enables direct evaluation of the thermographic data. IR thermography easily provides the relative temperature enhancement, while the absolute value of the temperature can be evaluated only when the exact values of the emissivity and reflectivity of all the objects imaged

by the IR sensor are known. Due to the complexity of the measurement scene (presence of shaved skin and fur, metal tools with high infrared reflectivity), we decided to evaluate the temperature enhancement respect to the intact skin and not the absolute temperature. In order to do this, the mean temperature of the exposed tissue was measured before laser treatment. The temperature enhancement was evaluated as the difference between the laser induced peak temperature and the mean value measured previously.

The mathematical model
A mathematical model was developed in order to study the laser-induced temperature enhancement in the deep skin. We based our study on the approach proposed by Babilas et al. (20). A bidimensional axial model of an intact rat skin was developed. The study was performed by using a commercial software (Comsol Multiphysics 3.5a, Comsol AB, Stockholm, Sweden). The skin was schematically described as composed by two different domains, the epidermis and the dermis, having different optical and thermal properties. The parameters used for modelling were taken from literature (21,22).
The skin characteristics (melanosomes concentration, haemoglobin concentration) of the two different animal models were taken into account, as it was possible to have a complete characterization of the animals from the seller datasheets. The propagation of light radiation was studied in the diffusion approximation and described by the following equation:
eq 1

$$\frac{\partial \Phi_\lambda(r,z,t)}{\partial t} - \nabla\left(a_\lambda^n \nabla \Phi_\lambda(r,z,t)\right) = -c_{n,\lambda}\mu_a^{n,\lambda}\Phi_\lambda(r,z,t)$$

where:
eq 2 $\quad a_\lambda^n = \dfrac{c_{v,\lambda}}{3\left(\mu_a^{n,\lambda} + (1-g_{n,\lambda})\mu_s^{n,\lambda}\right)}$

is the diffusion coefficient; $\mu_a^{n,\lambda}$ and $\mu_s^{n,\lambda}$ (1/m) are the n-th subdomain (epidermis, dermis) absorption and scattering coefficient respectively, at the wavelength

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λ; $g_{n',\lambda}$ is the optical anisotropy factor and $c_{n,\lambda}$ (m/s) is the light velocity in the n-th medium at the wavelength λ.
$\Phi\lambda$ (r,z,t) is the photon number per unit area and time, and it was written as:

eq 3 $\Phi_\lambda(r,z,t) = \Phi_\lambda(r,z)P_\lambda(t)$

Φ_λ (r,z) describes the photon diffusion in space for the two different wavelengths λ, while $P_\lambda(t)$ describes the different pulse widths and trains of the two laser emission modalities. Thanks to the symmetry of the problem, we described the geometrical model in axial symmetry, where r is the horizontal axis and z is the vertical axis (z=0 is the external skin surface, directly irradiated by the laser light, and the light propagates in the positive direction of the axis). The time dependent problem was solved by choosing a time step (50 μs) that enabled to correctly describe the pulse trains in time. The light source was imposed as a boundary condition, i.e. it is the photon flux at the directly irradiated surface (z=0 in Figure 1).
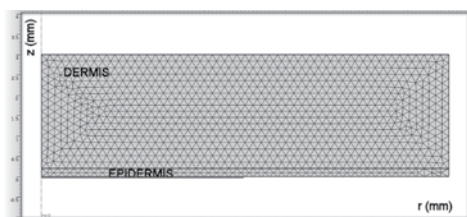

Figure 1: The 2D geometrical model and triangular mesh of the current study. The external skin layer is at z=0. The light propagation is in the z positive direction. We suppose that the laser probe is in close contact with the tissue (at z=0), and the contact surface is evidenced by the horizontal red line.

The temperature enhancement due to the illumination was calculated by solving the bio-heat equation:

eq 4

$$\rho_n C_n \frac{\partial T(r,z,t)}{\partial t} - \nabla(k_n \nabla T) = Q_{met} - Q_{perf} + Q_{ext}$$

T (r,z,t) describes the temperature dynamics due to all the heat sources of the problem: the contribution due to the metabolism (Q_{met}), to the blood perfusion (Q_{perf}), and to the external light sources (Q_{ext}). The term due to blood perfusion is described as:

eq 5 $\quad Q_{perf} = \rho_b C_b \omega_b (T_b - T)$

where ω_b (1/s) is the blood perfusion rate and T_b (K) is the arterial blood temperature, while T(r,z,t) (K) is the local tissue temperature.
The heat source due to the absorption of the light emitted by the two diode modules, is considered to be the sum of the single contribution of the two absorbed wavelengths:

eq 6

$$Q_{ext} = \mu_a^{n,\lambda} \Phi_{\lambda 1}(r,z,t)h_p \nu_{\lambda 1} + \mu_a^{n,\lambda} \Phi_{\lambda 2}(r,z,t)h_p \nu_{\lambda 2}$$

where h_p (6.626076 x 10^{-34} Js) is the Planck constant and $\nu_{\lambda i}$ (1/s) is the i-th light source frequency. In the bio-heat equation (eq 4) ρ_n is the density (kg/m^3), C_n (J/kgK) is the specific heat and k_n (W/mK) the thermal conductivity of the n-th subdomain. In all the above equations the optical parameters depend on the tissue characteristics and on the wavelength of the light source. We supposed that at the tissue/light source interface (z=0 in Figure 1) the laser handpiece was in close contact to the epidermis: thermal insulation is thus the boundary condition at z=0 in the thermal modelling in an area corresponding to the handpiece dimensions (r= 5 mm), while free convection with ambient air was supposed in the not irradiated tissue external surface. Light flux is the boundary condition for the diffusion equation at the same surface. At the other surfaces towards the surroundings the boundary conditions are heat and diffusion flux in the outwards direction. The diffusion and the bio-heat equations were simultaneously solved with the Finite Element Method, provided by the commercial software. The geometry was meshed with a triangular extra fine mesh with 2076 elements. We considered two different emission modalities, as reported in §2.1. By the use of the FEM analysis, we also studied the temperature dynamics in a human skin, considering three different pigmentation: light skin, moderately pigmented and dark skin. In order to do this, the concentration of the melanosomes was varied, as reported in Jacques (3). The melanosome concentration is related to the skin phototype (23): thus we considered 3.8% melanosomes volume fraction in the epidermis for a light skin, 13.5% for a moderately pigmented skin and 30% for a dark skin.

RESULTS

3.1 Photothermal effects in animal models

The thermal camera detected the temperature dynamics soon after irradiation and on the skin surface. It was evidenced that the temperature rise was higher in darkly pigmented mice then in albino mice, as it would be expected (see Figure 2). The postprocessing data of the modelling study evidenced that the thermal effects penetrate in depth (see Figure 3) and are higher in case of darkly pigmented mice respect to albino mice, as it was expected. The same model can thus reasonably be used to describe the laser induced thermal effects in human subjects. The results are described in the followings.
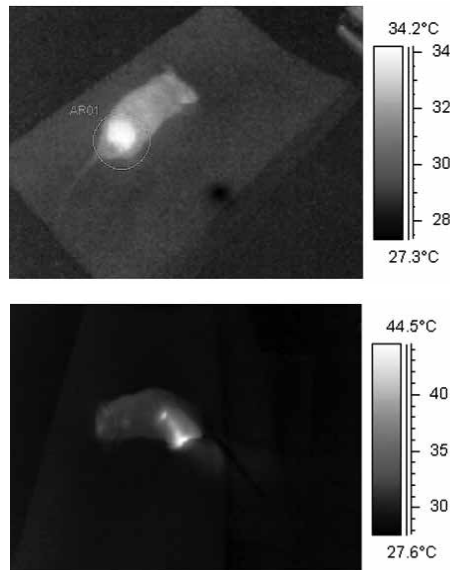

Figure 2: Thermograms soon after laser treatment of a white (up) and black (down) mouse, posterior leg.
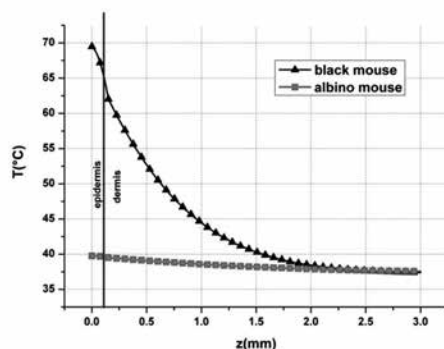

Figure 3: Modeling analysis of temperature dynamics in the deep tissue, at the end of the laser treatment. Differences in black and albino mouse are evidenced.

3.2 Photothermal effects in humans

In the modelling study we firstly supposed that the handpiece is kept in the same fixed position for 5s. In this condition the thermal effect was studied, evidencing the different values of temperature induced in the human tissue in dependence of the skin type and on the treatment settings. The results for different skin types are reported in Figure 4 for the same treatment condition, evidencing also the heat propagation in depth in Figure 5. In Figure 6 different settings for the same skin type (moderately pigmented) are reported. However, it has to be considered that the handpiece for delivering the laser light is kept in continuous movement on the skin surface during a typical treatment in human subjects. We then modelled this condition and we evaluated the thermal elevation during a standard treatment, where the handpiece is moved onto the external skin surface. We have to notice that a prolonged CW treatment in a single trigger point is very rarely considered. By considering the typical settings of the treatment for arthrosis, we studied the temperature enhancement in the deep tissue for a dark skin. The results are depicted in figure 7: in the starting phase of the treatment, the handpiece is kept in a fixed position for a time of around 2s for a dark skin; this is enough to induce a modest effect. In the lasting part of
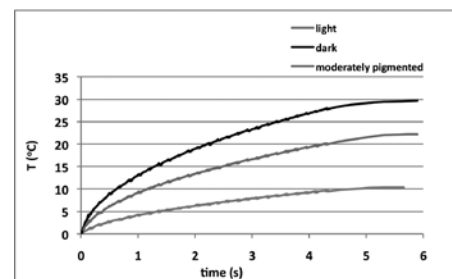
Figure 4: Temperature graphs in skin tissues with different concentration of melanosomes: light, moderately pigmented and dark skin.
The graph represents the temperature distribution on the external skin surface during a 5s treatment time (continuous wave configuration).
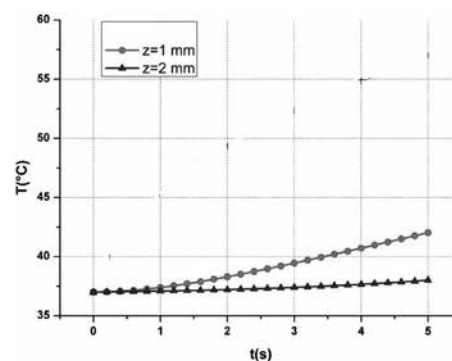
Figure 5: Temperature elevation along the symmetry axis (r=0) in a moderately pigmented human skin, at two different depths (1, 2mm), during the continuous wave treatment.

the procedure the probe is moved onto the surface with a speed that is around 2 mm/s. In the model we considered to move the probe in a line and to treat the same volume in subsequent steps. This movement enables to cool the external surface, where the highest temperature rise is detected, and in the meantime to accumulate the temperature rise in the deep tissue. During the whole procedure is thus possible to maintain a quasi-constant value in depth in the interested volume.
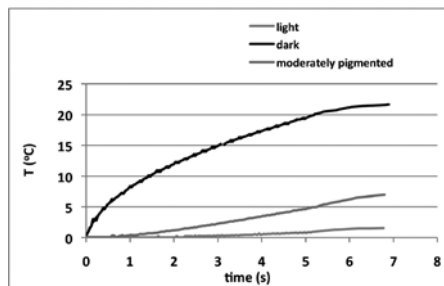
Figure 6: Temperature rise at z=1 mm in a light pigmented human skin, in two different treatment modalities (continuous wave and pulsed mode at 700 Hz) lasting 5s.
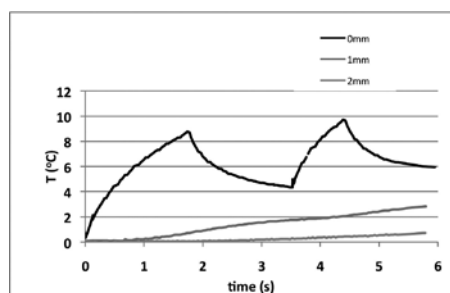
Figure 7: Temperature elevation at different depth in a light (A) and dark (B) skin, during the arthrosis treatment, considering the handpiece gently moved onto the external surface. The epidermis (z=0) is cooled by the ambient air when the handpiece is moved, while inside the tissue thermal confinement is achieved, resulting in a thermal enhancement and in the possibility to control an asymptotically quasi constant temperature value, around 40°C during the whole procedure time.

4. Discussion and conclusions

In this paper we present a modelling study of laser-tissue interaction in the specific applications of lasers for rehabilitation and pain relief. The study was supported by a preliminary experimental measurement session in animal model, evidencing a good agreement between the simulation study and the measured data. The model was thus used to study the same laser-tissue interactions in humans. Different skin types were studied, evidencing that in dark pigmented skin the thermal effects are more intense, because of the presence of a higher concentration of natural chromophores. This result is particularly true when considering the first superficial layer (the epidermis), where

the main natural chromophores of the skin are localized, i.e. the melanosomes. As reported in literature (3), the volume fraction of melanosomes in the epidermis is in the range 1.3-6.3% for a light-skinned adult, in the range 11-16% in moderately pigmented adults and 18-43% in darkly pigmented adults. This study evidenced that, if the handpiece used for delivering the laser light is maintained fixed in the same position onto the external skin surface for 5s and more, very high temperatures are induced in a dark pigmented subject, with a high risk of thermal damage or induced pain (the threshold of thermal damage is around 60°C), while in a light pigmented skin very modest temperatures are reached in the same time. This should be taken into account when treating patients with different pigmentation, and particularly during the treatment of the trigger points, where the light probe is maintained in a fixed position. In this case the handpiece is in close contact with the skin and acts as an adiabatic wall, inducing accumulation of the temperature at the interface handpiece/tissue. If the probe is maintained far (at least few millimetres) from the skin surface, natural convection due to the temperature difference with the ambient air (that is supposed to be cooler than the human body) or, moreover, the presence of an air flux (not considered in the present study) may help in cooling the external surface without significantly affect the thermal distribution inside the tissue. The same model was used to evaluate a treatment modality close to the real clinical protocols, which can include both fixed (trigger points) and scanned irradiation of the skin: in the first seconds of the treatment the handpiece is maintained in the same position, in contact with the skin, so as the temperature rises to a some effective but not harmful values. In the last part of the procedure the handpiece is scanned onto the skin. A very slow motion has to be maintained, in order to transfer an homogeneous temperature rise in the

deep skin. The duration of the starting phase and the speed of the motion strongly depend on the skin pigmentation, as evidenced in figure 7: in a dark skin very few seconds (2s) and a high speed linear motion (2mm/s) enable to reach and then to maintain a modulated and therapeutic effect, when the temperature has to be kept in the range 40-43°C in depth. This effect is reached because during the movement of the probe the external surface that is not in contact with the handpiece may be cooled, while in the deep tissue there is thermal confinement, so that we observe thermal accumulation effects while preserving the epidermis from thermal damage. In conclusion in this modelling study, that was supported by experimental evidence, we evaluated the temperature enhancement in the tissue during the irradiation with IR laser used in rehabilitation therapies. The results evidenced that it is possible to induce a therapeutic effect inside the tissue, modulating the thermal effect by the control of a continuous linear motion of the handpiece onto the external skin surface. Particular care should be taken into account when treating patients with different skin pigmentation, selecting very short exposure time in dark pigmented skin when the handpiece is maintained fixed in close contact with the tissue, and a higher speed when the handpiece is kept in continuous movement. The modelling approach used in this study may thus be proposed as an useful tool to draft guidelines to be included in clinical protocols.

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Laser-tissue interaction principles: tissue optical properties in the light therapeutic window (invited review)

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ABSTRACT

The ability of light to penetrate a tissue and deposit energy in tissues is key to therapeutic applications. In this context, the knowledge of the optical properties of the various biological tissues is mandatory, since the efficacy of laser treatment depends on photon propagation and fluence rate distribution within irradiated tissues. Photon propagation in biological tissue is characterized by the basic optical properties of absorption, scattering and refractive index variations. These properties govern the numbers of photons that are transmitted between points on the tissue surface and deep into the tissue. Even for thin, sub-millimeter sections of tissue, injected photons are likely to be scattered several times before they reach the boundary. As a consequence a coherent, collimated input laser beam will be effectively incoherent and isotropic after traversing a few tissue millimeters, as scattering does not generally preserve coherence.

1. Introduction to tissue optical properties

An exact modelling of the inhomogeneous and turbid tissue is not presently feasible. The tissue is therefore generally represented as an absorbing bulk material with scattering particles randomly distributed over the volume. Further, it is usually assumed to be homogenous (e.g. with constant density), even if this is approximation is not always a good model. The parameters used to characterize the optical properties of the tissue are: the absorption coefficient (μ_a), the single scattering coefficient (μ_s), the transport coefficient $\mu_t = \mu_a + \mu_s$ and the phase function $p(s, s')$. For the interpretation of μ_a, μ_s and μ_t we refer the reader to previous articles [1,2]. We remind also that units for μ_t, μ_a and μ_s are usually cm^{-1}.

The function $p(s, s')$ is the probability density function, giving the probability that a photon undergoes scattering from

an initial propagation direction s to a final direction s'.

The optical coefficients μ_a and μ_s are related to the absorption and scattering cross-sections (σ_a and σ_s respectively) by $\mu_a = \rho \, \sigma_a$ and $\mu_s = \rho \, \sigma_s$. We remind that σ is proportional to the probability that a photon undergoes an absorption (σ_a) and scattering (σ_s) event respectively, while ρ is the particle volume density. In this context independent scatterer approximation is implicit. This corresponds to the assumption that scattering from one particle is not influenced by scattering from other particles, so that the so called "multiple scattering" is neglected.

The average cosine of the scattering angle is denoted by g. It is also referred to as the anisotropy parameter, and represents the average propagation direction of a photon after one scattering event. The value of g ranges from –1 to +1, where g = 0 corresponds to isotropic scattering (i.e. all scattering directions have equal probability), g = +1 corresponds to ideal forward scattering (i.e. the beam continues propagating straightforward) and g = –1 corresponds to ideal backward scattering (i.e. the beam is back-reflected). The coefficients μ_a , μ_s and g may be interpreted as follows. The absorption and scattering coefficients equal the average number of absorption and scattering events per unit path length of photon travel in the tissue, respectively. Any additional scattering event tends to randomize the photon direction, according to the value of g. For example, it can be found that a photon acquires random direction after about 1/(1 – g) scattering events, which is only five for g = 0.8. Typical values of g for biological tissues vary from 0.7 to 0.99, so that 3-100 scattering events are necessary to obtain photons with random directions if a collimated beam penetrates into the tissue. An exact calculation of the cross-sections for the poorly characterized absorbing and scattering centers located in a biological tissue is not feasible. The average optical constants of relatively

homogeneous tissues can be calculated from experimental data by fitting the experimental results with appropriate models. The scattering seen in tissue is mainly due to cells and is dependent on the cell morphology. Scattering can be caused by the cell nuclei, mitochondria, lysosomes, and the Golgi apparatus. At small incident angles the cells themselves are responsible for scattering, whereas at larger incident angles the nuclei of cells may be responsible for it. Cell refraction indices must be considered to apply the scattering theory. To model this, Mie theory is often used, treating the scattering particles as individual spheres distributed either monodispersely or polydispersely with an incident planar electromagnetic wave as a function of the distance between the observer and the particles, the scattering angle, the refractive index and the diameter of the particles.
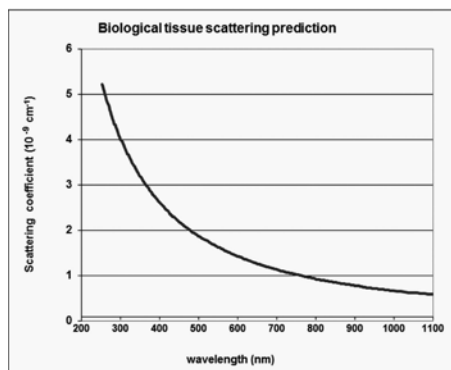

Figure 1: Theoretical prediction for the scattering coefficient of a human tissue, mean values.
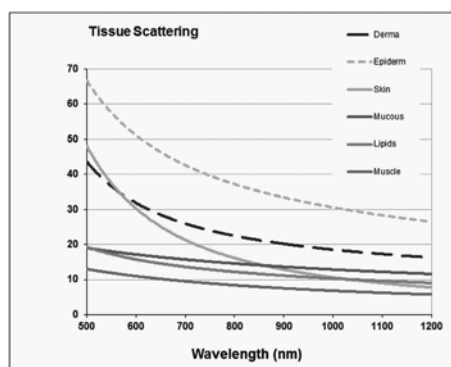

Figure 2: Scattering coefficient of various tissue types (adapted from 3).

The absorption coefficient varies greatly over the visible spectrum, while the scattering coefficient of tissue decreases monotonically as the wavelength increases (Figures 1,2). The presence of chromophores affects the absorption coefficient. There are a variety of chromophores, both natural and exogenously supplied, which can contribute to μ_a. Usually, blood and water will dominate the absorption. In relation to the visible part of the spectrum, melanin, fat, bilirubin and beta-carotene must be considered. Other chromophores present minor contributions. If one is interested in spectroscopic detection, then the minor contributions are important. If one is interested in understanding light penetration into a tissue for some therapeutic protocol, then the minor contributions usually do not significantly perturb the light transport.

Let us conclude this part by analyzing the most important tissue chromophores and their contribution to μ_s and μ_a.

Blood

The major contribution to blood optical absorption is due to hemoglobin, both in its oxygenated and deoxygenated forms. The absorption spectrum for deoxy-hemoglobin and oxy-hemoglobin are distinctly different, thus resulting in difference in total absorption as a function of oxygen saturation. The absorption spectrum of oxy-hemoglobin peaks between 400 nm and 600 nm and deoxy-hemoglobin peaks between 400 nm and 850 nm. The absorption coefficient of whole blood is represented in Fig. 3, which shows fully oxygenated and deoxygenated blood. Reliable data beyond 1000 nm wavelength is difficult to find in the literature. Beyond 1000 nm water absorption (Fig. 4) might begin to dominate over hemoglobin absorption.
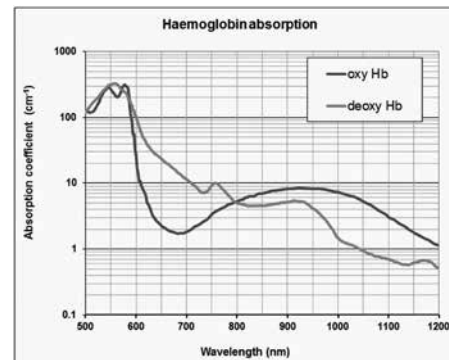
Figure 3: Hemoglobin absorption coefficient in the range 500-1200 nm (adapted from Prahl 2012, cited in 5).
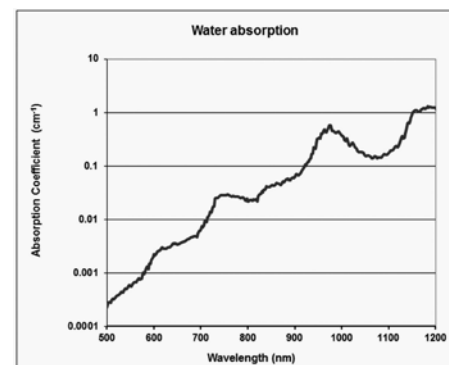
Figure 4: Water absorption coefficient in the range 500-1200 nm (adapted from 4 and 5).

Water

Although water is nearly transparent in the range of visible light, it becomes absorbing over the near-infrared region. Water is a critical component since its concentration is high in human tissue. The absorption spectrum of water in the range 500-1200 nm is shown in Fig. 4 [3,4,5 and bibliography therein]. Although absorption is rather low in this spectral range, it still contributes to the overall tissue attenuation.

Melanin

Melanin is the chromophore of the human skin epidermal layer responsible for protection from harmful UV radiation. When melanocytes are stimulated by solar radiation, melanin is produced. Melanin is one of the major light absorbers in some biological tissue (although its contribution is smaller than other components). There are two types of melanin: eumelanin which is black-brown and pheomelanin which is red-yellow. The molar

extinction coefficient spectra corresponding to both types are shown in Fig. 5.

Figure 5: Absorption coefficient for Eumelanin (orange curve) and Pheomelanin (blue curve) – adapted from 5.

Yellow pigments: Bilirubin and Caroten

The yellow pigments, bilirubin and β -caroten, are sometimes present to a small degree in the absorption spectra of tissues. Bilirubin absorption in the skin is routinely used to detect hyper-bilirubinemia in neonates. β -carotene can also give a yellow hue to tissues. The extinction coefficients spectra (data not shown) for bilirubin and β -caroten in vivo are in the violet-blu range of the visible light, adding their contribution to the main absorption band of other pigments like haemoglobins and melanins.

2. Physical properties and structure of the investigated tissues

Following our analysis of the main tissue chromophores, let us now consider the most important tissues involved in a therapeutical approach with light: skin, muscle and the mucous membrane. In the following we will briefly present literature data about tissue optical properties. It has to be considered that the complexity and variability of the tissue structure may lead to different results, as for example corrections for water content (i.e. water absorption/scattering) may have been applied.

2.1 Skin

The skin presents a complex heterogeneous medium, where the blood and pigment content is spatially distributed with depth variations. The skin consists of three main visible layers from the surface: stratum corneum (~20μm thick), epidermis (100μm thick, the blood free layer), dermis (1–4mm thick, vascularized layer). We remember that the optical properties of the layers are characterized by the absorption and scattering coefficient, which equals the average number of absorption and scattering events per unit path length of photon travel in the tissue, and by the anisotropy factor, which represents the average cosine of the scattering angles.

2.1.1 Epidermis

The epidermis can be subdivided into the two sublayers: corneous tissue and living epidermis. The stratum corneum (about 10-40 μm thick) consists of only dead squamous cells, which are highly keratinized with a high lipid and protein content, and has a relatively low (~20%) water content. Living epidermis (~100μm thick) contains most of the skin pigmentation, mainly melanin, which is produced in the melanocytes occurring in the stratum basale. There are two types of this pigment: the red/yellow phaeomelanin and a brown/back eumelanin. The relative percentage of these two pigments varies from person to person also depending from the race.

2.1.2 Dermis

Dermis is a vascularized layer and the main absorbers in the visible spectral range are the blood hemoglobin, β-carotene and bilirubin. In the NIR spectral range, absorption properties of skin dermis are defined by absorption of water. The scattering properties of the dermis are mainly defined by the fibrous structure of the tissue, where collagen fibrils are packed in collagen bundles and have lamellae structure. This layer is highly backscattering. The blood volume fraction

in the skin varies from 0.2% to 4%. The volume fraction of water in the dermis is estimated ranging from 65 % to 76%. Fig. 6 shows the penetration depth calculated by these parameters. Because of its thickness, dermis is the skin layer contributing the most to skin optical properties.
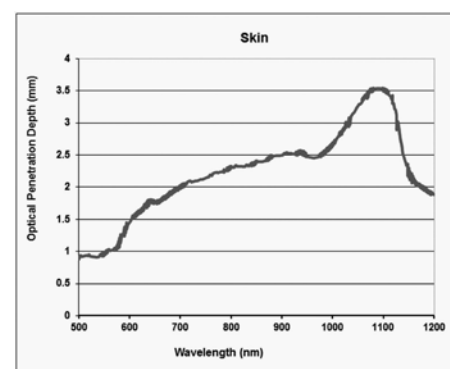
Figure 6: Skin optical penetration depth in the range 500-1200nm, (adapted from 3)

2.1.3 Subcutaneous adipose tissue

The subcutaneous adipose tissue (1-6mm thick depending from the body site) is formed by aggregation of fat cells (adipocytes) containing stored fat (lipids) in the form of a number of small droplets for normal (not obese) humans. In the spaces between the cells, there are blood capillaries (arterial and venous plexus), nerves, and reticular fibrils connecting each cell and providing metabolic activity of fat tissue . [6] Absorption of the human adipose tissue is defined by absorption of hemoglobin, lipids, and water (about 11%) [7] (Figure 7).
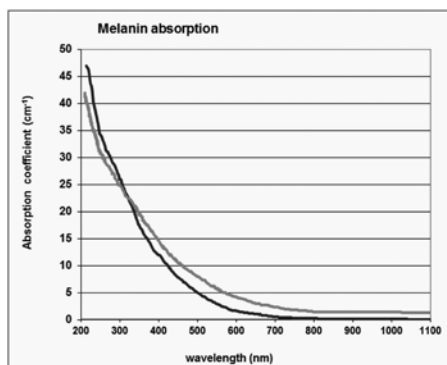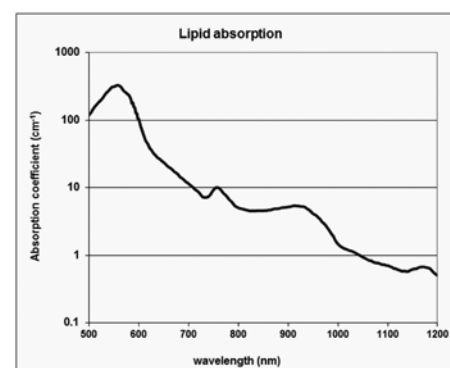

Figure 7: Lipid absorption coefficient in the range 500-1200nm (adapted from 4)

To sum up, the average scattering properties of the skin are defined by the scattering properties of the reticular dermis because of the relatively large thickness of the layer (up to 4mm) and of the comparable scattering coefficients of the epidermis and the reticular dermis. Absorption of hemoglobin (both oxy- and deoxy- forms) and water of the skin dermis and lipids of the skin epidermis define absorption properties of the whole skin.

2.2 Muscle

Muscle is one of the most abundant tissues in the human body. It is well understood that muscle is made up of individual components known as muscle fibers. These fibers are made from myofibrils, which are long cylinders of few μm diameter.
Absorption of the muscle tissue is defined by the absorption of hemoglobin and of water, ranging from 52 % to 73 % according to the specific muscle type and conditions (data from the literature) (52% from [7] or 73% from [8] and [9]).

2.3 Mucous membrane

The proper layer of the mucous membrane is similar in structure to connective tissue, consisting of collagen and elastin fibrils. The interstitial fluid of the mucous membrane contains proteins and polysaccharides and is similar in composition to the interstitial fluid of most of the connective tissues. Figure 8 shows the optical penetration depth in human mucous membrane.
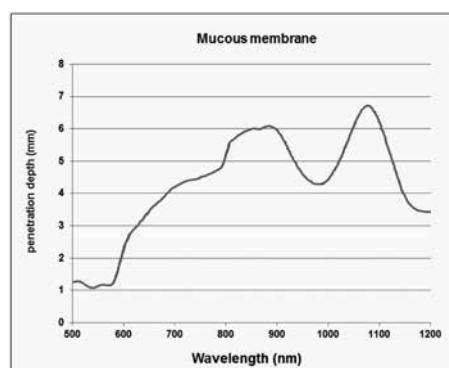
Figure 8: Penetration depth of 500-1200nm light in human mucosa (adapted from 3)

CONCLUSIONS

In this article we have presented a brief review of the physical principles at the basis of the light-tissue interaction, together with experimental data on tissue and tissue chromophore scattering and absorption.
The concepts of absorption and scattering are fundamental and relatively simple, but stem for much complex studies of laser interaction with human tissues. After the introduction of the optical coefficients μ_a, μ_s and g, we have analyzed the main tissue chromophores and, then, tissue optical properties. Most light therapies are concentrated in few but fundamental organs like skin, muscles and the mucous membrane. Even if most literature in this field is based on experimental data, nevertheless the understanding of the basic physical principles remains a fundamental step to understand and plan future applications.

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Nd:YAG laser in the management of low back pain.

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ABSTRACT

It is well established that low back pain is a common musculoskeletal disorder in adult population and has high social-economic impact. Among therapeutic methods we chose to test those suggested by international literature as effective, such as high intensity laser therapy, versus standard physiotherapy protocol (ultrasound, T.E.N.S., massage, mobilization and exercises). We designed a trial with three therapy groups. The main objective was to compare the efficacy of a specific Nd:YAG laser (HILT) with a standard physiotherapy protocol on low back pain and a combination of the two methods. It was within the scope of this paper to address in detail the variation of pain through pain type and localization for low back pain patients. Analyses showed that a significant number of patients with acute pain gained full recovery (28,8%), while patients affected by chronic pain obtained a significant improvement (31,1%) of pain symptoms, but not healing. Focal pain was healed more effectively than widespread pain. Final assessment showed that standardized physiotherapy (group A) did not manage to bring full recovery to patients, but just improving. Hilterapia® (group C) had better results in comparison with the standardized physioterapeutic approach and showed early analgesic effects. Patients treated with both HILT and standardized physiotherapy showed the higher clinical-functional improvement (group B), when compared with the other groups of patients. The results showed that a therapeutical protocol based on the combination of HILT and standardized physiotherapy may be successfully used to obtain improvement of pain symptoms and early healing in patients with non-specific pain of the lumbar area.

INTRODUCTION

Low back pain is a widespread musculoskeletal disorder in adult population and therefore has high social-economic impact.

Most of the patients are experiencing symptoms every year and 50-80% of general population will experience at least once in their lifetime affecting their quality of life [1]. It is the most common cause of functional disability among employees [2]. The past 15 years have seen an intensive research effort to identify effective treatments and management strategies for low-back pain [3].

Non-specific low back pain is a painful and self-limiting condition. Apart from severe pathology cases, current guidelines recommend pain management interventions plus reassurance and advice to stay active [4]. The aim of conservative treatments is usually to relieve pain and reduce associated disability. Several treatment options are recommended, but there is sound evidence for only a minority of them [3,5].

Physiotherapy approach uses analgesia and anti-inflammatory effects to reduce pain symptoms.

Methods such as ultrasound, T.E.N.S., massage, mobilization and exercises, yielded mixed results [6,7]. Among physiotherapeutic modalities, there is little evidence to support the therapeutic effectiveness of ultrasound and T.E.N.S. in low back pain [8,9]. The total effect of these modalities on duration of the symptoms and among the different types of pain, vary and is quite limited [10,11]. Recent bibliography suggested that physical therapy has not provided stable results due to variability of the causes of low back pain and different modalities in the application of therapies [8,12,13]. Many studies have shown physiotherapy to be effective in improving the symptoms, proposing physiotherapic treatments as an acceptable physical modality for trigger points or muscle spasms [13]. Several studies reported the effectiveness of laser therapy in the treatment of low back pain, especially combined with exercises [14,15]. High intensity laser therapy (HILT) may be beneficial for pain relief and improved disability in patients with acute or chronic symptoms [16]. The wavelength (1064 nm), high intensity (up to 15,000 W/cm^2) and high energetic impact characteristic of HILT, can effectively cure even the deepest chronic lesions [17]. It is well known that the variety of interaction mechanisms has positive effects and may occur when applying laser light to biological tissue for repair and pain management [18-20]. Laser radiation alters cellular functions involved in tissue repair. The photothermal processes produce tissue heating that reduces muscular spasm and supports relaxation, therefore with a general analgesic and sedative effect against pain.

For our study, among therapeutical methods commonly used, we chose to

test those suggested by international literature as effective, such as laser therapy, versus ultrasound, T.E.N.S., massage, mobilization and exercises. The main objective in this article is to compare the efficacy Nd:YAG laser (HILT) with a standard physiotherapy protocol on low back pain and also evaluate combination of the two methods. It is within the scope of this paper to address in detail the variation of pain through pain type and localization for low back pain patients.

METHODS
Participants & procedure
The recruitment and therapies took place in ARCOS, a private practice of medical rehabilitation in Athens, from October 2012 to January 2013. All patients had been recently diagnosed with non-specific low back pain. We made classification according to localization of the pain in focal and diffuse, and for the type of the pain in acute and chronic. All patients attended clinical practice and were asked to participate the study. After a detailed briefing, a written consent was asked to confirm their participation. The recruited patients aged 18 to 70 years and had Greek ethnicity.

Exclusion criteria concerned patients diagnosed with tumor or any neoplasmic disease, use of NSAID's or heavy analgesic treatment, patients with psychiatric history, recent surgical procedure in the lumbar area, dermatoses or tattoo marks in the same area.

Forty-five patients (25 woman and 20 man) suffering with non-specific low back pain were recruited for the study. Participants were randomized into 3 groups: Group A) physiotherapy (n=15), Group B) Hilterapia® and physiotherapy (n=15), Group C) Hilterapia® (n=15). The 10-sessions treatment protocol lasted four weeks. The therapy sessions for each group included: Group A: 10 physiotherapy sessions. Group B: 10 laser

treatments +10 physiotherapy sessions. Group C: 10 laser treatments.

Randomization and blinding
The patients during their first visit for physiotherapy were randomly assigned to therapy groups A, B or C, using random numbers generated by an online generator (www.randomizer.org), which is based on a computer algorithm. Randomization, baseline and final assessments were not blinded.

Protocols and measures
Each group was provided with verbal and written information concerning physiotherapy assessment and laser effects. The 10-sessions protocol was completed for each patient in 4 weeks. Sessions were administered as follows: 3 treatments/week for the first who weeks and 2 treatments/week for the following two weeks.

Visual Analogue Scale (VAS) is a pain score with scale 0-10, which was used to evaluate the subjective pain symptomatology before each application. Furthermore, after the 10-session protocol each patient received a total physiotherapy assessment and the level of recovery was evaluated in a 4-point scale (cure, improve, unchange, worse).

The physiotherapy assessment included a standard protocol with ultrasound application, electrotherapy through T.E.N.S., massage, active and passive mobilization and exercises, all specific for the lumbar area [21]. Physiotherapy treatment lasted 60-90 min and modifications were performed only in mobilization and exercises section according to patients needs.

The laser applications were performed with Hilterapia®. The source was a pulsed Nd:YAG laser Hiro 3.0 device (ASA s.r.l., Vicenza, Italy), with the standard handpiece for pain therapy, which was oriented vertically to the surface of the patient's body. The treatment lasted 30 minutes. The preferred protocol

was chosen by a physiotherapist who is experienced in using the device (Table I). The protocol included three phases (initial, intermediate, final). The initial phase, divided into three sub-phases and considered as a "cold" treatment, requires fast scanning and helps activate muscular relaxation and analgesia. The intermediate phase, which is static and purely analgesic, is divided into four sub-phases and carried out on the trigger points or pain points. The final phase, which is also divided into three steps and carried out with slow scanning, creates a slight local hyperaemia. In this manner, wash out of catabolites is activated and the antalgic effects, muscular relaxation and the articular range obtained during the previous phases improve. When necessary, according to the painful symptoms of each patient, modifications on the Hilterapia® protocol were done by shortening the intermediate phase.

Phase	Subphase	Fluence (mJ/cm²)	Frequency (Hz)	Mode	Total energy (J)
Initial	Step 1	710	11	Fast	500
	Step 2	970	9	Fast	500
	Step 3	1070	4	Fast	500
Intermediate	Step 1	710	4	Static	(Depended on the number of treating points)
	Step 2	970	4		(Depended on the number of treating points)
	Step 3	1070	3		(Depended on the number of treating points)
	Step 4	710	5		(Depended on the number of treating points)
Final	Step 1	710	11	Slow	500
	Step 2	970	9	Slow	500
	Step 3	1070	4	Slow	500

Table I: Treatment protocol for Hiliterapia.

Characteristics	Percentage (%)	Group A (n)	Group B (n)	Group C (n)
Age (mean ± SD)	47,07 ± 14,11			
17-32	6 (13,3)	2	2	2
33-47	19 (42,2)	5	9	5
48-62	12 (26,6)	5	3	4
63-77	8 (17,7)	3	1	4
Sex				
Woman (n[%])	25 (55,6)	10	9	6
Man (n[%])	20 (43,5)	5	6	9
Cause				
Acute (n[%])	22 (47,8)	4	9	9
Chronic (n[%])	23 (51,1)	11	6	6
Location				
Diffuse (n[%])	20 (43,5)	5	11	9
Focal (n[%])	25 (55,6)	10	4	6

Table II: Baseline characteristics of the 45 participants.

Statistical methods

Group characteristics were presented as means, SD and percentage values. The mean values ±SD were used as outcomes. The statistical calculations were performed using the SPSS for Windows (version 20) statistical software (SPSS Inc., Chicago IL).

Results

Among the 45 participants, 5 did not complete the 10-sessions protocol, three because of pain worsening and two due to early recovery. The mean age was 47,07 years, with the 42,2% being in between 33-47 and the highest percentage were women (55,6%). Often pain location was focal (55,6%) and cause of pain 51,1% was chronic (Table II).

By analysis of results according to the type of pain, we observed that more than half of the patients with acute pain gained full recovery (28,8%) in contrast to chronic pain patients, who had generally an improvement (31,1%) in pain symptoms (Table III). As regards pain location, the final results showed that focal pain was healed more effectively than widespread pain (Table IV).

In each treatment session, the Mean VAS scores of patients were evaluated for each group (A, B and C). As shown in Figure 1, at the beginning all groups ranged into the same pain levels. On 4th session, group B (Hilterapia® and physiotherapy) showed signs of early improvement, which continued over the next sessions. The final assessment showed minimum pain level with VAS 2,64 for group B, VAS 3,57 and 4,33 for group C and A, respectively.
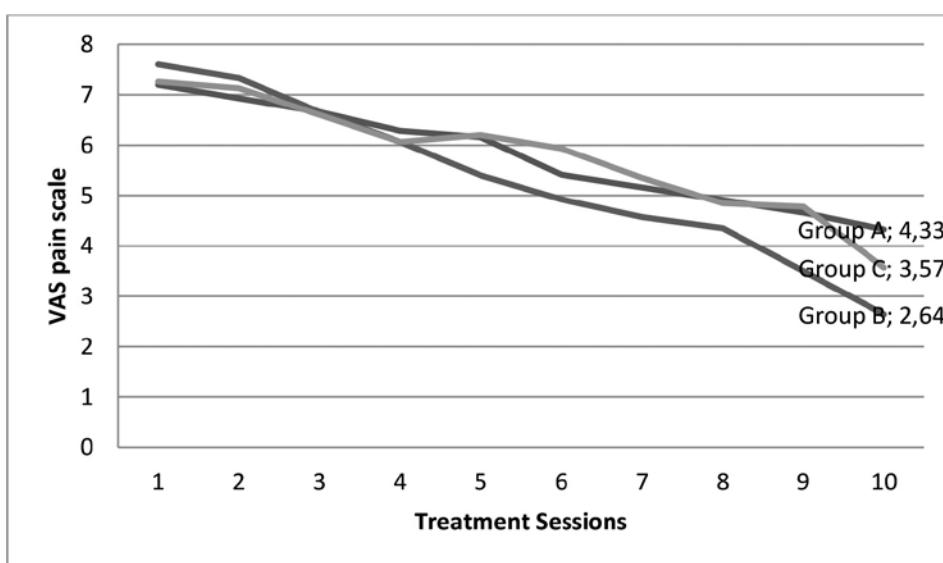
Figure 1: Pain levels through sessions per therapy groups

		Assessment				Total n
		Full recovery	Improve	Unchange	Worse	
Pain Type	Acute	13 (28,8%)	9 (20%)	0 (0%)	0 (0%)	22 (48,8%)
	Chronic	0 (0%)	14 (31,1%)	6 (13,3%)	3 (6,6%)	23 (51,1%)
Total n		13 (28,8%)	23 (51,1%)	6 (13,3%)	3 (6,6%)	45 (100%)

Table III: Crosstab of final assessment and pain type [n(%)].

		Assessment				Total n
		Full recovery	Improve	Unchange	Worse	
Localization	Focal	11 (24,4%)	13 (28,8%)	0 (0%)	1 (2,2%)	25 (55,5%)
	Diffuse	2 (4,4%)	10 (22,2%)	6 (13,3%)	2 (4,4%)	20 (44,4%)
Total n		13 (28,8%)	23 (51,1%)	6 (13,3%)	3 (6,6%)	45 (100%)

Table IV: Crosstab of final assessment and pain localization [n(%)].

		Assessment				Total n
		Full recovery	Improve	Unchange	Worse	
Therapy groups	Physiotherapy	0 (0%)	12 (26,6%)	2 (4,4%)	1 (2,2%)	15
	Hilterapia® & physiotherapy	8 (17,7)	6 (13,3%)	0 (0%)	1 (2,2%)	15
	Hilterapia®	5 (11,1%)	5 (11,1%)	4 (8,8%)	1 (2,2%)	15
Total n		13 (28,8%)	23 (51,1%)	6 (13,3%)	3 (6,6%)	45

Table V: Final assessment through therapy groups [n(%)].

Finally physiotherapy assessment per therapy groups (table V) showed that physiotherapy (group A) failed to provide full recovery to patients, but led to an improvement. Hilterapia® (group C) had better results, but some patients did not show significant changes of the symptomatology. The combination of Hilterapia® with the standard physiotherapy protocol was the most effective therapeutic approach in relieving low back pain.

DISCUSSION

This study was designed to evaluate the effectiveness of HILT against and within standard physiotherapy treatment in non-specific low back pain. It has been reported by other authors that HILT is effective in reducing low back pain [15,22,23]. In our study, the patients treated with Hilterapia® showed an improvement that persisted over time and, on average, was higher than the result observed in patients treated with standard physiotherapy protocol. Pain levels strongly and significantly decreased in patients exposed to a combination of HILT and standard physiotherapy protocol. The linear regression chart revealed that this combined treatment appeared the most effective therapy up from early sessions. Patients with focal pain were treated more successfully than the ones

with widespread pain. We speculated that, being the duration of the treatment the same (30 min), laser radiation is more effective on a limited area because the energy delivered to the tissue is higher and photothermal effects increase. The increased articular and muscular recovery made it easier for the therapist to carry out the typical rehabilitation exercises and manual therapy. Therefore the application of HILT resulted a very useful tool for the management of acute pain, (such as that caused by sports injuries).

This study has some limitations:

a) Low back pain is a disease characterized by complex symptomatology and many possible causes. Therefore categorization of symptoms in homogeneous groups, diagnosis and classification are difficult. In literature, many different systems of classification have been proposed on the basis of symptoms, involved structures, duration of the disease, etc. and there is no agreement on what is the best [24,25]. We chose to recruit patients according to symptoms and not by exact diagnosis.

b) An estimated bias arises from the arbitrarily chosen number of sessions: 10 treatments.

CONCLUSION

Each group of patients showed an improvement of the clinical parameters, but the subjects treated with both therapies (standardized physiotherapy and lasertherapy) showed a more evident improvement. This therapeutic approach can be recommended as the most effective and mainly in acute and focal pain types. Moreover, on the basis of our findings, HILT resulted more effective in improving pain symptoms and promoting an earlier healing of disease than the only standardized physiotherapic approach, when applied at patients with non-specific pain of the lumbar area. Future studies should extend these findings using differentiation of lumbar disorders according to diagnosis, examining other similar therapeutical tools and using objective analytical techniques to evaluate the effectiveness of the treatments.

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Laser Acupuncture in the management of muscolo-skeletal pain and hemophilic arthropathy: a brief analysis of theorical basis.

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ABSTRACT

The article discusses the possibility and the potentiality of treating hemophilic arthropathy with laser acupuncture. Laser acupuncture is a modern technique to stimulate acupoints without needling. Laser acupuncture is a safe and painless tool to manage osteoarticular pain. Parameters, dosages and modality are discussed: laserpuncture needs lower frequency and lower dosage than other laser's protocols. In hemophilic arthropathy it could be a good non-pharmacological treatment in the management of chronic pain.

INTRODUCTION

Hemophilia is a hereditary disease that affects coagulation system, which causes internal or external bleeding episodes. Patients with more severe forms of hemophilia suffer severe and frequent bleeds, while patients with mild hemophilia usually suffer minor symptoms. Repeated bleeding into joints, mainly knees, elbows and ankles, which occurs after minor injuries, can lead to arthropathy ("haemophilic arthropathy"), with associated pain, loss of range of movement and loss of function. Deep internal bleeding may affect deep-muscles, leading to swelling, numbness and pain of a limb. Musculoskeletal and articular problems are therefore common manifestations of hemophilia.

Despite severe arthropathy development, such patients limit the use of analgesics due to the risk of bleeding. The orthopedic state is correlated with the quality of life, and hemofilic arthropathy, with its load of pain and functional limitation, is an important factor of disability of the hemophilic patient (1.2). The difficulty in pharmacological treatment of joint pathology, that limits the field to a few drugs, is noticeable. Also, infiltrative intra-articular therapy, which is effective and widely used in chronic arthritis, may be difficult to perform for the risk of intra-articular bleeding. For the same reasons also kinesitherapy must be performed carefully and by expert operators.

A recent review of the European Hemophilia Therapy Standardisation Board (3) notes that evidence-based guidelines on the management of pain in hemophilic patient are not yet available, despite the fact that analgesics are prescribed in 80% of cases. As haemophilia is a potentially disabling condition, any treatment that can reduce the intake of analgesic drugs, and control pain, would be considered useful.

Acupuncture today is considered a good non-pharmacological tool in treatment and pain control in osteoarthritic disorders: the mechanisms of action are well known and studied for decades by the international scientific community (4,5). Acupuncture uses the stimulation of specific points of the body, variably based upon the situations, to obtain, through local reactions and general reaction, a lowering of pain and the thrust to healing. The most classic method of stimulation of points is, as the name says, with dry needles (particular and very thin needles). The acupuncture point was recently defined as NAU ("Neural Acupuncture Unit"), which corresponds to an anatomy set particularly dense of neuronal and neuroactive components, present in skin, muscle and connective tissue (6-7). From the viewpoint of western medicine, acupuncture is a sensory stimulation technique (through the activation of peripheral fiber A-delta and C) applied at

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specific points, acupuncture points and/or trigger points, which activates the pain pathways of the SNC, causes the release of pain-relieving substances and induces a rebalancing of the muscular contractility and of the sympathetic nervous system. The WHO's documents, which became milestones in the acupuncture literature, show several pathological situations in which acupuncture is proven, that in the osteoarticular field mainly concern headache, knee pain, back pain, cervicalgia, epicondylitis, shoulder pain, and rheumatoid arthritis (8.9).

Acupuncture and hemophilia.
In the archives of Pubmed only 3 articles are recoverable that deal with the topic of acupuncture in heamophilia (10,11,12). In the study of Lambing (2012) nine subjects participated: six of the nine subjects reported an improvement in pain scores by at least 50%, no one of the subjects experienced bleeding, and Authors conclude that acupuncture therapy can be a safe additional modality for pain management therapies in persons with hemophilia. Wallny and Co. (2006) applied acupuncture in twelve hemophilic subjects, and ten of 12 patients showed an improvement of their pain perception, without side effects. Rosted (2002) presented a case report of an hemophilic patient with pains due to arthropathy, successfully treated with acupuncture. Even if very preliminary, these works show the effectiveness of acupuncture on pain from hemophilic arthropathy, together with the absence of side effects.
In a recent review (26) Young et CoA. underline that despite being a pervasive problem, chronic pain is suboptimally treated in subjects with hemophilia and propose a multimodal approach, which includes non-pharmacological interventions, including complementary treatments.
The need for the use of needles makes however acupuncture potentially harmful in hemophilic subjects, even if the risk of bleeding seems to be minimal.

Laser acupuncture.
Actually, to stimulate the acupoints the risk of bleeding could be entirely eliminated: in fact it is known that the acupuncture points also react to types of stimulation different from the needle. A form of modern stimulation, which could be alternative to the classic treatment with needles, is Laser-acupuncture (13), which consists in the treatment of acupuncture points with a laser light beam. The laser acupuncture is defined as "stimulation of the traditional acupuncture points with laser irradiation at low intensity, which does not induce heat". The generated light ray is directly applied on the skin at the site of acupuncture points and/or trigger points (ASHI points). The points for laser treatment are selected using the same rules for the selection of classical acupuncture. The scientific literature on laser acupuncture is broad enough even if not conclusive, and it is not yet clear whether there are differences between laser and acupuncture or not (14-20). The stimulation of acupuncture points with laser beam seems to produce similar effects to the classical acupuncture, both at clinical and neuro-molecular level. The effects of laser acupuncture are in part attributable to the peripheral nerve stimulation, with modulation of afferent input on spinal neurons, and to the increase of analgesia due to endogenous opioids, through action on central mechanisms. The laser also acts at the cellular and local level, with evidence of immune processes modulation.
Some of the researches suggests that laser acupuncture can be even more effective than the classical acupuncture. In fact laser acupuncture combines the effects of acupuncture to the stimulation effects of the laser beam. The "photo-biostimulation", i.e. the trigger function of specific wavelengths laser on the acupoint, can cause biochemical, electrochemical and structural changes at the cellular level, which are specific to the laser light (and not the needle), triggering

additional factors on the outcome of the disease. The most important factor of the effectiveness of the laser acupuncture is the depth of action, and the technical parameters have recently been defined (21). The power of the laser beam in laser acupuncture is between 5-500mW (laser Class IIIb), while we remember that the lasers used in surgery exceed 100W. The therapeutic effect of laserpuncture is obtained for wavelength range between 600 and 1000 nm. With the red laser (600-700 nm) there is a poor tissue penetration, so it is usable for the treatment the surface points (points Jing Well) on the tip of the fingers of the hands and feet, while the IR laser (800-1000 mm) are required for the points on the arms, legs, back, and Ashi points. A laser beam of 5-20 mW directed on the skin does not produce pain, nor heat, nor other feelings. The stimulation frequency is very low (10 Hz) and continuous or pulsed mode evoke different reactions (22). For additional treatment details see TABLE I.

TABLE I:
Possible parameters of laser acupuncture in hemophilic arthropaty.
In acupuncture the depth of acupoints differs from each other, and the necessary depth of needle insertion for each point is specified in acupuncture tests. Therefore the laser ray must change in relation to the depth of the point to stimulate: as transmission in skin varies accordingly with wavelength, use 600-700 nm laser emission for superficial points (such as Ting points) and 800-1000 nm emission for deeper point (such as trunk and limb points). Different is also the light emission modality, because continuous or pulsed modality produce different effects, hyperpolarization or depolarization of nervous fibers: according to Chinese acupuncture we recommend continuous modality to obtain "tonification" and modulated modality to obtain "dispersion". In laser acupuncture also beam diameter, which should be 2-4 mm, is very important.

Joint	Main ACUPOINTS in meridians	LASER PARAMETERS Wavelenght, frequency, power	LASER PARAMETERS Dosage, treatment time
Knee	34 GB, 34 ST, 35 ST, 36 ST, 10 SP, 9 SP, Ro Ting point, Xiyan points, ASHI points	600-1000 nm 10 Hz 5-500mW	0.2 -0.5 J/cm^2 (for superficial points, i.e. Ting point) up to 4-8 J/cm^2 (for deep points, i.e. trunk and limb points) Stimulation time: from 10 sec up to few minutes
Elbow	10 TB, 11TB, 11 LI, 8 SI, 3 LU, 10 LI, 5 SI, 3 SI, 4 LI, ASHI points	600-1000 nm 10 Hz 5-500mW	0.2 -0.5 J/cm^2 (for superficial points, i.e. Ting point) up to 4-8 J/cm^2 (for deep points, i.e. trunk and limb points) Stimulation time: from 10 sec up to few minutes
Ankle	6 SP, 3 KID, 5 KID, 5 SP, 4 KID, 41 ST, 60-62 UB, 3 LIV, ASHI POINTS	600-1000 nm 10 Hz 5-500mW	0.2 -0.5 J/cm^2 (for superficial points, i.e. Ting point) up to 4-8 J/cm^2 (for deep points, i.e. trunk and limb points) Stimulation time: from 10 sec up to few minutes
Shoulder	3 SI, 5TB, 10-11-13 SI, 13-14 TB, 15 LI, 21 GB or 2 LU, 14-15 LI, 5-7 LU, 10 LI, ASHI points	600-1000 nm 10 Hz 5-500mW	0.2 -0.5 J/cm^2 (for superficial points, i.e. Ting point) up to 4-8 J/cm^2 (for deep points, i.e. trunk and limb points) Stimulation time: from 10 sec up to few minutes

SI = Small Intestine, LIV= Liver, GB = Gall Bladder, TB= Triple Burner, LU = Lung, LI = Large Intestine, SP = Spleen, ST = Stomach, UB = Urinary Bladder, KID = Kidney.

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Only recent research has begun to analyze the possibility of using the high intensity laser acupuncture to reproduce the stimulation of the needle (23).

The other treatment parameter is the treatment time on each point, which is a function of the laser power and of the type of pathology. In general, the higher the power the lower is the treatment time, more time is needed for treatment of joint pain with respect to soft tissue pain, and more time is needed in chronic than in acute conditions. The dosage is expressed in J/cm^2 (density): 1W= 1J/sec. Knowing the type of laser we have we can calculate the time needed. To decide the dosage on the point it must also be taken into account the area of the light beam in cm^2. In laser acupuncture is necessary to keep the tip in contact with the skin to obtain a few millimeters diameter of the beam. Acupuncture points are then treated with different dosages on the basis of the location and the depth of the point to be addressed: in the literature there are prevailing indications on 0.2 -0.5 J/cm^2 up to 4-8 J/cm^2, with time exposure variables depending on the type of laser. The dose should always be adjusted in relation to the assessment of the pathology and the individual response. Laser acupuncture is applied in painful pathologies, acute and chronic pains, lumbago, pain in shoulder, pain from arthritis of the hip, knee, hands, feet, epicondylitis, carpal tunnel syndrome, and in general, in all fields of application of the somatic acupuncture, ear acupuncture and other acupuncture microsystems (scalp, hand). Acupoints must be chosen accordingly with meridian theory of Chinese medicine, which nevertheless includes always the so-called ASHI points, that is painful points. The use of laser acupuncture has grown considerably in recent years due to to its painless nature and to the absence of side effects. Laser Therapy is considered a safe and not dangerous tool, even in hemophilic subjects (24), reserve the operator best-practice.

Laser acupuncture and hemophilia.
Acupuncture, and consequently the laser acupuncture, is a very individualized therapy and, in order to maximize the results, it must be carried out by operators with specific preparation. In acupuncture is not possible to use standard protocols for each patient, however, in joints diseases some points are considered essential, and effective in general. You can then process the treatment plans for each joint, in particular for knee, shoulder, elbow, ankle. With defined protocols laser acupuncture can be easily associated with standard laser treatments, in the same sitting, allowing for greater efficiency, greater speed of action and a smaller number of sittings.

Some practical suggestions, only as example: in painful arthropathy the treatment acupoints could be the following (25): 34 GB, 34 ST, 35 ST, 36 ST, 10 KID, 9 SP, Ro Ting point, Xiyan points, for the knee; 3 SI, 5 TB, 10-11-13 SI, 13-14 TB, 15 LI, 21 GB or 2 LU, 14-15 LI, 5-7 LU, 10 LI, as local points for shoulder pain; and 6SP, 3 KID, 5 KID, 5SP, 4 KID, 41 ST, 60-62 UB, 3 LIV for ankle arthritis. For elbow arthropathy we could se acupoints as 10 TB, 11TB, 11 LI, 8 SI, LU, 10 LI, 5 SI, 3 SI, 4 LI. Never forget that acupuncture doesn't treat only local points but always adds very distal points related to the main involved meridians, in order to re-equilibrate the whole system, so that a knowledge of acupuncture theory and practice is required to obtain the maximum result.

CONCLUSIONS
Some experimental and clinical data suggests an hypothetical importance of laser therapy and laser acupuncture in the management of the arthropathy and musculoskeletal problems in hemophilic patients. This topic certainly deserves in-depth analysis, research programs and clinical validation.

Persons with haemophilia experience persistent joint pain as a result of repeated haemarthrosis; within a multimodal and multidisciplinary approach, the use of laser therapy and specifically of laser acupuncture, could be a safe tool for the control of chronic pain in hemophilic arthropathy and for challenging the musculoskeletal deterioration, in order to improve the patient's quality of life as final purpose.

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