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 cm^2). 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 $$\frac{\partial \Phi_n(r,z,t)}{\partial t} - \nabla(\alpha^n \nabla \Phi_n(r,z,t)) + \mu_a^n \Phi_n(r,z,t)$$

where:

eq 2 $$\alpha_n = \frac{c_n}{3(\mu_a^n + (1-g_n)\mu_s^n)}$$

is the diffusion coefficient; μ_a^n and μ_s^n (1/m) are the n-th subdomain (epidermis, dermis) absorption and scattering coefficient respectively, at the wavelength

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.
Φλ (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).

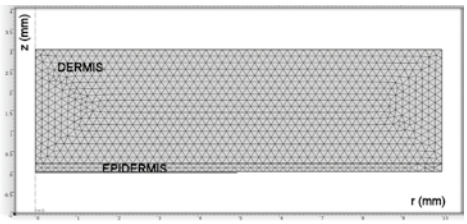

Figure 1: The 2D geometrical model and triangular mesh of the current study. The external skin layer is at z=0. The light propagation is in the z positive direction. We suppose that the laser probe is in close contact with the tissue (at z=0), and the contact surface is evidenced by the horizontal red 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 $Q_{perf} = \rho_b C_b \omega_b (T_b - T)$

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

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.

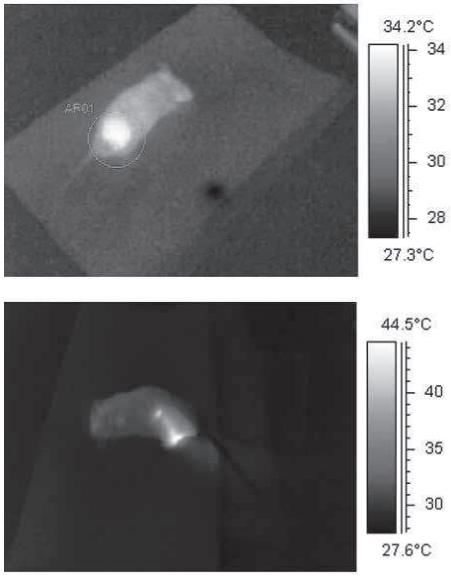

Figure 2: Thermograms soon after laser treatment of a white (up) and black (down) mouse, posterior leg.

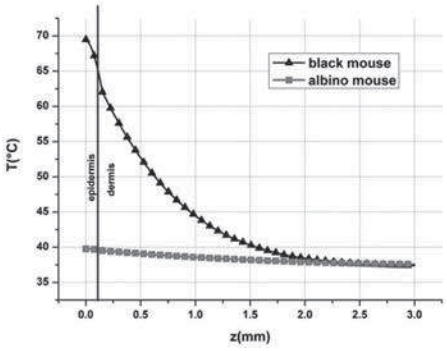

Figure 3: Modeling analysis of temperature dynamics in the deep tissue, at the end of the laser treatment. Differences in black and albino mouse are evidenced.

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

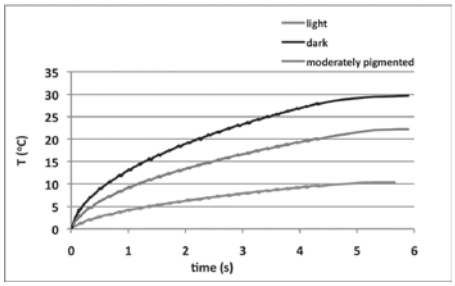

Figure 4: Temperature graphs in skin tissues with different concentration of melanosomes: light, moderately pigmented and dark skin.
The graph represents the temperature distribution on the external skin surface during a 5s treatment time (continuous wave configuration).

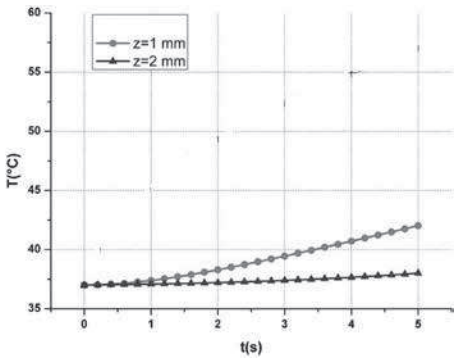

Figure 5: Temperature elevation along the symmetry axis (r=0) in a moderately pigmented human skin, at two different depths (1, 2mm), during the continuous wave treatment.

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.

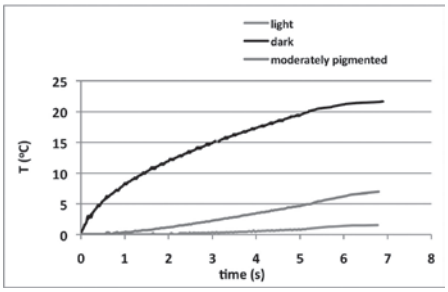

Figure 6: Temperature rise at z=1 mm in a light pigmented human skin, in two different treatment modalities (continuous wave and pulsed mode at 700 Hz) lasting 5s.

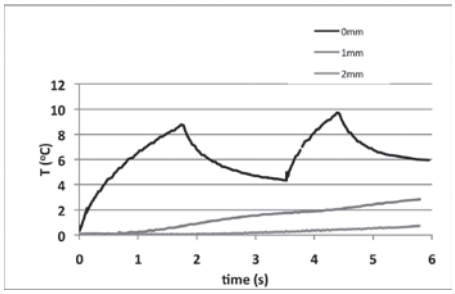

Figure 7: Temperature elevation at different depth in a light (A) and dark (B) skin, during the arthrosis treatment, considering the handpiece gently moved onto the external surface. The epidermis (z=0) is cooled by the ambient air when the handpiece is moved, while inside the tissue thermal confinement 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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