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ACKNOWLEDGEMENTS
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1. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature, 2003, 423: 337-342.
Reference to a book:

2. Michaeli W. Extrusion Dies. Hanser Publishers, Munich, Vienna, New York, 1984.

Reference to a chapter in an edited book:
3. Gmünder FK, Cogoli A. Effect of space flight on lymphocyte function and immunity. In: Fregly MJ, Blatteis CM, eds. Handbook of Physiology. Oxford:University Press, 1996, vol. 2, pp 799-813.

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Contents

Key words: Reflectance, medical lasers, laser safety, UVA-visible-IR

Material reflectance in laboratories with medical lasers: study in the UVA-visible-IR range

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ABSTRACT

Despite the wide diffusion of medical lasers, the interest in laser risk management in the clinical practice is not as rapidly growing. One of the major points of interest is the knowledge of the adverse effects for the personnel using laser sources in the medical practice; this mainly involves the study of the optic behavior of the materials which may interact with the laser beam. These include not only tissues but also tools, clothing, fabrics and in general all the different surfaces which may cause harmful effects to the skin and the eyes, due to their properties of reflecting laser light in an uncontrolled way.

Particular attention should then be paid both for monitoring all the surfaces interacting with lasers, and for analyzing their reflectance at the wavelength corresponding to the laser being used in the specific case.

In this communication, the reflectance of various materials (fabrics, plastics, metals) utilized in a biomedical laboratory was measured as a function of the wavelength of the impinging light, ranging from UVA to the visible and infrared (IR) range. Both specular (45°) and diffuse reflectance spectra were measured by a spectrophotometric technique, as well as small angle reflectance in the IR range by a Fourier Transform IR spectrometer. Results indicate that both bulk material and surface properties play an important role in reflectance properties, which in any case are wavelength dependent. A simple parameter can be defined, which answers to the question "Is this surface more diffusive or specular ?"

INTRODUCTION

The new Italian Normative and Law on Safety [1-4] (and in particular the Italian DLgs. 81/2008) introduces for the employer the compulsory evaluation of the risks deriving from the presence of physical agents [1]. In case, the employer must adopt all the technical and / or organization measures to minimize those risks; this leads to the necessity of a very accurate analysis of all the fonts of risk, in particular for the eye and the skin, in rooms where medical lasers are used. Particular attention should be paid both for monitoring all the reflecting surfaces interacting with lasers in the clinical practice, and for the analysis of their reflectance at the various wavelengths corresponding to the specific laser emission. Nowadays only few experimental studies have been performed in this field, and an exhaustive database of surface reflective properties for a risk management purpose does not exist.

MATERIALS AND METHODS

In this study, samples of various origin were considered among the materials and tools mainly used in a biomedical laboratory, comprising also furniture and fabrics, wall and floor coverings, plastic and metal samples. The goal is the measurement of the reflectance in the spectral interval UVA- visible-near and medium Infra-Red (IR). In particular materials with different surface treatment were selected, such as satin-finished and bright steel.

If laser light strikes a surface, the portion which does not penetrate into the material is partially reflected following the specular reflection laws (specular reflection) and partially scattered in all directions (diffuse reflection) [5]. The balance between the two phenomena depends on the laser emission characteristics (e.g. the wavelength) and the surface properties (material type and roughness degree).

The reflectance measurements consisted in: (i) total and specular (45°) reflectance in the range 250-2500 nm; (ii) small angle reflectance (<10°) in the range 1280-25000 nm.

The sum of the specular (in our case with an incidence angle of 45o: R45) and diffuse reflectance (R_d) is defined as the total reflectance (R_{tot}):

$$\mathbf{R_{tot} = R_{45} + R_d} \quad (1)$$

In equation all the quantities are wavelength dependent.

Total and specular reflectance were measured by a Spectrophotometer (Lambda900, Perkin Elmer) with integrating sphere and a Xenon arc lamp. Small angle reflectance was measured by

a FTIR Spectrometer (Perkin Elmer). In both cases the spectral resolution was of about 1nm.

In order to correlate the reflectance measurements with a single parameter describing the mean reflectance properties of the sample, the following procedure was considered.

Generally speaking (see equation 1), the measurement of R_{tot} does not allow discrimination between R_S and R_d. In addition, the reflected light intensity is not equally distributed in space: different directions are characterized by a different reflected light intensity. That's why separate measurements of R_{45} and R_d were performed and used to define a single parameter (Lmb), which describes how a certain surface is mostly a specular one ($R_{tot} \approx R_{45}$) or, on the other side, a diffusive one ($R_{tot} \approx R_d$):

$$\mathbf{Lmb = 100 \cdot (R_{45} / R_{tot})} \quad (2)$$

As Lmb is wavelength dependent, it can be suggested to perform a mean over a range such as the visible or UVA; alternatively it is possible to calculate Lmb for a specific wavelength, depending on the laser(s) of interest.

If a surface is perfectly specular, then Lmb=100 as $R_{45}=R_{tot}$. In the case of a perfectly diffusing surface, R_{45} will be only a minimal portion of R_{tot}. The exact measurement was performed by considering Spectralon® (LabSphere), which is known to be a perfectly diffusing material, whose optical behavior is called "Lambertian" [6]; this indicates that Spectralon® (LabSphere), diffuses light homogeneously in all space directions.

In addition to the above cited techniques, spectral measurements of human skin (Caucasian type, forearm region) have been performed by a portable spectrometer, opportunely designed and constructed to be easy to handle e.g. in a medical laboratory. This instrument consists of a white light source (halogen lamp), whose emission spectrum lies in the region 400-1100nm. The illumination light is collected by an optical fiber and conveyed to the sample. The specularly reflected light is collected by a second optical fiber and analyzed by a spectrometer, whose resolution is of about 0.5 nm.

RESULTS

The obtained results are in the form of reflectance spectra (Fig. 1-4), whose reproducibility was estimated to be within 5% on the whole spectral range considered. In Fig.1,2,3 different bulk materials and surface characteristics are taken into consideration. In Fig.4 the specular reflectance spectrum for human skin is shown.

Out of the spectra shown in Fig.1, Lmb values were calculated by applying equation 2. In Table I, Lmb mean values over the range 400-1100 nm are shown for the various samples considered. The case of Spectralon® (LabSphere), was considered as well for the calculation of Lmb (data not shown).

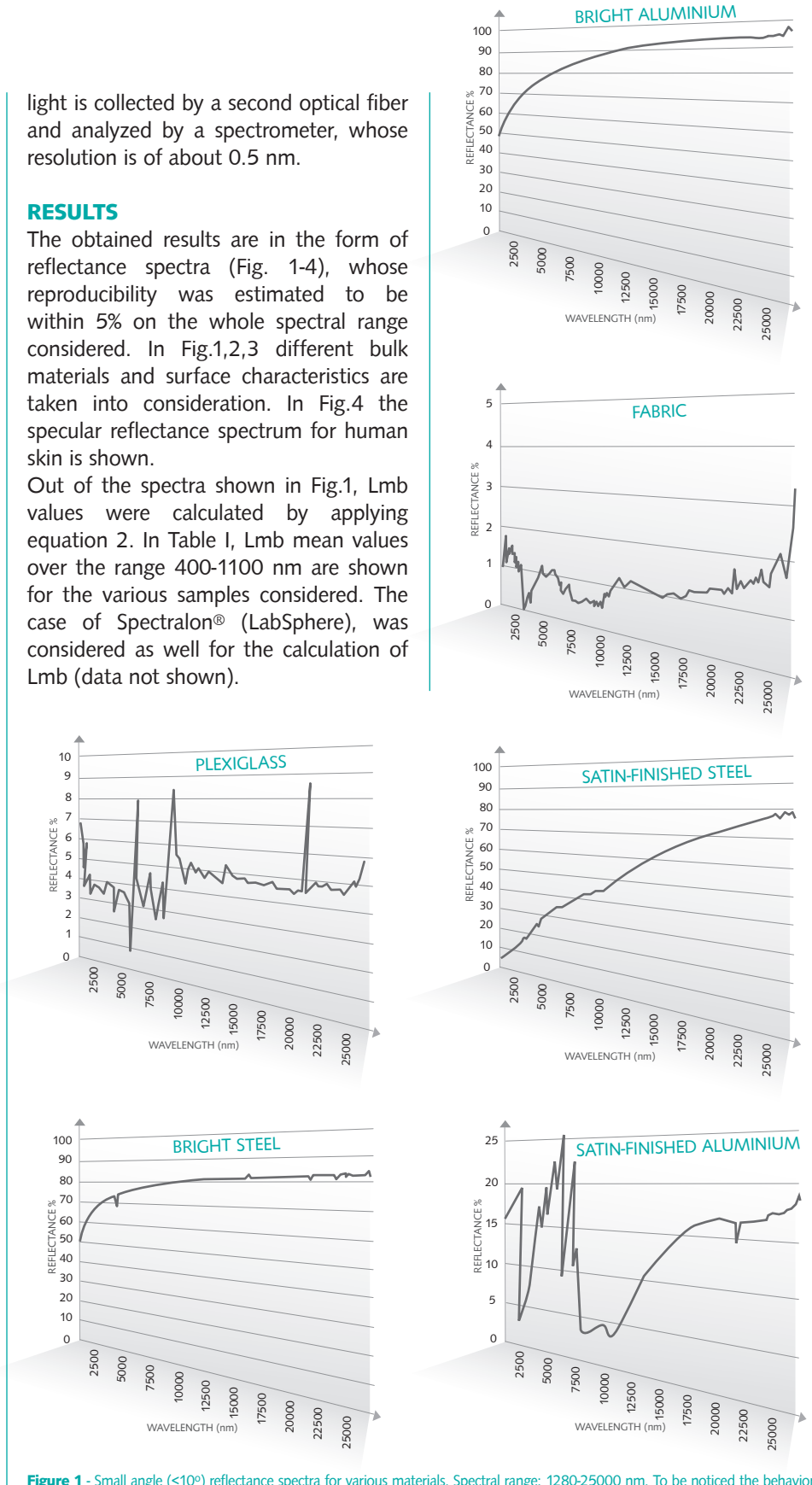

Figure 1 - Small angle (<10°) reflectance spectra for various materials. Spectral range: 1280-25000 nm. To be noticed the behavior of satin-finished aluminium with respect to the other curves for metals. Fabric: in the inset a second example of a different kind of tissue.

DISCUSSION

Reflectance spectra show a great variability: the surface properties (bulk material, surface treatment) play an important role (Fig.1,2,3). Also the wavelength dependency has to be carefully considered: reflectance properties in the UVA or IR range cannot always be deduced from properties in the visible one, as can be observed in figures 1,2,3.

Bright metals, as expected, show a much higher specular reflectance with respect to satin-finished ones; significant differences can also be noticed, for a fixed sample, between the near IR and the medium IR (up to λ=25000 nm). Satin-finished metals in particular show a small-angle reflectance which is greater than 30% in the 10000-25000 nm range, which includes the CO_2 laser emission. The same samples show up to 10% specular reflectance in the visible wavelengths, which in many cases cannot be neglected for risk management. In the cases of fabric samples (which include for instance white and green coat: samples S1-7 in Table I), the specular reflectance is very low (<1%) in the whole UVA-visible-IR range: their behavior is diffusive, as can be noticed from their Lmb values. Metals show the opposite behavior, being mostly good specular reflectors, even if aluminium is more diffusive than steel (figures 1 and 3). Plexiglass has a mainly specular behavior, even if total reflectance is always below 10%.

The results for the reflectance spectrum of skin (Fig.4) indicate that only a very small fraction (<1%) of the impinging light is specularly reflected. This indicates that almost the totality of the impinging light is either absorbed or diffusely reflected in the environment [7,8].

The obtained value for Lmb in the case of Spectralon® (LabSphere) was about 1. This indicates that Lmb for a given material ranges from 1 to 100: the more Lmb is close to 1, the more diffusing is the sample, while for a specular behaviour Lmb is close to 100. The results shown in Table I reflect the results in Fig.1: plexiglass is the mostly "specular" material (Lmb=86), while for fabric samples (S1-S7) Lmb=1÷2, indicating a highly diffusive behavior. Lmb values for metals indicate that aluminium is roughly more diffusive than steel, both in the bright and in the satin-finished form. To be noticed that the Lmb parameter is not related to absolute diffusive or specular properties of the sample, being proportional to the ratio between R_{45} and R_{tot}.

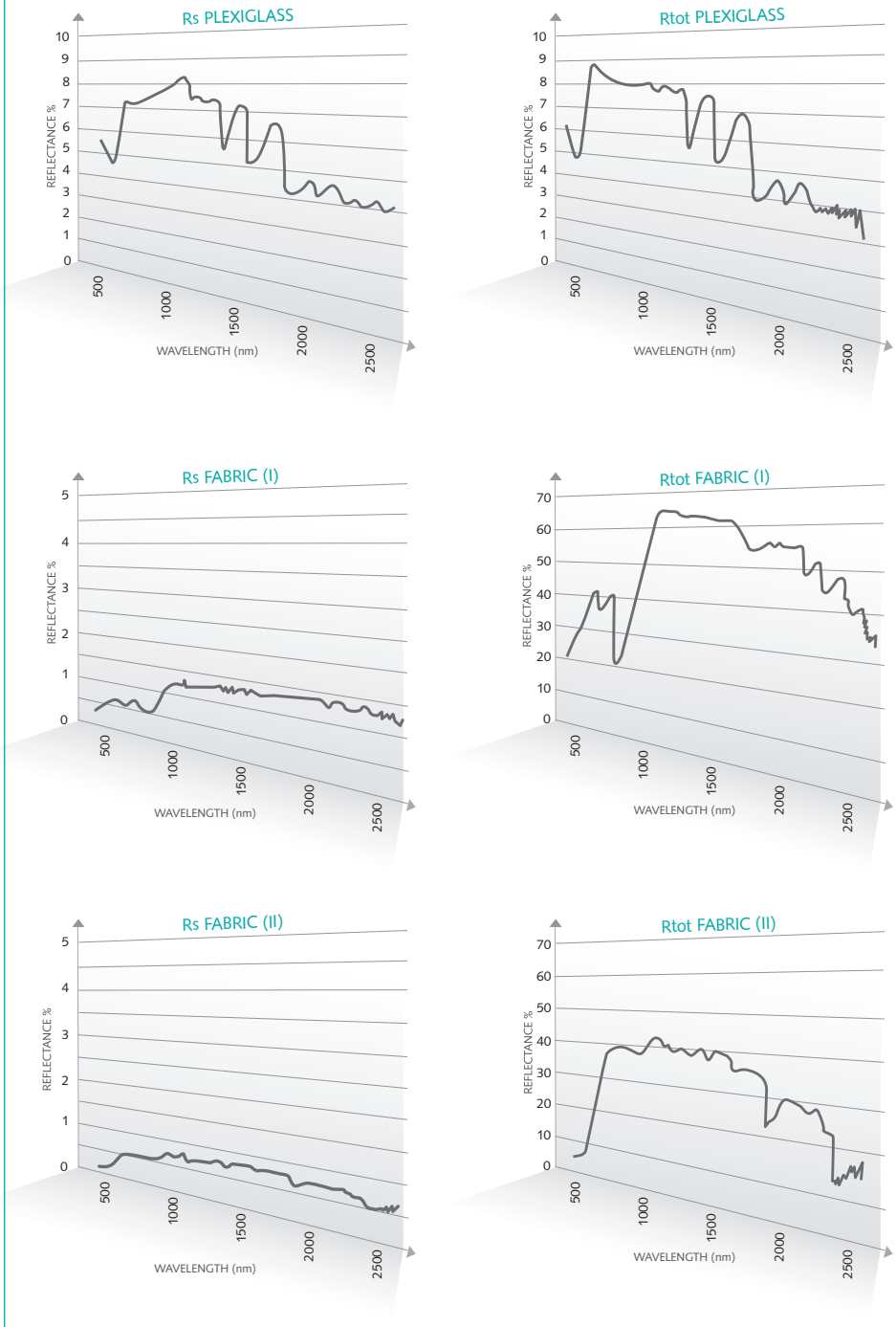

Figure 2. Specular (Rs) and total (Rtot) reflectance spectra for plexiglass and fabric samples (two different kinds are shown, among all the samples studied). Incidence angle for Rs is 45 degrees. Spectral range: 250-2500 nm.

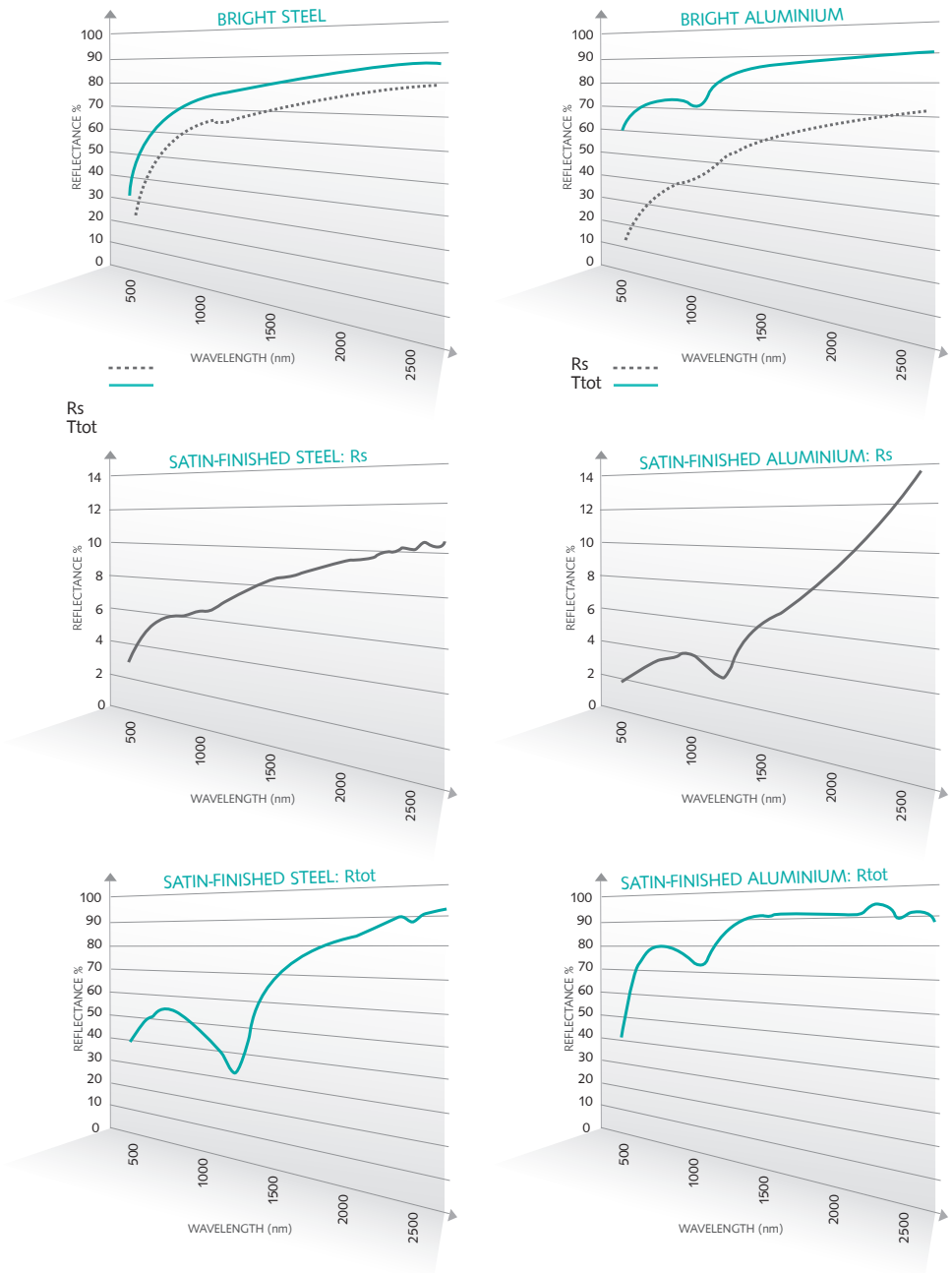

Figure 3. Specular (Rs) and total (Rtot) reflectance spectra for metal samples. Incidence angle for Rs is 45 degrees. Spectral range: 250-2500 nm.

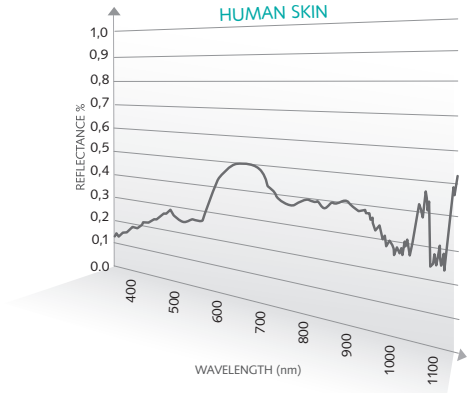

Figure 4. Specular reflectance spectrum (45°) of human skin (Caucasian type). Spectral range: 400-1100 nm.

CONCLUSIONS

Reflection properties of materials in medical laboratories are of great interest for risk management when laser light is used for clinical or research practice. When the light from a laser source impinges on a surface, it produces both specular and diffuse reflections, which may cause harmful effects mainly to the eyes and the skin. Consequently, it becomes important to measure the reflectance properties of various materials (fabric, plastic, metals) which are present in bio-medical laboratories. Skin reflectance must be a concern as well.

Specular, total and small angle reflectance have been measured in the UVA-visible-IR range by spectrophotometric (250-2500 nm range) and FTIR techniques (1280-25000 nm range). Out of the obtained results it turns out that the reflectance properties in the non-visible range can greatly differ from those in the visible one, having a strong dependence on the specific surface properties (material type, surface roughness).

A portable spectrometer has been used to measure skin reflective properties in the visible-near IR range. By considering that skin total reflectance (Caucasian type) in the 400-1100 nm range is between 30% and 70%, the obtained results indicate that a considerable fraction of the impinging light is diffusely reflected by the skin.

A simple parameter (Lmb) has been defined to determine, in a simple and practical way, if a certain surface behavior is mainly a diffusive or a specular one. Particular interest lies in the confrontation between different Lmb values, calculated in correspondence to the laser wavelengths which are the most widely used in the clinical practice. The creation of a database of reflective properties is now being initiated, starting from the presented results.

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Key words: Low-reactive level laser therapy, laser treatment, photobioactivation, tissue welding

A new effect-based classification of laser treatment based on the concept of auto-simultaneous laser treatment.

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- Professor, Department of Plastic and Reconstructive Surgery, Keio University,
- Shinanomachi; and Director, Japan Medical Laser Laboratory, Shinanomachi, Tokyo, Japan

ABSTRACT

Different tissue reactions can be obtained simultaneously with a single laser 'shot': irreversible and destructive photoreactions in the tissue (high-reactive-level laser treatment, HLLT), as in laser surgery for incision, excision, vaporization tissue coagulation and protein breaking, all above the cell survival threshold; if some irreversible damage occurs together with reversible photodamage, as in tissue welding, the author refers to this as mid reactive-level laser treatment (MLLT), where many cells are not irreparably damaged; if the level of reaction in the target tissue creates no cellular damage whatsoever, then the author calls this low reactive-level laser therapy (LLLT). All three of these reactions can occur simultaneously in the one target, and fall under the umbrella of laser treatment (LT). Auto-simultaneous laser treatment (ASi-LT) is the author's term to describe a treatment situation whereby 2 or more tissue reaction types are achieved simultaneously in tissue with the one laser. This is the essential component allowing the successful surgical application of the laser in many fields compared with conventional modalities, due to the beneficial actions on the wound healing process of the zone of photobioactivated cells at the periphery of any HLLT or MLLT application. The author herein presents his new categorization of laser treatment, under the three sub-classifications of Mono-type, Multi-type and Concomitant-type laser treatment (Mo – LT, Mu – LT and Cc – LT, respectively) and their sub-types, whereby understanding control of the incident laser beam can achieve the desired range of bioeffects, delivering tailor-made treatment on an individual basis and enabling others to achieve the same result.

INTRODUCTION

Dr Theodore Maiman developed the first practical ruby laser in 1960, delivering a new tool to clinicians. The ruby laser opened up the way for other systems, and from 1960 to 1964 all of the major lasers used to this day in medicine and surgery were developed, including the argon, helium-neon (HeNe), neodymium yttrium aluminium garnet (Nd:YAG) and the carbon dioxide (CO_2) laser. I first started using the ruby and argon lasers in Japan in the mid 1970s, and by this time the literature was reporting that procedures performed with a laser offered better wound healing and less postoperative pain compared with the same procedure performed by conventional means. This was particularly true where operations normally performed with the scalpel could be performed with the CO_2 such as tonsillectomies, laser conization for carcinoma in situ of the uterine cervix, and any procedure where the laser could justifiably replace the scalpel, where there was a large chance of blood loss from a highly vascularized field such as the lip. When verrucae are excised using the conventional scalpel they often reappear along the incision line. However, when a focused infrared or visible light laser is used to excise and vaporize warts, the reappearance rate is dramatically less,[1] and the same phenomenon has been reported in gynaecology for the removal of condyloma accuminata, genital warts. [2] There appeared to be a virocidal effect seen with the laser which was not the case with the conventional scalpel, and because the laser is simply a generator of light, the light energy must have been responsible for the beneficial differences seen between laser and conventional surgeries.

Many different examples of this interesting and useful phenomenon have appeared in the literature, such as the more effective use of the laser to remove tattoos compared with dermabrasion [3]. My personal experience with the beneficial effects of laser versus conventional surgery dates back to the late 1970's [4]. In figure 1a, a haemangioma simplex lesion (port-wine stain) had been treated in another institute with electrocautery. Particularly in large lesions of this type, it was very difficult to achieve a homogeneous treatment effect, and the result was

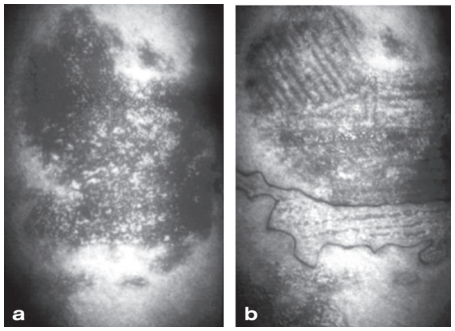

Fig 1: Haemangioma simplex previously treated elsewhere with electrocautery. (a): Findings before laser treatment. Much of the colour of the haemangioma remains together with pin point raised scarring corresponding to the needle treatment points. (b): During treatment with the argon laser in Ohshiro's modified zebra method. The argon laser has not only succeeded in removing the abnormal colour of the lesion, but has also treated the areas of scarring, thus illustrating the beneficial simultaneous photoactivative effect associated with laser treatment.

typical 'tapioca skin' comprising tiny areas of lustred and hypertrophic scarring corresponding to the points where the electrocautery needle had been inserted, surrounded by untreated areas of the lesion. When the argon laser was used to treat this lesion, using my first version of the zebra technique for lesion revision, it not only removed the remaining colour of the naevus, but also controlled the configurational problem of the lustred scarring. An untreated area in Figure 1b shows the original condition of the lesion, surrounded by treated areas. As early as 1980, I had classified the compound photobiological reactions in skin depending on the survival threshold of cells, ranging from the death zone through the recuperatory zone to the photoactivated zone, all occurring simultaneously in laser-irradiated tissue [4].

The case which really sparked my attention and encouraged me to investigate the 'photoactivated effect' in more detail concerned a lady who had intractable postherpetic neuralgia (PHN) for which she had received many treatments but without effect. The pain was so severe that she was contemplating suicide, and, to save her relatives any embarrassment after her death, decided to have a hemangioma simplex lesion on her chest removed (Figure 2a) and consulted my clinic. After a test treatment (seen as the small square area on the lower left of Figure 2b), the author started treatment with his modified zebra technique as seen on the upper portion of the lesion. When the patient returned to continue the treatment, she announced that her PHN had totally resolved.

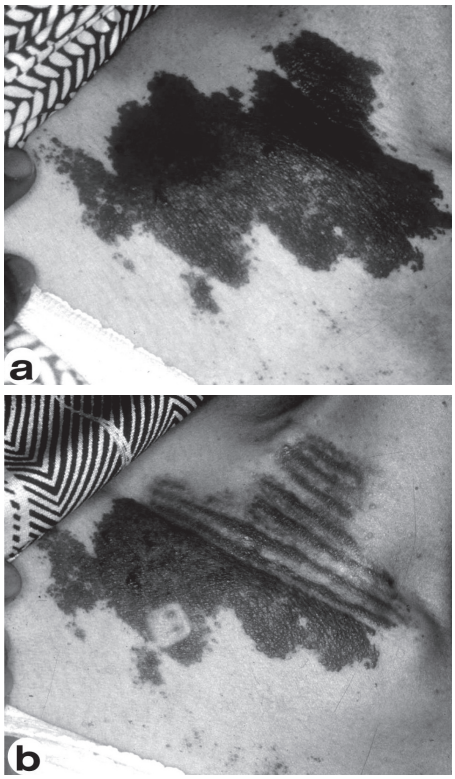

Fig 2a: Hemangioma simplex on the chest of a female patient, pre treatment. The patient suffered from severe and intractable postherpetic neuralgia (PHN).
Fig 2b: During argon laser treatment. When the patient returned to have a further treatment session, she reported that the pain from her PHN had completely gone. This was the main impetus for the author to explore simultaneous laser therapy in detail.

This made me think very carefully, and led to my hypothesis of the simultaneous range of reactions associated with the photodestructive application of the argon laser as mentioned above. When a laser is used in the surgical mode, it creates a typical pattern in stained tissue specimens (typical example in Fig 3), consisting of the ablated tissue at the target, a layer of carbon char if the temperature is high enough (1 in Figure 3), a zone of necrotic coagulated tissue (2 in Figure 3), a zone where the protein is at first broken (3) and then denatured (4) as

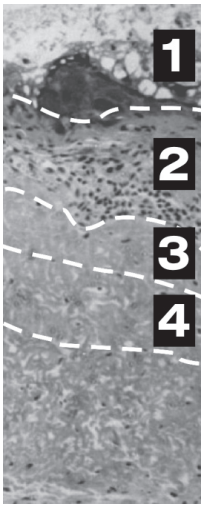

Fig 3: Clearly demarcated zones of photothermally-mediated biological changes in living tissue following a surgical laser impact. (1) Ablated tissue with char. (2) photocoagulation. (3 and 4) Tissue degradation and denaturation, also referred to by the author as the tissue welding and tissue breaking phenomenon. The lowest layer of tissue exhibits normal architecture.

the photothermal gradient drops in the irradiated tissue, giving a zone of tissue in which the damaged cells gradually merge towards normal tissue, as seen by the basophilic to eosinophilic changes in the stained specimen. I already understood that the photon energy did not stop there, however, but continued on into the normal tissue and somehow athermally modulated the behaviour of the various cell types, including axons as in the case of the patient already mentioned, thereby bringing around pain relief. This is shown schematically in Figure 4, with the peripheral photon energy of the argon laser penetrating down to and involving the intercostal nerves.

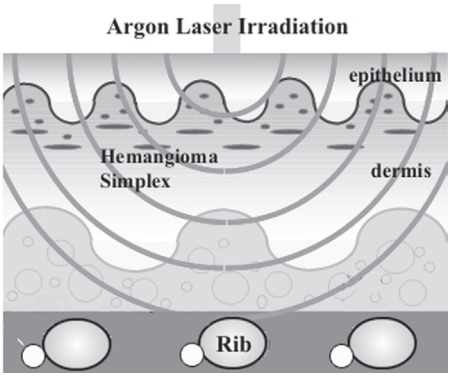

Fig 4: The author's explanation for the mechanism involved in the removal of the PHN pain of the patient seen in Fig. 2. As the photon energy from the argon laser penetrates into tissue, it becomes progressively weaker till the effect is almost totally athermal in nature. The incident photon densities at the area of the intercostal nerves are thus acting on the nerves which are in a state of hyperexcitability and hypersensitivity due to the PHN, thus removing the painful stimuli.

With a fuller understanding of laser-tissue interaction, I and others have now recognized that these phenomena are due to the simultaneous induction in the target tissue of a range of effects, culminating in a zone of athermally photobiomodulated tissue at the outermost periphery of the thermal or other destructive reactions. From my 1980 term of the 'photoactivated zone', I then coined the term 'simultaneous laser therapy' in the mid 1990's [5], and I have now evolved a new effect-based classification for this simultaneous generation of the range of tissue effects with the one laser as auto-simultaneous laser treatment (ASi-LT), and it is the photoactivated zone of ASi-LT which underpins the efficacy of the surgical laser when compared with a variety of conventional approaches.

CLASSIFICATION OF LASER TREATMENT FROM CELL SURVIVAL

Before looking at laser treatment effects under the umbrella of auto-simultaneous laser treatment (ASi-LT), and how my new classification system can help others to achieve these effects in a consistent manner, I would like to explain how my thinking has been changed since 1980 by a better understanding of what actually happens at a cellular level when biological tissue is heated using the laser.

Figure 5 shows the development of my understanding. In 1980 I had a rather simple view of the reaction, because the worldwide clinical applications of the laser were still comparatively in their infancy, and I had a rather fuzzy concept of the 'cell survival threshold' being somewhere at the upper range of what I called the 'recuperative zone' of the photothermal effects. Even then, however, I had obtained a clear concept of the 'photobioactive zone' at the periphery of the laser effects in tissue, and based on this I was investigating dedicated laser diode therapeutic systems for pain attenuation [6]. By the mid-1990's, a greater level of sophistication had been obtained through better photobiological research at a cellular level [7], and I developed my first classification of laser treatment based on the levels of cellular damage and survival, as already seen in Figure 5 [5]. This allowed me to take the histological tissue findings as seen in Figure 3 and marry them to the concepts seen in Figure 5, arriving at the schematic seen in Figure 6 that also has aspects of what I call the 'laser apple', which will be discussed elsewhere in this review.

I thus categorize laser treatment very broadly under three aspects. The photodestructive reactions of vaporization/carbonization, tissue coagulation and protein breaking all come under high reactive-level laser treatment, or HLLT within the temperature ranges of from approximately 56°C to 100°C and over, where the level of activity generated by the incident light is above the survival threshold of the cells, and they die: in the case of tissue breaking, however, a few damaged cells may survive. When level of activity induced in the target cells is just below their survival threshold, at temperatures between approximately 50°C and 55°C, the phenomenon known as protein welding occurs, whereby the heat-labile hydrogen bonds holding collagen fibres together totally denature, allowing the fibres they were linking to comingle with each other. When the temperature drops to normal, the denatured H2 bonds renature, but not with their original partner and the architecture of the tissue has irreversibly altered, even though the component cells survive, albeit with a degree of thermal damage in some of them. I refer to this as mid reactive-level laser treatment, or MLLT. At temperatures from approximately 40°C to around 50°C, the denaturation phenomenon still occurs, but only partially, and below 40°C there is a totally athermal and atraumatic energy exchange between incident photons and the skin cells which have absorbed them. This range I class as photoactivation, otherwise known as low reactive-level laser therapy or LLLT. These three aspects of ASi-LT are shown in Figure 6.

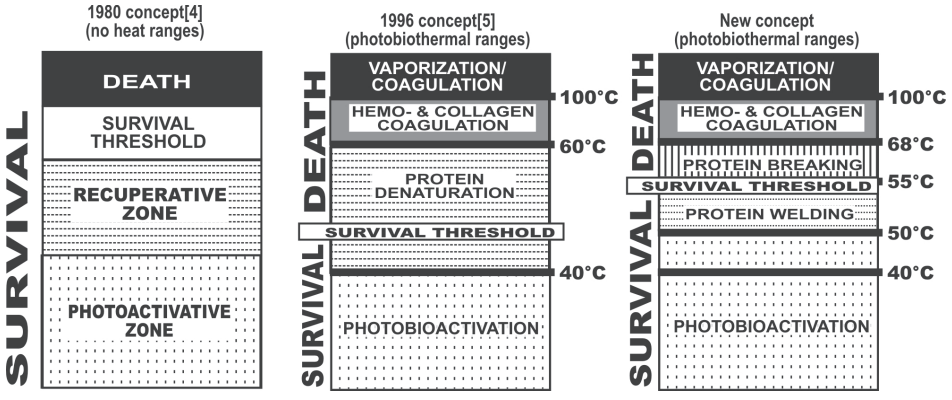

Fig 5: Illustration showing how the author's understanding of the cell survival threshold influenced his concept of all aspects of laser treatment. [Left panel, adapted from reference 4; middle panel, adapted from reference 5]

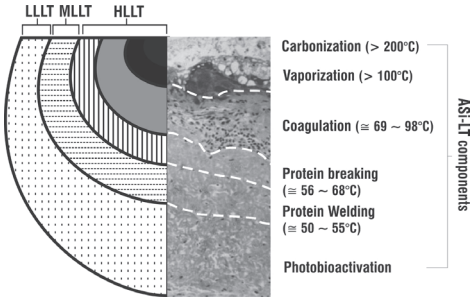

Fig 6: The concepts from the authors latest understanding as illustrated in the right panel of Fig 5, coupled with the histological findings from Fig 3 to produce the first iteration of a laser apple-like schematic of a surgical laser impact in living tissue, showing the three aspects of ASi – LT, namely HLLT, MLLT and LLLT. Please note that the temperature ranges are illustrative only, since much will depend on other factors such as wavelength and the exposure time during which the tissue is irradiated.

THE OHSHIRO ASI-LT LASER APPLES

I first introduced the concept of the 'laser apple' in the mid-90's [8]. I noted that the HeNe laser produced an apple-like pattern in translucent plastic block, and

from that I worked out my basic laser apple as a way of graphically expressing the tissue effect and parameters of a laser 'all in one' schematic. Figure 7 shows my new basic laser apple, based on the reactions discussed above. The one singe graphic can show the type of laser used, its wavelength (nm), the power incident on the tissue in W and the irradiated area in cm² (from which the power density can be calculated in W/cm²), and the irradiation time in sec (which will allow calculation of the dose or energy density in J/cm²).

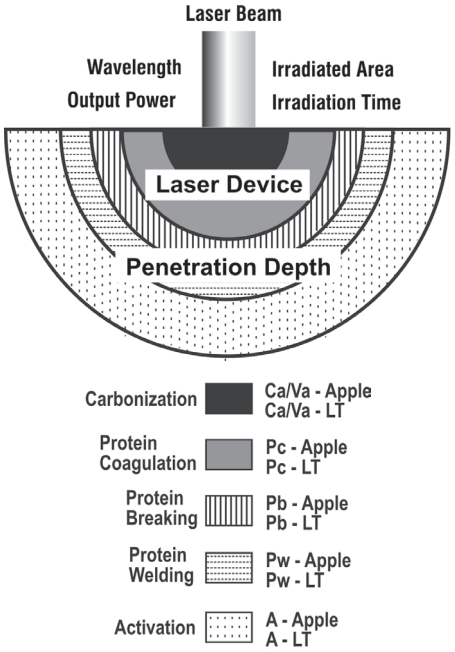

Fig 7: Ohshiro's basic laser apple, enabling all relevant laser parameter, tissue penetration and tissue effect data to be shown in one single graphic.

The concentric patterns can show the range of reactions, and the approximate penetration depth of the laser is also shown. How this can be used in practice is illustrated in Figure 8. Two very different laser apples are shown, but with the same laser, the CO_2. In Figure 8a, the CO_2 is being used at 2 W with a 100 mm spot, and can be used for tissue vaporization at these parameters (power density 25,700 W/cm²), so it can be depicted with a CO_2 Va - apple, delivering Va - LT. In Figure 8b, the output power is now 10 W, but the spot size is 10 cm, and at these parameters,

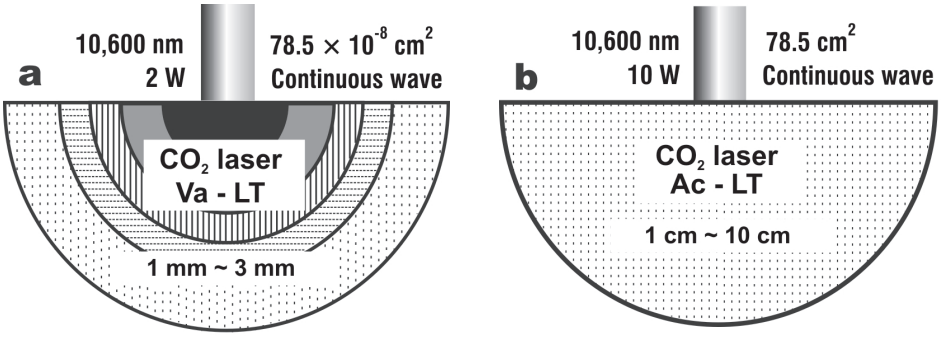

Fig 8: Two CO2 laser apples showing how the same laser can induce quite different tissue effects, and why the tissue effect should be used to categorize reactions, and not the laser hardware used to produce the effect. In 2a the incident power is only 2 W, but at the parameters shown the CO2 laser energy is capable of producing the full range of LT reactions from Va – LT all the way to Ac – LT. This is an HLLT effect for laser surgery. In 2b, on the other hand, the incident power is now 10 W but at the parameters shown it produces only an Ac – Apple, namely LLLT for laser therapy.

the CO_2 laser can be used for photoactivation (power density 257 mW/cm²), so it is depicted with a CO_2 Ac – apple to give Ac – LT. This shows very clearly why terminology like 'low power laser' should not be used, as 10 W is certainly not low power, but the effect is pure photobioactivation. On the other hand, 2 W is a low incident power, but it can produce a surgical effect with the appropriate parameters. Figure 9 shows the compete set of ASi-LT apples.

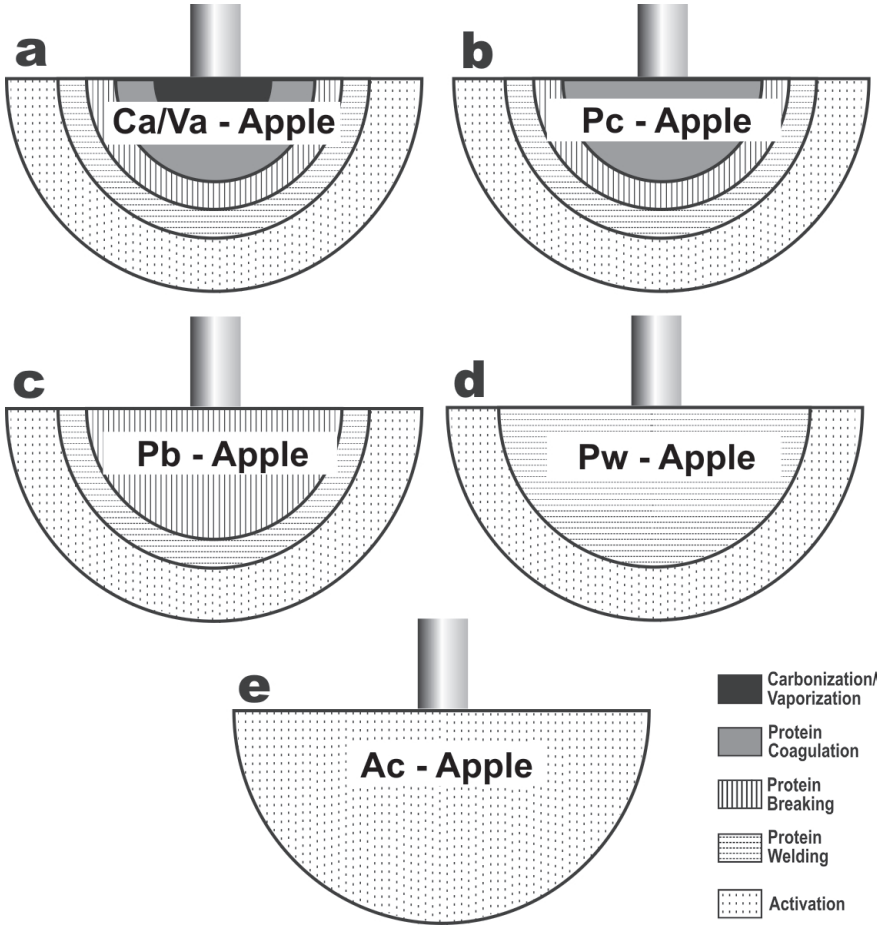

Fig 9: The entire family of ASi – LT laser apples illustrated with the range of tissue effects produced by each.

LASER CLASSIFICATION

Under my effect-based categorization, laser treatment falls into three main classifications: mono-type laser treatment (Mo-LT), multi-type laser treatment (Mu-LT) and concomitant-type laser treatment (Cc-LT) (Table 1 and see also Figure 9 above). Mono-and multi-type LT are further subclassified, and these will now be explained in detail.

TYPE OF LT	ABBREVIATION
1 Mono-type Laser Treatment:	Mo-LT
1-1: Pure-LT	Pu-LT
1-1-1: Activative LT	Ac-LT
1-2: Auto-simultaneous LT	Asi-LT
1-2-1: Welding LT	We-LT
1-2-2: Destructive LT	De-LT
1-2-2-a: Protein Welding LT	Pw-LT
1-2-2-b: Protein Breaking LT	Pb-LT
1-2-2-c: Protein Coagulative LT	Pc-LT
1-2-2-d: Vaporizational LT	Va-LT
1-2-2-e: Carbonizational LT	Ca-LT
1-2-2-f: Others	
2: Multi-type Laser Treatment	**Mu-LT**
2-1: Combined LT	Cb-LT
2-2: Compound LT	Cp-LT
3: Concomitant LT	Cc-LT

Table 1: Breakdown of the three main classes of laser treatment

1. Mono-type Laser Treatment (Mo-LT)

As the name suggests, Mo-LT is used to describe the use of a single laser, either in laser therapy (LLLT), laser welding (MLLT) laser surgery (HLLT) or a combination of the three. Mo-LT contains two sub-sets, pure LT (Pu-LT) and auto-simultaneous LT (ASi-LT). Pu-LT describes a single reaction achieved with a single laser, for example pain attenuation with a GaAlAs diode laser therapy system for pure LLLT in activative laser treatment (Ac-LT) ASi-LT is used to describe two or more simultaneous reactions achieved with the one laser, such as has been discussed already in Figure 6 above where the complete range of therapeutic and surgical reactions occur simultaneously. ASi-LT is subdivided into two categories, Welding LT and Destructive LT. Destructive LT is further subcategoorized as protein welding LT (Pw-LT); protein breaking LT (Pb-LT); Protein coagulative LT (Pc-LT); vaporizational LT (V-LT); carbonizational LT (C-LT) and other reactions such as photo-osmotic, photodynamic, photoelectric effects and so on. Any of these destructive type reactions will almost always be combined with an Ac-LT reaction at the outer periphery of the beam, due to the attenuation of the incident photon density as it penetrates the target tissue.

It is important to remember that the present review encompasses both laser therapy and laser surgery under the single heading of laser treatment (LT), and that the lasers may be used alone, in combination with themselves or with each other, depending on the classification type. The examples given, while only used hypothetically in this review, are drawn from the author's experience and will be illustrated in subsequent clinically-based studies.

2: Multi-type Laser Treatment (Mu-LT)

(Table 2) Mu-LT has two main sub-sets, combined LT (Cb-LT) and compound LT (Cp-LT). Each of these sub-sets contain homogeneous-combined and compound LT (HoCb-LT, HoCp-LT) and xenogeneous-combined and compound LT (XeCb-LT, XeCp-LT). The sub-types of combined laser treatment (Cb-LT) will now be discussed in detail, but the reader should bear in mind that they can be applied identically to compound laser treatment (Cb-LT). In the interests of space, Cb-LT will thus not be illustrated.

Type 2-1: Combined LT (Cb-LT)

Cb-LT describes treatment of the one disease existing in one or multiple sites with the same or different lasers, simultaneously or on successive applications or sessions.

Type 2-1-1: Homogeneous Cb-LT

When the same lasers are used in combination, it is referred to as homogeneous Cb-LT, Ho.Cb-LT. If the

TYPE OF LT	ABBREVIATION
2-1: Combined LT	Cb-LT
2-1-1: Homogeneous Cb-LT	Ho.Cb-LT
2-1-1-a: Homo-simultaneous Cb-LT	HoSi.Cb-LT
2-1-1-a-i: Same reactive HoSi.Cb-LT	Sr.HoSi.Cb-LT
2-1-1-a-ii: Different reactive HoSi.Cb-LT	Dr.HoSi.Cb-LT
2-1-1-b: Homo-successive Cb-LT	HoSu.Cb-LT
2-1-1-b-i: Same reactive HoSu.Cb-LT	Sr.HoSui.Cb-LT
2-1-1-b-ii: Different reactive HoSu.Cb-LT	Dr.HoSu.Cb-LT
2-1-2: Xenogeneous Cb-LT	Xe.Cb-LT
2-1-2-a: Xeno-simultaneous Cb-LT	XeSi.Cb-LT
2-1-2-a-i: Same reactive XeSi.Cb-LT	Sr.XeSi.Cb-LT
2-1-2-a-ii: Different reactive XeSi.Cb-LT	Dr.XeSi.Cb-LT
2-1-2-b: Xeno-successive Cb-LT	XeSu.Cb-LT
2-1-2-b-i: Same reactive XeSu.Cb-LT	Sr.XeSui.Cb-LT
2-1-2-b-ii: Different reactive XeSu.Cb-LT	Dr.XeSu.Cb-LT
2-2: Compound LT (Same classifications as Cb-LT)	Cp-LT

Table 2: Breakdown of Multi-type LT (Mu-LT)

same laser types are used at the same time, it is referred to as homo-simultaneous combined laser treatment, or HoSi.Cb-LT. In this case, the reaction produced by the lasers may be the same (same-reactive HoSi.Cb-LT, Sr.HoSi.Cb-LT), or they may produce different reactions (different-reactive HoSi.Cb-LT, Dr.HoSi.Cb-LT) as illustrated in the following examples.

In a patient with severe headache, two GaAlAs diode lasers may be applied simultaneously (Ac-Apples), one to the occipital zone and the other to a point on parietal zone: in both cases the reaction is the same, namely the relief of pain.[9] This is an example of same-reactive homo-simultaneous combined laser treatment (Sr.HoSi.Cb-LT), and is illustrated in Figure 10. In Figure 11, a soft tumour in a haemangioma simplex lesion is being excised with a focused CO_2 laser (Ca/Va – Apple), and at the same time a pulsed dye laser (PDL) is used to control haemorrhage of vessels and treat shallower vessels in wound bed through protein breaking, including haemocoagulation (Pb – Apple). This is different-reactive homo-simultaneous combined laser treatment (Dr.HoSi.Cb-LT).

The same lasers may be applied at different times, rather than at the same time, either in the one treatment session or in different treatment sessions. This is referred to as homo-successive combined laser treatment, or HoSu.Cb-LT. Once again the reactions produced may be the same, (same-reactive HoSu.Cb-LT, Sr.HoSu.Cb-LT), or the reactions may differ (different-reactive HoSu.Cb-LT, Dr.HoSu.Cb-LT).

For example, in the treatment of a very large tumour, one area of the tumour is treated with the Nd:YAG laser at high power densities to achive vaporization (Va – Apple). At a later stage, the Nd:YAG laser is applied again to a different area of the tumour, again in Va – LT. This is same-reactive HoSu.Cb-LT. On the other hand, a focused CO_2 laser is first used to

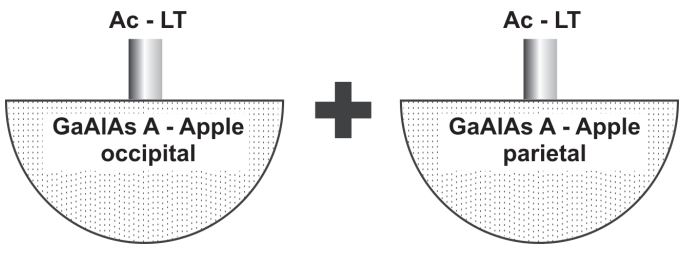

Fig 10: An example of same-reactive homo-simultaneous combined laser treatment (Sr.HoSi. Cb – LT) with two GaAlAs diode lasers being used in Ac – LT, where the LT effect is the same. See text for details.

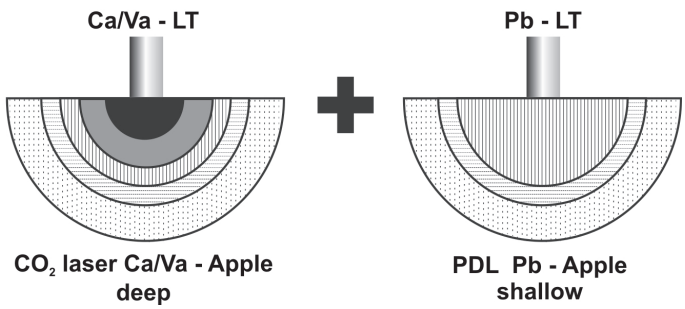

Fig 11: Different-reactive homo-simultaneous combined laser treatment (Dr.HoSi.Cb-LT) shown for CO2 and PDL lasers in Ca/Va – LT and Pb – LT. The effect obtained from each laser is different. See text for details.

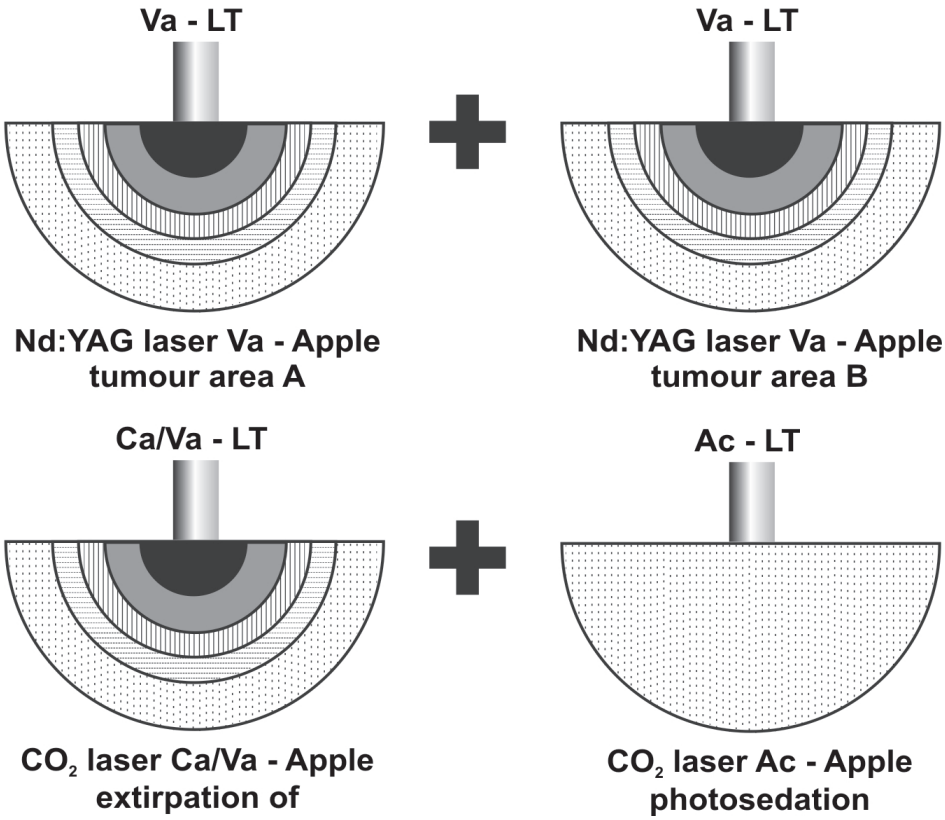

Fig 12: Same-reactive xeno-simultaneous combined laser treatment (Sr.XeSi.Cb – LT) with the Nd:YAG shown in the upper panels giving the same effect with the lasers applied at different times. Different-reactive xeno-simultaneous combined laser treatment (Dr.XeSi.Cb-LT) with the CO_2 laser is seen in the lower panels. Although the lasers are the same, the effect is different. See text for details.

extirpate a lipoma in Ca/Va – LT. The CO_2 laser is subsequently used in defocused mode to avhieve photosedation through Ac – LT. because the tissue reaction is different, this is an example of different-reactive HoSu.Cb-LT. These are illustrated in Figure 12.

Type 2.1.2: xenogeneous Cb-LT

Rather than the same laser type, different laser types may also be combined in laser treatment of the one disease, known as xenogeneous Cb – LT, Xe.Cb – LT. Xe.Cb – LT has the same sub-types as Ho.Cb – LT already discussed above, namely xeno-simultaneous combined laser treatment (XeSi.Cb – LT) and xeno – successive combined laser treatment (XeSu.Cb – LT), both with same- or different reactive subtypes.

For treatment of a headache, the GaAlAs laser is applied to the occiput (GaAlAs Ac – Apple), while the HeNe laser is applied to the pharynx (HeNe – Ac Apple), each laser targeting different control centres. This is same-reactive XeSi.Cb – LT. On the other hand, as an example of different-reactive XeSi.Cb LT, a focused CO_2 laser is used to vaporize a cavernous haemangioma (CO_2 Va – apple), and on the same beam axis an Nd:YAG laser is used to control bleeding with haemocoagulation through protein breaking (Nd:YAG Pb – Apple).

When the lasers are applied successively rather than together (XeSu.Cb LT), examples would be first of all the treatment of rheumatoid arthritis with same-reactive XeSu.Cb LT. The GaAlAs laser is first used in photosedation, and then the HeNe laser is applied to treat the microvilli on the joint cartilage. In both cases the Ac – Apple is being used to achieve Ac – LT. Compare this with the use of the CO_2 and GaAlAs lasers in the treatment of a fibroma. The CO2 laser is first used in focused mode to excise the fibroma (CO_2 Ca/Va – LT) followed by the GaAlAs laser to treat postoperative inflammation in GaAlAs Ac – LT (GaAlAl Ac – Apple).

Type 2-2: Compound LT (Cp-LT)

In Cp-LT the same subdivisions exist as for combined laser treatment, the difference being that in Cp-LT two or more diseases are being treated with the same type of laser (HoCp-LT) or different laser types (XeCp-LT), with exactly the same sub-types as for Cb-LT. In the interests of space, no examples or details are given since the subclassifications follow exactly those of Cb – LT. All of the above example of the type 2 Mu-LT are summarized in Table 2.

TYPE 3: Concomitant Laser Treatment (Cc - LT)

The final subdivision of my laser treatment classification is concomitant laser treatment, Cc – LT, in which any of the above 2 LT types can be used in combination with each other or with conventional treatment methods. Each disease is different, and each patient is an individual; furthermore the same disease may exist in different stages or degrees on the one patient site-by site. Accordingly the author has developed over the last 30 years his Total Treatment Concept, which calls for the flexible design of a treatment protocol based on each patient's individual needs. This concept requires the use of all of the LT types already discussed and where necessary used concomitantly with conventional treatment methods. An example is my treatment for that very difficult to treat disease, Ohta's naevus. I sometimes use compounded snow dry ice sticks for the superficial pigment components, followed by epithelial peeling. I might then use a small spot focused visible light laser such as the argon or KTP 532 to reach the deeper pigment, with minimal damage to the overlying epidermis. The Q-switched

ruby could then be used in focused mode for the remaining scattered superficial or intermediate pigment. The GaAlAs laser would be used after each session for photosedation to counteract ant intra-procedure pain, to ensure rapid and enhanced wound healing and to minimize any side effects such as hypertrophic scar or secondary hyperpigmentation formation.

CONCLUSIONS

Accuracy and consistency in the reporting of any laser-based procedure is of prime importance. Without accurate reporting and the use of appropriate and consistent terminology other researchers will unable accurately to repeat clinical trials or experimental studies to corroborate data and validate findings, and the concept of evidence-based medicine becomes hazy. This leads to lack of scientific acceptance of the specific study or trial in particular and of the laser in surgery and medicine in general. Because laser surgery has a much longer history and is used in many more specialities than is the case with laser therapy, therapeutic uses of laser and light have taken longer to gain full scientific acceptance. As I already mentioned above, incorrect and inaccurate terminology such as the ever-popular 'low power laser' does nothing to help the acceptance of laser therapy into the medicoscientific literature, especially in the USA. I am firmly of the opinion, that while there may definitely be lasers with low output powers and lasers with high output powers, they can actually both be used to produce an entire range of tissue reactions, irrespective of the output power at which the laser is set (Figure 8 and related text, above).

I would like to reiterate that the tissue reaction should be used to classify laser systems, and not any aspect of the hardware, including the output power. A laser is simply a device which is capable of generating a beam of laser energy. It is the reaction with the tissue which gives this beam of special energy its clinical utility, whether for laser surgery or for laser therapy. That is why I have developed this classification system based on the tissue reaction, and I also designed the 'laser apple' concept so that a single pictorial representation is capable of giving all salient characteristics of a laser beam which not only includes all the physical parameters associated with the laser itself, but also goes further and graphically represents the laser tissue reactions and the beam penetration. When the laser apple is coupled with my laser treatment classification criteria as explained in this review, and in particular the auto-simultaneous laser treatment (ASi-LT) concept, the laser clinician can report in one simple schematic the entire range of parameters necessary for other colleagues to repeat the work.

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Key words: Denervated muscle, peripheral nerve, nerve cells, laser phototherapy

Muscle and Peripheral Nerve Response to Laser Phototherapy Review of 30-years experience

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ABSTRACT

Posttraumatic nerve repair and prevention of muscle atrophy represent a major challenge of restorative medicine. Considerable interest exists in the potential therapeutic value of laser phototherapy for restoring or temporary preventing denervated muscle atrophy as well as enhancing regeneration of severely injured peripheral nerve.

Low power laser irradiation (laser phototherapy) was applied for treatment of rat denervated muscle in order to estimate biochemical transformation on cellular and tissue levels, as well as on rat sciatic nerve model after crush injury, direct or side-to-end anastomosis and neurotube reconstruction. Nerve cells' growth and axonal sprouting were investigated on embryonic rat brain cultures. The animal outcome allowed clinical double-blind, placebo-controlled randomized study which measured the effectiveness of 780-nm laser phototherapy on patients suffering from incomplete peripheral nerve injuries for 6 months up to several years.

In denervated muscle, animal study suggests that the function of denervated muscles can be partially preserved by temporary prevention of denervation–induced biochemical changes. The function of denervated muscles can be restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury.

In peripheral nerve injury, laser phototherapy has an immediate protective effect, maintains functional activity of the injured nerve for a long period of time, decreases scar tissue formation at the injury site, decreases degeneration in corresponding motor neurons of the spinal cord and significantly increases axonal growth and myelinization. In cell cultures, laser irradiation accelerates migration, nerve cell growth and fiber sprouting.

In a pilot, clinical, double-blind, placebo-controlled randomized study in patients with incomplete long-term peripheral nerve injury, 780-nm laser irradiation can progressively improve peripheral nerve function, which leads to significant functional recovery.

780-nm laser phototherapy temporarily preserves the function of a denervated muscle, accelerates and enhances axonal growth and regeneration after peripheral nerve injury or reconstructive procedures. Laser activation of nerve cells, their growth and axonal sprouting can be considered as potential treatment of neural injury. Animal and clinical studies show the promoting action of phototherapy on peripheral nerve regeneration, which makes it possible to suggest that the time for broader clinical trials has come.

INTRODUCTION

When muscles are denervated, in cases of complete peripheral nerve injury, they deteriorate progressively. Although some muscle regeneration does occur [1], it is at a level insufficient to replace the degenerative loss. There is a need to find effective methods for muscle preservation and nerve regeneration enhancement, especially after surgical nerve repair [2,3]. Surgical repair is the preferred modality of treatment for the complete or severe peripheral nerve injury [4-10]. In most cases, the results can be successful if the surgery is performed in the first six months after injury, in comparison to long-term cases where surgical management is less successful. Nonetheless, in related literature, there are several publications of surgical treatment of long-term injuries (most of which were severe, incomplete and with minimal or partial preservation of muscle activity) of the brachial plexus and peripheral nerve [11-14]. The reason for early surgical intervention has to do with the fact that between 1 and 3 years post-injury, denervated muscle undergoes progressive degeneration, which leads to loss of muscle fibers and their replacement with fat and fibrous connective tissue. For most patients who suffer from long-term peripheral nerve injuries, spontaneous recovery is often unsatisfactory. The usual results after such an injury are degeneration of the distal axons and retrograde degeneration of the corresponding neurons of the spinal cord, followed by a very slow regeneration. Recovery may eventually occur, but it is slow and frequently incomplete. The secondary effects of peripheral nerve injury are wasted muscles and high incidence of pressure sores. Therefore, numerous attempts have been made to enhance and/or accelerate the recovery

of injured peripheral nerves and decrease or prevent atrophy of the corresponding muscles. Among the various proposed methods for enhancing nerve repair, phototherapy has received increasing attention over the last two decades. The term phototherapy refers to the use of light for producing a therapeutic effect on living tissues. Although a pioneering report on the effects of laser phototherapy on the regeneration of traumatically injured peripheral nerves was published in the late 1970s [15], it is only since the late 1980s that scientific interest was kindled in this therapeutic approach for neural rehabilitation, leading to the publication of a number of studies that have shown positive effects of phototherapy on peripheral nerve regeneration [16,17].

The possible mechanism of action of phototherapy on the nervous tissue with respect to peripheral nerve regeneration has been provided by the in vitro studies which showed that phototherapy induces massive neurite sprouting and outgrowth in cultured neuronal cells [18], as well as Schwann cell proliferation [19]. Also, it has been suggested that phototherapy may enhance recovery of neurons from injury by altering mithochondrial oxidative metabolism [20], and guide neuronal growth cones in vitro, perhaps due to the interaction with cytoplasmic proteins and, particularly, to the enhancement of actin polymerization at the leading axon edge [21]. Phototherapy alters nerve cell activity, including upregulation of a number of neurotrophic growth factors and extracellular matrix proteins known to support neurite outgrowth [22]. A possible molecular explanation was provided by demonstrating an increase in growth-associated protein-43 (GAP-43) immunoreactivity in early stages of rat sciatic nerve regeneration after phototherapy [23]. Another study [24] showed that application of phototherapy upregulates calcitonin gene-related peptide (CGRP) mRNA expression in facial motor nuclei after axotomy. By altering the intensity or temporal pattern of injury-induced CGRP expression, phototherapy may thus optimize the rate of regeneration and target innervation and neuronal survival of axotomized neurons.

In this paper we report the results of an experimental study aimed at investigating how laser phototherapy affects long-term denervated muscles by examining acetylcholine receptors (AChR), which play a special role in neuromuscular transmission, and creatine kinase (CK) content, which is an important enzyme for supplying a source of energy to the muscle. The results of this investigation supplement our previous studies [17] pertaining to the effectiveness of laser phototherapy in treating severely injured peripheral nerve after crush injury, neurorraphy, side-to-end anastomosis or neurotube reconstruction, based on our 30 years of research.

PHOTOTHERAPY IN DENERVATED MUSCLE PRESERVATION

Using as a model of study the denervated rat gastrocnemius muscle (in vivo), we investigated the influence of low power laser irradiation on CK activity and the level of AChR in denervated muscle in order to estimate biochemical transformation on cellular and tissue levels. Much of the literature on the effects of long-term denervation of mammalian skeletal muscle has focused on experimental studies of total sciatic section in rats [25,26]. In our study [27], rats were chosen for investigation in the vast majority of cases due to their availability, good survival record and ease of treatment. For the surgical procedure Wister rats were anesthetized and complete denervation of the gastrocnemius muscle was done (cut and removal of 1cm segment of the sciatic nerve). After operation, the rats were divided into 4 groups: Group I - denervated non-irradiated group (15 rats); Group II - denervated laser treated group (15 rats); Group III – intact non-irradiated group (15 rats), and Group IV – intact irradiated group (15 rats). The rats underwent laser treatment (HeNe laser, 35mW, 30 minutes) every day, for 14 days. Low power laser irradiation was delivered transcutaneously to the gastrocnemius muscle of denervated Group II and intact Group IV. Under general anesthesia, the rats were sacrificed and the gastrocnemius muscle was homogenized.

CK activity was measured by the specific spectrophotometrical method using spectrophotometer at 340nm and a Sigma kit [28,29] 7, 30, 60 and 120 days after denervation in both denervated and intact muscles.

Internal and membrane inserted AChR was quantitated by 125I-alpha-bungarotoxin on the same homogenates [30,31] 7, 30, 60 and 120 days after denervation in both denervated and intact muscles. The data obtained was evaluated as cpm of bound 125I-a-BuTX/mg protein. Radioactivity was assessed with Auto-Gamma Counter in denervated and intact muscles.

A. Creatine Kinase (CK) Activity in Intact and Denervated Rat Gastrocnemius Muscle

Muscle contraction and relaxation require the action of CK. Phosphocreatine, formed by the reaction of this enzyme, constitutes a reservoir of high energy phosphate which is available for quick resynthesis of ATP. This high concentration of ATP is then accessible for muscle contraction. Following muscle denervation, the level of CK and muscle weight decreases [32]. Like others [33], we found [32] that in the control non-irradiated group, denervation of the gastrocnemius muscle reduces creatine kinase activity. The decrease of CK activity in both groups (non-irradiated and laser-treated) progresses to a similar value for 7 days after denervation and is followed by a sharp fall in the non-laser treated group in comparison to the delayed and attenuated decrease of the CK activity in the laser-irradiated group. Thus, in the control non-irradiated group, 30 days after denervation, the amount of CK decreased markedly to 41% of the normal value (intact muscle). In the same time delayed and attenuated decrease of the CK activity was observed in the laser treated group. The CK activity of the laser

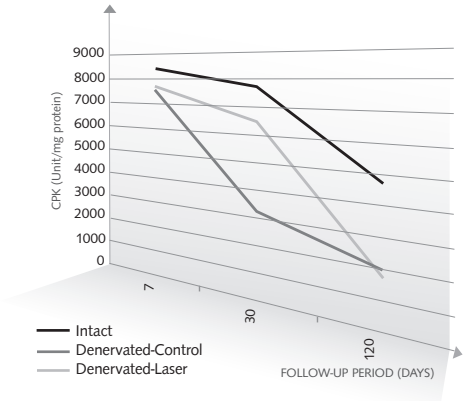

Fig. 1. Graph illustrating the results of study eveluating creatine kinase (CK) activity (unit/mg protein) in intact and denervated rat gastrocnemius muscle. Graph showing content CK (unit/mg protein) during 7, 30 and 120 days in intact and denervated muscles with and without laser treatmet.

treated denervated muscle decreased only by 17% of the normal value. The analysis of CK activity in the denervated laser treated group, compared to the control denervated group showed statistically significant difference (p = 0.008). After the 30-day period the CK activity gradually began to decrease in both groups and 4 months after denervation it reached similar levels (Fig. 1).

It's known that in denervated muscle the protein degradation rate is accelerated [32]. The temporary prevention of denervation–induced biochemical changes may be prompted by a trophic signal for increased synthesis of CK, thus preserving a reservoir of high energy phosphate available for quick resynthesis of ATP. This data supports Bolognani [34], which shows that laser irradiation increased ATP production in the mitochondria.

B. Acetylcholine Receptors (AChR) Synthesis in Intact and Denervated Rat Gastrocnemius Muscle

Acetylcholine receptors (AChR), which play a special role in neuromuscular transmission, are concentrated at the neuromuscular junction of the adult muscle. A nerve impulse triggers the release of acetylcholine, producing a much larger end-plate potential, which excites the muscle membrane and leads to muscle contraction. The amount of AChR in neuromuscular junction appears to increase their number and to cover the entire extra-junctional area following muscle denervation. In the denervated muscle, the amount of AChR increases prior to the muscle degeneration [35].

In the control non-irradiated group, 7 days after muscle denervation, as expected, the amount of AChR increased to 161% of the normal value (intact muscle). In contrast, the amount of AChR of the laser irradiated denervated muscle remained near normal value. Thirty days after denervation in laser-treated group amount of AChR increased to 180% compared to 278% in non-laser group. It is interesting that 4 months after denervation, in spite of progressive muscle atrophy, the amount of AChR in laser- treated group remains 53% of normal value compared to only 27% in the non-irradiated group. Statistical analysis showed borderline significance (p = 0.056) between denervated laser-treated and non-irradiated denervated muscles (Fig. 2).

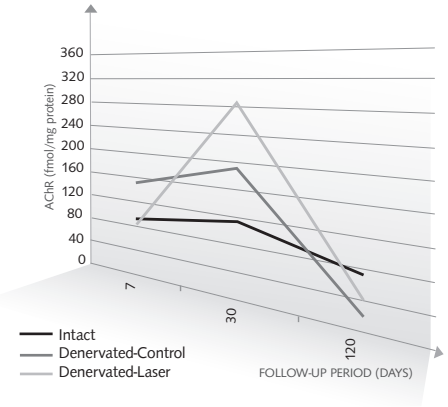

Fig. 2. Graph illustrating the results of study evaluating the level of acetylcholine receptors (AChR) in intact and denervated rat gastrocnemius muscle. Graph showing content AChR (fmol/mg protein) during 7, 30 and 120 days in intact and denervated muscles with and without laser treatmet.

Our findings suggest that in early stage of muscle degeneration laser treatment may temporarily preserve the denervated muscle close to its physiological status before injury and during progressive stages of muscle degeneration partially maintain the amount of AChR in the denervated muscle compared to the non-laser treated muscle.

C. Is Laser Phototherapy Damaging to the Muscle?

During 4 months of follow-up period we found no evidence of laser induced damage after irradiation. Moreover, in laser irradiated intact muscle group we found significant increase in CK activity 60 days into the follow-up period (p=0.008) and increasing amount of AChR (p=0.0008) compared to non-irradiated intact muscle. These findings suggest a possible positive therapeutic effect of laser phototherapy on the muscle.

D. Can Laser Phototherapy Prevent Denervation Muscle Atrophy?

Late denervation has been widely studied in animal models. In rats it has been shown that for the first 7 months after denervation myofibers exhibit a net loss of nuclear domains followed by nuclear groupings [36]. If not re-innervated, the regenerating myofibers undergo atrophy and degeneration [37]. For decrease or temporary prevention of this process, especially in cases of complete peripheral nerve injury, where affected nerve is reconstructed by grafts, tube or primary anastomosis, laser phototherapy can be an effective tool that preserves denervated muscle until nerve sprouting into the muscle occurs. This experimental study suggests that the function of denervated muscles can be restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury. These findings could have direct therapeutic applications for possible treatment of denervated muscles.

PHOTOTHERAPY IN PERIPHERAL NERVE REGENERATION

Posttraumatic nerve repair continues to be a major challenge of restorative medicine. Although enormous progress has been made in surgical techniques over the past three decades, functional recovery after severe lesion of a major nerve trunk is often incomplete and sometimes unsatisfactory.

A. Incomplete Peripheral Nerve Injury

1. Experimental Peripheral Nerve Crush Injury

Under general anesthesia the rat sciatic nerve was exposed and crushed with applied pressure of 6.3±0.7 MPa of an ordinary closed hemostat for 30 seconds. Studies investigating the effects of low power laser irradiation on injured peripheral nerves of rats have found that it provides:

1) immediate protective effects which increase the functional activity of the injured peripheral nerve [38];
2) maintenance of functional activity of the injured nerve over time [39];
3) decrease or prevention of scar tissue formation at the site of injury (Fig. 3) [40];

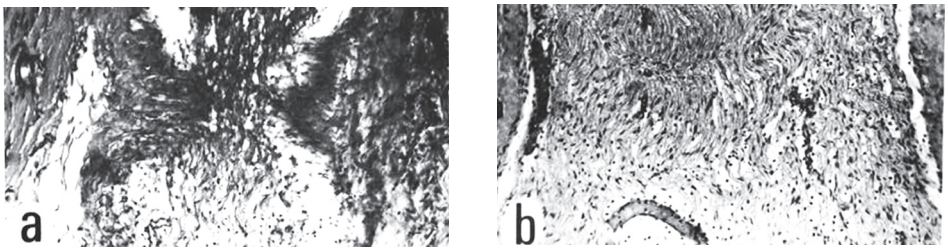

Fig. 3. Histological section of the crush area of the rat sciatic nerve showing the response of the nerve to laser phototherapy.
a. Nonirradiated nerve. Note of the scar of fibrous tissue; **b.** Laser-treated nerve shows no visible scar. H & E, original magnification X 150.

4) prevention or decreased degeneration in corresponding motor neurons of the spinal cord (Fig. 4) [41];

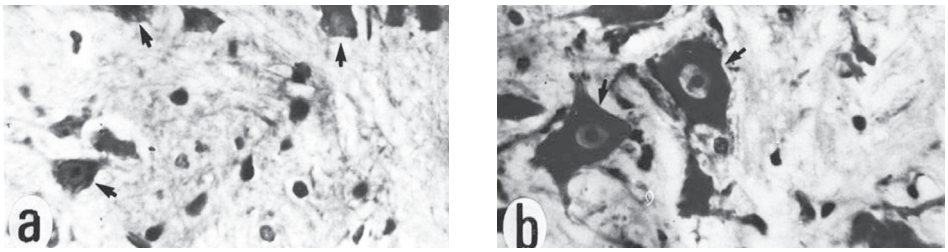

Fig. 4. Paraffin section from the anterior horn of corresponding segments of the rat spinal cord 14 days after crush injury to the sciatic nerve, showing the spinal cord response to laser treatment of the injured peripheral nerve.
a: Section from a control animal shows extensive chromatolysis and cytoplasmic atrophy found in 40% of the motor neurons (arrows).
b: Section from a laser-treated animal shows minimal degenerative changes found in 20% of the motor neurons (arrows). Stained by cresyl fast violet, magnification X800.

5) increase in rate of axonal growth and myelinization (Fig. 5) [39]. Moreover, direct laser irradiation of the spinal cord improves recovery of the corresponding injured peripheral nerve [42]. These results, as those of Anders [43], suggest that laser phototherapy accelerates and improves the regeneration of the incomplete injured peripheral nerve.

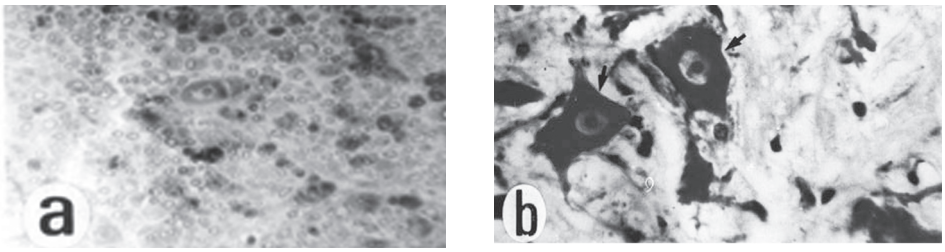

Fig. 5. Photomicrographs of semithin sections stained with toluidine blue showing the axonal response to laser treatment of the injured (crushed) peripheral nerve in rat. One group of rats was treated using laser phototherapy for 20 consecutive days after injury. Twenty-one days after injury the nerves were excised and stained. **a:** Site of crush injury in an untreated nerve showing nerve fibers that appear to be smaller and mostly nonmyelinated. Numerous macrophages and phagocytes are observed.
b: Site of crush injury in a laser-treated nerve demonstrating that most axons are ensheathed with myelin and a very few infiltrating macrophages are observed. Magnification X300.

B. Complete Peripheral Nerve Injury

1. Regeneration of the Transected Sciatic Nerve in the Rat after Primary Anastomosis

In acute cases where a peripheral nerve is completely transected, the treatment of choice is direct anastomosis. Means of enhancing regeneration are essential, since degeneration is always inevitable in severely damaged peripheral nerves.
The therapeutic effect of 780-nm laser irradiation on peripheral nerve regeneration after complete transection and direct anastomosis of rat sciatic nerve was evaluated in double-blind randomized study [44]. After surgery, 13 of 24 rats received post-operative laser treatment, applied transcutaneously for 30 minutes on a daily basis, for 21 consecutive days - 15 minutes to the injured sciatic nerve and 15 minutes to the corresponding segments of the spinal cord. Positive somato-sensory evoked responses were found in 69.2% of the irradiated rats (p=0.019), compared to 18.2% of the non-irradiated rats.

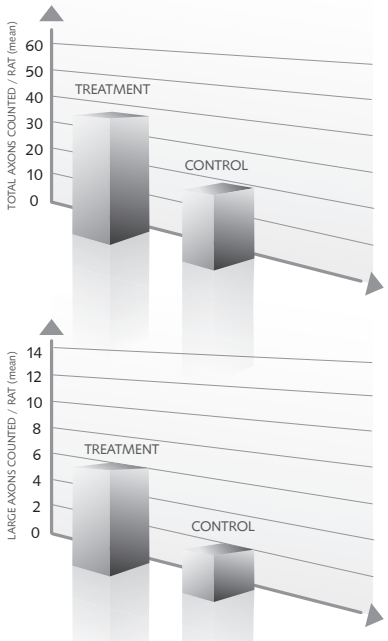

Fig. 6. Bar graphs illustrating the results double-blind randomized study evaluating regeneration of the transected rat sciatic nerve after suturing and postoperative low power laser treatment.
a. Graph showing a statistically significant increase in the total number of axons in the laser-treated group (p=0.026), compared with the nontreated control group.
b. Graph showing a statistically significant increase in large diameter axons in the laser-irradiated group (p=0.021), compared to the non-irradiated control group.

Immunohistochemical staining in the laser-treated group showed an increased total number of axons (p=0.026) and better quality of regeneration process, which became evident by an increased number of large diameter axons (p=0.021), compared to the non-irradiated control group (Fig. 6). The study suggests that postoperative laser phototherapy enhances the regenerative processes of peripheral nerves after complete transection and anastomosis.

2. Regeneration of the Sciatic Nerve in the Rat after Complete Segmental Loss and Neurotube Reconstruction

In cases where peripheral nerve is injured and complete segmental loss exists, the treatment of choice is nerve reconstruction using an autogenous nerve graft. The use of a regenerating guiding tube for the reconstruction of segmental loss of a peripheral nerve has some advantages over the regular nerve grafting procedure.

This double-blind randomized study was done to evaluate the efficacy of 780-nm laser phototherapy on the acceleration of axonal growth and regeneration after experimental peripheral nerve reconstruction by guiding tube [45]. The 5mm segment of the right sciatic nerve was removed and proximal and distal parts were inserted into an artificial neurotube (Fig. 7).

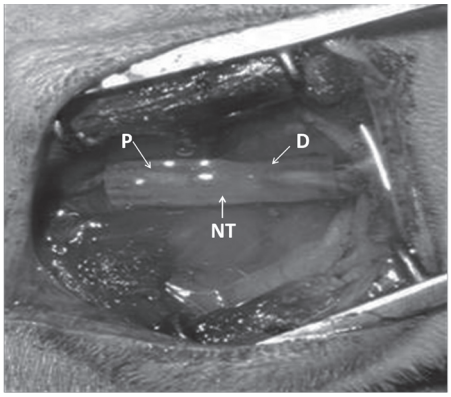

Fig. 7. Intraoperative photograph of the neurotube (NT) reconstruction procedure. A neurotube placed between the proximal (P) and the distal (D) parts of the rat sciatic nerve for the reconnection of 0.5 cm nerve defect.

The rats were divided into two groups - laser treated and non-laser treated. Postoperative low power laser irradiation was applied transcutaneously for 30 minutes: 15 minutes on the transplanted peripheral nerve area and 15 minutes on corresponding segments of the spinal cord, during 14 consecutive days. Conductivity of the sciatic nerve was studied by stimulating the sciatic nerve and recording the somato-sensory evoked potentials (SSEP) from the scalp.

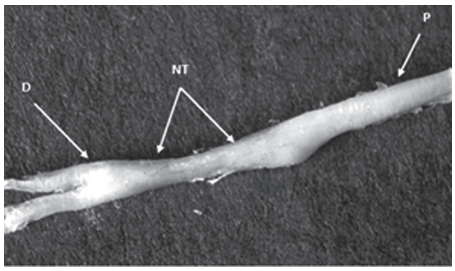

Fig. 8. Photograph of the sciatic nerve of an adult rat 3 months after neurotube (NT) reconstruction. The neurotube recreated the anatomical connection of the previously transected and divided nerve, and a distance of 0.5 cm was recreated.

Three months after surgery SSEP were found in 70% of the rats in the laser-treated group in comparison with 40% of the rats in the non-irradiated group. Morphologically, the transected nerve had good reconnection in both groups and the neurotube had dissolved (Fig. 8). The growth of myelinated axons, which crossed through the composite neurotube, was found and the continuation of axonal sprouting through the area of the tube to the distal part of the nerve was recognized. The laser treated group showed more intensive axonal growth compared to the non-irradiated control group.

PHOTOTHERAPY ON NERVE CELL GROWTH IN VITRO AS A POTENTIAL PROCEDURE FOR CELL THERAPY

Neuronal loss and degeneration resulting from peripheral nerve injuries has lead us to explore the possibility of using laser phototherapy on cells, as a method of preventing or decreasing this phenomena. Rochkind [46] investigated the effect of 780-nm laser phototherapy on sprouting and cell size of embryonic rat brain cells, which were grown on microcarriers (MC) and embedded in neurogel.

Cell cultures: Whole brains were dissected from 16-days old rat embryos (Sprague Dawley). After mechanical dissociation, cells were seeded directly in neurogel, or suspended in positively charged cylindrical MC. Single cell-MC aggregates were either 780-nm laser irradiated within one hour after seeding, or cultured without irradiation.

Neurogel (hyaluronic acid and laminin) was enriched with growth factors: BDNF and IGF-1 [47].

780-nm Low Power Laser irradiation of 10, 30, 50, 110, 160, 200 and 250 mw were used to optimize energy density for activation of nerve cell cultures. Dissociated cells or cell-MC aggregates embedded in neurogel, were irradiated for 1, 3, 4 or 7 min.

Fluorescent staining: Cultures were fixed with 4% paraformaldehyde and incubated with antibodies against neural cell marker: Mouse anti Rat microtubule associated protein. Cells were then washed and incubated with Texas Red conjugated goat anti mouse IgG.

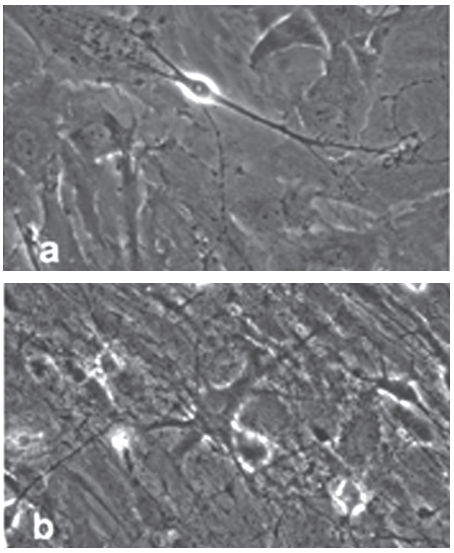

Fig. 9. Photographs of the effect of 780-nm laser irradiation treatment on perikarya and fibers of nerve cells derived from rat embryonic brain.
Dissociated brain cells were embedded in NeuroGel and were either exposed to single radiation dose of 50 mW for 3 min (b), or were non-irradiated controls (a). Large neural cells exhibiting thick fibers were observed after 8 days in vitro irradiated cultures (b). Original magnification: 200X.
(Lasers Surg Med, in press, 2009)

A rapid sprouting of nerve processes from the irradiated cell-MC aggregates was detected already within 24h after seeding. The extension of nerve fibers was followed by active neuronal migration. Differences between controls, and irradiated stationary dissociated brain cultures, became evident at about the end of the first week of cultivation - several neurons in the irradiated cultures exhibited large perikarya and thick elongated processes (Fig. 9). Furthermore, during the next 2-3 weeks of cultivation, neurons in the irradiated cultures developed a dense branched interconnected network of neuronal fibers. The sprouting of long processes from large cell body was mainly observed in immunofluorescent MAP-2 staining (Fig.10).
This study suggests that laser phototherapy may play a role in prevention of neuronal loss and accelerate axonal regeneration.

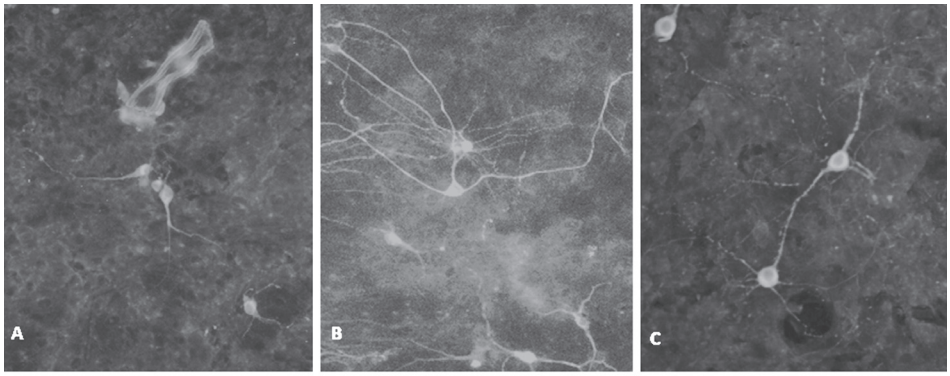

Fig. 10. Immunofluorescent staining of controls and laser irradiated neuronal brain cells migrated from cell-MC aggregates in NeuroGell. **a:** non-irradiated control. **b:** 50 mW 1 min irradiation. **c:** 50 mW 4 min irradiation. In the irradiated cultures note large perikarya bearing long interconnected fibers, which are positively stained for the neuronal marker MAP-2. Original magnification: 200X.

780-nm LASER PHOTOTHERAPY IN CLINICAL TRIAL

Based on the outcome of animal studies, a clinical double-blind, placebo-controlled randomized study was performed to measure the effectiveness of 780-nm low power laser irradiation on patients who had been suffering from incomplete peripheral nerve and brachial plexus injuries for 6 months up to several years [48]. Most of these patients were discharged by orthopedics, neurosurgeons and plastic surgeons without further treatment. In this study 18 patients with a history of traumatic peripheral nerve / brachial plexus injury (means duration from injury to treatment: 7 months in laser-treated group and 11.5 months in placebo group) with a stable neurological deficit and a significant weakness, were randomly divided to receive either 780-nm laser or placebo (non-active light) irradiation.
The laser or placebo (non-active light) treatment was applied transcutaneously; each day for 21 consecutive days, 5 hours daily (3 hours to the injured area of the peripheral nerve and 2 hours to the corresponding segments of the spinal cord). The laser or placebo device were placed approximately 40 cm from the skin treated point, focused on the injured area of the peripheral nerve or corresponding level of the spine (area of corresponding segments of the spinal cord).

Laser dosage:

Spinal cord area - laser irradiation was performed transcutaneously directly above the projection of the corresponding segments of the spinal cord, which was divided into 2 intravertebral levels. Each level was irradiated for 60 minutes a day (150 J/mm2), totaling in 120 minutes a day (300 J/mm2).
Peripheral nerve area - laser irradiation was performed transcutaneously directly above the projection of the injured nerve, which was divided into 3 parts: proximal, injured area and distal. Each section was irradiated for 60 minutes a day (150 J/mm2), totaling in 180 minutes a day (450 J/mm2). The irradiating spot size was 3x2 mm (6 mm2). The penetration of the near-infrared 780-nm wavelength was approximately 4 cm. Analysis of results of this trial in the laser-irradiated group showed statistically significant improvement in motor function in the previously partially paralyzed limbs, compared to the placebo group, where no statistical significance in neurological status was found (Fig. 11).

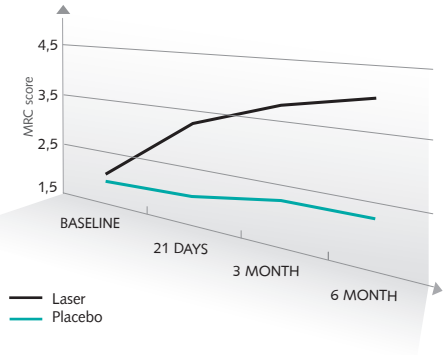

Fig. 11. Graph of the motor function follow-up in injured patients who underwent 780-nm laser phototherapy or placebo treatment.
Mean motor function (+SD) of all affected muscles was examined in injured patients using the Medical Research Council (MRC) Grading System. The analysis of the results showed that at baseline the 780-nm laser-treated and placebo groups were in clinically similar conditions (p=0.887). The analysis of motor function during the 6-month follow up period compared to baseline showed statistically significant improvement (p=0.0001) in the laser-treated group compared with the placebo group.

Mean motor function of the most influential (functionally dominant) muscle for movement of the affected limb showed a statistically significant improvement in the laser-irradiated group compared to the placebo group. The function was improved mostly by increasing power of the dominant muscles and not intrinsic muscles. Electrophysiological observation during the trial supplied us with important diagnostic information and helped to determine the degree of functional recovery in nerve-injured patients. The electrophysiological analysis also showed statistically significant improvement in

recruitment of voluntary muscle activity in the laser-irradiated group, compared to the placebo group (Fig. 12).

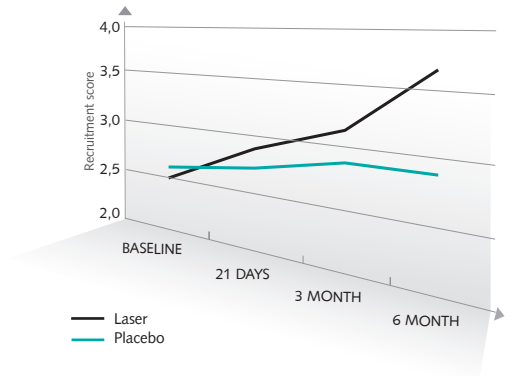

Fig. 12. Graph of the motor unit recruitment in injured patients who underwent either 780-nm laser phototherapy or placebo treatment. Motor unit recruitment, the mean of all examined muscles (+SD), was monitored in injured patients. The 780-nm laser-treated and placebo groups were in similar conditions at baseline (p=0.934). In the laser-treated group, statistically significant improvement (p=0.0006) was found in motor unit recruitment during the 6-month follow up period, compared with the placebo group.

This study is not the ultimate word regarding 780-nm laser phototherapy in peripheral nerve injured patients. The disadvantages of this study are the small amount of patients, different nerves and etiology of injury. Nevertheless, this pilot study suggests that in peripheral nerve injured patients 780-nm low power laser irradiation can progressively improve peripheral nerve function. That leads us to continue this study with perspective to multicenter trial.

CONCLUSIONS

Results of the experimental study on denervated muscles suggests that laser treatment can restore its function to a substantial degree when initiated at the earliest possible post-injury stage. These findings could have direct therapeutic applications for preserving the function of denervated muscle after peripheral nerve injury.

The extensive review of published articles reported in this paper as well as in previous ones published in Muscle and Nerve [16] and Neurosurgical Focus [17], revealed that most of experimental studies showed phototherapy to promote the recovery of the severely injured peripheral nerve. This review makes it possible to suggest that a time for broader clinical trials has arrived. The significance of the experimental and clinical studies is the provision of new laser technology for treatment of severe nerve injury.

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Key words: Osteoarthritis, High Intensity Laser Therapy, Low Level Laser Therapy, Ultrasound.

Clinical experience using Hilterapia® in "knee arthrosis".

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ABSTRACT

The aim of this study is to compare the efficacy of High Intensity Laser Therapy "HILT" against Low Level Laser Therapy "LLLT" and therapeutic ultrasound "US", in combination with exercises, in relieving knee pain, increasing walking distance without pain and squatting in patients with knee early osteoarthritis (OA).

Thirty subjects with knee early OA, males and females, age between 40 and 72 years, were enrolled. Participants were randomly and equally classified into three groups. All participants received exercise program for knee in combination with one of the therapeutic modalities compared (HILT, LLLT, US). All participants received six treatments for three weeks (two-sessions/week).

The results show that HILT is significantly more effective than LLLT and therapeutic US in inhibiting pain, increasing walking distance without pain and improving the ability to squat in people with knee early OA. No differences between LLLT and therapeutic US effectiveness in the treatment of early OA have been found.

INTRODUCTION

Knee osteoarthritis (OA) is a complex disease whose pathogenesis includes the contribution of biomechanical and metabolic factors which gradually lead to articular joint tissue destruction [1,2]. Knee OA is a common musculoskeletal disorder in the Saudi population and is a leading cause of physical disability. Pain and functional disability "like walking and squatting", are considered the most common complaints by OA patients. Knee OA affects both males and females and is one of the two major skeletal disorders (OA and osteoporosis) with strong social impact. It has been reported on 1999, that about 50% of adults with knee OA were "unable" or had "much difficulty" in crouching, stooping, or kneeling [3]. More than 30% of adults with knee OA were "unable" or had "much difficulty" in walking a quarter of a mile; more than one-fourth was unable to take 10 steps without resting. More than 25% were "unable" or had "much difficulty" in lifting or carrying 10 lbs [3].

In addition to NSAIDs (nonsteroidal anti-inflammatory drugs), routine therapy may use instrumental therapeutic modalities such as ultrasound or low level laser for their analgesic effects in combination with daily exercise. Regular exercise is recommended for middle-aged and older people, but the effect of exercise on the development of OA in older people is unclear, especially if they are overweight. Some studies have suggested that exercise has a protective effect [4,5]. The efficacy of weight bearing exercises on increasing the thickness of normal knee cartilage has been assessed using MRI (la prima volta scrivere per esteso) [6]. It has been reported that exercises were associated with an increase in tibial cartilage volume, free from cartilage defects. Also, moderate physical activity, including regular walking, was associated with a lower incidence of bone marrow lesions [6].

Controlled clinical studies of LLLT effectiveness in knee OA showed different and sometime contradictory results [7] . In a double blind, placebo-controlled study, no difference was found between the LLLT and placebo groups in relieving knee pain [8].

The results of clinical trials on the effectiveness of ultrasound in OA treatment were also controversial. In clinical trials carried out on 294 patients no difference between US and placebo were found for the outcomes of pain and patient-assessed improvement [9]. On the other hand, some studies suggest that therapeutic US is a safe and effective treatment modality in pain relief and improvement of functions in patients with knee OA [10].

High intensity laser therapy (HILT-or Hilterapia) is an advanced therapeutic approach of Orthopedic Physical Therapy. Its characteristic is a high peak power laser pulse, featuring peculiar frequencies and pulse width. As reported by some research, HILT has the capability to induce indirect photomechanical effects, which can act on the lymph drainage pump, performing their action on the inflammatory process and on cells of the connective tissues, stimulating the production of matrix molecules, like collagen and proteoglycans [11].

The aim of the study is to compare the efficacy of HILT against LLLT and US in

combination with exercises in relieving knee pain, walking distance without pain and squatting in patients affected by knee early OA.
The comparison between High Power- and Low Level - Laser Therapies (very different as regards the energy release into the tissue) is, in our opinion, particularly interesting because of the different mechanisms at the basis of the two therapeutic strategies: mostly phototermical-photomechanical in the former case and mostly phototermical-photochemical in the latter one.

MATERIALS AND METHODS

Patients suffering for knee OA were recruited for this trial from Physical Therapy Department, King Faisal Specialist Hospital & Research Center at Riyadh, Kingdom of Saudi Arabia. Thirty subjects with knee early OA, aged 40-72 years, were included. Informed consenst was obtained. Inclusion criteria required the presence of knee/s pain no more than two years, with clinical and radiological confirmation of the diagnosis of OA. Exclusion criteria were: knee joint disease other than OA, OA of the hip joint or of the foot joints, presence of knee varus or valgus deformities, knee surgery.
Patients were randomly and equally classified into three groups. All participants received exercises program for knee in combination with one of the therapeutic modalities compared (HILT, LLLT, US). Patients of group (1) were treated with HILT, group (2) with LLLT, and group (3) with US therapy. No control group was included for ethical reasons. All participants received six treatments for three weeks (two treatments/week). Equipments used were 3.0 HIRO (Pulsed high power Nd:YAG laser, λ 1064 nm) (ASA S.r.l., Vicenza, Italy), IDEA PULSED (IR laser, λ 905 nm), (ASA S.r.l., Vicenza, Italy), Ultrasound Unit (Zimmer Medizin System). Measurement parameters were perceptive pain using VAS, walking distance without pain (in meters), and squatting with/without pain. Measurements were taken pre-treatment and after completion of six sessions.

Therapeutic Protocol:

Hilterapia protocol	6 treatments, two treatment/week. Total energy of treatment 3000 Joule (J) (500 J. antero-lateral window; 500 J. antero-medial window; 500 J postero-lateral window, and 500 J postero-medial window; 500 J medial patella; 500 J lateral patella, according to the individual optical windows). Surface area: 24 cm^2 for all anterior and posterior windows. The total treatment duration is 15-20 min.
Low level laser therapy	6 treatments, two treatment/week. Surface area: 7-9 cm^2 for each compartment, 20 cm^2 for popliteal fossa. The session duration is 13 min. Energy 1,365 J.
Ultrasound therapy	6 treatments, two treatment/week. (Patella, medial and lateral compartments). Pus 8.1 W, era 4.1 cm. Power 1.7 w/cm^2. Dimensions of the treated surface: 12 cm^2. The treatment duration is 7.5 min.
Exercise program (for all patients)	Quadriceps muscle strengthening and hamstrings'stretch. Three sessions/day.

STATISTICAL ANALYSIS

Data obtained from the three groups have been compared using ANOVA test.

RESULTS

Comparing the baseline and after-treatment data, results showed the following:
HILT had a more significant analgesic effect than LLLT and therapeutic US in inhibiting pain ($p<0.0001$)
HILT was more effective in increasing walking distance without pain and improvement of the ability to squat without pain than LLLT/US in people with early OA ($p<0.0001$).
No difference were found between LLLT and therapeutic US in inhibiting pain, increasing walking distance without pain and improving of the ability to squat in people with early OA

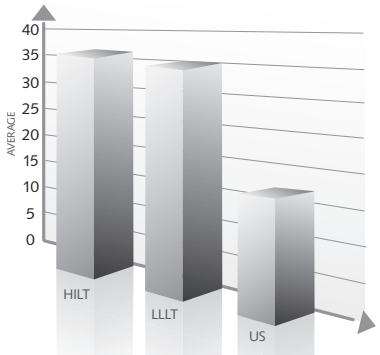

Figure 1. Effect of HILT, LLLT, and US on VAS. HILT shows the greatest effectiveness in pain inhibition in comparison with the other modalities: LLLT and US.

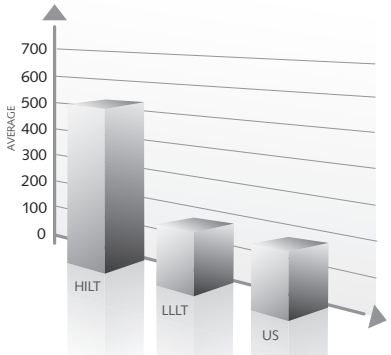

Figure 2. The effect of HILT, LLLT and US on walk. HILT had a significant effect in increasing walking distance without pain. No differences between LLLT and US were found.

Finally, the patients' satisfaction index is shown in Figure 5. Better results were achieved in group H, compared to group C also for this parameter. In group H, 9 patients were very satisfied (60%) and 6 satisfied (40%). In group C, 6 patients were very satisfied (40%), 7 satisfied (46%), 1 a little satisfied (7%) and 1 not satisfied (7%).

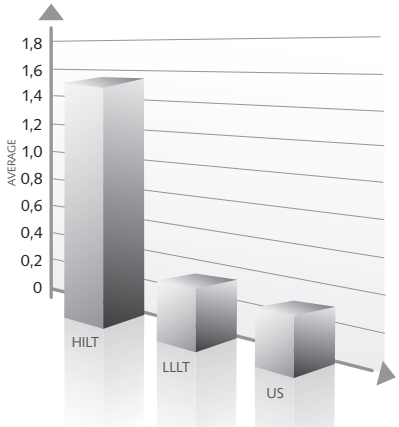

Figure 3. The effect of HILT, LLL and US on squat. As shown, HILT is more effective that LLLT and US (at p<0.0001).

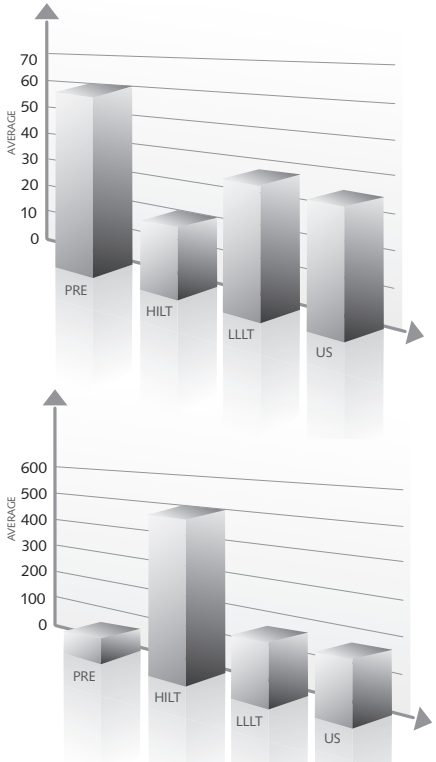

Figure 4-5. The effect of HILT, LLLT and US on VAS and walk. HILT had a very significant effect on pain reduction and improved the ability of knee OA patients to walk for longer distance whitout pain. No significant differences between LLLT and US on pain inhibition or function improvement in people whit kneeOA was found.

DISCUSSIONS

The results from our study may support the importance of HILT application for analgesic and anti-inflammatory effects in patients with symptomatic knee OA, over other therapeutic modalities as LLL and US. In general, in patients with knee OA, an association between pain severity and physical disabilities "as walk and squat" is observed. With the significant effect of Hilterapia on pain inhibition, the patient's ability to walk and squat without pain is improved.

In knee OA, pain control represents one of the principal tasks in order to get over acute phases. In this study, patients enrolled in the in Hilterapia group reported very early (often after the first treatment) a significant pain reduction. In patients who received other physical Therapies "LLLT or US", in combination with exercises, little or no significant pain reduction was observed. The effectiveness of LLL and US has been often investigated with different results. LLLT showed some limits in the treatment of knee OA, possibly due to low penetration and a low intensity of the light radiation [12]. A recent Cochrane review [13] didn't succeed in demonstrating a sure effect of laser therapy, mainly due to methodological problems in the studies, as doses and wavelength of laser.

In the current study, US therapy appears to have no benefit over placebo for people with knee OA. In a Cochrane review of three studies with a total of 147 participants assigned to compare US to placebo, there was no benefit of US therapy for pain relief, range of motion or functional status. These conclusions are limited by overall poor methodological quality of the comparative trials [14].

Hilterapia has proved his efficacy in the treatment of early knee OA, as shown in figures (1,2,3), in pain inhibition and improvement of knee functions. This data may come along with other studies which support its efficacy in different musculoskeletal diseases and it is believed to have anti-inflammatory, anti-edema and analgesic effects [15]. It seems that Hilterapia laser may overcome difficulties and limitations in LLLT, and prove its efficacy over LLLT.

For the effects of exercises on pain and disabilities of patients with early OA, it seems that quadriceps muscle strengthening may be attributed to pain inhibition and improved function. Research work suggested that quadriceps strength is strongly associated with knee pain and physical disability, even when activation and psychological factors are taken into account [16,17]. These deficits in physical capacity may be corrected with exercise training. By decreasing pain and increasing joint movement, knee exercises for strengthening quadriceps muscles and stretching hamstrings muscles may help patients to maximize their ability to overcome their physical disabilities as walk and squat.

From our data, Hilterapia appears to be the an elective treatment tool for pain reduction and improvement in functional disabilities in people with knee OA.. This study may come along with other preliminary studies indicating that Hilterapia can be more effective than LLLT in pain and flogosis control, due to its more intense and deeper effects [18,19,20].

CONCLUSIONS

Because of its efficacy in relieving pain and improving function, Hilterapia in combination with exercises can be considered a very suitable therapeutic tool in treatment of early knee OA.

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