Volume 29 - N. 2 - June 2022
ISSN: 1884-7269
Indexed in PubMed and Scopus

Laser Therapy

International Journal of Laser PhotoBioModulation, Biophotonics and Surgery

Official Journal of

World Federation of Society for Laser Medicine and Surgery (WFSLMS)
International Society for Laser Surgery and Medicine (ISLSM)
International Academy Laser Medicine and Surgery (IALMS)
International Phototherapy Association (IPTA)
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filo diritto editore DAL 2008

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Indexed in PubMed and Scopus

ISBN 979-12-80225-47-4
ISSN: 1884-7269
DOI: 10.26352/GJ06_1884-7269_LASER-THERAPY_VOL.29_JUNE2022

First Edition June 2022

filodirittoeditore.com
inFOROmatica srl, Via Castiglione, 81, 40124 Bologna (Italy)
inforomatica.it
tel. 051 9843125 - Fax 051 9843529 - proceedings@filodiritto.com

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ISSN: 1884-7269
Indexed in PubMed and Scopus
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EDITORIAL

It is with great pleasure that we announce the resumption of the peer journal "LASER THERAPY" publications after two years of silence. In fact, due to the terrible pandemic that has devastated our world, has taken away friends and family members and has deeply changed our lifestyles, the latest issue was published online in the Spring of 2020.

Prof. Toshio Ohshiro, founder of "Laser Therapy" charged us to manage the Journal and we want to thank him for the trust placed in us, as well as for the enormous work done during these many long years. However, he will remain our point of reference as "Old Editor-in-Chief and Founder".

We want to remember our friends who have supported the newspaper over the years and who are no longer with us: Kenij Yoshida, Abraham Baruchin and Kazuhiko Atsumi: may they rest in peace.

The realization of this issue was full of difficulties and required a lot of work on our part, but we did our best to get to the final result because we think that it may represent a sign of recovery of a normality after the COVID emergency.

For this issue we have decided to offer readers open access to all articles and authors the opportunity to publish at no cost.

The publisher is an Italian company, FILODIRITTO (www.filodiritto.com), and with the help of the Head of the Organizational Secretariat, Ms. Roberta Miano, we want to be able, for the Autumn issue, to inaugurate a new website and an automatic submission system.

Last but not least, we want to thank all the Institutions, Societies and Associations that have supported LASER THERAPY in these years. We ask them to go on to choose it for next years.

We thank all of you, Authors, Board Members, Reviewers and Readers, with the prayers of giving us the great help we need.

Enjoy the reading!
Sincerely

Leonardo Longo, Editor-in-Chief

Carlo Fornaini, Co-Editor-in-Chief

ISSN: 1884-7269
Indexed in PubMed and Scopus Volume 29 - N. 2 - June 2022

Potential role of Er:YAG laser and fluoride in the dental enamel remineraliation: a raman spectroscopy preliminary *ex vivo* study

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ABSTRACT

Background and aims: Dental caries are a widespread oral disease and a serious public health problem, starting by teeth demineralization, which is a loss of minerals such as calcium and phosphate. Modern caries treatment is aimed at preventing the disease progression by teeth remineralization which is a supply of minerals to the enamel. The most popular remineralization method is the treatment of teeth with fluoride. Er:YAG laser has also gained research attention as a method for improving the uptake of fluoride and phosphate by introducing chemical and morphological changes into the structure of enamel but, while some researchers described it as effective, others found no significant effect from its application. This work aimed to further study the effect of Er:YAG laser, alone or combined with fluoride, to dental enamel.
Materials and Methods: Twenty upper central human incisors, extracted for periodontal reasons, were used in the study. Samples were demineralized by acetic acid and divided into four groups: a) control, b) fluoride + Er:YAG laser, c) Er:YAG laser alone and d) fluoride alone. The remineralization rate of teeth was estimated by Raman Spectroscopy.
Results: In comparison with the control group, the phosphate peak's intensity increased notably for the teeth treated by fluoride, but decreased slightly for the teeth treated with Er:YAG laser and with a combination of the laser and fluoride.
Conclusions: With the limits of this study, due to the limited samples number, Er:YAG laser, alone and combined with fluoride, seems to be not effective, at the parameters used, for the enamel remineralization.

Key words: Dental decay, Enamel remineralization, Er:YAG laser, Fluoride, Raman spectroscopy

[*]***Running Head:***
ER: YAG laser for dental remineralization
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Introduction

Dental caries is the most common disease of dental tissues and the second most common infectious disease after respiratory diseases. Its cause is multifactorial, and its pathogenesis consists of the disruption of the balance between demineralization and remineralization of the teeth [1]. The most critical factors for tooth demineralization are the acid produced by cariogenic bacteria [2] and the loss of enamel's inorganic structure due to erosive processes. One of the most high-risk factors for tooth erosion is excessive consumption of soft drinks with erosive properties able to decrease salivary function and perturb the enamel structure. The inorganic contents of tooth hard structures are lost due to the exposure to acid with a bacterial and nonbacterial origin, so decreasing tooth hardness [4]. The tooth hard tissue loss is caused by two important processes, the dissolution of enamel's hydroxyapatite crystals and the distribution/dissolution of calcium, phosphate, and hydrogen ions into and out of the enamel microstructures. Tooth enamel demineralization is a dynamic process beginning with the formation of incipient subsurface lesions: when the demineralization process persists, incipient clinical lesions result in enamel cavitation [5].
The remineralization of decalcified enamel and the restoration of its surface hardness are two important and challenging aims in dentistry. Topical applications of fluoride have been the primary method for stabilization of early carious lesions through remineralization, and thus, fluoride has had a profound effect on reducing caries prevalence. However, fluoride has only a partial cariostatic and remineralization potential [6] and dental fluorosis remains an issue of concern, especially in young children. Combinations with other methods and non-fluoride agents have been suggested as alternatives in this regard [7]. An adjunct method in the prevention of caries is the use of laser [8]. The caries preventive effect of this laser can be related to reducing the permeability of the enamel by re-crystallization of enamel crystals into larger ones and removal of the enamel inter-rod substance [6]. Laser irradiation causes longitudinal exposure of the enamel rods as a consequence of the micro-explosion, resulting in morphology changes in the enamel through melting and re-crystallizing processes, and creation of larger hydroxyapatite crystals on the surface. This reduces the enamel permeability of acid penetration and modify the mineral composition of its deeper layers [9-11]. Moreover, the heating and melting processes results in significant reduction of carbonate content in hydroxyapatite up to a complete loss resulting in enamel composition that is more resistant to demineralization [12].
Several studies have been performed to observe the behaviour of different laser wavelengths, alone or in combination with fluoride application, on dental enamel remineralization. Thus, Fekrazad *et al.*, [6], Fornaini *et al.*, [14], Zezell *et al.*, [15], Al-Maliky *et al.*, [16] have found a positive effect on the remineralization from the CO_2, Er:YAG, Nd:YAG, and 445 nm diode lasers respectively. On the other hand, Moghadam *et al.*, [17] have found that although the application of Er:YAG laser was effective for remineralization, its combination with fluoride has not shown a significant performance improvement.
Moreover, Delbem *et al.*, [18], Nair *et al.*, [19], and Ulkur *et al.*, [20] have not found any significant contribution from the Er:YAG laser to the demineralization prevention.
The aim of this preliminary ex vivo study is to evaluate, by means of Raman spectroscopy, the effects of Er:YAG laser irradiation, alone and in combination with fluoride, on human permanent teeth.

Materials and methods

Teeth treatment

Twenty human incisors, extracted for periodontal reasons were utilized in this study: they were polished and subjected to the demineralization by immersing them into a demineralizing solution (2.9 g of NaCl, 0.12 g of $CaCl_2$, 0.13 g of NaH_2PO_4, 5cc of NaF (100 ppm), 5 cc of NaN_3 (2% ww), 1.5 cc acetic acid. The pH of the solution was maintained at 4.5. The solution was renewed daily to prevent the accumulation of materials produced by demineralization and the consequent pH change. After 48 hours, the surface of each sample was washed with a syringe containing artificial saliva and placed for storage in the container with the artificial saliva (2.9 g of NaCl, 0.12 g of $CaCl_2$, 0.13 g of NaH_2PO_4, 5cc of NaF (100 ppm), 5 cc of NaN_3 (2% ww)). Subsequently, by means of a diamond burr, a square sample was obtained from the crown of each of them and as shown in Fig. 1., the nineteen samples (one was destroyed and was not able for the research) were divided into four groups: four teeth in group 1 and five teeth in all other groups. After that, each group of teeth has been subjected to the following treatment:

- Group 1 – four teeth were irradiated by the Er:YAG laser (0.5 W power; 2940 nm wavelength; 0.6 mm spot diameter; 120 µs pulse duration, 20 Hz repetition rate)
- Group 2 – five teeth that were not treated and used as a control
- Group 3 – five teeth that were treated by the fluoride at concentrations between 0.5 and 10 ppm and simultaneously subjected to Er:YAG laser (same parameters)
- Group 4 – five teeth treated by fluoride only (same concentration)

After the treatment all teeth have been fixed on a plate and subjected to Raman measurement. Because this is a blind study, the researcher who performed the Raman analysis did not know what was the treatment for each group.

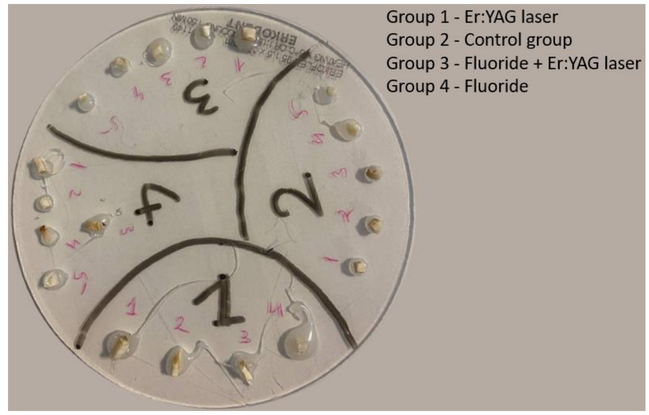

Fig. 1. Four groups of teeth treated by different methods

The laser used in this study is LiteTouch (Light Instruments, Yokneam, Israel), emitting at 2940 nm. It was used in contact mode with the handpiece AS7825X and the sapphire tip AS7073X (1.0 x 14 mm). This is a particular dental laser device where the optical resonator is located very close to the handpiece, in this way avoiding the problems related to the delivery system of the laser beam and mainly due to the great absorption of this wavelength by water. The irradiation was performed with one passage of the beam on enamel in "lawn cut" way, under an "air/water spray".

Remineralization measurement by Raman Spectroscopy

Raman Spectroscopy has gained attention for the biomedical application due to its ability to provide a quantitative and qualitative chemical analysis of the biological sample [22].

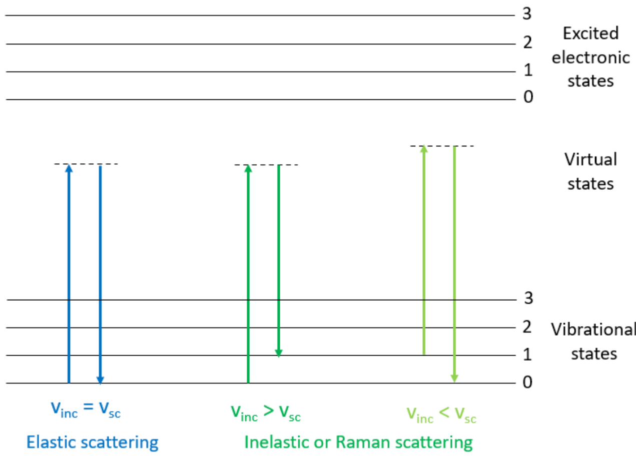

Fig. 2. Schematics of the Raman Scattering

The working principle of Raman Spectroscopy is based on the inelastic scattering of light in combination with a vibrational excitation of the molecular structure. Inelastic scattering occurs in rare cases when the molecule excited by the incident light returns one vibrational state lower or higher than its original state (see Fig. 2). This results in a frequency difference between the incident and scattered light, called Raman shift, that is specific to each molecule. Therefore, the position of the Raman peak can be used to discriminate the type of molecule present in the sample and its intensity indicates the concentration of the molecule [22].

Figure 3 (a) illustrates the schematics of the Raman spectrum of the tooth enamel [23]. As can be seen, there are several Raman peaks in the range from 550 to 1150 cm^{-1}, but the most prominent one is a peak around 960 cm^{-1} associated with the phosphate molecule ions (PO_4^{2-}). Silveira *et al.*, have used Raman Spectroscopy to measure the demineralization and remineralization of the enamel due to the application of the whitening product and saliva respectively [24]. They have shown that the intensity of the 960 cm^{-1} Raman peak decreases when the teeth are demineralized and increases with the remineralization process. In this work, the degree of enamel remineralization due to the application of three types of treatment (fluoride, laser, laser + fluoride) are going to be measured by the 960 cm^{-1} Raman peak's intensity. The teeth samples have been measured by HORIBA LabRam HR Evolution spectrometer with 785 nm wavelength at 5 different points each. The HORIBA software has been used to remove the background of the Raman spectra. The average of the five obtained spectra has been found for each tooth and the average of all teeth inside the group has been calculated. The intensity of the Raman peak at 960 cm^{-1} has been compared in the final representative spectrum of each group.

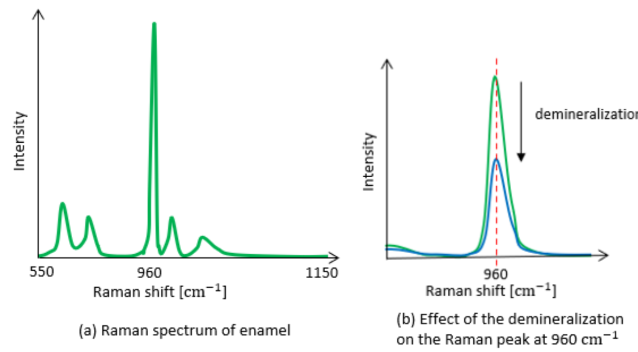

Fig. 3. Schematics of the Raman spectra of enamel

Results

Figure 4 illustrates the results of the experiment: an average of the Raman spectra of each group of teeth. As can be seen, all spectra correspond to the Raman spectrum of enamel found in the literature (Fig. 3, a) and have the highest peak around 960 cm^{-1}. The magnification of this region shows that the intensity of the peak increases with respect to the control only for the teeth treated by fluoride, which proves the remineralization abilities of this method. However, laser irradiation alone and in combination with fluoride resulted in the reduction of the peak's intensity. The difference between the control group and laser-irradiated teeth is very small, so it could be assumed that the laser has no effect on the teeth remineralization. However, a considerable difference between the fluoride treatment alone and with an application of laser implies that the laser has an influence on the peak's decrease. One of the explanations for such behaviour can be the morphological changes that the laser is known to introduce to the teeth. Thus, the microscopic image of the teeth from groups 3 and 4 shown in Fig. 5., illustrates the difference in the teeth structure of teeth treated by the fluoride and laser or by fluoride alone. It can be also seen that the focus on the right image is worse, which can significantly decrease the Raman signal intensity.

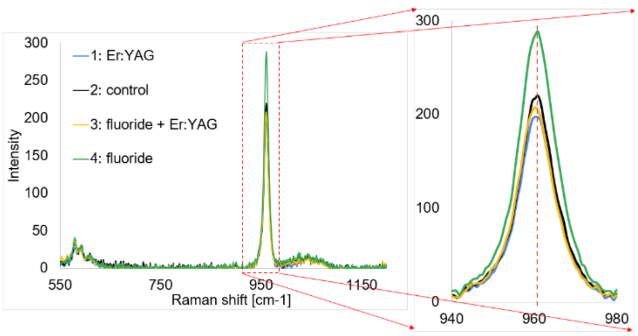

Fig. 4. Average Raman spectra of each of the four groups of teeth

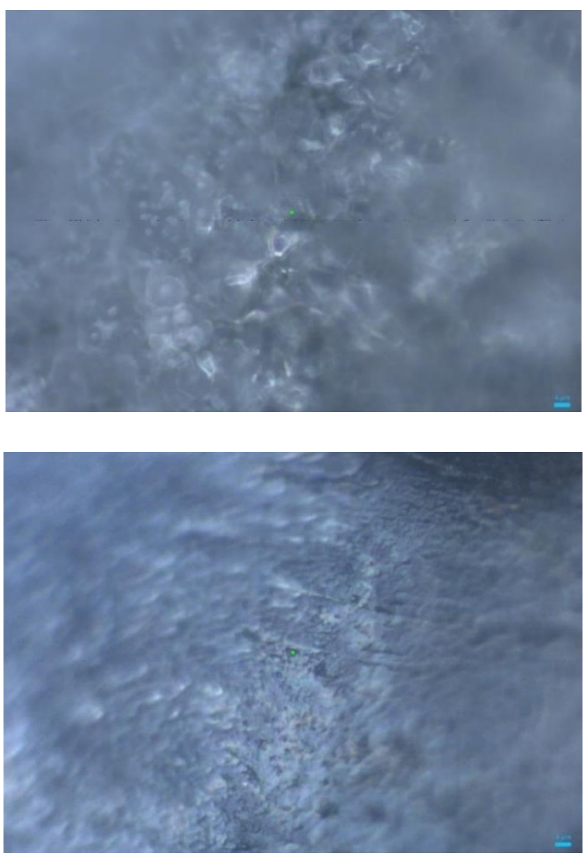

Fig. 5. Microscopic images of the sample teeth from the group 3 (left) and 4 (right)

Fig. 6 shows that the variation of the data from the teeth in the same group is considerable, which makes it difficult to differentiate between groups 1, 2, and 3. To the best of our knowledge, there is only one paper that has evaluated the effect of the laser treatment of the teeth using Raman Spectroscopy [25]. Kerr *et al.*, have applied Microwave energy to the teeth followed by the treatment with remineralization protocol. The results are not consistent: two out of four teeth are characterized by an expected increase of the Raman peak's intensity, but the peak has decreased for the other two teeth. This and the challenges with a focus due to the changed morphology can indicate that the Raman Spectroscopy is not a perfect tool for the remineralization assessment after the laser application.

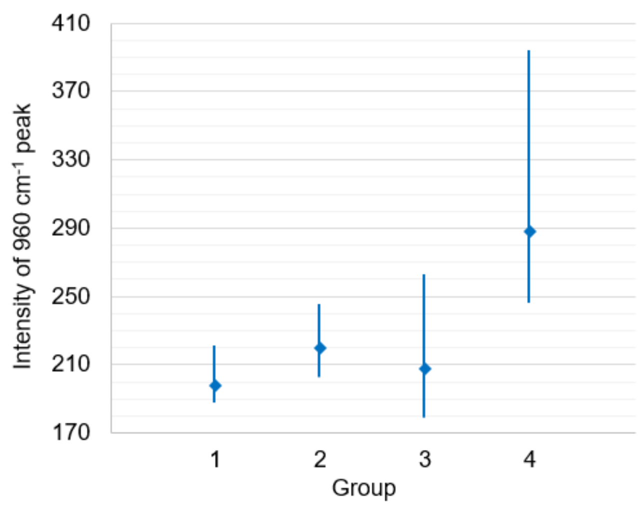

Fig. 6. Variation of the 960 cm^{-1} peak's intensity for all teeth

Discussion

Untreated dental caries is the most prevalent and common factor affecting health [26] and, on the global scale, it is estimated that they are prevalent among 2.3 billion adults with secondary dentition, and among 530 million children with deciduous dentition [27]. In 2015, the global cost of oral diseases was reported to exceed 540 billion dollars, consequently leading to major health and financial burden [28] and, for this reason, a great number of

research aiming to find new ways for decay prevention, has been performed in these last years.
Dental decay has a multi-factorial etiopathogenesis consisting in three main factors, the microorganisms (the dental plaque), the substrate (the fermentable carbohydrates in a diet), and the host susceptibility (the teeth and saliva) [29], and for each of them, several preventive approaches have been proposed. The two main methods suggested for obtaining a re-mineralization of tooth enamel are today fluoride and laser but opinions about their effectiveness are discordant. Xue and coll, comparing the effects of combined treatment of topical fluoride and semiconductor laser, concluded that lasers enhance the effect of fluoride on remineralizing but not on preventing enamel caries [30]. AlShamrani and coll. investigated the effects of combining erbium, chromium: Yttrium-scandium-gallium-garnet laser irradiation with fluoride application and concluded that acidulated phosphate fluoride application as well as laser irradiation prior to fluoride application increases enamel surface microhardness, so preventing the progression of enamel erosion [31]. Soltanimehr and coll. evaluated the efficacy of diode and CO_2 lasers along with calcium and fluoride-containing compounds for the remineralization of primary teeth and concluded that, while the highest microhardness was achieved after remineralization with CPP-ACP, the efficacy of the diode and CO_2 lasers was the same and no synergistic effect was found between materials and lasers [32]. Ceballos-Jiménez and coll. studied the acid resistance of dental enamel treated with remineralizing agents, Er:YAG laser and combined treatments and concluded that, although the combination of Er:YAG laser plus NaF and the single application of NaF showed values suggesting superior resistance to demineralization of dental enamel compared to all the other groups in the study, no statistically significant differences were found to support this assertion [33].
In our study, the conventional fluoride treatment method has shown expected results: the 960 cm^{-1} Raman peak's intensity has increased considerably showing the growth of the mineral content. The laser application for teeth remineralization has shown a questionable effect in different literature, which is also in agreement with our findings. Thus, the laser treatment has slightly decreased the Raman peak with respect to the control group, while the peak reduced significantly for the laser and fluoride treatment compared with the fluoride application only. One of the possible explanations is that the morphological changes induced by the laser may complicate the focus of the Raman laser and hence deteriorate the overall signal intensity.

Conclusions

With the limits due to the limited number of samples, we may conclude that this preliminary study of the Raman Spectroscopy application for the evaluation of the remineralization effect of the Er:YAG laser treatment demonstrated a good effectiveness of fluoride application. Use of Er:YAG laser did not seem to achieve the expected remineralization of enamel, as well as the association laser and fluoride. Future work is aimed at evaluating a larger number of the samples and conducting the XRD measurement before and after treatment for the monitoring of morphological changes.

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Acknowledgements
This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 860185.

Cite: *Issatayeva A, Fornaini C, Msino M, Cucinotta A.*
Potential role of Er:YAG laser and fluoride in the dental enamel remineralization: a Raman spectroscopy preliminary ex vivo *study.*
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 5-12.
DOI: 10.26352/GJ06F5001-June2022

ISSN: 1884-7269
Indexed in PubMed and Scopus
Volume 29 - N. 2 - June 2022

Dual Laser Circumcision: a novel technique to improve traditional surgery

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ABSTRACT

Circumcision represents one of the oldest and routinely performed urological surgery. It implies the exeresis of the prepuce due to ethnic, religious and medical reasons. Different authors reported straightforward advantages of this procedure including increased penile hygiene, reduction of penile cancer risks and lower rates of urinary tract infections. Circumcision can be easily performed and reproduced according to different techniques. Nowadays, different types of lasers have been developed and this technology is gaining consensus in the urological community. Aim of this article is to describe our surgical technique for laser circumcision with the Lasemar Eufuton 1500, clarifying the surgical steps and the benefits of this technology.

Key words: Circumcision, Laser, Phimosis, Paraphimosis, Technological Innovations, Diode Laser

Introduction

Circumcision represents one of the oldest urological surgeries and it has been routinely performed since the Sumerian era.[1] It implies the exeresis of the prepuce, the skin covering the penile glans, and it can be performed for ethnic, religious and medical reasons. Major surgical indications are: paraphimosis, phimosis, posthitis, recurrent balano-posthitis/balanitis, lichen sclera-atroficus and certain infectious diseases of the genitalia[2]. There are no reported specific contraindications to circumcision, however patients with active infection, suspected penile carcinoma or anatomic genitalia

**Running title:*
Dual Laser Circumcision: novel technique

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malformations should be further investigated prior to surgery. Different authors reported, within the scientific literature, straightforward advantages of this procedure such as increased penile hygiene, reduction of penile cancer risks and lower rates of urinary tract infections. Circumcision can be easily performed and reproduced according to different techniques. Fabrice D'Acquapendente[3] in 1666, firstly proposed a procedure based on a circular incision while Celse *et al.*,[4] in 1754 suggested a longitudinal approach. Moreover, Bell and Ricord, respectively in 1796 and 1838, produced new surgical tools to ease the procedure.[5,6]

Maiman developed the laser technology in 1959 and, since then, its application in medical surgery grew exponentially, due to its capacity to vaporize and sharply cut tissues. Nowadays, different types of lasers have been developed and this technology, once applied only to treatment of common skin diseases and aesthetic dermatology, is gaining consensus in the urological community.[7]

Aim of this article is to describe our surgical technique for laser circumcision with the LASEmaR® Eufoton® Mini DUAL 980/1470, clarifying the surgical steps and the benefits of this technology.

Material and Methods

LASEmaR® Eufoton® Mini DUAL 980/1470

LASEmaR® Mini DUAL 980-1470 (Eufoton® S.r.l.) (**Fig. 1**) is a new, fifth generation high power Diode portable laser with a power capacity output between 7-12 Watts and a dual working wavelength ranging from 980nm to 1470nm, with both pulsed or continuous energy transmission settings available.

Fig. 1. LASEmaR® Mini DUAL 980-1470 (Eufoton® S.r.l.)

Thanks to these technical specifics, its use has been already validated in different soft tissue surgical procedures (aesthetic medicine, plastic, gynaecology, general, proctological and endovenous phlebological surgery).[8-11] As consequence of its specific working wavelength (**Fig. 2**) it is a fast and efficient laser for cutaneous incisions, allowing an atraumatic haemostasis, skin tightening and contouring with improved fractional rejuvenation of the skin.[11]

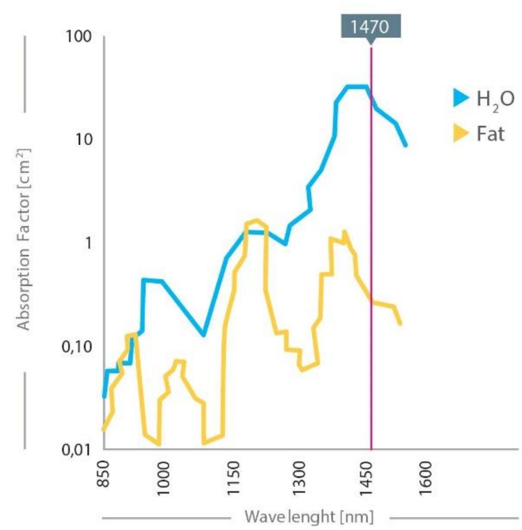

Fig. 2. Thanks to the high-water absorption, it allows a precise hemostasis, applying lower energetic levels LEED (Linear Endovenous Energy Density)

Moreover, is known as the present gold standard for the EVLA (Endovenous Laser Ablation) in the outpatient elimination of saphenous reflux thanks to the DPC (Dynamic Pulse Control), that allows a selective photocoagulation of water and fat, permitting a better endovenous occlusion at the lowest energetic levels LEED (Linear Endovenous Energy Density), and reduced thermal damages to neighbouring tissues[8]. In Gynaecology, due to the development of a specific Ladylift protocol it allows a safe treatment of vaginal atrophy and stress urinary incontinence, improving the tone of the vaginal walls and accelerating the production and the remodelling of mucosa's collagen.[9,10]

The machine is extremely compact, multifunctional and enriched by numerous headpieces and pre-set protocols making the laser immediately usable for different specialistic treatments. For circumcisions, we use the surgical handpiece (**Fig. 3**), designed to improve ergonomics and dexterity.

Fig. 3. surgical handpiece, designed to improve ergonomics and dexterity, equipped with a visible aiming beam that permits pinpoint accuracy, reducing damages to surrounding tissues during the procedure

It is a pen-like device providing a natural scalpel-like feel, equipped with a visible aiming beam that permits pinpoint accuracy, reducing damages to surrounding tissues during the procedure.

The device is both CE marked and FDA approved.

Surgical Technique description

Informed consent should always be acquired providing the patient with detailed knowledge of the procedure ahead of time. Specifically, all information regarding possible complications such as bleeding, infection, hematoma formation, poor cosmetic results and change in sensation during intercourse should be given. The patient should also be enlightened those erections, occurring in the post-operative setting, could cause pain and disruption of the suture line requiring re-intervention. Full recovery generally requires between four to six weeks of abstinence from both genital stimulation and intercourse. The procedure can be performed in the outpatient setting or after hospital admission, according to patients' comorbidities and preferences. Preparation of the surgical site includes a thorough surgical scrub of the genital area with povidone-iodine preparation.

Shaving and clipping of the pubic hair should be avoided to minimize the possibility of infection.

Sterile draping of the area should be used to identify the surgical field. Subsequent adequate local or regional anaesthesia should be administered. We usually perform a dorsal penile nerve block with or without a circumferential ring penile block according to patient's response to the first injection.

The first step of the procedure requires the retraction of the prepuce over the glans penis with frenulum exposure and incision (**Fig. 4**).

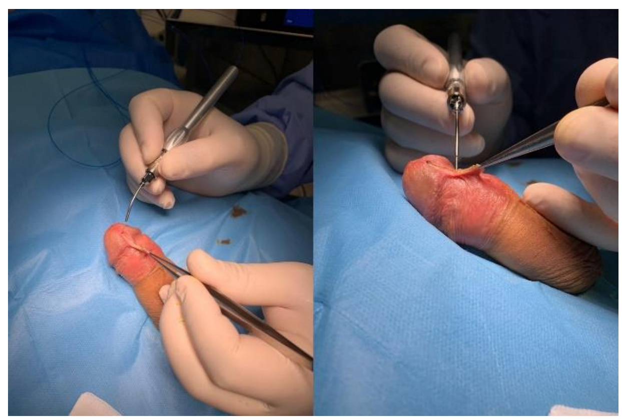

Fig. 4. frenulum exposure and incision

Then a circumferential cut is made around the shaft as far back as the scar line is to be placed, usually few millimetres distal to the corona (**Fig. 5**).

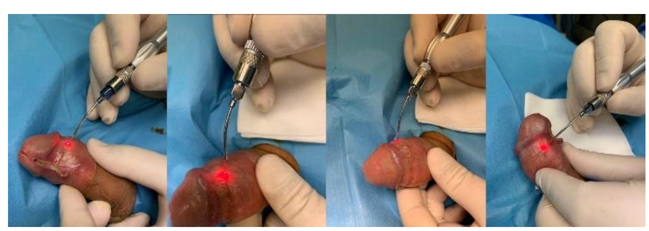

Fig. 5. circumferential cut around the shaft few millimeters distal to the corona

ISSN: 1884-7269
Indexed in PubMed and Scopus
Volume 29 - N. 2 - June 2022

A second circumferential incision is made around the shaft according to the prepuce tissue that has to be removed (**Fig. 6**).

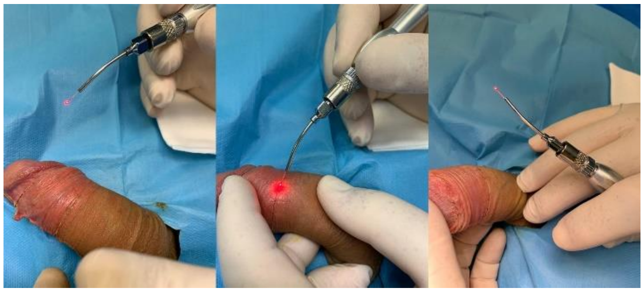

Fig. 6. second circumferential incision

A longitudinal cut is then made between the two previous circumferential ones (**Fig. 7**) and the excisional procedure is completed with the progressive bloodless incision and stripping of the rebounding prepuce (**Fig. 8**).

Fig. 7. a longitudinal cut is made between the two previous circumferential ones

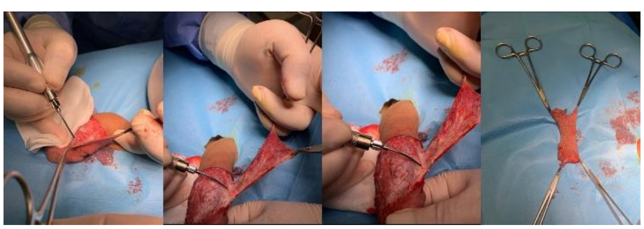

Fig. 8. the excisional procedure is completed with the progressive incision and stripping of the rebounding prepuce.

The free raw edges are re-approximated with four initial quadrant sutures placed on the dorsum, both sides and frenulum with the remaining foreskin sutured through simple interrupted sutures every four to seven mm intervals (**Fig. 9**).

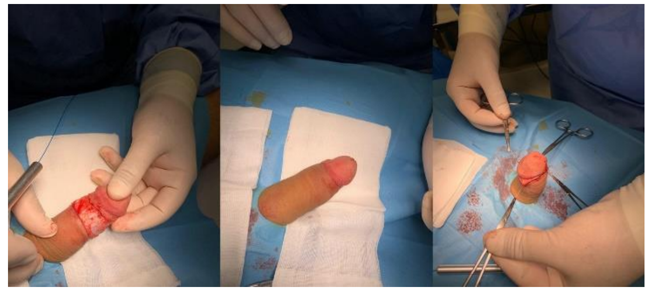

Fig. 9. the remaining foreskin sutured through simple interrupted sutures every four to seven mm intervals

The final appearance of the penile shaft is depicted in **Fig. 10**.

Fig. 10. finale appearance with compressive sterile dressing

While many surgeons use no dressing at all following the procedure, we usually apply a petroleum sterile jelly gauze wrapped circumferentially around the sutured area, followed by sterile gauze and lightly closed with self-adherent stretch gauze. This dressing is removed within 24 to 48 hours after surgery with no need of further dressing; the patient is then advised to gently wash the wound twice daily for the next seven days. After one month from the procedure the patient should be scheduled for a follow up consultation to verify the complete healing of the surgical site.

Discussion

Circumcision is one of the most common urological procedures, routinely performed in different

countries for medical, religious and cultural purposes. The benefits include both the reduction of urinary tract infections and sexually transmitted diseases, balanitis, posthitis, phimosis and paraphimosis prevention. In addition, when performed by trained physicians, it is a safe and reproducible surgery with low complication rates.[12]
Even though considered a simple procedure, some risk of complications needs to be taken into account. The most common one is bleeding, with an incidence varying between 0.1% to 3.1%, which can be easily controlled by ligature or electrocoagulation.[13] Other rarely reported complications are local edema and tenderness, hematoma, infection, wound dehiscence and scarring.[14] Within the scientific literature, different conventional techniques have been described including the dorsal slit, the sleeve and the guillotine approaches. [15] During recent years, technological advances such as CO_2 or YAG lasers have been proposed alternatively to the standard scalpel.[16, 17]
Moreover, various non-invasive circumcision devices have been increasingly been used due to reduced recovery periods, faster operative times and shorter learning curves.[18] The CO_2 laser was used for the first time in this field in 1964 by Patel and collaborators, reporting promising outcomes thanks to its high-water absorption, precise incision, excision and thermo-coagulation of small blood vessels with both improved haemostasis and a dry surgical field.[19] Gorgulu *et al.*, [20] demonstrated the association between the adoption of CO_2 laser and shorter operative time thus reducing bleeding complications and post-procedural pain, when compared with the traditional Guillotine technique. Xu *et al.*, [21] reported similar outcomes regarding the safety and efficacy of a modified CO_2 laser circumcision approach compared with the standard dorsal slit-sleeve one. Other Authors [22] confirmed these findings, publishing their personal experience in various case series.
The Holmium YAG laser has been proposed as a substitute technology for circumcision thanks to a molecular absorption of water particles around 2140 nm with a penetration depth of 0.4 mm, allowing both incision and coagulation, providing shorter operative time, less postoperative pain, less blood loss and fewer complications compared to traditional techniques.[16]
A compelling development in this field could be the application of diode laser to further improve laser circumcision outcomes. To the best of our knowledge, we present the first study describing the efficacy and safety of a circumcision technique using diode laser. Thanks to this technology, it is possible to incise and obtain an optimal haemostasis through the accurate cauterization of small vessels. Moreover, due to its beneficial physical properties, it provides enhanced precision in both cutting and sealing surfaces with reduced blood losses and less postoperative pain. This is of paramount importance considering the potential risks for sub-clinical penile injuries caused by an excessive use of mono-polar electrocoagulation. Tuncer *et al.*, described electrical burns, penile tissue damages and necrosis contributing to both erectile tissue dysfunction and irregular wound edges with poor cosmetic outcomes due to conventional mono-polar electrocoagulation.[23] For these reasons, they suggested to perform haemostasis with bipolar or laser technologies, as lower power (W) settings, are associated with reduced thermal transmission to surrounding tissues. Furthermore, diode laser could provide improved cosmetic appearances inducing both wound healing and skin remodelling, while maintaining the advantages of cutting and coagulating at the same time. Moreover, the ergonomic handpiece can be held close to tissues increasing both the stability of the surgeon's hands and treatment precision.
This work represents the first application of diode laser technology to safely and effectively perform circumcisions. It is a personal modified technique of

a surgeon (P.R.) with more than 20 years' experience with both traditional and laser circumcision techniques. Further prospective studies and randomized trials are advocated to both confirm the promising results of this new approach and compare the outcomes with traditional procedures.

Conclusions

We present for the first time a personal modified technique with Diode laser for circumcisions. This laser allows a precise cut and cauterization of surgical margins at the same time, reducing wound irritation with a better cosmetic appearance.
Moreover, the diode laser technology allows an accurate haemostasis implementing the surgical view during the procedure with a dry surgical field.

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Cite: *Piero Ronchi, Simone Scarcella, Stefano Manno, Valerio Beatrici, Lucio Dell'Atti.*
Dual Laser Circumcision: a novel technique to improve traditional surgery.
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 13-19
DOI: 10.26352/GJ06F5002-June2022

ISSN: 1884-7269
Indexed in PubMed and Scopus Volume 29 - N. 2 - June 2022

Use of laser in oral frenectomies: a case report series

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ABSTRACT

The aim of these case report series concerns the use of laser technology in oral frenectomies, by discussing different methods as well as comparing different laser wavelengths and underlining the role of photobiomodulation in the healing process.

Key words: Laser, Oral Surgery, Frenectomy, Orthodontics, Periodontics

Introduction

In a not so far time, rotative devices for conservative treatments and bone surgery and cold blades for soft tissues surgery were the only instruments used even if several critical points have been described by many authors [1, 2].
Recently, the use of new technologies, such laser, piezo-surgery and quantic molecular resonance has significantly increased and laser technology, thanks to its possibility to reduce or eliminate the use of anesthetic injection, to avoid use of sutures and to make a better and faster healing process, seems to be able to play an important role for discomfort reduction and, consequently, patient compliance increase [3].
Frenectomy is a minor oral surgical procedure that may concern different locations and situations: lingual, upper vestibular and lower vestibular.
Lingual frenectomy is suggested in presence of an abnormal short lingual frenulum, also called "anchyloglossia" or "tongue-tie": this is a condition in which the tip of the tongue cannot protrude over the incisors and it is often associated to several

[*]***Running title:***
Laser in oral frenectomies
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orthodontic diseases such as total open bite and class third malocclusion [4].
This anomaly may be classified in function of the distance measure between frenulum insertion and tongue tip while patient is touching the palate with mouth opened, allowing to distinguish four different classes of severity [5]. Anchyloglossia has a great importance in the deglutition and phonation functional re-education: speech therapists suggest surgical frenectomy when patients cannot do the exercises due to the tongue movement limitation [6].
Upper vestibular frenectomy is indicated, when related to inter-incisive diastema, if frenum is attached to the papillary gingiva (positive traction test) or if the distance between central incisors is larger than 6-8 mm. The intervention, beyond the elimination of frenulum insertion, must also cut the inter-incisive fibers until to reach the periosteum, in order to allow the space closure [7, 8].
In some cases, when the intervention is done choosing a correct timing during denture development, it may be sufficient to reach a good result without a classical orthodontic treatment and without appliance wearing [9].
Lower vestibular frenum abnormal insertion is often related to periodontal problems. In fact, In the mandibular anterior area, gingival recession can be associated with a minimal amount or lack of attached gingiva, a shallow vestibule, and high frenum insertion and these anatomical features may preclude the use of traditional root coverage procedures [10].
When the laser energy is emitted on the target tissue it may be reflected, transmitted, diffuse or absorbed and this last interaction, absorption, is necessary to obtain the surgical effect of tissue ablation. This is possible because biological soft tissues contain water, proteins and some coloured pigments such haemoglobin and melanin, each of them able to absorb a specific range of laser wavelengths. Even if most of lasers used in dentistry emit in infrared portion of the spectrum, in the last time also visible wavelengths, particularly green and blue, have been proposed for oral surgery, thanks to their great absorption in biological tissues [11-13].

Material and methods

Case Report 1: Lingual frenectomy performed by Diode 808nm laser.

ST, a 17-year-old girl, was sent to our clinics by a physiotherapist who was taking care of her for a spinal column pain. Recently in fact, several Authors have described a relation between anchyloglossia and postural diseases and lingual frenectomy has been proposed to improve physiotherapy treatment [14]. Due to physical examination had showed an abnormal insertion of the lingual frenum, with a 2nd class ankyloglossia of the Kotlow classification **(Fig. 1)**, it was decided for a frenectomy by an 808nm diode laser (LAseMAr 800, Eufoton, Trieste, Italy).

Fig. 1. Abnormal insertion of lingual frenum (Class 2nd of Kotlow classification)

The intervention had a duration of 145 sec, anaesthetics injection and sutures were not requested, and only a topic cream EMLA (AstraZeneca AB, Sodertalje, Sweden) was applied on the mucosa for five minutes before laser irradiation for pain controlling. **(Fig. 2)**

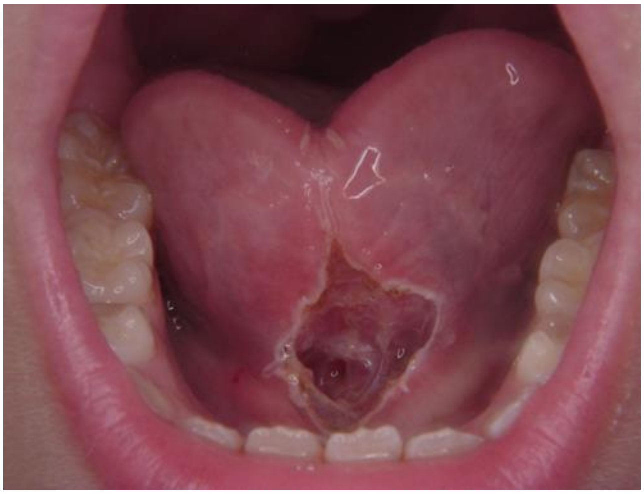

Fig. 2. Surgical area after intervention

Laser was used in contact mode (320 μm diameter fiber) with an output power of 3W CW (continuous mode).

Subsequently, it was used an "at home" device (B-Cure, Israel) for 10 min photobiomodulation; this device was also given to patient for domestic use in the next week.

Just after intervention, it was noticed that the tongue was able to protrude over the lower lip and the patient was instructed to repeat this exercise also in the postoperative days, in order to avoid the risk of relapse. Follow-up was without complications and, after two weeks healing process was completed. **(Fig. 3-4)**

Fig. 3. Partial healing process one week after with fibrine deposition

Fig. 4. Complete healing two weeks after

Case Report 2: Upper vestibular frenectomy performed by KTP laser.

PP, a 13-year-old boy, was sent to our clinics by his orthodontist who had suggested to cut the abnormally inserted upper vestibular frenum before starting the orthodontic treatment. **(Fig. 5)**

Fig. 5. Clinical examination: abnormal insertion of the upee vestibular frenum

After applying topic anesthetic in the surgical area (AstraZeneca AB, Sodertalje, Sweden) **(Fig. 6)**, frenectomy was performed by KTP laser, emitting at 532 nm (LaseMar 500, Eufoton, Trieste, Italy) in the visible portion of the spectrum, with green light **(Figg. 7-8)**. The optic fiber, used in contact mode, had a diameter of 320 μm and the output power of 3W CW (continuous mode) was used.

Fig. 6. Topical anaesthetics applied to the mucosa

Fig. 7. 320μm fiber before irradiation

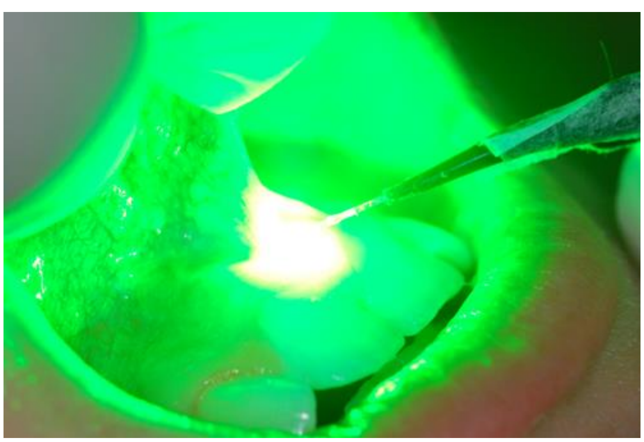

Fig. 8. 320μm fiber during KTP laser irradiation

The intervention had a duration of 110 sec, sutures were not applied due to the bleeding absence and 10 min of photobiomodulation were performed by "at home" device (B-Cure, Israel) just after KTP laser irradiation and also in the next week. **(Fig. 9)**

Fig. 9. Surgical area after intervention

Healing process was completed after ten days without any sort of problems. **(Fig. 10)**

Fig. 10. Not complete healing process a week after

Case Report 3: Lower vestibular frenectomy performed by 445nm diode laser.

RM, an 18-year-old girl, came to our clinics because she had noticed an important gingival recession on the central lower incisors.

Clinical observation revelled an abnormal insertion of the frenum and so it was decided for its ablation by means of a laser device. **(Fig. 11)**

Fig. 11. Abnormal insertion of lower vestibular frenum associated to gingival recession of lower incisors

The surgical intervention was performed by a 445nm diode laser (Ermes, Gardalaser, Verona, Italy) emitting in the blue portion of the electromagnetic spectrum with an output power of 1W in CW and a 320 μm fiber diameter. Due to the low energy used and the great absorption of this wavelength in the tissue no anaesthetics was needed and the irradiation time was very short (75 sec) **(Fig. 12)**.

Fig. 12. Surgical site after intervention

Just after the blue laser irradiation, it was used an "at home" device (B-Cure, Israel) for 10 min photobiomodulation; this device was also given to patient for domestic use in the next week.
The healing process was completed in a week. **(Figg. 13-14)**

Fig. 13. Healing process three days after with fibrine deposition

Fig. 14. Complete healing one week after

Discussion

Frenectomy is one of the minor oral surgical interventions where are more evident the advantages given by laser technology.
In fact, a good compliance of patients, due to the possibility to generally eliminate pain and discomfort without anesthetic injection as well as to

avoid the use of sutures, associated to the short duration of intervention, make this approach very interesting in pediatric and "special need" patients [15].
Moreover, the possibility to move tongue and lips just after intervention, as well as in the next days as suggested to patients, results very effective in relapse avoiding.
Regarding the choice of the proper laser, in these last years a great attention has been focused in the advantages given by visible wavelengths, particularly blue light.
In fact, its great absorption in haemoglobin **(Fig. 15)** makes possible to perform a very effective ablation with a very low energy, this resulting in pain absence, faster healing process and better patient compliance [16].

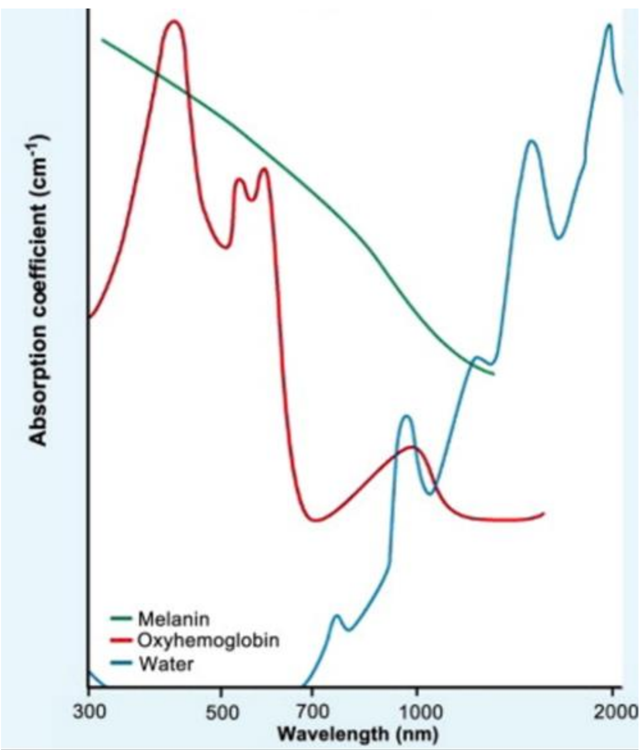

Fig. 15. Absorption of different chromophores by different wavelengths

An important aspect is related with the correct instructions to patients, seeing as laser surgery is very different from conventional, even in the follow-up.
Patient must be informed that, due to sutures are not used, the healing process is reached by the fibrine apposition and so, the presence of a yellow-brown aspect of the lesion in the days after intervention, must not be attributed to an infection but to a regular healing process.
Moreover, it must be informed to avoid brushing in this area as well as not to assume acid, salted and spicy food.
Finally, we must underline the great help to improve comfort in the follow-up given by photobiomodulation [17] both with traditional devices and with "at home" appliances able to be self-administered by patients themselves.

Conclusion

Laser technology may give several advantages in surgical frenectomies, particularly by using short visible wavelengths and associating photobiomodulation after surgery and in the follow-up.

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Cite: *Jean-Paul Rocca, Nathalie Brulat-Bouchard, Christine Voha, Hèlène Raybaud, Caaroline Bertrand, Carlo Fornaini.*
Use of laser in oral frenectomies: a case report series.
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 20-26
DOI: 10.26352/GJ06F5003-June2022

ISSN: 1884-7269
Indexed in PubMed and Scopus
Volume 29 - N. 2 - June 2022

Therapeutic processes for eradicating cancerous or benign tumours by laser beams using the excitonic approach of peptide groups

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ABSTRACT

The aim of the present study was to develop a protocol for the treatment of cancerous or benign tumours making use of laser rays, also demonstrating that the destruction process remains exclusively confined in the defective organ.
Thermal effects of lasers on biological tissue have been elucidated using vibrational excitations approach of peptide groups (PGs). It was proposed a Hamiltonian which integrate excitations induced by laser pulses and it was shown that the system is governed by a nonlinear equation with strong nonlinearity. It was also exactly described what happens in polypeptide chain once the unwanted organ is irradiated by the Neodymium-doped yttrium aluminium garnet, chosen as incident laser.
It was shown that, the advent of incident laser beams contributes to a sudden reinforcement of the vibrational excitations of PGs frequencies and amplitudes.
It was also demonstrated that the heating process leads to transverse and longitudinal deformation of the polypeptide chain and these sudden changes lead to the denaturation and subsequently to the destruction of the bulky organ. The drawn curves make it possible to estimate the spatial expansion of the denaturation, in order to effectively control the spread of the heat. Laser irradiation leads to a drastic increase in the vibration amplitudes of the PGs and subsequently results in the destruction of the undesirable tissue. An appropriate choice of the laser can make it possible to circumscribe the destruction only in the defective zone and to protect healthy cells.

Key words: Cancer, radiotherapy, heat transport in biological tissues, protein denaturation, peptide groups

[*] ***Running title:***
Peptide groups laser excitonic approach for cancer treatment
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Introduction

Radiotherapy is a fascinating topic tackled by researchers in several directions, with the common objective to easily and effectively remove the excess substance from the body, alleviate the suffering of the patient and even completely overcome the disease. Schnelzer and coll. [1] took into consideration smoking on radon-associated risk for lung cancer mortality among German uranium miners: they investigated the increase in risk with a linear excess relative risk model while considering smoking as a multiplicative factor and concluded that lung cancer mortality risk increased essentially with radon exposure increasing and also that the impact of smoking led only to marginal changes.
Cancers manifest themselves in different forms and, whatever the mode of treatment, the primary wish of doctors is to achieve an early diagnosis and screening. Diagnosis is a crucial step in the process of eradication of all forms of cancer and it fundamentally determines what happens later on. In this spirit, Hamim and coll. [2] developed an automated system for diagnosing oral lesions at initial stages with the result to be able to quickly intervene and save the patient's life. A quick assessment of the extent of the damage can make doctors be more efficient and, according to these authors, early diagnoses can significantly reduce the mortality resulting from oral cancer.
As earlier mentioned, the main object is to rid the body of foreign and unwanted substances as well as to protect healthy cells against collateral damages.
The present work represents a platform for the description of biological tissue thermal process under laser pulsed radiation and, in this contribution, it intends to exactly describe what happens in polypeptide chain once the unwanted organ is irradiated by a pulsed laser. In this context, a complete diagnosis must be made to determine, with precision, the exact size of the unwanted organ to eliminate and it is important to clearly define the framework of this operation. As soon as these conditions are fulfilled, the physician could easily remove the unwanted substance and, at the same time, protect healthy cells from the violence of laser beams. In fact, special care must be taken so that heat does not reach healthy substances. Thus, the development proposed in this paper will make it possible to determine:

- The most suitable type of laser that will allow the operation to be efficiently carried out;
- The required intensity of the selected laser;
- The duration of the laser irradiation.

Such predictions could be done only if the transfer process of heat generated by laser beam through biological tissue as well as the mode of degradation of the tissue under heat action are fully understood. In this model the process of denaturation and total destruction of biological tissue based on the formalism of vibrational excitations of PGs is described. Indeed, the long polypeptide chain is made up of PGs which, thanks to the vibrations to which they are subjected, generate the energy necessary for the proper functioning of the organism. It turns out that, during laser beam surgery, PGs receive an energy excess and this is likely to excessively multiply the frequencies and amplitudes of vibrational excitations, leading inevitably to uncontrollable situations.
Radiotherapy is a very delicate and risky exercise and precautions should be taken to avoid or limit unwanted effects. In this sense, a survey by a Korean group [3] was performed in view of improving the risk management system of radiation therapy departments in the Republic of Korea. It should be noted, in passing, that collateral damages which could occur during radiation therapy are of two types: they are inherent to the patient, as we have just underlined but they could also concern physician. Indeed, physician faces great risks while manipulating laser beams and this aspect of the

problem has been very well developed by Fadi and coll. [4] in a quite interesting contribution.
Our model was built following laser treatment processes developed by Simo in a very recent book [5]: in this contribution, he proposed protocols for overcoming certain diseases making use of the technique of radiotherapy, namely, the angioma, the condyloma and the tracheal tumours. In the same vein, Simo and coll. [6] proposed an innovative model of laser production using the technics of high-order harmonic generation. Given that, lasers so generated could also be used in the medical area.

Materials and methods

The denaturation process of human tissue that occur during laser beam surgery can be explained using vibrational excitations approach.

Tissue functioning in normal mode, without irradiation

The PGs constituting the giant polypeptidic chain are in perpetual vibrational movement around their equilibrium positions. The biological tissue consists of protein molecules with long chains of polypeptidic giant molecules, the constitutes of which being the PGs. PGs are constantly vibrating in our body and the idea is that energy is stored as vibrational energy in the C=O stretching mode (amide-I) of a polypeptidic chain. This vibrational excitation propagates from one group to the next because of the dipole-dipole interaction between the neighbouring groups [7-12]. Indeed, energy which results from these vibrations ensures the good functionality of the human body. These vibrations are permanently in progress since they are at the origin of the life. Indeed, these vibrations generate energy which punctuates the life of the human being. Human beings need energy to cover various functionalities that condition their existence. All the vital functions of human beings require energy for a perfect accomplishment of the tasks which are devoted to them. We can list, for instance, breathing activities, walking, muscle contraction, etc. The organism is therefore installed in a regime of residual vibrational excitations which maintain life.
The overall Hamiltonian of the system is made up of two essential components: the phonon Hamiltonian and the exciton Hamiltonian:

(i) The phonon Hamiltonian describes the pure lattice vibrations. This reflects the slow movements of the entire molecule around its equilibrium position. The expression of which is given by:

$$H_{ph} = \sum_n \left[\frac{P_n^2}{2M} + \frac{M\omega_0^2}{2}(Q_n - Q_{n-1})\right] \qquad (1)$$

Where Q_n, P_n, m, are the displacement of the n^{th} low frequency vibration from the equilibrium position, the momenta and mass of the molecules respectively. ω_0 is the characteristic frequency,

(ii) The exciton Hamiltonian describes intramolecular vibrational excitations. This Hamiltonian takes the following form:

$$H_{ex} = \sum_n [J_0\, B_n^+ B_n + M_0(B_n^+ B_{n+1} + H.C)] \quad (2)$$

$\boldsymbol{B_n^+}$ and $\boldsymbol{B_n}$ are the corresponding creation and annihilation operators. M_0 is the dipole-dipole interaction energy between nearest-neighbour molecules. J_0 is the free-molecule excitation energy due to an intramolecular vibration.

The biological tissue is subjected to laser irradiation

As we mentioned earlier, prior to tissue irradiation, PGs are subjected to a continuous vibration mode naturally maintained. The sudden absorption of laser pulses corresponds to a sharp and highly significant increase in the energy of the PGs. The additional supply of laser energy strengthens vibrational excitations and generates an excess of energy which causes increased heating. So, the occurrence of the laser energy causes the PGs to oscillate with very high amplitudes and frequencies. This could subsequently subject these PGs to critical resonances which obviously gives rise to the process of denaturation. Consequently, a drastic collapse of the unwanted tissue can be observed. At the same time, healthy cells must be protected.

Finally, as soon as the PG absorbs photonic energy, it accelerates the vibrating process from its equilibrium position. Therefore, the amplitudes of vibrational excitations are rapidly amplified over time. The heat source involved in surgical operation is derived from the Beer-Lambert Law: according to the latter, the intensity of the incident radiation decreases exponentially with the penetration depth (13-14). Let's notice in passing that, we consider collimated irradiation at normal incidence and irradiation is assumed to be constant over time.

Then, the Hamiltonian of the vibrational excitations induced by pulsed laser is given by

$$H_{lex} = \sum_n (1-R)\mu_a E_\nu e^{-\mu_a Q_n} B_n^+ B_n \qquad (3)$$

where E_ν stands for the incident energy flux at tissue surface and μ_a is the absorption coefficient. The parameter R represents the Fresnel tissue surface reflectance. Thus, H_{lex} describes the coupling between laser manifestations and vibrational excitations.

In this context, the total Hamiltonian of the system is determined by the contribution of the three components described by the formulas (1-3):

$$H = H_{ph} + H_{ex} + H_{lep} \qquad (4)$$

Next, we introduce state vectors which are products of a normalized one-exciton state and a coherent phonon state,

$$|\Psi(t)> = \sum_n \beta_n (t) B_n^+ |0>_{ex} \left[exp\left(\frac{1}{i\hbar}\sum_n (u_n(t)P_n - \pi_n(t)Q_n)\right)\right] |0>_{ph} \qquad (5)$$

Discrete equations of motions satisfied by β_n and u_n are given as

$$i\hbar \frac{\partial \beta_n}{\partial t} = J_0\beta_n + M_0(\beta_{n+1} + \beta_{n-1}) + (1-R)bE_\nu e^{-bu_n}\beta_n \qquad (6)$$

$$M\frac{\partial^2 u_n}{\partial t^2} = (u_{n+1} + u_{n-1} - 2u_n)M\omega_0^2 - (1-R)bE_\nu e^{-bu_n}|\beta_n|^2 \qquad (7)$$

$$J_0 = 0{,}205\ eV \equiv 0{,}328.10^{-19}\ J;$$
$$M_0 = -7{,}8\ cm^{-1} \equiv -1{,}549.10^{-22}\ J;$$
$$v_0 = a\omega_0 = 4{,}6.10^3\ ms^{-1};$$
$$v = 4{,}5.10^3\ ms^{-1}; R = 2{,}4\%$$

$$E_\nu = 50031\ Wcm^{-2};$$
$$\mu_a = 20\ cm^{-1} \equiv 3{,}971.10^{-22}\ J;\ \ M = 114\ m_p \qquad (8)$$

Exact analytical solutions of the nonlinear coupled differential-difference equations are unobtainable.

In the rest of this work, we will be interested in smooth waves or waves with long wavelengths compared with the lattice constant, a. In this context, we may adopt a continuum approximation and Eqs. (6-7) turn into:

$$i\beta_t = -A_0\beta_{xx} - \mu\beta + (1-R)bE_\nu e^{-bu}\beta \qquad (9)$$

$$mu_{tt} = C_0 u_{xx} - (1-R)b^2 E_\nu e^{-bu}|\beta|^2 \qquad (10)$$

The subscripts *t* and x denote partial differentiation with respect to time and space, respectively. Now, we differentiate Eq. (10) once with respect to the spatial coordinate. After replacing u_x by η, we arrive at these two equations:

$$i\beta_t = -A_0\beta_{xx} - \mu\beta + (1-R)bE_\nu e^{-b\int \eta dx}\beta \qquad (11)$$

$$m\eta_{tt} = C_0\eta_{xx} - (1-R)b^2 E_\nu \left[e^{-b\int \eta dx}|\beta|^2\right]_x \qquad (12)$$

In order to propose a solution to these master equations governing the system, we introduce the ansatz

$\beta = \Phi(s)exp[i(kx - \omega t)], \eta = \eta(s), s = x - v$ (13)

Substituting Eq. (13) into Eqs. (11-12), we obtain after some algebras:

$$\Phi_{ss} = a_1\Phi + a_2 e^{-b\int \eta dx} \quad (14)$$

$$\eta_s = b_1 e^{-b\int \eta dx}\Phi^2 \quad (15)$$

Here,

$$a_1 = -\frac{\omega + \mu - A_0 k^2}{A_0};\ a_2 = \frac{\mu_a(1-R)E_0}{A_0};$$
$$A_0 = -\frac{M_0 a^2}{h} \quad (16)$$

$$b_1 = -\frac{(1-R)b^2 E_0}{mv^2 - C_0}; \mu = -\frac{J_0 + 2M_0}{h} \quad (17)$$

The above system of equations (14–15) is not easy to solve. We will approach it following the work done by Simo and coll. [8] in biological models. So, for the sake of simplicity, we assume that η is of the form:

$$\eta = Nsech^2\left(\frac{s}{\Delta}\right) \quad (18)$$

where N is the amplitude of the pulse solution and Δ is its width. The numerical values considered in the computations being: N=1.24 and Δ= 2.32 Å. This will significantly easier the process under consideration. Substituting (18) into (14-15) we obtain, after some algebras:

$$\Phi_{ss} = a_1\Phi - \frac{2a_2 N}{\Delta b_1} sech^2\left(\frac{s}{\Delta}\right) tanh\left(\frac{s}{\Delta}\right)\frac{1}{\Phi^2} \quad (19)$$

Results and discussion

Numerical analyses of the action of laser rays on biological tissues: search for conditions for effective treatment preserving healthy cells

Studies are made on the basis of master equation (19). The starting point is the irradiation of the biological tissue by a laser ray, followed by the absorption of the photonic energy by the matter.
This energy reinforces the vibrational motility of the PGs forming the polypeptide chain and these strong vibrations are accompanied by a significant release of heat. So, the energy carried by photons is converted into thermal agitation of the molecules and the thermal energy so released is transported into the tissue by dipole-dipole interaction. So, these reinforced vibrations move along the polypeptide chain producing important damages throughout its course. In this study, we consider as incident lasers the Neodymium-doped yttrium aluminium garnet (Nd:YAG).
The capital fact that should be emphasized is that tissue degradation must be formally controlled in order to protect healthy tissues during this operation. The incident heat that arrives at the site, n, via the laser beam does not stagnate at this position but it is intended to propagate along the polypeptide chain and this is called heat contamination. Once the appropriate type of laser has been chosen, we want to examine the impact of the intensity and the duration of the irradiation on the extent of the heated tissue. The calculations proposed in this contribution make it possible to determine, beforehand, the set of physical parameters associated to each configuration. Thus, we determine the intensity and the time period that allow the heat to be confined exactly in the defective tissue thickness. It is therefore a technique which makes it possible to treat the patient while protecting its healthy cells.
The expansion of forced vibrations is visible on Fig. (1). Three typical situations are deeply analysed.
Here, the thickness of the tissue impacted by heat is indicated for three different values of the intensity of the incident laser. These are specifically:

$I_1 = 1{,}5\ W/cm^2$; $I_2 = 1{,}75\ W/cm^2$ and $I_3 = 2{,}0\ W/cm^2$.

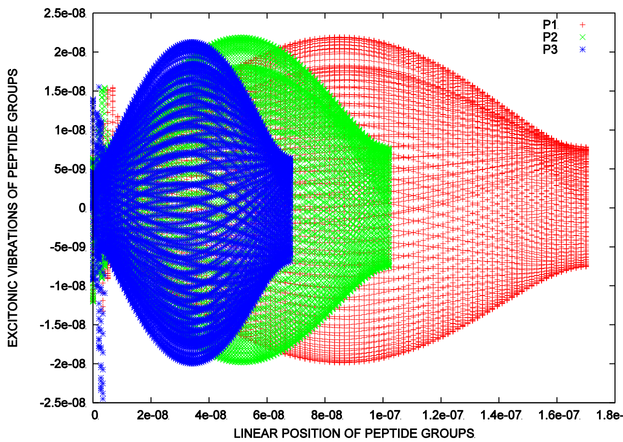

Fig. 1. Thickness of the tissue impacted by heat for three different values of the intensity of the incident laser: $I_1 = 1{,}5\ W/cm^2$ (curve in blue), $I_2 = 1{,}75\ W/cm^2$ (curve in green) and $I_3 = 2{,}0\ W/cm^2$ (curve in red).

It clearly appears that the thickness of the heated tissue increases with the intensity increase.

It should also be noticed that, following the irradiation of biological tissues, the amplitudes of vibrational excitations are rapidly amplified over time.

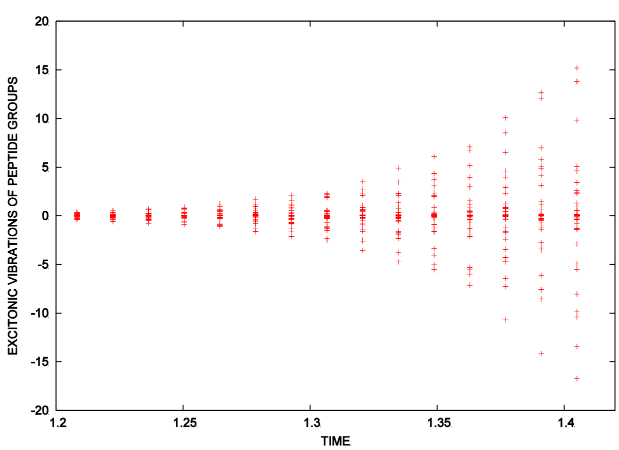

Fig. 2. The variation of vibrational excitation amplitude as a function of time after the initiation of laser beam irradiation. Here, the time period is multiplied by $1{,}5.10^{12}s$ while the amplitude of vibrations is multiplied by $1{,}25.10^{7}m$.

Fig. (2) depicts the abrupt change of vibrational excitation amplitudes with time as soon as the irradiation starts. In this figure, are first represented the typical vibrational excitations, in a normal and natural situation of life. Amplitudes suddenly increase almost exponentially, this leading to a chaotic process and subsequently to the degradation and destruction of the tissue.

The heating process is also accompanied by a strong elongation of the chain. Figs. (3-5) depict the rate of change in chain length of the biological tissue for three different values of the laser intensity: For the value $I_1 = 1{,}5\ W/cm^2$, the rate of length variation is around 1.2; For $I_2 = 1{,}75\ W/cm^2$, it is around 2.5. While, for a somewhat higher value $I_3 = 2{,}0\ W/cm^2$, it turns to the value 3.5. These results tell us that the rate of length change grows as the intensity of the incident laser grows.

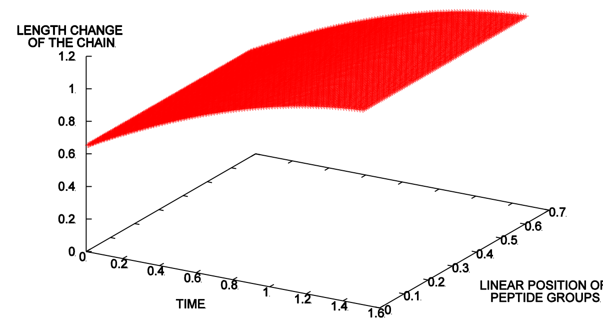

Fig. 3. The rate of change in chain length for a laser intensity $I_1 = 1{,}5\ W/cm^2$. Here, the time period is multiplied by $1{,}5.10^{12}s$ while the position of the PG is multiplied by $1{,}25.10^{7}m$.

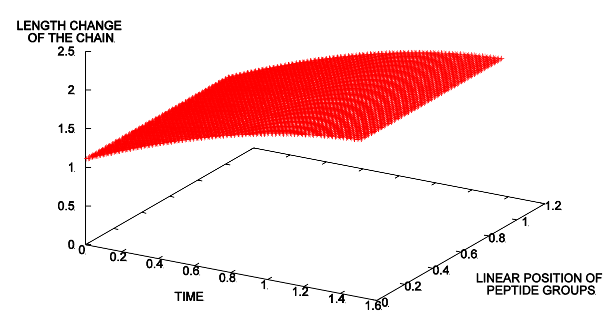

Fig. 4. The rate of change in chain length for a laser intensity $I_2 = 1{,}75\ W/cm^2$. Here, the time period is multiplied by $1{,}5.10^{12}s$ while the position of the PG is multiplied by $1{,}25.10^{7}m$.

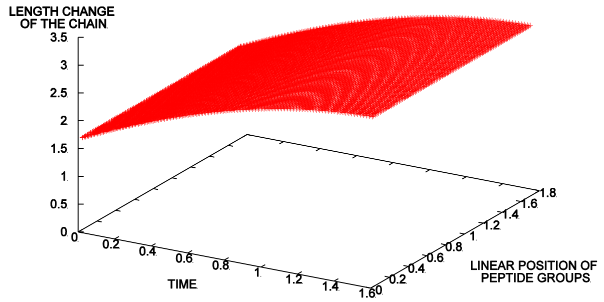

Fig. 5. The rate of change in chain length for a laser intensity $I_3 = 2{,}0\ W/cm^2$. Here, the time period is multiplied by $1{,}5.10^{12}s$ while the position of the PG is multiplied by $1{,}25.10^7m$.

Our investigations demonstrates that heat input causes both transverse and longitudinal deformation of the chain and these abnormal increases in the amplitudes of motion result in denaturation and total destruction of the tissue, as we mentioned earlier.

The ambition pursued while carrying out these calculations can be summed up as follows: In the presence of a perfectly determined and elucidated anomaly, we must be able to select the appropriate incident laser and all the corresponding physical parameters.

Conclusion

The therapies proposed in this paper use laser beams to overcome certain forms of cancers. The main objective here is to destroy the mass of undesirable, inconvenient or cumbersome organ making the carrier of this default uncomfortable.

The PGs constituting the giant polypeptidic chain are in perpetual vibrational movement around their equilibrium positions. So, in a normal life condition, the biological tissue is constantly submitted to the primary heating resulting from natural vibrational excitations of PGs. These vibrations release energy which ensures various functionalities of the organism. So, the main function of these vibrations is to give life to the cell and subsequently to the organism. It is clear that energy concerns are at the heart of the life. Life ceases as soon as the energy disappears. There is a source of persistent energy in the human body which allows one to exist. The local energy recorded around the PG irradiated by the laser beam is extremely high, to abruptly multiply the amplitudes of the vibrational excitations. The occurrence of a dramatic increase in the amplitudes and the frequencies of vibrational excitations gives rise to the process of denaturation. Consequently, a drastic collapse of the unwanted tissue can be observed. Therefore, denaturation occurs in an abnormal situation and alters the protein molecule.

It becomes imperative to protect healthy cells while confining heat only in the defective area.

The formalism developed in this paper aims to display a total control on the process of heat diffusion in human tissue. We have built a Hamiltonian describing the action of laser rays on biological tissue. We have obtained that; higher values of laser intensity are associated with higher length change of the biological tissue. The results presented in this paper also show that the heated area is a function of the intensity and the period of irradiation. This survey enables one to determine the type of appropriate laser for this operation, its physical characteristics and the duration of irradiation. By doing so, we could eradicate the unwanted substance while protecting healthy cells.

The novelty that can be highlighted in this model is the coupling of the energy induced by irradiations of biological tissues with the natural energy generated by the hydrolysis of the ATP molecule.

And this, in order to elucidate the denaturation and the destruction of the undesirable organs by making use of the excitation concept of PGs.

This model obviously has some limitations. Indeed, risks cannot be completely eradicated. We can only strive to limit them as much as possible and reduce them to their minimum portion. First of all, we

must point out that laser beams are dangerous and difficult to handle. Possible risks can be recorded not only for the patient but also for the doctors.
Moreover, it is an expensive technique, not within everyone's reach; especially if we refer to Third World countries where people live below the poverty line, in indescribable precariousness. They cannot afford this type of care.
This model has been built by nourishing the wish that the proposed analyses will improve the protocols of treatment of certain anomalies by laser rays. Doctors should do their utmost to treat while preserving healthy organs.

Acknowledgments
The authors of this paper greatly appreciate the fruitful discussions with Professor Jean-Guy CAPUTO of the Laboratoire de Mathématiques de l'INSA de Rouen-France, about the numerical code.

Conflict of interest
The authors declare no conflict of interest.

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Cite: *Jose Ivan Charles Zambe, Elie Simo, Dylan Kouam Kuicheu, Péguy Rompavel Fono Fotso, Hakim Souleman Abdel.*
Therapeutic processes for eradicating cancerous or benign tumours by laser beams using the excitonic approach of peptide groups.
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 27-34
DOI: 10.26352/GJ06F5004-June2022

ISSN: 1884-7269
Indexed in PubMed and Scopus
Volume 29 - N. 2 - June 2022

ENDOLIFT® and multi-wavelength laser photobiomodulation: a randomized controlled trial study on 96 subjects, treating skin laxity of the lower third of the face

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ABSTRACT

Background and aims: Many patients look for procedures that can achieve similar results to surgical lift avoiding the risks and possible complications of surgery. Endolift® and multi-wavelength (MW) Laser photobiomodulation (PBM) are considered two of the most effective procedures in the treatment of skin laxity and superficial wrinkles.
We aimed to investigate the reduction of skin laxity of the lower third of the face treated with the Endolift® technique alone or in combination with MW Laser PBM.
Materials and Methods: From June 2016 to June 2018, 96 subjects (18 M; 78 F) were included in this randomized controlled trial (RCT) conducted in two Italian institutions. The area treated was the lower third of the face. The subjects were classified using the Ptosis Scale (grade I-V) at T0, with a Pinch Test <1.9mm. We selected only subjects' grade 4, both males and females, 45-55-years old. Thirty-two subjects (6M; 26F) (group 1) were treated with the Endolift® procedure followed by a session of MW Laser PBM using a simultaneous nonsurgical laser therapy emission of three different wavelengths (532nm, 808nm, 1064nm). The MW Laser PBM was repeated after 7, 14 and 21 days. Thirty-two subjects (6M; 26F) (group 2) underwent only the Endolift® session. 32 subjects (6M; 26F) (group 3) received only MW Laser PBM. Follow up visits were performed at days 60 (T60), 120 (T120) and 540 (T540) after the treatments. The study ended in December 2019 when all the subjects had been followed for 18 months.
Results: The results of the three groups were evaluated using the Ptosis Scale at T0, T60, T120 and T540. The combination of the two treatments (group 1) achieved more rapid effects, while MW Laser PBM alone treatment (group 3) achieved the slowest effect. The median test for

[*]***Running title:***
Enfolift® and treatment of face skin laxity
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independent samples showed that all the differences between groups are statistically significant at each follow-up (p=0.000).
Conclusions: The Endolift® laser in the last 15 years allows to achieve exceptional results in the treatment of skin laxity reaching the desired 'soft' rejuvenation model which is increasingly desired. MW Laser PBM, in combination with Endolift®, obtains better results. Although significant at T540, the effects of MW Laser PBM alone are slower to reach.

Key words: Endolift, laser therapy, skin laxity, face

Introduction

Endolift® represents the most innovative interstitial connective remodelling technique based on a laser micro-fiber 1470nm. However, best results are obtained in several months, although lasting for more than two years.
Multi-wavelength (MW) Laser photobiomodulation (PBM) is a non-invasive, non-thermal trans-dermal therapy which stimulates cells to generate more energy and undergo self-repair through the use of visible and near infrared light (NIR) with no side effects. MW Laser PBM is not as efficacious as Endolift® in deeper layers and takes longer to attain best results.
Until the beginning of the millennium good cosmetic results needed surgery, but techniques such as botulinum toxin, thread lifts, radiofrequency or more recently the micro-focalized ultrasound have proved to be breakthrough treatments in achieving 'soft' lifting and rejuvenation. Indeed, only the introduction of recent generation fiber lasers, have allowed to achieve excellent results. The mechanism of action of the subcutaneous laser is twofold; both acoustic/mechanical and selective photothermolysis of the targeted tissue. Over the last 20 years various studies [1] have published on laser liposculpture and in 2006 the FDA demonstrated that approximately 50% of 2200 subjects reported superior 'skin tightening' after laser liposuction than patients treated with traditional liposuction [2]. Indeed, it is well established in the literature that it is possible to obtain excellent results with the primary aim of achieving skin remodelling and retraction [3].
Following this trend of success, Dr. Dell'Avanzato developed the Endolift® technique in 2005, which gained popularity in the global market largely since it is a relatively simple and safe procedure, producing immediately visible results whilst also being reproducible and repeatable [4], [5], [6].
Endolift® technique represents nowadays one of the most innovative procedures using a micro-fiber connected to a diode laser 1470nm. The name Endolift® reflects the basic distinctive concept of the methodology; ENDO meaning 'internal' and LIFT 'to raise'. In such a way, the technique aims to lift tissues from deeper layers using an optical fiber as thin as a human hair inserted under the skin in the superficial-middle hypodermis. Here it delivers the laser energy, without the need to cross the superficial layers of the skin (epidermis and dermis). This is one of the many advantages that Endolift® has over its counterparts such as radiofrequency which deliver targeted energy through the superficial cutaneous layer and therefore undergo dispersion as well as causing more pain. Moreover, it uses a naked fibre avoiding any additional cannulas.
MW Laser PBM is also known as low-level laser therapy (LLLT) and seems still highly appreciated in the scientific community. MW Laser PBM could be performed also with non-coherent sources, as LED or IPL, but these sources do not allow a precise dosage of radiation.

The first non-surgical laser stimulation effect was discovered in 1967 by Endre Mester when trying to repeat an experiment first conducted by Paul McGuff in Boston, who had successfully used the newly discovered ruby laser to cure malignant tumours in rats [7]. Mester's custom-made ruby laser possessed only a very small fraction of the power possessed by McGuff's laser, and despite not curing tumours he observed a heigh rate of hair growth and better wound healing in the rats [8].
This was the first indication that LLLT could have a beneficial use in medicine [9]. Nowadays, LLLT or MW Laser PBM are continuing to receive consent [10], [11] and represent an effective non-invasive, non-thermal trans-dermal therapy to stimulates cells to generate more energy and undergo self-repair through the use of visible and NIR with no side effects, as anti-inflammatory and to increase the venous-lymphatic drainage [12].

Materials and Methods

From June 2016 to June 2018, 96 subjects (18 M; 78 F) were included in this randomized controlled trial (RCT) in two different institutions in Italy, the Medical Department of Espace Chenot Health Wellness SPA at L'Albereta Relais and Chateaux and the Institute of Laser Medicine of Florence. The 96 subjects were selected with a Pinch Test <1.9mm, in order to not treat subjects with large amount of extra fat. The area treated was the lower third of the face. The subjects were classified using the Ptosis Scale (grade I-V) at T0. We selected only subjects' grade 4, both sex, age 45-55 years old. Thirty-two subjects (6M; 26F) (group 1) received the Endolift® procedure as first, then underwent a session of MW Laser PBM using a simultaneous nonsurgical laser therapy emission of three different wavelengths (532nm, 808nm, 1064nm). The Laser PBM was repeated after 7, 14 and 21 days. Thirty-two subjects (6M; 26F) (group 2) underwent only an Endolift® session. Further 32 subjects (6M; 26F) (group 3) received only MW Laser PBM. Follow up visits were programmed at days 60 (T60), 120 (T120) and 540 (T540) after treatment. The study was closed in December 2019 when all subjects had been followed for 18 months.
The Endolift® session is performed using a 300micron FTF (fiber -to-fiber) micro-fiber with radial emission, connected to a diode laser 1470nm, inserted, without any incision or anesthetic, directly into the superficial middle-hypodermis **(Fig. 1)**.

Fig.1: The Fiber is easily inserted, without any incision or anaesthetic drug

Once the fiber has been inserted into the correct plane, represented by the superficial and medium hypodermis, the positioning of the fiber is identifiable due to a red aiming beam visible through the skin. The fiber is moved throughout the intervention area in a fan-like manner, delivering energy firstly in a retrograde then anterograde fashion, creating micro-tunnels oriented mainly along the anti-gravitational vectors. During the procedure the skin surface temperature should never exceed 40 °C (measured through an external thermal sensor). Cumulative energy of Endolift® ranging from 500 J to a maximum of 1400 J with a mean of 800 J for the entire area. The session of MW Laser PBM is then performed. The MW Laser PBM energy delivered is around 4J/cm^2 for each emission with a total of 12J/cm^2 considering the overlapping technique in each zone, with a mean total of 360 J/cm^2 for each subject. The dose of each laser irradiation was established following the parameters accepted by the international scientific community regarding the laser biomodulation of the skin and soft tissues [9], [10]. We use more wavelengths because each one has a different penetration in the tissue and a different antiphlogostic activity, anti-oedema and regenerative, increasing the metabolic exchange in each irradiated tissue. The antiphlogistic effect occurs at 808nm and 1064nm, the regenerative effect at 532nm and 808nm [11], [12]. The three different wavelengths have also different penetration, so that they can be absorbed by more tissue layers. The subjects after 7, 14 and 21 days, received the same amount of J/cm^2 as in T0. After the procedure all subjects were able to immediately resume their daily life activities; there were no cutaneous marks or signs, just a moderate erythema (2% of cases) and oedema (3%) that resolved in a few hours, in all the 3 groups.

Statistical Analysis

In all groups data distribution was non normal as evaluated by the Kolmogorov-Smirnov's single sample test. Thus, changes of median scores between groups were examined using the non-parametric median test for independent samples.
The non-parametric related samples sign test was used to analyse variations of median values in the same group through the follow-ups. The IBM SPSS Statistics for Windows, (version 26.0; IBM Corp, Armonk, NY, USA) was utilized for calculations.
The significance level was set at p<.05.

Results

The results of the three groups were evaluated using the Ptosis Scale at T0, T60, T120 and T540. The majority of subjects shifted from grade 4 to grade 2 and 1 **(Fig. 2)**.

Fig 2: Endolift® + MW Laser PBM

The median value comparison within groups showed a strong modification in group 1 between T0 (4) and T60 (1, p=0.000) while no modification was observed through the other follow-up visits at T120 and T540 (p=0.157).
However statistical significance was observed in the T0-T540 difference (p=0.000) **(Table 1a)**.

Table 1a: Kolmogorov-Smirnov's single sample test was used

	T0	T60 (p in groups T0-T1)	T120 (p in groups T1-T2)	T540 (p in groups T2-T3)	p in groups T0-T3
Group 1	4	1 p=0,000	1 p=0,157	1 p=0,157	0,000
Group 2	4	2 p=0,000	1 p=0,008	1 p=0,180	0,000
Group 3	4	4 p=1	2 p=0,000	2 p=0,157	0,000
p between the groups	0,000	0,000	0,000	0,000	

In group 2 **(Fig. 3)** this value changed from 4 to 2 at T60 (p=0.000), and from 2 to 1 at T120 (p=0.008).

Fig 3: Endolift®

At T540 it remained the same but did not reach any statistical significance (p=0.180). However, the difference between values at T0 the baseline and at T540 are equally significant (p=0.000).

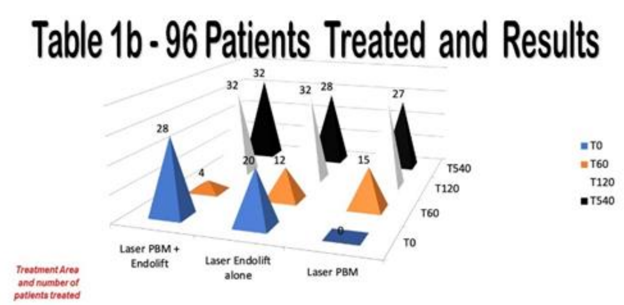
In group 3 **(Fig. 4)** values are equal at T60 (4, p=1) while a significant change was found between T60 and T120 (median value from 4 to 2, p=0.000). No statistically significant difference was found through T120 and T540 (p=0.157). The difference T0-T540 was significant (p=0.000).

Fig 4: MW Laser PBM

The median test for independent samples showed that all the differences between groups are equally statistically significant at each follow-up (p=0.000).

Discussion

Follow-up was excellent in all groups **(Table 1b)**.



The procedure causes remodelling of collagen and connective tissue photobiomodulation, resulting in the 'skin tightening' effect. Skin laxity is reduced, and superficial wrinkles are smoothed resulting in an overall compacting of the skin which is immediately visible and continues to progress over the following period. The laser acts simultaneously on the collagenous fibrous septa, resulting in further skin retraction and tissue compaction.

As in many other procedures evaluating symmetries can be difficult with the naked eye, i.e., for the mandibular contours, due the natural physiological differences between the body sides. Thus, while skin changes visibly appreciated by the operator are the main endpoint, the amount of laser energy emitted during the Endolift® treatment represents a secondary clinical end point. The combination of the 2 procedures Endolift® and MW Laser PBM allows to obtain the best results in a faster time.

Conclusions

In recent years, laser has become one of the most important innovations in medicine and surgery, of similar importance than laparoscopic or robotic-assisted surgery. It has several clinical indications including aesthetic thanks to devices and techniques which are now more easily reproducible, safer and minimally invasive. We consider the use of Endolift® laser technology a breakthrough treatment aimed at lifting tissues directly within the skin. The use of MW Laser PBM alone could be useful in patients with early lesions. The use of MW Laser PBM in combination with Endolift® showed a significant role in achieving the desired 'soft' rejuvenation model which is increasingly required but needs the concomitant use of different cosmetic techniques to achieve the best results in a shorter time, longer duration of the effects and conservation of the natural expression of the face.

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Cite: *Leonardo Longo, Roberto Dell'Avanzato, Diego Longo. ENDOLIFT® and multi-wavelength laser photobiomodulation: a randomized controlled trial study on 96 subjects, treating skin laxity of the lower third of the face.*
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 35-40
DOI: 10.26352/GJ06F5005-June2022

ISSN: 1884-7269
Indexed in PubMed and Scopus
Volume 29 - N. 2 - June 2022

Effects of laser therapy and Grimaldi's muscle shortening maneuver on spasticity in central nervous system injuries

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Background an Aims: For 2003 year until today we treated hundreds of patients with Central Nervous System Injuries (CNSI), using Non-Surgical Laser Therapy (NSLT) obtaining good results in terms of sensibility and movement. In order to increase muscle strength and to further explore new emerging synergies, we have also started using a physical therapy practice based on the most current knowledge about the motor control, called Grimaldi's Muscle Shortening Maneuver (GMSM). Spasticity is often the most disabling symptom and the current therapies are still not able to heal it at all. The goal of our study is to suggest a new way of treatment of spasticity, supporting it with objective measurements of muscle thresholds.

Materials and Methods: In 2016-2017, 36 patients with traumatic or degenerative CNSI were enrolled. Lasers used were 808 nm, 10600 nm, and 1064 nm, applied with a first cycle of 20 sessions, four a day. Patients were subjected to Grimaldi's Muscle Shortening Maneuver (MSM) twice a day, ten sessions at all, working selectively on hypertonic muscles and their antagonists. Before treatment, tonic stretch reflex thresholds (TSRTs) in Gastrocnemius Lateralis (GL) were assessed through a surface electromyography (sEMG) device paired with an electrogoniometer. Antagonist muscle force (Tibialis Anterioris) was assessed by some electronic hand-held dynamometers. For the clinical measure, we used the Modified Ashworth Scale (MAS). All tests have been performed at the baseline (before starting treatments), after one week (at the end of the last treatment) and after a month.

Results: Results were considered positive if the instrumental assessment procedure showed modifications in TSRT values and subjects improved their antagonist muscle strength. Results showed modifications in TSRT values at every follow up. The average comparison between the follow-ups was always statistically significant (p .000). The increase in Tibialis Anterioris muscle strength was statistically significant as well (p .000). MAS showed some differences between follow-ups but not all of them are statistically significant (T0-T1 p .063, T1-T2 p .001, T0-T2 p .000).

[*]***Running title:***
NSLT and GMSM on spasticity in CNSI
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Encouraging results suggest a possible correlation between laser and MSM therapies and modifications of TSRT in spastic muscles.

Conclusion: Associating laser treatment and Grimaldi's Muscle Shortening Maneuver (MSM) seems to be effective on spasticity in patients affected by traumatic or degenerative CNSI. Obviously, this kind of study design showed a lot of limits however this clinical series could be an important hint for every researcher working in the complex field of spasticity, a symptom that is poorly defined and hardly treated.

Key words: Laser Therapy, Grimaldi Muscle Shortening Maneuver, spasticity, tonic stretch reflex threshold, laser biomodulation, motor control.

Introduction

From December 2003 until December 2018, we treated hundreds of patients with Central Nervous System Injuries (CNSI), using Non-Surgical Laser Therapy (NSLT) (1) obtaining good results in terms of sensitivity and movement (2). To increase muscle strength and to further explore new emerging synergies, we have also started using a physical therapy practice based on the most current knowledge about motor control (3, 4), called Grimaldi's Muscle Shortening Maneuver (GMSM) (5-8). Spasticity is one of the most common and disabling symptoms that results from a first motor neuron injury and afflicts over 12 million people worldwide (9). As reported by Bhimani (10), it has been estimated the prevalence of lower limb spasticity for stroke from 40 to 60 per 100,000, for multiple sclerosis from 2 to 350 per 100,000, for cerebral palsy from 260 to 340 per 100,000 and for spinal cord injuries from 22 to 90 for 100,000. The annual incidence of the spastic of the lower limb, however, is estimated for stroke from 30 to 485 per 100,000, for traumatic brain injury from 100 to 235 per 100,000 and for spinal cord injuries from 0.2 to 8 for 100,000. For Blanchette *et al.*, (11) the prevalence of spasticity in post-stroke subjects is highly variable, ranging between 17.0% and 42.6% in patients in the chronic phase of recovery.

The importance of spasticity by the fact that it interferes with functional recovery and leads to secondary complications such as muscle retractions, weakness and pain [12)]. It is responsible for insomnia and muscle fatigue and can interfere with mobility, transfers, self-care, activities of daily life and society. Clinical factors such as urinary tract infections and bedsores are a cause of increased spasticity (13).

Because of these invalidating causes, spasticity is the burden on caregivers exponentially.

The definition of spasticity is common suggested by Lance: "A motor disorder caused by a speed-reflex increase of the stretch tonic reflex with exaggerated tendon response, resulting from a hyperexcitability of the stretch reflex as a component of the syndrome of the first motoneuron" (14). However, the definition of the term spasticity is still not well established and is not commonly accepted.

For the symptom of spasticity, given the lack of agreement on the definition and the lack of consistency of the most used evaluation methods available, nowadays there are great difficulties in validating measurement systems and in suggesting any kind of treatment. The literature review allows us to point out that the most commonly used methodologies often do not collate with the latest neuro-physiological and motion control discoveries or the most common spasticity definitions. The framing of tone disorders within the threshold control theory allows a parameterization of the

spasticity symptom which, in turn, can represent a valid unit of measurement. Several authors have experienced this type of measurement through portable devices, achieving good results in terms of intra and inter-rater reliability.
The stretch reflex threshold (SRT) is an integral part of the lambda model of motor control. It represents the joint angle at which motoneurons and respective muscles of the joint begin to be recruited. Research in animals and in chronic stroke subjects suggests that SRT may be altered by descending pathways, including cortico-spinal systems with or without a concomitant alteration in the reflex gain. (3)
SRT depends on velocity of stretch. Taking this into account, it is called the dynamic SR threshold (DSRT). The tonic SRT (TSRT) represents a specific value of the DSRT for zero velocity. DSRTs and TSRT are expressed in relation to the actual configuration of the joint within a body frame of reference. In particular, when the threshold lies within the biomechanical range of the joint and the patient has no ability to shift this threshold angle, it separates the joint configurations in which muscles are spastic from those in which they are not, thus quantifying an important, spatial aspect of the motor control impairment (3, 4).
The goal of our study is to suggest a new way of treatment of spasticity, supporting it with objective measurements of muscle thresholds.

Materials and Methods

2.1 Subjects

In 2017 – 2018, 36 patients (23 men) with traumatic or degenerative CNSI – with injuries sustained at least one year before laser treatment and documented by NMR or CT, ESSP, and ESMP – were enrolled (mean age 30,97 ± 8,91; range 18-52) **(table 1)**. Selection criteria are shown in **table 2**.
Informed consent was obtained from all individual participants included in the study.

SUBJECT	SEX	AGE	PATHOLOGY
1	M	32	SCI
2	M	28	SCI
3	W	29	SCI
4	M	42	SCI
5	M	52	CP
6	M	22	SCI
7	M	43	Stroke
8	M	18	CP
9	M	30	SCI
10	M	39	Stroke
11	W	41	SCI
12	W	36	SCI
13	W	32	SCI
14	W	39	CP
15	W	27	CP
16	W	26	Stroke
17	M	18	Stroke
18	M	45	SCI
19	M	18	SCI
20	M	22	Stroke
21	M	28	SCI
22	W	29	SCI
23	M	22	SCI
24	M	36	SCI
25	M	21	SCI
26	M	39	SCI
27	W	41	SCI
28	M	20	SCI
29	M	25	SCI
30	M	41	SCI
31	W	37	SCI
32	W	23	Stroke
33	M	20	SCI
34	M	31	SCI
35	W	36	Stroke
36	W	27	SCI

Table 1. Characteristics of participants

36 Participants

Inclusion Criteria:	Exclusion Criteria:
Both sex, 18 – 40 years old	- Surgical intervention contra-nature
- CNSI occurred at least one year before	- Orthopedic complications (deformity, pain)
- Spasticity in ankle joint (MAS 1-3)	- Inability to join the program in its entirety (economic, logistic, voluntary, etc)
	- Cognitive impairments

Table 2. Inclusion and exclusion criteria

2.2 Evaluators

Three physiotherapists (two males and one female) evaluated each subject. The evaluators had similar amounts of clinical experience (4.5 and 5 years). All had been specializing in neurorehabilitation for at least three years prior to the study. Evaluators were not blinded to the objectives of the study.

2.3 Experimental protocol

Before treatment, TSRTs were assessed through a surface electromyography (sEMG) device (Enraf Nonius Myomed 632X) paired with an electro-goniometer (Jtech Medical Commander Echo). All tests have been performed at the baseline (before starting treatments), after one week (at the end of the last treatment) and after a month.

2.3.1 Clinical testing

For the clinical measure, we used the Modified Ashworth Scale (MAS). The MAS is a 6-point ordinal scale, ranging from 0 (no increase in tonus) to 4 (rigid limb), based on the subjective impression of the examiner of the resistance felt to stretching the knee extensors at a low velocity (15).
Bohannon and Smith (1987) reported an inter-evaluator agreement of 86.7% with no more than one grade difference between evaluators (s=0.85, p<0.001). Since the MAS is more reliable than the original Ashworth Scale for the evaluation of spasticity in the elbow flexors (15), this test was only performed by one evaluator. Three stretches were performed at a velocity of approximately 80-100°/s, and the highest MAS score was recorded.

2.3.2 Instrumented testing

After cleaning the skin, two disposable square silver-silver chloride self-adhesive electrodes (center-to-center distance of 3.5 cm) were placed over the motor points of the agonist muscle. The reference electrode was placed over a bony prominence near the observation point. The axis of rotation of a calibrated electro-goniometer was positioned above the rotation axis of the joint in question. The electro-goniometer arms were aligned with the subject's bone segments and fixed in place with self-adhesive straps.

The determination of DSRT proceeded as follows: Step 1: subject, evaluator and session codes were entered in the database. Step 2: the baseline EMG was established with the subject completely relaxed and the EMG gain was adjusted to ensure that the signal was not saturated. This was done by stretching the muscle three to five times at high velocity and monitoring the EMG response. Once the gain was set, it was not readjusted during the remainder of the evaluation. Step 3: the starting angle of the joint was chosen. Step 4: A trial consisting in a very slow passive stretch (0.5 °/s) of the agonist muscle was performed. When muscles started to show EMG activity, the experimenter noticed the angle value showed by the electro-goniometer previously fixed to the examined joint.
That value is rated as the DSRT of the spastic muscle and it could be compared with its TSRT because of the very slow velocity of stretching. Antagonist muscle strength was assessed too through handheld electronic dynamometers (Jtech Medical Commander Echo). This kind of evaluation was also administered one week and two months later the first one.

2.4 Intervention

Lasers used were 808 nm (Eufoton, Trieste, Italy), 10600 nm (General Project, Firenze, Italy) and 1064 nm (Eufoton, Trieste, Italy), applied with a first cycle of four sessions per day for a total of 20 sessions **(table 3)**.

	Treatment of Inflammation and Edema	Support of Nerve Regeneration	Muscle Tone	Anti-inflammatory Muscle Tone
Laser	diode 808 nm wavelength	diode 808 nm wavelength	CO_2 10,600 nm wavelength	Nd-YAG 1064 nm wavelength
Output power	10 W	10 W	15 W	5 W
Spot size	5 cm	5 cm	10 cm	6 mm
Fluence	12 J/cm^2	4 J/cm2	36 J/cm^2	35 J/cm^2/passage
Total Energy	720 J	240 J	variable	variable
PW Repetition Frequency	1000 HZ	10 HZ	100 HZ	1 HZ
Tissue Target	Spinal Lesion	Nerve Trigger Points Coherence Domains	Around the lesion	Area of Lesion and muscle-tendon unit
Sessions per day	4	4	4	4
Sessions per Cycle	Cycles of 20 sessions, with interval of 1 month			

Table 3. Parameters of laser treatment

Different laser wavelengths were used because each wavelength has a different penetration 16,17). Details about the dosage are specified in **table 3**. One dosage of 720 Joules/cm2 in total used with laser 808 nm, aimed at achieving an anti-inflammatory effect, according to current knowledge 1, 16, 18, 19, 20). Another dosage of 240 Joules/cm2 in total was used for regenerative purposes 1, 16, 21, 22), always with the same laser 808. Lasers of 10600 nm and 1064 nm were used at dosages of 36 Joules/cm2 that could influence muscle tone 23, 24, 25).

The patients participated in specific physical therapy training (Grimaldi's Muscle Shortening Maneuver – GMSM) twice a day, for a total of ten sessions, working selectively on ankle' s plantar and dorsiflexor muscles (Tibialis Anterioris and Gastrocnemius Lateralis). GMSM is a technique which works on neuromuscular spindles with the aim of producing stimulation. It intentionally balances the shortening and lengthening of the muscle simultaneously 5-8). This process produces an informational catastrophe in the neuromuscular spindles forcing them to set new muscle thresholds. This kind of maneuver is an active but involuntary training for the patient.

The participants were lying on their backs on a physiotherapy table with the knee stabilized at about 90° flexion by means of a roller cushion and quick release restraining straps, with the foot resting outside the lower edge of the bed and the ankle resting on a 5 cm thick U-shaped bearing fixed to the same edge using adhesive materials.

A first skin protection bandage was applied to the patient (using skin-saving plaster covered with simple absorbent paper held together and fixed to the limb by strips of adhesive bandage plaster) starting from the distal third of the leg to cover the entire foot. Secondly, the flexible rod was fixed (a harmonic steel rod 60 cm long, 6 cm wide and 0.5 cm thick, covered with 1 cm high foam rubber for the whole extension on one of the two sides and covered in soft neoprene type material) to the plantar surface of the subject's foot using simple adhesive tape so that the rod protrudes for about 5 cm from the rear edge of the heel. From this position the experimenter manually imprinted dorsiflexion stresses in the center of the plantar arch in a rhythmic manner and with a frequency of about 2 Hz for a duration of 15 min. At the upper end of the shaft (the one facing the patient's fingers) small parallelepiped iron weights (1.2 or 3 kg) of the same width as the rod and with max height of 1 cm could be fixed by total coverage with bandage plaster, to increase the elastic return of the harmonic steel in the case in which the ankle excursion was so reduced by spasticity that it could not provide the necessary space for the oscillation.

2.5 Statistical analysis

In statistical analysis we used the Kolmogorov-Smirnov Test to analyse the distribution of values for TSRT, MAS and Force variables. For evaluating the muscle strength, TSRTs and MAS scores we compared the results obtained for each parameter in three observation times (T0, T1, T2). For this reason, we used a non-parametric Wilcoxon Test for paired samples, which let us know if there are significant differences between the follow-ups for continuous variables (strength, TSRTs). For ordinal ones (MAS) we used the sign test with the same aim.

2.6 Ethical Considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The authors declare that they have no conflict of interest.

Results

Regarding TSRTs, results showed modifications in TSRT values at every follow up.
TSRTs decreased by an average of 5,66° in the treatment group at the first follow up and 4,81° at the second. In this way, after one month we found an increase of 10,47° with respect to the baseline **(Fig. 1)**

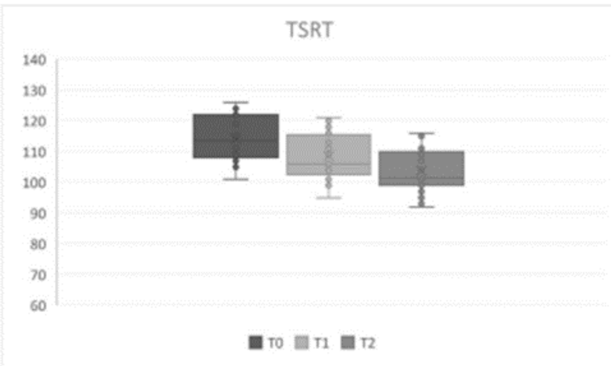

Fig. 1. Decrease of Tonic Stretch Reflex Threshold values through follow-ups

This decrease was statistically significant, in addition, the TSRT change obtained between follow-up, (T0-T1) (T1-T2) (T0-T2) are statistically significant (p.000) **(table 4)**.

TSRT

		T0	T1	T2
36 SUBJECTS	AVERAGE	114,19	108,53	103,72
	STANDARD DEVIATION	7,210	7,213	6,696
	STANDARD ERROR	1,202	1,202	1,116
	WILCOXON TEST	T0-T1 0,000	T1-T2 0,000	T0-T2 0 ,000

Table 4. Results regarding TSRT average values through the follow-ups p<0.05

Muscle strength was assessed in one movement: ankle dorsiflexion. Force increased by an average of 7,8 N at the first follow up and 8,12 N at the second. In this way, after one month we found an increase of 15,92 N with respect to the baseline **(Fig. 2)**

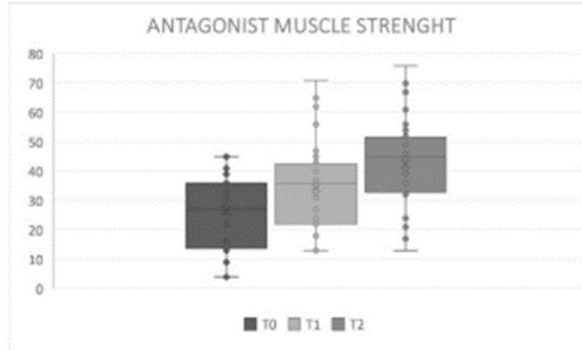

Fig. 2. Increase of antagonist muscle strength through follows-ups

This modification in strength obtained between follow-up, (T0-T1) (T1-T2) (T0-T2) is statistically significant (p.000) **(table 5)**.

ANTAGONIST MUSCLE STRENGHT

		T0	T1	T2
36 SUBJECTS	AVERAGE	26,64	34,44	42,56
	STANDARD DEVIATION	12,036	14,941	15,044
	STANDARD ERROR	2,006	2,490	2,507
	WILCOXON TEST	T0-T1 0,000	T1-T2 0,000	T0-T2 0 ,000

Table 5. Results regarding antagonist muscle strength average values through the follow-ups p<0.05

Modified Ashworth Scale was administered to all subjects at every follow-up, assessing the passive resistance to stretch of plantar flexors muscles. This parameter showed some differences between follow-ups but not all of them are statistically significant (T0-T1 p.063, T1-T2 p.001, T0-T2 p.000) **(table 6)**.

SUBJECT	T0	T1	T2
1	3	3	2
2	3	3	3
3	3	2	2
4	3	3	2
5	2	1	1
6	2	2	1
7	3	3	2
8	2	2	1
9	2	2	2
10	2	2	2
11	3	3	2
12	3	3	3
13	3	3	3
14	3	3	2
15	3	3	2
16	2	2	2
17	2	2	2
18	2	2	2
19	3	3	3
20	3	2	2
21	3	3	3
22	2	2	2
23	3	2	2
24	3	3	3
25	2	2	2
26	2	2	1
27	1	1	1
28	2	2	1
29	3	3	3
30	1	1	1
31	1	1	1
32	2	2	1
33	1	1	1
34	2	1	1
35	2	2	2
36	2	2	2
MEDIAN	**2**	**2**	**2**
SIGN TEST	MAS T0-T1 0.063	MAS T1-T2 0.001	MAS T0-T2 0.000

Table 6. Results regarding MAS median values through the follow-ups $p<0.0$

Discussion and Conclusion

Decreasing values of TSRT through the follow-ups suggest that both treatments had some direct effect on spasticity in Gastrocnemius Lateralis thus freeing some degrees of freedom in the movement of the affected ankle. This improvement in motor control is also supported by an increase in data's regarding muscle strength of the antagonist muscle.
Clinical data emerged from MAS scores have also some statistical significances but no correlation was observed relating them to TSRT scores suggesting that this kind of scale is not a real measure of spasticity but rather an assessment of muscle resistance to passive stretch, as found in literature.
Obviously, this study could not state any kind of cause/effect ratio because of its many limits in all the study design. The lack of a control group, wide inclusion criteria and the combination of two different kinds of treatment are issues needing to be solved through a higher-level study design.
However, we think that this clinical series could be an important hint for every researcher working in the complex field of spasticity, a symptom that is poorly defined and hardly treated.

Declarations

Funding: all this study was self-funded by the authors themselves.

Conflicts of interest/Competing interests: the authors declare that they have no conflict of interest.

Ethics approval: all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Consent to participate All the subjects signed an informed consent to participate according to the Italian laws and the ethical standards of the institutional and/or national research committee.
Consent for publication All the subjects signed an informed consent to the publication of results accordingly to the Italian laws and the ethical standards of the institutional and/or national research committee.

Availability of data and material: all data and material are available at the corresponding author institution address.

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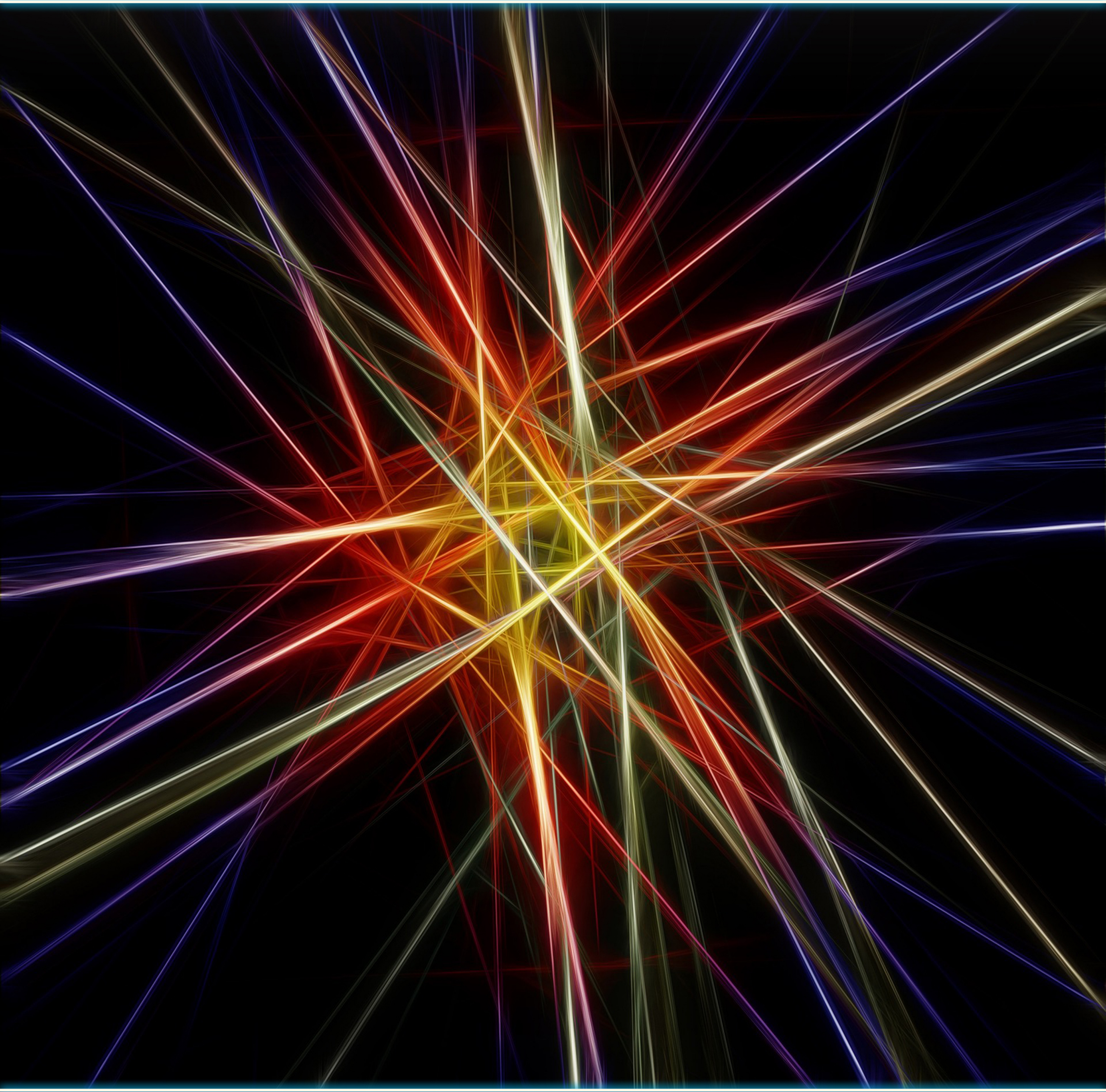
Cite: *Diego Longo, Giulio Cherubini, Vanessa Mangè, Paolo Lippi, Leonardo Longo, Daniela Melchiorre, Maria Angela Bagni.*
Effects of laser therapy and Grimaldi's muscle shortening maneuver on spasticity in central nervous system injuries.
Laser Ther. Vol. 29 N.2 - June 2022, pagg.: 41-48
DOI: 10.26352/GJ06F5006-June2022

Laser Therapy

International Journal of Laser PhotoBioModulation, Biophotonics and Surgery



ISBN 979-12-80225-47-4