Energy for Health
International journal of information and scientific culture

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I.O.V. – I.R.C.S. - Padova, Italy
e-mail: luigi.corti@unipd.it

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e-mail: monica.monici@unifi.it    monica.monici@asalaser.com

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Emeritus Professor of Surgery, and past incumbent of the chair of Plastic Surgery, Beth Israel Deaconess Medical Center, Boston, P O.B. 2338, Savoy, 56530 Israel
e-mail: ikaplan90@gmail.com

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Application of Concentrated Growth Factors (CGF) and Mphi Laser to Treat Defects in the Oral and Maxillofacial Region. A two - case report.

N. Doan1,2, L. Nguyen-Pham2, C. Liang1, Q. T. Duong 1,2
1 Private practice, Brisbane, Queensland, Australia
2 Department of Biomedical Engineering the University of Queensland, Brisbane, Queensland, Australia

Abstract
The application of Concentrated Growth Factors (CGF) in oral and maxillofacial surgery (OMS) and, in general, in regenerative medicine is steadily increasing. The purpose of this study is to present a review and case reporting on the use of CGF for tissue regeneration in the oral maxillofacial region. Two case reports on the surgical regenerative management of oral lesions CGF and Mphi laser were described. Postoperative recovery was uneventful. Laser was effective in reducing postoperative pain, swelling, bleeding, speech impairment, and cosmetic appearance. The CGF technique is minimally invasive and well tolerated. The advantage of the Mphi laser over the traditional lasers is its dual effect: tissue coagulation and promotion of wound healing. The use of Mphi lasers in conjunction with CGF applications in oral surgery is beneficial due to its safety, no side effect, easy technique, cheap, and efficacy for the patients [20, 21].

Materials and methods
This study attempted to carry out a literature review and cases report on the use of Mphi laser in conjunction with CGF applications in the oral and maxillofacial region. Literature search was carried out using Medline search and manual search using the keywords: “concentrated”, “growth factors”, “tissue engineering”, “regenerative medicine” and “blood”. The review followed the method recommended by PRISMA and included clinical studies with adequate information. Papers with lack of data were excluded. Additionally, authors’ experience on this topic was reported with the description of two relevant cases. In both patients, bony defects were filled with autologous fibrous rich CGF and synthetic alloplastic materials, and then treated with Mphi laser. Results: There were no published data on the combined use of Mphi laser and CGF in clinical applications in the OMS region. Two case reports on the surgical regenerative management of oral lesions CGF and Mphi laser were described. Postoperative recovery was uneventful. Laser was effective in reducing postoperative pain, swelling, bleeding, speech impairment, and cosmetic appearance. The CGF technique is minimally invasive and well tolerated. The advantage of the Mphi laser over the traditional lasers is its dual effect: tissue coagulation and promotion of wound healing. The use of Mphi lasers in conjunction with CGF applications in oral surgery is beneficial due to its safety, no side effect, easy technique, cheap, and efficacy for the patients [20, 21].

1. Introduction
Growth factors are molecules capable of facilitating several biological activities, such as cell proliferation, differentiation and repair. The use of concentrated growth factors (CGF) in dentistry is a relatively new concept. Since its inception, CGF have been used to enhance regeneration and healing for a variety of procedures, such implant placement, socket preservation, bony reconstruction and tissue regeneration [1]. The use of Multiwave Locked System laser devices, such as Mphi laser [2], represents an innovative adjunctive therapy for the enhancement of wound healing after CGF treatment. A unique feature of Mphi laser Therapy is the patented wave technology involving the use of two different and synchronized emissions: one with continuous/intermittent mode and 808 nm wavelength, the other with pulsed mode and 905 nm wavelength, that makes it one of the most efficient strategies for wound healing. Mphi laser has many therapeutic indications: sprains, muscle tears, tendinitis, brachial neuritis, craniofacial pain, bunions, lumbago, arthritis, articular pain, edema, hematomas. Mphi Laser Therapy produces its effects through anti-inflammatory and analgesic properties [3]. These effects are beneficial in the enhancement of wound healing and on review on the use of Mphi laser in conjunction with CGF applications in oral surgery is beneficial due to its safety, no side effect, easy technique, cheap, and efficacy for the patients [20, 21].

Materials and methods
Literature search was carried out using Medline search and manual search using the keywords: “concentrated”, “growth factors”, “tissue engineering”, “regenerative medicine” and “blood”. The review followed the method recommended by PRISMA and included clinical studies with adequate information. Papers with lack of data were excluded. Additionally, authors’ experience on this topic was reported with the description of two relevant cases. In both patients, bony defects were filled with autologous fibrous rich CGF and synthetic alloplastic materials, and then treated with Mphi laser. Results: There were no published data on the combined use of Mphi laser and CGF in clinical applications in the OMS region. Two case reports on the surgical regenerative management of oral lesions CGF and Mphi laser were described. Postoperative recovery was uneventful. Laser was effective in reducing postoperative pain, swelling, bleeding, speech impairment, and cosmetic appearance. The CGF technique is minimally invasive and well tolerated. The advantage of the Mphi laser over the traditional lasers is its dual effect: tissue coagulation and promotion of wound healing. The use of Mphi lasers in conjunction with CGF applications in oral surgery is beneficial due to its safety, no side effect, easy technique, cheap, and efficacy for the patients [20, 21].

2. Materials and methods
This study attempted to carry out a literature review and cases report on the use of Mphi laser in conjunction with CGF applications in the oral and maxillofacial region. Literature search was carried out using Medline search and manual search using the keywords: “concentrated”, “growth factors”, “tissue engineering”, “regenerative medicine” and “blood”. The review followed the method recommended by PRISMA and included clinical studies with adequate information. Papers with lack of data were excluded. No publication was found on this topic. The authors presented their experience with the above mentioned technique by describing two cases on the application of Mphi laser in conjunction with CGF in the regeneration of bony patients with large craniofacial bone defects in the upper and lower jaw respectively. CGF was prepared (Figure 1) according to the procedure using patient’s blood through venepuncture (total blood collected 40 ml), which was allocated into two red and two white sterile reaction tubes. These tubes were instantly centrifuged with Medifuge MP 200 (Sliedrecht, Italy) following the manufacturer’s recommendation. After spinning, sedimentation of the Vacutainer’s content was allowed for 20 min until further processing. The top 2ml layer comprised the platelets poor plasma (PPP) containing no anticoagulant, the next 2ml layer was platelets rich plasma (PRP), the third 0.7ml was the stem cells/white layer, while the fibroinrich mass with CGF was in the central part of the tube constituting the CGF clot, the remaining 4ml and 1ml precipitated portions were red blood cells and sedimentary blood fragments, respectively. The final CGF mixture was collected by tipping off the top PPP and meticulously collecting the clot layers. Using a sterile Petri dish, the CGF clot

Key words: Concentrated Growth Factors, Mphi Laser, Regeneration, Wound Healing.
was detached from the red blood portion using scissors (12). Fabrications of CGF sticky bone and membrane were followed by the manufacturer’s protocol. For sticky bone, Geistlich BioOss® (bovine demineralized freeze dried bone, Geistlich Biomaterials) and Osteon (hydroxyapatite and calcium phosphate, Genoss®) were used instead of calcium phosphate. Digital radiographic and clinical assessments were performed at one, three and six month review. The patients were monitored and were asked to report post operative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing.

CASE REPORT

Case 1
A female patient, 55 year old, was presented to a private specialist Oral Surgery clinic for a complaint of pain and swelling in the anterior lower jaw region. Her medical history was unremarkable. Clinical assessment indicated inflammation in the lower vestibule, tender and fluctuant to touching in the lower right lateral incisor and first premolar area.

As the involved teeth were splinted together in a fixed porcelain fused to metal bridge, no mobility was detected (Figure 2). After clinical assessment, cone beam computed tomography (CBCT) scans were acquired, displaying two well outlined round cystic defects (diameter ~ 3 cm), located in the right anterior area of the mandible (Figure 3).

Figure 1: White and Red Vacutainer® tubes showed different colours and layers after spinning, used for CGF preparation of sticky bone and membrane (Adapted from Dr Ezio Gheno, 2015).

Ideally, there should be a control treatment site on a contralateral side to compare the effectiveness of Mphi laser and CGF treatment to the sham/control side. However, due to lack of suitable cases for a split mouth study, these two cases were treated as clinical audit.

RESULTS

The search found only six CGF related articles that, however, did not meet completely the inclusion criteria. These six articles illustrated the application of CGF in the oral and maxillofacial region (OMR) including: sinus lift, ridge augmentation, gingival recession, implantology, maxillofacial reconstruction. The cases reported below showed good results, indicating that the innovative clinical application of CGF in OMR is promising.

No post-operative complications associated with CGF procedures were reported in the two cases. Mphi laser was effective in reducing postoperative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing. Apparently, there was no difference in wound healing after one and three months.

Figure 2: Intra-oral photo of lower right lateral and first premolar splinted together by porcelain fused to metal bridge.

Figure 3: Cone beam computed tomography (CBCT) and 3D reconstruction of patient’s oral and maxillofacial region shows defects at the lower right anterior section of the mandible.

Figure 4: Outline of treatment for two dental cysts using CGF sticky bone and membrane.

Figure 5: Postoperative peri-apical radiography showed cystic defects were filled CGF containing sticky bone and membrane.

Figure 6: Left x-ray showed peri-implantitis defect at coronal third of upper first premolar and molar. Right x-ray Illustrated defects after CGF treatment.

The surgical management involved total elimination of two cystic defects (lower right lateral incisor and second premolar), apicoectomy of the involved teeth and concealing the deficiency with CGF mixture of fibrin rich sticky bone and membrane. After CGF clot preparation, surgical process was carried out under local anaesthesia.

Subsequently, a mucoperiosteal buccal flap was raised between lower left first incisor and lower right second premolar. An interchanging duffled and piercing division was used to detach and eradicate the two cystic lesions from bone. After enucleation/cystectomy, apicoectomies of the involved teeth were performed. The resultant bony deficiencies were rebuilt by packing of CGF fibrinrich dot sticky bone reinforced with Geistlich BioOss® and Osteon, which totally covered the cystic defects (Figure 4).

Ultimately, the flap was sutured in place with resorbable chromic sutures. At the end of the treatment, Mphi laser was applied at the surgical site following the manufacturer’s protocol. The total CPW energy used was 0.637 Joules at an energy density of 1.27 J/cm², frequency of 1500 Hz, lasting time per application 0.04 second at 25% intensity.

Two laser applications, at the apical and coronal half, were employed at each implant on buccal and lingual side. Postoperatively, the patient was given thorough oral hygiene instruction and dietary program. Prescription of antibiotics (Amoxicillin/Clavulanic and metronidazole) and analgesics (combined paracetamol and ibuprofen) were given. Postoperative follow-ups were uneventful and were done at first day, first week, one month, three months and six months. No post-operative complications associated with CGF treatment were noted. Digital radiographic and clinical assessments were performed at one, three and six month review.

The patient was monitored for postoperative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing. Mphi laser helped to reduce postoperative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing. There was no difference in wound healing after one and three months. Through the ensuing six months, an even and stable building of the deficiencies by freshly produced bone was noted.

Case 2
A healthy 81 years old lady with no significant medical history, presented to a specialist Oral Surgery Clinic for surgical management of peri-implantitis of her two existing implants in the upper first premolar and molar area. Though the patient did not complain of any particular troubles, digital imaging indicated areas of bone loss surrounding the coronal third of her two implants spreading bucco-lingually.

Clinical evaluation indicated slight gingival inflammation in the buccal aspect of the two implants though the sulcular mucosa appeared normal. A digital peri-apical imaging of the implants showed moderate bone loss in their coronal thirds (Figure 5).

Treatment options for the defect encompass conservative and radical surgical management. Conservative treatment includes debridement followed either by traditional or CGF bone and tissue regeneration (GBR and GTR).

Radical treatment involved surgical removal of the two mentioned implants. The patient chose conservative CGF GBR and GTR. This treatment comprised of raising a buccopalatal mucoperiosteal flap followed by a total removal of soft granulated peri-implantitis tissue, debridement of the infected bone, removal of two remaining GBR screws and smoothing of exposed implant titanium threads. The exposed implant surfaces were conditioned with CGF stem cells and PRP prior to application of CGF sticky bone and membrane to restore the entire periimplant defects (Figure 6). The CGF sticky bone and membrane were prepared instantaneously, prior to operation, as explained in case 1, using four 10 ml Vacutainer® tubes. Once the defects were completely filled with sticky bone and membrane, the flap was closed using chromic resorbable sutures then coated with PRF to create a fibrin seal to enhance wound healing (Figure 7).

At the conclusion of the procedure, Mphi laser was used to irradiate the surgical wound following the manufacturer’s protocol. The total CPW energy used was 0.637 Joules at an energy density of 1.27 J/cm², frequency of 1500 Hz, lasting time per application 0.04 second at 25% intensity. Two laser applications, at the apical and coronal half, were employed at each implant on buccal and lingual side. Similar prescription antibiotic regimen (Amoxicillin/Clavulanic and metronidazole) and analgesics (combined paracetamol and ibuprofen) and post surgical oral hygiene instruction were given. Postoperative follow-up were uneventful and were carried at first day, first week, one month, three months and six months. The patient was observed and reported for post operative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing. No post-operative complications associated with CGF therapy were recorded.

Digital x-ray assessments were performed at one, three and six month review. Mphi laser helped to reduce postoperative pain, swelling, bleeding, speech impairment, analgesic use, trismus and wound healing. There was no difference in wound healing after one and three months.

Figure 7: Outline of treatment for periimplantitis for upper right first premolar and first molar using CGF sticky bone and membrane.
DISCUSSION
Traditionally, regeneration of bony defects following eradication of big cyctic defects in the oral and maxillofacial region, such as maxilla and mandible, may, at times, be coupled with complications. For example, shrinkage of blood clot, serum exudation, and development of Webers gaps, as well as a risk of minor infection, considerably affect the regenerative courses of the jawbones. The above issues have brought attention to the medical scientific community through research and publications [1,8]. Conventionally, it is not uncommon to have a total eradication of cystic defects and covering the finishing bony lesion with primary wound suturing. The main dilemma of the surgeon is to find the best way for bone defect reconstruction. According to the available literature [8], large bony defects are commonly filled and reconstructed with autotransplants obtained from the iliac ridge, ribs or donor sites in the oral cavity. Application of autotransplants enables primary wound healing, preservation of bone contours and fast regeneration. However, a drawback of this approach is the need for additional surgical procedure, highly specialized personnel, general anaesthesia and very high expenses [8,9]. Application of growth factors in guided bone regeneration procedure has been well-known for an extended period of time. This technique is applicable in implantology, specially due to its versatility in various augmentation techniques, and in unavoidable anatomic situations (horizontal and vertical augmentation, sinus lift etc.) [14]. CGF can be applied alone or mixed with bone graft material. The two cases presented in this article demonstrate the efficiency of CGF in significantly shortening bonehealing time, particularly in massive bone defects, reducing the incidence of postoperative complications, and enabling better restoration of surrounding periodontium. The method is relatively simple, with minimal risk of infection and allergic reaction, and economically feasible. In an ideal scientific study, there should be a control group. However, the current cases were not suitable for a split mouth study and were treated as clinical audit. More research should be carried out, particularly, prospective split mouth study to confirm CGF and Mphi laser effectiveness and efficacy in tissue regeneration for the oral and maxillofacial region.

CONCLUSION
Majority of current regenerative technologies in OMR still faced with three main issues: compatibility, failure and cost. The use of autologous materials such as CGF may represent a potential solution to these dilemma. The combination with Mphi laser application may represent a valuable aid in managing post operative discomfort. Further research should be conducted, especially, prospective split mouth study to validate the effectiveness of the CGF and Mphi laser combined treatment in tissue regeneration for the oral and maxillofacial region.

REFERENCES
ABSTRACT
Tendon diseases are widespread in the population and constitute a high percentage of the consultations to the physician for musculoskeletal disorders. These are painful, debilitating, and negatively affect patient’s quality of life. Some tendons are particularly vulnerable to degenerative pathologies; these include the Achilles, elements of the rotator cuff, patella, foot extensors and tibialis posterior tendons. Although tendinopathies are common, their treatment is not easy and often combined therapies are needed to alleviate symptoms, promote functional recovery and prevent recurrence. Among the resources available for treating tendinopathies, laser therapy showed to be effective in some in vivo studies on the efficacy of laser therapy in the treatment of Achilles tendinopathies, the following results have been reported: modulation of inflammatory response following trauma, analgesic effect, antioxidant effect, stimulation of healing process by increasing collagen I production and tenocyte proliferation [23].

INTRODUCTION
Tendinopathies are common diseases with an incidence of about 30% of all physician examinations for musculoskeletal disorders [1]. Such conditions have an adverse impact on patient’s quality of life, because therefore, nowadays, do not affect only athletes but also the general and elder population [2,3]. Although tendinopathies may involve tendons of any joint, the most affected are those of wrist and hand (for example, finger flexor tenosynovitis), elbow (epicondylitis, or tennis elbow), shoulder (cuff, rotator tendinopathy), ankle (Achilles tendinopathy) and knee (patellar tendinitis and popliteal tendinitis) [4-6].

Physiologically, tendon is composed of densely arranged collagen fibers, elastin, proteoglycans, and lipids. These elements are produced by tenoblasts and tenocytes, elongated fibroblasts and fibrocytes, located among the collagen fibers, that represent about 90-95% of the cellular elements in the tendon. The remaining 5-10% includes chondrocytes, synovial cells, endothelial cells and smooth muscle cells. Tendon is sheathed by the epitenon, a particular connective tissue which contains the tendon neurovascular supply and facilitates the gliding of collagen bundles against one another during tendon movement. Muscular force is transmitted to the skeleton at the point where the tendon inserts into the bone. The osteotendinous junctions, as well as the musculotendinous junctions, are the areas most susceptible to mechanical stress and consequently to tendon injury [7,8]. Mechanical etiopathogenesis is, in combined therapies, the most common cause of tendon pathologies; in particular, insertional tendinopathies, tenosynovitis, peritendinitis and tenosynovitis, also associated among them. These diseases may arise from an imbalance of the microvasculature, following an acute trauma or, even more frequently, to repeated microtraumas of exogenous origin (exercise machines, footwear...) and/or endogenous (congenital abnormalities, primary or secondary skeletal disorders, functional overuse, leg length discrepancy, muscle tension). Tendinopathies classification remains difficult and encompasses a variety of histopathologic entities. A possible classification is: (I) acute tendinitis alone: tendon injury with inflammation; (II) chronic tendinosis alone: tendon injury with degeneration at cellular level and no inflammation; (III) chronic tendinosis with acute tendinitis [9]. Although cases of tendinopathies with true inflammatory component exist, many patients have symptoms for a long time and wait for a long time before contacting the family doctor, so that, acute inflammation has probably subsided and it has been replaced by degeneration of collagen fiber structure. Histologic descriptions of tendinopathies have demonstrated disordered collagen arrangement together with non-collagenous matrix increase, cellular alterations and neo-angiogenesis [10]. This process seems to be one of the causes of pain symptoms: in Achilles tendinopathies, the presence of sensory nerves associated with new formed vessels could be responsible of the production of nociceptive and proinflammatory substances [11]. Since it is unclear if these chronic degenerative changes are preceded by an acute inflammatory response, the term tendinosis is more appropriate to describe these clinical aspects in absence of evidence of acute inflammation [12].

Conservative or physical therapies are generally accepted as the first line approach for managing tendinopathies with the purpose of alleviating symptoms, promoting functional recovery and prevent their recurrence [13-16]. These therapies can be used more appropriate to describe clinical aspects in absence of evidence of acute inflammation [12].

MATERIALS AND METHODS
Patients
Seventeen adult patients, 13 M and 4 F, mean age 47 years (range 15-80 years), were treated on an outpatient basis, with an average of 8 sessions/patient. The patients suffered from acute or chronic tendon diseases in the following anatomical districts: knee (5 patients: 3 Pes Anserine Tendinopathy -Gracilis, Sartorius and Semitendinosus tendons and 2 patellar tendon), shoulder (4 patients: 2 acromioclavicular joint, 1 supraspinatus tendon and 1 long head of biceps tendon), ankle (3 patients: Achilles tendon) and foot (5 patients: 4 foot extensor tendon and 1 tibialis posterior tendon) see Fig. 1.

Physical therapies include eccentric exercises, electrotherapeutic modalities, such as Extracorporeal Shock Wave Therapy, soft tissue therapies, splints and orthosis. Among the resources available for treating tendinopathies within the field of physical therapy, laser therapy showed positive effects in some in vivo studies on the efficacy of laser therapy in the treatment of Achilles tendinopathies, the following results have been reported: modulation of inflammatory response following trauma, analgesic effect, antioxidant effect, stimulation of healing process by increasing collagen I production and tenocyte proliferation [23].

This study aimed to evaluate the effectiveness of a high power, dual wavelength NIR laser source in the treatment of patients affected by tendinopathies.

MLS® Laser Therapy in the treatment of patients affected by Tendinopathies

L. Vignali1, G. Caruso2, S. Gervasi3, F. Cialdai1

1 AS-Scampus Joint Laboratory, ASA Research Division, Dept. of Experimental and Clinical Biomedical Sciences, University of Florence, Florence
2 Caruso Physiotherapy outpatients, La Fontina, Ghezzano, Pisa
3 ASAcampus Joint Laboratory, ASA Research Division, Dept. of Experimental and Clinical Biomedical Sciences, University of Florence, Florence

Key words: tendinopathies, MLS® therapy, NIR radiation

Fig 1: Distribution of tendon diseases according to the anatomical district (Big pie chart); Small pie charts show, within each district, the specific tendons involved.
Inclusion criteria required the presence of symptomatic tendinopathies assessed following clinical and instrumental evaluation. Exclusion criteria were: therapy with oral anticoagulants, non-compliant patients (cognitive impairment or psychiatric disorder), neoplastic pathology, skin diseases. Before treatment, all the patients were informed about the technique and laser beam properties, and they signed an informed consent to the treatment. The evaluation of each patient was performed by means of pain VAS scale [24]. The VAS is a visual analog test which evaluates the subjective painful symptomatology; the score ranges from 0 (lack of pain) to 10 (worst imaginable pain). It was administered to the patients before and at the end of the whole treatment. The patients were treated 3 times / week, for a total of 8 session/patient.

Laser treatments
The laser source was a Multwave Locked System laser (MLS®, ASA Srl, Vicenza, Italy). It is a commercially available laser source built in compliance with EC/EU rules, which received FDA approval and is widely used in clinics. MLS® laser is a class IV NIR laser with two synchronized sources (laser diodes). These emit at different wavelengths, peak power and emission mode. The first one is a pulsed 905 nm laser diode with 25 W peak optical power. The pulse frequency may be varied in the range 1-2000 Hz, thus varying the average power delivered to the tissue. The second laser diode (808 nm) may operate in continuous (power 1.1 W) or pulsed mode (repetition rate 1-2000 Hz, 550mW mean optical power, with a 50% duty ratio independently of the repetition rate). The two laser beams are emitted synchronously and the propagation axes are coincident.

Treatment modality
The patients received the following energy dose: Knee: 5.27 J/cm²; Shoulder: 5.63 J/cm²; Ankle: 14.69 J/cm²; Foot: 14.69 J/cm².

Data Analysis
The data were analyzed using paired Student’s t-test to compare the values found pre and post treatment in all the patients and into each subgroup. The level of significance was set at 0.05.

RESULTS
Seventeen patients affected by tendinopathies were enrolled in the study; one of them (patient affected by tibialis posterior tendinitis) underwent only the first session and decided to interrupt the treatment, therefore the related values were excluded. Patients consisted of 13 males and 4 females; mean age was 47.6 (15-80) (Table I). At the end of the treatment patients showed improvement in pain symptoms: mean value changed from 6.58 ± 2 to 3 ± 2.4, with a 56.9% reduction in the VAS score after treatment (Fig.2). In order to analyze the data in more detail, the patients were divided, as described in “Material and Methods” section, into subgroups based on anatomical district and anatomical structures affected by the disease (Table II). For each group, VAS score differences were evaluated. The score of patients affected by knee tendinopathies highlighted a statistically significant improvement (p<0.005) at the end of the treatment compared to basal score; the mean value decreased from 6.8 ± 1.4 to 3 ± 2.2. Although in the other anatomical districts considered the mean changes in VAS score did not result statistically significant, however there was an improvement in pain symptoms and a corresponding decrease in the average VAS score. In particular, in patients affected by shoulder, ankle and foot tendinopathies the average score changed from 7.25 ± 1.5 to 3.5 ± 2.64; from 5.3 ± 2.5 to 2 ± 2.6 and from 7 ± 2.94 to 2.75 ± 3.2, respectively (Fig.3 and Table III). In percentage, after treatment VAS score was reduced by 55% in the subgroup of patients affected by knee tendinopathies and by 51% in the subgroup of patients affected by shoulder tendinopathies. Higher percentages were found in the subgroups of patients affected by ankle and foot tendinopathies, where VAS score was reduced by 69% and 51%, respectively. VAS score for single patients treated with MLS® Laser Therapy.

Table I - Group baseline characteristics

<table>
<thead>
<tr>
<th>PAT. NUMBER</th>
<th>MEAN AGE</th>
<th>SEX</th>
<th>VAS Before treatment (mean)</th>
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<tbody>
<tr>
<td>17</td>
<td>47.6 (1580)</td>
<td>13 M, 4 F</td>
<td>6.58 ± 2</td>
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Table II - Subgroups baseline characteristics

<table>
<thead>
<tr>
<th>ANATOMICAL DISTRICT</th>
<th>PAT. NUMBER</th>
<th>MEAN AGE</th>
<th>SEX</th>
<th>VAS Before treatment (mean)</th>
</tr>
</thead>
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<tr>
<td>KNEE</td>
<td>5</td>
<td>44.2</td>
<td>4 M, 1 F</td>
<td>6.8 ± 1.48</td>
</tr>
<tr>
<td>SHOULDER</td>
<td>4</td>
<td>53.25</td>
<td>4 M</td>
<td>7.25 ± 1.5</td>
</tr>
<tr>
<td>ANKLE</td>
<td>3</td>
<td>71.5</td>
<td>2 M, 1 F</td>
<td>7.6 ± 2.94</td>
</tr>
<tr>
<td>FOOT</td>
<td>5</td>
<td>37</td>
<td>3 M, 2 F</td>
<td>6.58 ± 2</td>
</tr>
</tbody>
</table>

Table III - Mean VAS Score for patients divided into subgroups before and after treatment application.

<table>
<thead>
<tr>
<th>ANATOMICAL DISTRICT</th>
<th>VAS Before Treatment</th>
<th>VAS After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNEE</td>
<td>6.8 ± 1.48</td>
<td>3 ± 2.2</td>
</tr>
<tr>
<td>SHOULDER</td>
<td>7.25 ± 1.5</td>
<td>3.5 ± 2.6</td>
</tr>
<tr>
<td>ANKLE</td>
<td>7 ± 2.94</td>
<td>2.64 ± 2.64</td>
</tr>
<tr>
<td>FOOT</td>
<td>6.58 ± 2</td>
<td>2.75 ± 3.2</td>
</tr>
</tbody>
</table>

Fig 2: Mean VAS Score for all patients before and after treatment application.

Fig 3: Mean VAS Score for patients divided into subgroups before and after treatment application.

Fig 4: VAS Score of single patients before and after treatment application.
scores decreased by 62% and 60%, respectively. No patients reported adverse events.

**DISCUSSION**

The results obtained in this study show that the treatment with a high power, dual wavelength NIR laser source is effective in inducing inhibition of pain referred by patients affected by tendinopathies. Since predisposing factors to pain and following rate of response to treatment are different depending on anatomical area, patients were divided into subgroups and results were evaluated considering the subgroup and the scores of each patient individually. The subgroup of patients affected by knee tendinopathies was the only subgroup where a significant change (p < 0.005) of VAS score was reported at the end of the treatment, compared to the basal score (from 6.8 ± 1.4 to 3 ± 2.2).

It is important to point out that a patient of this subgroup (see fig. 4, patient 1) was affected by congenital joint laxity; therefore, at the end of the treatment, its score decreased only from 7 to 6 points of the VAS scale (Fig. 4). Excluding the values of this patient, in the knee subgroup VAS score decreased from 6.75 ± 1.7 to 2.25 ± 1.7. A positive result in terms of pain reduction, even though not statistically significant, was obtained also in the subgroup of patients affected by ankle tendinopathies, where the mean VAS score decreased from 7.25 ± 1.5 to 3.5 ± 2.64. In this subgroup, the lack of statistical significance can be attributed to a small sample size and to a single patient poorly responsive to the treatment (see fig. 4, patient 4, VAS score from 8 to 6). During the treatment, this patient did not follow the doctor’s advice and continued sport activity. This behavior partially nullified the effect of therapy and continued sport activity. This behavior partially nullified the effect of therapy and treatment and, therefore, at the end of the treatment, its score decreased only from 7 to 6 points of the VAS scale (Fig. 4). Excluding the values of this patient, in the knee subgroup VAS score decreased from 6.75 ± 1.7 to 2.25 ± 1.7.

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Hypothesis for a future application of a Laser-device in patients with symptoms of a developmental auditory processing disorder

Part I: Methodological basics

E. Friederichs
Centre for Development and Learning, Bambang
Business and Economic Education, University of Bamberg, Germany

ABSTRACT

This article provides a conceptualisation of research studies which should evaluate whether changes of electrophysiological late event related potential pattern (latency, amplitudes) could be used to reflect clinical changes from therapeutic intervention with LASER light in patients with symptoms of central auditory processing disorder. The contingent negative variation (CNV) of event related potentials reflects a synchronization of together firing neural assemblies, responsible for auditory processing, suggesting an accelerated neuromaturation process when applying a LASER device stimulation. It will be discussed whether a LASER stimulation might be useful for a clinical improvement of distraction symptoms caused by auditory processing deficits. A model is presented explaining these effects by inducing the respiratory chain of the mitochondria.

INTRODUCTION

It is well accepted that an acoustic environment (noise and reverberation) in classroom conditions is a critical factor in the educational achievement of many children. Such populations being at risk for academic failure encompass children with language impairment, dyslexia, attentional deficits and general developmental delay [1]. An increasing number of children appear to have hearing impairment in spite of normal auditory thresholds. Parents and teachers describe difficulties in listening in the presence of background noises and difficulties in understanding rapid or degraded speech. In these cases, listening problems result from dysfunctions of auditory processing inside the brain and will be considered as central auditory processing disorder (CAPD) [3, 4, 5]. It will be assumed that improper neural acoustic representation will lead to serious problems in the maturation of the auditory pathways and hence the development of auditory processing ability. Recent research results suggest that neuroplasticity and neuromaturation are dependent on stimulation [1, 3, 7]. Therefore, comprehensive management of CAPD should include auditory stimulation to achieve functional changes within the central auditory nervous system. Thus, young children would be expected to benefit from a great degree of neuroplasticity. Stages of neuromaturation are i) neurostimulation, ii) neuromodulation ii) neurorelaxation and iv) neurodifferentiation [7, 8]. This article refers mainly to the neurostimulation part of a relevant circuit is turned on, it fires and the blood flows to this circuit. The brain scan (Functional Magnetic Resonance Tomography (fMRT)) is running like this. Neurostimulation is effective in proving the brain's ability to build new circuits (internal neuroplastic stimulation). Energy based stimulation (light, sound, electricity, vibration, movement, substance) may help to reinitiate dormant circuits to achieve homeostasis from external and internal sources. The purpose of this article is to introduce a conceptualization of studies for auditory perception improvements of children with CAPD. The purpose of this article is to introduce a conceptualization of studies for auditory perception improvements of children with CAPD by turning on specific neural networks in order to improve auditory processing when applying laser light. A model will be presented to explain possible results using electron modelling and proton exchange inside the respiratory chain.

(RE)-WIRING A BRAIN

Already Francis Crick speculated the idea that sensory stimulation of the auditory centres of the brain is critically important, and influences the actual organization of auditory brain pathways [1, 15]. Increase in auditory stimulation may result in morphological alterations within the auditory part of the brain [3]. The ability of the auditory cortex to reorganize continuously throughout life reflects the ability to acquire new skills and behaviours. Long-term potentiation is related to increases in the synaptic activity and efficacy following strong and repeated stimulation of a sensory system. There have been reports of morphological and structural alterations within nerve cells including increase in size and postsynaptic density along with alterations in late event potentials [3, 4, 16]. Usually cells wire and fire together i.e. in rhythm. Neurons work usually in large groups of neuronal assemblies, communicating electrically through distributed networks throughout the brain [7, 8]. If the neurons are not synchronized, they cannot generate enough strong, sharp signals to stand out against the background noise of all the other neuronal signals inside the brain. Neurons do not necessarily fall silent, but they continue to fire at a slower rate. Therefore, these cells mess up the function of the “normal” cells. This occurs in epilepsy, Alzheimer disease, brain injuries and learning problems. Light frequencies carry energy and provide different types of information. In this context, it could be already shown that distinct frequencies from a low level laser might be used to turn off “sick” neurons. Light might be used to turn on specific clusters (classes) of neurons, thus wiring these neurons not by substance-based (chemical) signals rather than by physical signals. Energy from light use light sensitized molecules to transforming light into energy [10, 11]. Thus, different wavelengths of the light spectrum may have different effects on the organism. Wilden et al. [12] already reported, that LASER stimulation with distinct wavelengths may vitalize the cell by increasing the mitochondrial ATP (adenosine-tri-phosphate) production. With regard to regard phenomena and its enhanced electron flow in the cellular energy transfer (respiratory chain), these authors postulated already that the experimentally found increase of ATP-production could be explained by means of low-level laser light on a cellular level. These investigations are mainly based on patients with tinnitus and sudden hearing loss, while developmental hearing problems are not considered. Studies of developmental disorders show that sensory stimulation in the case of the visual centres of the brain is critically important, and influences the actual organization of visual brain pathways. Increase in visual stimulation result in morphological alterations within the visual parts of the brain [13, 14]. Strategies for management of auditory processing disorder are usually direct remediation, i.e. the environment modifications and compensatory strategies. One of a possible new strategy for reducing the deleterious effects of auditory noise is the use of Laser light, providing discrete wavelength light, resulting in morphological alterations within the visual parts of the brain [13, 14]. Studies of these effects on the neuronal assemblies, responsible for auditory processing, suggesting an accelerated neuromaturation process when applying a LASER device stimulation. It will be discussed whether a LASER stimulation might be useful for a clinical improvement of distraction
Ca²⁺ and cAMP stimulate DNA and RNA ATPase. ATP controls the cAMP level, both the construction of a photon gradient lead to an increase in ATP synthesis [21, 26]. The light function [17, 21, 28-30]. The energy from the respective centres of the retinal, leading to an increase in ATP synthesis [21, 26]. The light induced increase of ATP synthesis and the construction of a photon gradient lead to an increased activity of the Na⁺/K⁺ and Ca²⁺/Na⁺ Antipporter and other ATP driven ion carriers like the Na⁺/K⁺ ATPase and the Calcium ATPase. ATP controls the cAMP level, both Ca²⁺ and cAMP stimulate DNA and RNA synthesis which leads to an increased cell growth [26]. It has been already demonstrated by Kanu and others that the so called “antenna pigments”, the flavoproteins and the cytochrome a/a₃ of the cytochrome oxidase complex can be considered as sensitive light receptors on the cell membrane [22, 31, 32, 33]. Further investigations demonstrated that there is a variety of additional light absorbing molecules (chromophores) capable of absorbing photons at the membrane and mitochondria level [22, 34]. Altogether, specific wavelengths (multiwavelength sources, NIR wavelength range, low power and high power laser etc.) may induce the respiratory chain of sick neurons thus leading to a modulation of mitochondrial regulation contributing to a molecular and cellular ATP driven repair mechanisms [17, 25, 35]. Monaci et al. showed that laser treatment leads to a cytoskeletal rearrangement and expression of early differentiation markers [36] leading to a possible improvement of cell proliferation and/or modulation at the protein level (cytoskeleton organization). Further on it may be speculated that epigenetically DNA synthesis can be increased by using distinct wavelengths of the light and NIR spectrum.

**EVENT RELATED AUDITORY CORTICAL POTENTIALS (ERCP)**

Over a few studies have been found in literature having focused on the use of CNV and P300 potential in documenting changes in clinical status. Recording of the Contingent negative variation (CNV) requires the patient to pay active attention to a stimulus. AERP’s are presumed to be related to attention, recognition, and memory processes. Event related cortical potentials allow the evaluation of brain activities. The contingent negative variation (CNV) is a slow negative potential decrease, which will appear hundreds of ms before target stimulation. CNV is representing a large number of increasing synchronous self-regulatory excitatory activity of neuron populations and is preparing the brain for the following auditory stimulus. In this sense CNV is related to the synchronous firing of wired neurons in order to provide the ability of reaction capacity of a certain brain task. Other studies emphasized the feasibility of using P300 event related potentials to document levels of auditory dysfunction [37, 38]. There are several studies suggesting that P300 auditory event related potentials in children with CAPD showed longer latency times and smaller amplitudes compared to controls [4, 16, 39]. Jirsa [40] demonstrated a significant decrease in P300 latency time along with an increase in P300 amplitude in the evoked potentials obtained from children with CAPD following an intensive therapeutic 14-week intervention program. The children in the experimental group exhibited improvement on selected auditory tasks and positive changes in overall academic performance. These data were interpreted as indicating that neurocognitive and behavior deficits could be improved by auditory specific intervention and could be distinctly objectified by means of late event related potential measures. It seems reasonable to assume that changes in the morphology of the waveform correlates with changes of the clinical status. Because maturation processes in highly plastic brain in childhood should be enhanced through sensory stimulation, expectation of improvement of auditory processing abilities must be confirmed by follow up investigation.

**CONSEQUENCES**

The organic living brain is quite the opposite of an engineered machine with硬化 circuits that can only perform a limited number of actions, but during the day the brain is forming / unwrapping new flexible neuronal networks. A group of neurons will be used for different purposes at different times. Tasks can be performed using different coalitions (assemblies) of neurons [41]. Learning skills are encoded in the cumulative electrical patterns resulting from the neurons firing together [41, 42]. The pattern, i.e. the population is interesting, not the individual cell. Cells that are, on whatever reason, chronically inflamed, are more sensitive to red and near-infrared light than are well-functioning cells. To heal, the body often needs to create new cells. Because auditory neuroatunation and neural plasticity depend on distinctive stimulation of auditory neurons, dynamic management of CAPD should begin as early as possible. Studies have to address the question whether use of AERP’s may be more sensitive for prediction of treatment outcomes as it has already suggested by Waldkeib et al. in the case of auditory processing (44). Studies on AERP measures performed before and after specific Laser light stimulation are in progress, to demonstrate possible therapeutic advantages of such a device and will be presented in Part II of this article. Furthermore, we need more information about the distinction of the different kinds of auditory processing dysfunction in children [43].

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CONFLICTS OF INTEREST
The author declares that he discloses any financial and personal relationships with other people or organisations that could inappropriately influence (bias) this work.

ALL INQUIRES SHOULD BE DIRECTED TO:
Prof. Dr. Edgar Friederichs
Centre of Development and Learning
D-96047 Bamberg, Germany
Phone: (+49 951) 297 299 1
Fax: (+ 49 951) 297 299 3
Email: info@entwicklung-staerken.de
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Management of the articular degenerative disease of the dog: comparison of physical and pharmacological therapies

S. Meggiolaro, S. Tention, G.M. De Benedictis

Degenerative Joint Disease (DJD) is one of the most common and disabling orthopaedic conditions of pets. The most recent therapeutic approach consists in the combination of different therapeutic options, such as the use of conventional drugs, the use of alternative treatments (i.e. homeopathy, phytotherapy, acupuncture), the oral administration of chondroprotectors, i.e. nutraceuticals; dog weight control, rehabilitation and correct home management. This study compared the efficacy on articular pain control of a physical therapy protocol, including MLS® treatment and hydrotherapy, versus traditional nonsteroidal anti-inflammatory drug (NSAID) therapy. Sixteen Labrador dogs, older than 5 years and affected by osteoarthritis have been included in the study. After the baseline visit, the animals matching inclusion criteria have been allocated to one of the treatment groups. The treatment efficacy has been assessed at 15 and 45 days via pet owner’s evaluation, using the Liverpool Osteoarthritis in Dogs (LOAD) and Canine Brief Pain Inventory (CBPI) scale, and by the clinical assessment of a technical expert. In both groups, a general improvement in symptoms has been observed, confirming that both physical therapy with MLS® and drug therapy are valuable aids in the management of pain symptoms associated with degenerative joint disease. In particular, for the treatment of osteoarthritis, when long term treatments are necessary, MLS® laser therapy is a valid alternative to pharmacological therapy, allowing for treating old dogs without worsening the condition of other compromised organs.

Introduction
Degenerative Joint Disease (DJD) is a major condition affecting, especially old and/or obese dogs, those subjects presenting genetic bone abnormalities or bone conditions and active dogs that are prone to repeated microtrauma due to intense physical activity [1]. Secondary DJD caused by trauma, articular instability or osteochondral lesion is the most common [2]. DJD has a severe impact on quality of life, due to associated pain and biologic functional limitations. Pain is the main clinical symptom of OA, therefore pain management is of utmost importance in osteoarthritics (OA) treatment, allowing improvement in both physical and psychological quality of life of the subject. Currently, there is no resolute treatment for OA and several approaches have been investigated to treat pain inflammation and progressive degeneration, which are different aspects of the disease, leading to the so-called multimodal approach [3]. This recent approach consists in the combination of different therapeutic options, combining the use of drugs with less conventional treatments, such as homeopathy, phytotherapy and acupuncture; the oral administration of chondroprotectors, i.e. nutraceuticals; dog weight control; rehabilitation and correct home management by the pet owner. The pharmacological treatment consists in the combination of different chondroprotective drugs and other complementary medications. This study investigates the possibility of alternative therapies that may be more suitable, especially for older dogs with unpaired general health for which drug therapy may not be appropriate. Among the different physical therapy options that were taken into account, Multilaser® (MLS®) laser therapy was considered the most suitable for dog DJD treatment. MLS® therapy involves the use of two different and synchronised emissions: one with continuous/frequent mode at 808 nm wavelength, the other with pulsed mode at 905 nm. The average power of the device is 1,1 W with a peak power of pulsed emission of 25 W. MLS® has been clinically applied for the treatment of several pathologies, including shoulder pain, lumbar, carpal tunnel syndrome, etc. MLS® pulse has been extensively characterized and its effects are well documented [4-6]. In this study a protocol that used MLS® associated with hydrotherapy treatment, was compared with the traditional pharmacological approach in dog DJD. The aim of the study was the assessment of both approaches efficacy in OA treatment, based on dog owner’s feedback and the clinical examination results, and the comparison of the results of the two treatments.

Materials and Methods
During the study, all dogs were clinically assessed and the laser protocol was adjusted accordingly. After the session, the animal was clinically assessed and the laser treatment was performed. In this study, the following treatment parameters have been applied:
- Animals with pain at palpation
  > 4: muscle scan phase with 18 Hz frequency and fluence of 4,81 J/cm²
  < 4: muscle scan phase with 36 Hz frequency and fluence of 4,81 J/cm²
- Animals with muscle scan phase with 18 Hz frequency and fluence 3,99 J/cm²
- Animals with flexion-extension pain > 4: the treatment had been carried out on points, covering the whole articular surface with 18 Hz frequency and fluence 3,99 J/cm² for a total of 12,52 J/point
- Animals with flexion-extension pain < 4: the treatment had been carried out on points, covering the whole articular surface with 36 Hz frequency and fluence 3,99 J/cm² for a total of 12,52 J/point

In general, 100% intensity has been used. It has been reduced to 75% for animals with dark fur.
- Each trigger point had been treated from one to four times with the following parameters:10 Hz with 25% intensity and fluency 1 J/cm².

The physical therapy protocol was repeated three times a week for the first two weeks, twice a week for the following two weeks and once a week up to the end of the study. Group B was treated with NSAIDs using oral administration of Carprofen (Rimadyl) with a dosage of 4mg/kg once per day (SID) for the first 7 days, followed by a dosage of 2 mg/kg SID for other 7 days. For gastroprotection, omeprazole was orally administered 20 minutes before food intake with a dosage of 0,7 mg/kg SID. Assessment were performed at day 0 (enrolment and first treatment), 15 and 45 using:
- Pet owner’s evaluation with Liverpool Osteoarthritis in Dogs (LOAD) scale and the Canine Brief Pain Inventory (CBPI) scale.
- Technical expert clinical examination, with observation of: lameness degree (score from 0- no lameness, to 4- limb is lifted and no load bearing), muscle tonicity (score from 0 - tonic limb, to 3 - severe hypotonicity), flexion-extension pain by VAS scale (evaluation based on dog behaviour reaction), pain at palpation.
- Assessment of muscle groups by VAS scale, trigger point number. When more than one limb was affected, the one with the most severe condition was scored. All multidisciplinary tasks were performed by the same operator, blinded to the pet owner’s assessment.

During the visit at day 0, dog specific counselling was provided to the owner in terms of dietary advice and physical activity protocol. Data were analysed using the Shapiro Wilk test, data presenting a normal distribution were expressed as mean ± standard deviation, while not normally distributed data or ordinal data
Muscle tonicity (Figure 4) improved for pain at palpation statistically improved for 1,89 at T15 to reach the score of 3,62 ± 0,99 at T15 and 2,49 ± 0,99 at T45. Trigger point number (Figure 5) statistically decreased in Group A (5,87 ± 2,17 at T0; 3,75 ± 1,67 at T15 and 1,75 ± 1,39 at T45) while no change was detected in Group B (5,5 ± 2,72 at T0; 4,87 ± 2,64 at T15 and 5,15 ± 2,99 at T45).

The degree of lameness decreased in Group A and B in terms of owner’s questionnaire were compared and show no differences between treatment, time and their interaction. Statistical significance was set with p<0,05.

RESULTS
Each owner completed the LOAD and CBPI questionnaires at days 0, 15, 45. In both groups, an improvement in the animal condition was perceived by the owners, despite it did not reach statistical significance (Table I). The results obtained in group A and B in terms of owner’s questionnaire were compared and show no statistical difference (Table II).

Lameness degree was assessed by the technical observer and scored 2,625 ± 0,89 at T0, 1,61 ± 1,91 at T15 and 1,87 ± 1,46 at T45. The degree of lameness decreased in both groups reaching statistical significance with respect to baseline, while no difference was observed between the groups (Figure 1). Flexion-extension pain by VAS scale (Figure 2) statistically improved during time in Group A from 6,37 ± 2,2 at T0 to 5,25 ± 1,89 at T15 to reach the score of 3,62 ± 0,74 at T45. Group B showed VAS value at T0 (6,87 ± 1,72), T15 (5,25 ± 1,67) and T45 (4,5 ± 1,77), that do not indicate significant modifications from baseline. Pain at palpation statistically improved for both groups from T0 to T15 (Figure 3). The following scores were assigned respectively to Group A and B: B: 6,5 ± 1,77 at T0; 5,37 ± 2,13 at T15 and 4,62 ± 1,60 at T45 and 3,67 ± 1,59 at T0; 4,5 ± 1,85 atT15 and 5 ± 2,14 at T45. Moderate pain (Figure 4) improved for Group A (from 1,75 ± 0,71 at T0 to 1,25 ± 1,03 at T15, up to 0,37 ± 0,74 at T45), while no changes were observed for Group B (scored 1,25 ± 1,03 at T0, 1,25 ± 0,99 at T15 and 1,26 ± 0,99 at T45). Another key point for the study was the pet owner’s contribution, both in terms of home management of the dog, based on the instruction received during the inclusion visit, and in terms of evaluation of the treatment outcome assessed using the Liverpool Osteoarthritids in Dogs (LOAD) and the Canine Brief Pain Inventory (CBPI). LOAD questionnaire is more focused on animal mobility, while the CBPI questionnaire focuses on pain, therefore they appear to be complementary and were used together to assess the overall health status of the animal. A limitation in the use of these questionnaires is the subjectivity related to the owner sensitivity and his emotional relationship with his dog, that may alter the perception of the real health condition of the animal [12].

To balance these factors, a clinical examination by a trained expert had been included in the study and, in fact, in many occasions this clinical assessment did not correspond to the owner’s evaluation. This can be explained by the fact that the clinical examination considered the OA grade of the dog, while the owner was likely influenced in his assessment by the knowledge of the owner’s clinical and financial conditions. For instance, when the owner is not aware of OA treatment and, in conclusion, the results of this study demonstrate that both physical therapy and pharmacological therapy are able to improve the general clinical conditions of OA affected dogs. Physical therapy allows to treat with no side effects even old and compromised animals and can be proposed as a valid alternative to traditional pharmacological therapy.

ACKNOWLEDGEMENTS
The Authors would like to thank Elena Tognato for her support and ASA S.r.l. for the help in defining the protocol setting for the laser treatment.

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ALL INQUIRIES SHOULD BE DIRECTED TO: Mequiglaro S. Ambulatorio Veterinario Thermal Physiopet, Via Roma 71, 35036 Montegrotto terme (PD) - Italy, thermalphysiopet@gmail.com, tel. +39 049795278.
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CONCLUSIONS
They should be concise and effective, with reference to possible involvements in the future.

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CONCLUSIONS
They should be concise and effective, with reference to possible involvements in the future.

ACKNOWLEDGEMENTS
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