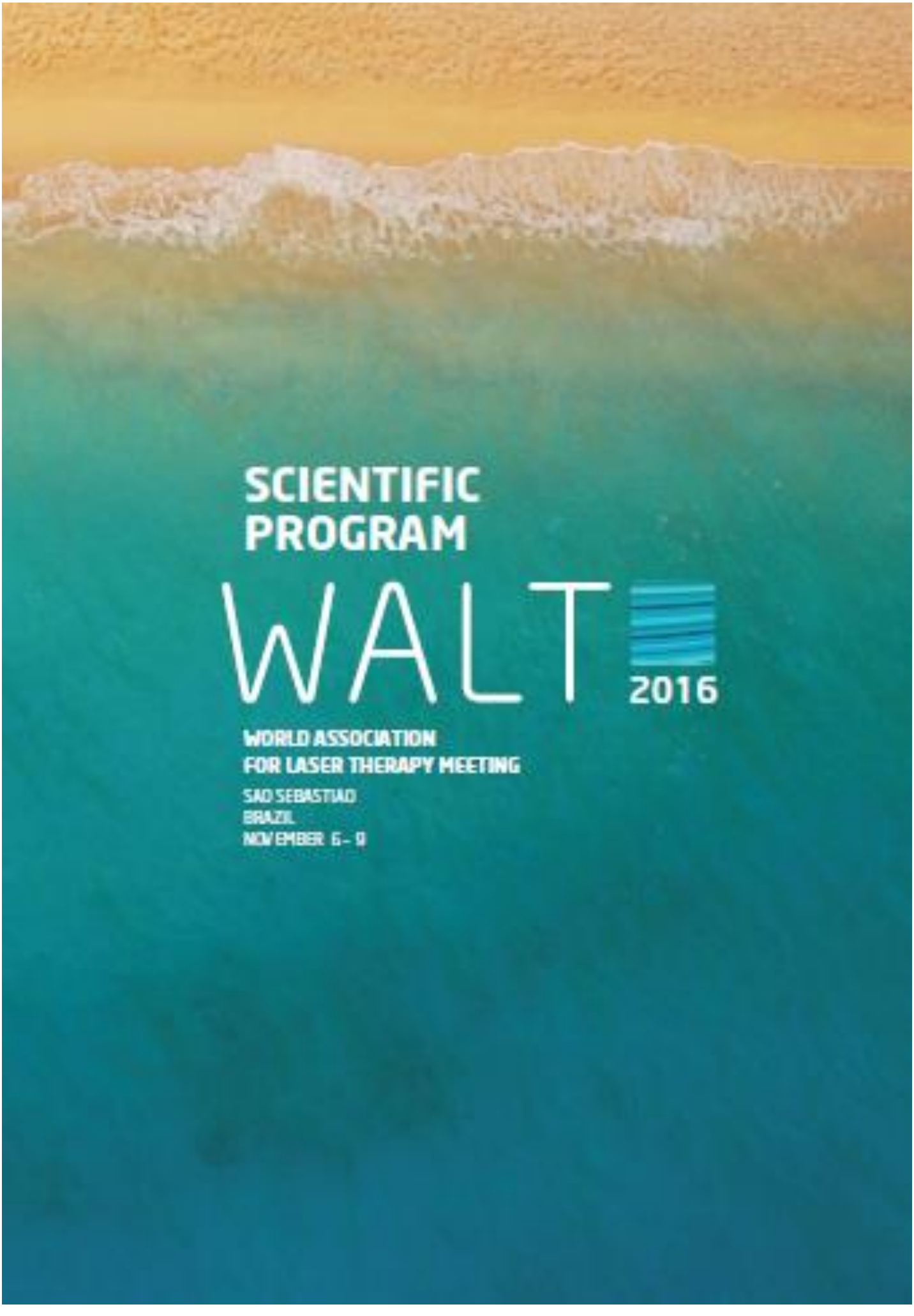

SCIENTIFIC PROGRAM

WALT 2016

WORLD ASSOCIATION FOR LASER THERAPY MEETING

SAO SEBASTIAO
BRAZIL
NOVEMBER 6 - 9

COMUNICAÇÕES ORAIS

CO.01

"Low-level laser therapy reverses orofacial pain and induces tissue healing of rats submitted to an experimental model of temporomandibular disorder induced by CFA"

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Introduction: Temporomandibular Disorders (TMD) is a collective term that embraces a number of clinical conditions that involve the masticatory musculature, temporomandibular joint and associated structures. Such disorders are a major cause of non-dental pain in the orofacial region. Experimental studies indicate that inflammatory process play important role in the development of tissue pathologies associated with this disorders. The therapeutics options to relief and control pain should be conservative and reversible. Therefore LLLT seems to be an appropriate option for the the treatment of TMD due to its photobiostimulatory effects, analgesic and anti-inflammatory action, and regeneration of damaged tissues.
Objectives: The aim of this study was to evaluate the analgesic and anti-inflammatory effects of LLLT in an experimental model of TMD in rats, as well as to evaluate part of the mechanisms involved in such effect.
Methods: TMD experimental model was induced by injection of complete Freund's adjuvante (CFA; 100 µl) in left masseter of rats. Fourty-four male Sprague-Dawley rats (180g-220g; CEUA 20/2014) were divided into four experimental groups: **Group 1:** saline; **Group 2:** CFA; **Group 3:** CFA + LLLT on; **Group 4:** CFA+ LLLT off. Evaluation of mechanical allodynia in the masseter muscle was evaluated by digital von Frey analgesymeter. After baseline measurements, animals were injected with CFA and, after 7 days, received daily applications of LLL (660 nm; 16 J/cm2; 30 mW; 15 seg; spot size: 0,2 $cm^{2.}$) for 14 consecutive days. On 21st day after CFA injection, samples of masseter muscle were removed and histological sections were obtained and stained in hematoxylin-eosin for analysis.
Results: Acute LLLT induced partial reversal of mechanical CFA-induced allodynia at 3h (CFA:46.39±3.27; CFA+LLLT on:56.76±3.18; n=11) and 6h (CFA:44.94±2.74; CFA+LLLT on:60.59±4.01; n=11) after CFA injection. Chronic treatment with LLLT for 14 days completely reversed mechanical allodynia induced by CFA at 7th day (CFA:48.36±2.45; CFA+LLLT on:75.16±3.96; n=11) and 14th day of evaluation(CFA 57.80±6.66; CFA+LLLT on:88.73±4.82; n=6), suggesting that consecutives applications are necessary for induction of analgesia. Histological analysis of rat´s masseter muscle demonstrated that CFA induced an intense inflammatory reaction with leucocytes infiltrate, necrotic areas and wide cellular degeneration that was reversed by LLL were myofibers with central nucleation, were observed, indicating the presence of immature regenerated muscle fibers suggestive of muscle repair.
Conclusion: CFA induced persistent nociceptive response in rats that was reversed by both acute and chronic treatment with LLLT. Besides reversing antinociception, chronic treatment of LLLT, induced healing process reinforcing the idea that LLLT is a potent and non invasive therapy for the treatment of TMD.

CO.02

ANALYSIS OF MECHANISMS OF ACTION OF LIGHT-EMITTING DIODE THERAPY INDUCING ANALGESIA IN ACUTE MODELS OF OVER NOCICEPTION IN MICE.

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Introduction: LED therapy produces the healing tissue injuries, decreased erythema, edema, and accelerates nerve regeneration. About the pain control with LEDT we can found gaps in the literature explained mechanisms. **Objective:** The present study aimed to evaluate the antinociceptive effect of LEDT in models of acute pain, as well as to investigate the possible mechanism adjacent to this effect in mice. Materials and Methods: We used female Swiss mice (n=8), 2 months (25-35 g) submitted to LED (Anodyne® device, wavelength 890nm infrared light with energy density of 20,8 J/cm², 390mW for 20 min) applications, and after the nociceptive response was evaluated by monitoring licking or biting behaviour following the formalin (2,5%, 20 µmol/paw), glutamate (20 µmol/paw), capsaicin (5.2nmol/i.pl., activator of TRPV1 channels), cinnamaldehyde (10 nmol/i.pl., activator of TRPA1 channels), menthol (1.2mmol, i.pl., activador of TRPM8 channels) or acidic saline (pH; 5.0, activator of acid-sensing ion channels, ASIC) administrations. Additionally, LEDT was able to prevent nociception induced by PLC/PKC and cAMP/PKA pathway activators, phorbol 12-myristate 13-acetate (PMA) (an activator of PKC, 50 nmol/paw), bradykinin (BK) (3 nmol/paw), forskolin (FKC) (an activator of AC, 50 nmol/paw) and prostaglandin E2 (PGE2) (3 nmol/paw). Finally, the participation of afferent C-fibers on LED analgesia was investigated by intrathecal injection of mice with capsaicin (10 µg/i.t) on formalin model. All experiments were previously approved by the UFSC's CEUA (PP00745).
Results: The LED significantly ($p < 0.05$) reduced the nociception caused by formalin (C=78.2±4.2, LED=51.4±1.3); glutamate (C=154.7±9.3,LED=103.3±6.8); capsaicin (C=72.1±5.8, LED=43,1±3.5); cinnamaldehyde (C=63.9±4.3, LED=25,9±3,7); menthol (C=228.8±41.1, LED=114.7±22.6); acidic saline (C=166.8±19.8, LED=84.3±16.2) as compared with control group. The LED significantly ($p < 0.05$) reduced the nociception caused by PMA (C=168±13, LED=117±16); BK (C=43.3±3.6, LED=26±6); FKC (C=161±12.4, LED=93.6±16.2); PGE2 (C=107.7±9.7, LED=57.4±10.5). In addition, the antinociception caused by LED was significantly prevented ($p > 0.05$) by pretreatment of mice with injection of capsaicin in formalin model (Des/Cap=57.2±5.6, LED=46±5.6) 1st phase and (Des/Cap=218.7±19.6, LED=179.4±35.5) 2nd phase.
Conclusions: Our results demonstrate, for the first time, that LED promotes important analgesic effect mediated by a reduction of the activation of nociceptors by TRPs agonist (i.e. cinnamaldehyde, capsaicin, menthol and acidified saline) and glutamate. We found as well, the PLC/PKC and/or cAMP/PKA signaling-dependent inhibition. Intrathecal treatment with capsaicin (for desensitization of C fibers) changed the nociceptive response of formalin and the desensitization of antinociceptive effect influenced LEDT.
Key words: LEDT, TRPs, glutamate, PKA, PKC, Afferent C-fibers
Financial support: **CNPq, CAPES, UFSC.**

CO.03

Lasertherapy Inhibits Neuropathic Pain of Diabetic Mice

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Introduction: Diabetic peripheral neuropathy (DPN) is one of the most common complications caused by diabetes mellitus and the development of chronic pain is the most prevalent symptom. Conventional treatments for DPN are still unsatisfactory, leading to the search for new therapies. Low level laser therapy (LLLt) arises as a new alternative target, once it is known to induce analgesic, anti-inflammatory and biomodulators effects leading a significant improvement of disabilities observed on DPN.
Objective: To evaluate the therapeutic potential of LLLt in a model of diabetic neuropathy induced by streptozotocin (STZ) in mice, as well as possible mechanisms involved in its effect.
Methods: 53 Male, C57BL/6 mice (20-26 g; 4 weeks old - CEUA-ICB Protocol n. 22/2014) were used thought this study. Animals received a single injection of STZ (225 mg/kg i.p.) and, after 14 days, mechanical allodynia was confirmed by von Frey filaments (J. Neurosci. Meth. 53:55 1994), applied to the left hind paw of the animals. Animals were then submitted to daily sessions of LLLt on the left paw (660 nm, 16 J/cm2; 30 mW; 15 sec, spot size 0.028 cm^2) for 21 consecutive days, being evaluated for mechanical sensitivity after 14 or 21 sessions of LLLt. Sciatic nerves were removed after 21 days of LLLt for nerve growth factor evaluation (NGF). Behavioral tests were analyzed by two way ANOVA followed by Bonferroni´s post-test; quantifications of NGF were analyzed by nonparametric t test. The significance level considered was $p < 0.05$.
Results: LLL significantly decreased mechanical hyperalgesia 14 after LLLt when compared to control group (**LLL** [n=11]: 0.934±0.191; **STZ** [n=11]: 0.248±0.098; $p<0.01$). Similar results were observed after 21 days of LLL treatment (**LLL** [n=11]: 0.811±0.120; **STZ** [n=12]: 0.261±0.144). Moreover, it was observed an increase on NGF levels in the sciatic nerve of mice treated 21 days with LLLt (**LLL** [n=5]: 0.016±0.0016; **STZ** [n=3]: 0.008 ± 0.0011).
Conclusion: LLLt significantly reverses mechanical hyperalgesia of mice and increases NGF levels on sciatic nerve of animals thus reinforcing the potential role of LLLt on the treatment of pain observed on diabetic neuropathy.
References: Chaplan, S.R., Bach, F. W., Pogrel, J. W., Chung, J.M. and Yaksh, T. L. **Journal of neuroscience methods**. v.53, n. 1, p. 55-63, 1994.

CO.04

LLLT energy in in vitro human tissue

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The absorption and penetration of energy from LLLT irradiation in biological tissue is an issue in photobiostimulation (PBS) of pathologies. The target structure for LLLT intervention in humans is often tendons, muscles and joints. The irradiated energy must penetrate the skin to interact with tissue pathologies.
In LLLT intervention typical wavelengths are λ=600nm-1000nm. Energy absorption in biological tissue decreases towards longer wavelengths in this spectre, as energy penetration is better with longer wavelengths. This is demonstrated in laboratory studies and in mathematical models such as radiation transport theory.
The effect from LLLT irradiation mode is less studied. Laser devised in LLLT operates in different irradiation mode: Continuous, chopped-pulsed or super-pulsed. In one of our studies in rat skin, we demonstrated that irradiation mode influenced lasers penetration capability, in addition to penetration time-profile. Lasers operating in continuous- and chopped-pulse mode have similar energy penetration ability and penetration time-profile. While lasers operating in super-pulsed mode has a different penetration capability and penetration time-profile.
Most studies on LLLT penetration in biological tissue are *in vitro* and *in vivo*. And so far, there are few studies on LLLT penetration on *in vitro* human tissue.
In a recent project, we have looked into the influence of irradiation mode for LLLT devises in *in vitro* human tissue.
In addition to this abstract for oral presentation, a master student will submit an abstract from a master thesis in this project on WALT-2016. The master thesis abstract includes numerical values.
This current presentation will illustrate penetration of energy from different LLLT devices in *in vitro* human tissue. Audio-/Video devices like infrared sensitive camera and Doppler on ultrasonography are used in the presentation, where the time-profiles for different laser devices will be demonstrated.

CO.05

Phototherapy and Therapeutic Ultrasound associated with eccentric exercise in intensity of pain in patients with Achilles and patellar tendinopathies: A Pilot study controlled, randomized and blind.

Santos MYO[1], Ventura MCA[1], Folha RAC[1], Sardim AC[1], Martinez BR[1], Pinfildi CE[1] - [1]Federal University of São Paulo - Department of Human Movement Sciences

Introduction: Tendinopathy is a painful condition that occurs in the tendon, most often due to overload. Achilles and patellar tendinopathy are among the most common of the lower limb. Although there have been advances in relation to the scientific evidence in the treatment of tendinopathy, there is still lack of studies to support the use of resources such as phototherapy and therapeutic ultrasound. **Objective:** Comparing the effect of phototherapy or therapeutic ultrasound associated with eccentric exercise in pain intensity in patients with achilles and patellar tendinopathy. **Methods**: Pilot study, controlled, randomized and blind, approved by the Research Ethics Committee of Unifesp, nº842.209. Fifteen volunteers (15) diagnosed with Achilles and patellar tendinopathies were randomized in 3 groups: EA: Eccentric exercise (EE) and stretching, EAF: EE, stretching and phototherapy and EAUS: EE, stretching and ultrasound. EE were made with 6 series of 15 repetitions, 3 times a week, 8 weeks. Ultrasound parameters: 1 MHz, 7W, 100Hz, 1.0W/cm^2, 50% SATA 0.5 W/cm^2, 4200J, 10 minutes. Phototherapy was performed with a cluster (13 diodes): 3 of 850nm, 7 of 670nm and 3 of 950nm, 265mW and 5J per point. The assessments were performed by the numerical pain scale in static position (palpation of the tendon) and dynamic situation (most painful activity and single hop test). Pretreatment, 4 and 8 weeks and follow-up of 16 and 24 weeks. Statistical analysis: For the statistical analysis to evaluate the behavior of the EA group, EAUS and EAF over the estimated time, according to the variables of interest, will be used the model ANOVA with repeated measures and the method of multiple comparisons Bonferroni. **Results**: Pain on palpation: pretreatment vs 4 weeks: reduction of 3.5 in EAF group, pretreatment vs 8 weeks: reduction of 3.83 in EAF group, 16 vs 24 weeks: decrease 3 in EAUS group. Assessment of pain during the most painful activity pre-treatment vs 4 weeks: decrease 2 in EAF group, pretreatment vs 8 weeks: reduction of 3 in EA and EAUS groups. Pain while performing the single hop test pretreatment vs 4 weeks: reduction of 2.5 in pain in groups EA and EAF, pre-treatment vs 8 weeks: reduction of 2.5 in the EAF and 16 vs 24weeks: reduction of 3 in EAUS group. **Conclusion**: The addition of phototherapy and therapeutic ultrasound to eccentric exercises decreased the pain of patients with Achilles and patelar tendinopathies in different periods of pain assessments.

Financial Support: Own Founding – Federal University of São Paulo

CO.06

Effects of photobiomodulation therapy over acute pain and inflammation on patients submitted to total hip arthroplasty

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Context: Musculoskeletal and joint deficiencies are among the more prevalent and symptomatic problems concerning the health of elderly people. The osteoarthritis (OA) is a chronic degenerative joint disease characterized by the erosion of articular cartilage, resulting in joint deformity and progressive loss of function. When the conservative therapy fails, the total hip arthroplasty (TKA) is indicated. The surgical trauma induces pain and immune response. The scientific literature has demonstrated the efficacy of phototherapy on tissue regeneration, modulation of inflammation and pain relief and can be used as part of the treatment.
Objective: The aim of this study is to analyze the effect of photobiomodulation on pain and inflammation in patients submitted to total hip arthroplasty.
Methods: The project was approved for ethical commission of Universidade Nove De Julho (UNINOVE) n. 066490. The study analyzed 18 patients, from both genders, aging 67±6.4 (placebo) and 69±5.6 (treated), between 8 to 12 hours after been submited to total hip arthroplasty (TKA). The participants were divided in 2 groups (9 placebos and 9 submitted to the photobiomodulation treatment). This patients were treated with 9 kinds of diodes: One superpulsed laser diodes with an average power of 905nm, four LEDs diode of 875 nm and four LEDs diode of 640 nm (spot 0,9cm2), operating on 1000 Hz, during 300 seconds of irradiation, delivering a dose of 39.3 Joules, with a 4 cm² diameter device. Those patients were analyzed before and after the photobiomodulation treatment, through analog visual pain scale and blood sample collect to observe TNF-α, IL-6 e IL-8 cytokines.
Results: After irradiation, we observed different values of analog visual pain scale between placebo and treated groups, while the firs presented 4.33±1.58, the second showed 4.44±1.51 P=0.03. Considering seric analysis of TNF-α, we observed 530.54±22.95 on placebo versus 451.58±26.92pg/mL, P=.0001 on treated group. On IL-8, we observed 190.67±12.08 pg/mL versus 182.25±15.66 pg/mL, P=0.04. Considering this results we can verify a significant statistic difference on those parameters, though the same was not observed on IL-6.
Conclusions: The photobiomodulation treatment showed efficacy in the pain relief as well as in the serum levels of TNF-α and IL-8 on postoperative patients submitted to total hip arthroplasty.

Keywords: Photobiomodulation therapy, low level laser therapy and total hip arthroplasty

CO.07

Evaluation of wound healing membranes with stem cells for photbiobiomodulation

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The photobiomodulation using low-energy laser for wound healing has positive effects including stimulated mitotic activity, fibroblast proliferation, and induced angiogenesis, while a wound dressing material serves as a physical barrier not only to absorb wound exudates, but also to protect against bacterial invasion during the repair process. In this study, stem cells have been incorporated onto a wound healing membrane to improve the wound healing properties during phototherapy. We hypothesized that the stem cell laden membrane would improve the fibroblast proliferation when treated with irradiation.

Materials and Methods: In this study, we fabricated wound healing membranes with different stem cells to evaluate their ability to repair wounds in combination with photobiomodulation using low level laser therapy (LLLT). The prepared membranes were characterized by biological assays and mechanical testers. Fibroblast proliferation and migration studies were performed under the laser-activated stem cell-laden wound healing membranes. For example, fibroblast cells covered by a the stem-cell laden membrane were irradiated with an InGaAsP diode laser prototype (780 ± 3 nm; 40 mW) with energy doses of 0.5, 1.5, 3, 5, and 7 J/cm^2. Cell number and metabolism activity were evaluated both by MTT and trypan blue assay. Cell migration test was performed using a standard boyden chamber setup.

Expected Results: The on-going study is expected to end at September 2016. The results might be presented in the WALT conference 2016.

Conclusions: This study indicates that a wound healing membrane with enhanced light penetration would be a promising approach to increase the usage of phototherapy for wound healing.

Financial support: This research was supported by KHIDI funded by the Ministry of Health & Welfare, Republic of Korea (HI16C0322)

Keywords: Photobiomodulation, Stem cell, Laser therapy, Woundhealing, Biomembrane

CO.09

EFFECTS OF LOW-LEVEL LASER THERAPY (808 NM) IN NEUROPATHIC PAIN CONTROL

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Introduction: Neuropathic pain was recently redefined by International Association for Study of Pain (IASP) as a "pain caused by a lesion or disease of somatosensory system", which can be classified according to its intrinsic cause or by the local of the nervous lesion – central or peripheral (Pain 152; 2204-2205, 2011). This pain's physiopathology is not yet completely clarified (Molecular Brain 4; 31, 2011). Thus, in an attempt to better comprehend its mechanisms, a great variety of studies with experimental models have been performed, as the sciatic nerve chronic constriction (CCI) surgery (Pain 33; 87-107, 1988). Physical therapy is pointed as an efficient treatment alternative and some of its resources can be highlighted, as low-level laser therapy (LLLT). However, its application parameters, particularly energy density, remain controversial (Lancet 374; 1897-1908, 2009). **Objectives:** We aim to investigate LLLT effect, in different energy densities, in controlling neuropathic pain in mice. **Methodology:** This study was approved by Ethics Committee in Animal Experimentation of Federal University of São Carlos (UFSCar - 026/2014). 50 male Swiss mice, weighing 25-30 grams, were used. Neuropathic experimental model was obtained by CCI surgery. Animals were randomly distributed in 5 groups: sham group (GS) – simulation of CCI surgery; placebo group (GP) – neuropathic pain induced by CCI surgery, with simulation of LLLT application; group with laser therapy 10 J/cm² (GL10) – neuropathic pain induced by CCI surgery alongside application of LLLT, in a energy density of 10 J/cm²; group with laser therapy 20 J/cm² (GL20) – neuropathic pain induced by CCI surgery alongside application of LLLT, in a energy density of 20 J/cm²; group with laser therapy 40 J/cm² (GL40) – neuropathic pain induced by CCI surgery alongside application of LLLT, in a energy density of 40 J/cm². The treatment of neuropathic pain by LLLT (808 nm) was performed three times per week, for 90 days. Nociceptive evaluations occurred every 15 days, in which it was used Hot Plate Test – for thermic hyperalgesia – and Randall and Selitto test for mechanic hyperalgesia. In order to quantify β-endorphin, we used ELISA. **Results:** In Hot Plate Test, we observed a significant reduction of pain in groups GL20 (15.6 s ± 2.5 s) and GL40 (14.6 s ± 2.8 s), starting at day 30 of treatment, if compared to GP (10.1 s ± 2 s); for GL10 (13 s ± 2.6 s), the difference started at day 75 of treatment. In Randall and Selitto test, we observed a reduced pain starting at day 45 of treatment for GL20 (157.7 g ± 41 g) and GL40 (151.6 g ± 40 g) if compared to GP (M:106.6 g SD: ± 21 g). GP did not show any improvement in pain until the end of the experiment in both evaluations. ELISA showed a significant increase of β-endorphin in groups GL20 (M: 0.2807 pg/mL; SD: 0.003pg/mL) and GL40 (M: 0.2815 pg/mL; SD: 0.004pg/mL) compared to GP (M: 0.2759 pg/mL; SD: 0.002 pg/mL), which agrees to functional evaluations. **Conclusion:** According to presented data, it is possible to conclude LLLT, 808 nm, acts in a positive way to reduce and control neuropathic pain, highlighting higher energy densities, as 20 J/cm² and 40 J/cm², are more efficient, also, they stimulate a higher β-endorphin production.

Financial Support: We would like to acknowledge the contributions of Brazilian funding agency CNPQ for the financial support of thisresearch.

CO.10

Low-level laser therapy associated with a resistive aquatic exercise in a model of knee osteoarthritis.

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Osteoarthritis (OA) is a chronic joint disease characterized by progressive degeneration of the extracellular matrix of articular cartilage, subchondral bone remodeling and inflammation of periarticular tissues. This process is directly associated to functional disability in elderly population. Many authors have been demonstrated that aquatic exercise and low-level laser therapy (LLLT) have positive effect on clinical rehabilitation of OA, however little is known about its effects on inflammatory and degenerative process of the cartilage tissue. Thus, the aim of this study was to evaluate the effects of a progressive exercise training and low-level laser therapy (LLLT) (associated or not) on degenerative modifications and inflammatory mediators on the articular cartilage using an experimental model of knee OA. This study was approved by the Ethical Committee of the Federal University of São Paulo (2013/814715).
Thirty male Wistar rats (weighing ±150 g, 8 weeks old) were randomly divided into 3 groups: knee OA – without treatment (OA); OA plus exercise training group (OAE); OA plus exercise training associated with LLLT (OAEL). Trained rats performed a water-jumping program carrying a load equivalent to 50-80 % of their body mass strapped to their chest. The laser irradiation (808 nm; 50 J/cm^2, 50 mW, spot size 0.028 cm^2, 28 sec) was used after the exercise training had been performed, at 2 points contact mode (medial and lateral side of the left joint). The treatments started 4 weeks after the surgery, 3 days/week for 8 weeks. The results revealed that all treated groups (irradiated or not) exhibited a better pattern of tissue organization, with less fibrillation and irregularities along the articular surface and improved chondrocytes organization. Also, a structural damage (OARSI score) and higher thickness values were observed in both treated groups ($p < 0.0001$). Additionally, OAE ($p = 0.0435$) and OAEL ($p = 0.0435$) showed a reduced expression in caspase-3 compared to OA. Furthermore, a statistically lower MMP-13 ($p = 0.0218$) and IL-1 β ($p = 0.0239$) expression were only observed in OAEL. These results suggest that aquatic progressive exercise training and LLLT were effective in preventing cartilage degeneration. Also, physical exercise program presented anti-inflammatory effects in the knees of rats after TCLA.
Financial Support: Fundação de Amparo à pesquisa do Estado de São Paulo - FAPESP grant number: FAPESP - 2014/13704-9

CO.11

SIX MONTHS FOLLOW-UP OF LOW POWER LASER THERAPY ASSOCIATED TO EXERCISES IN KNEE OSTEOARTHRITIS: A RANDOMIZED, DOUBLE-BLIND STUDY

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Introduction: Knee osteoarthritis (OA) is the most common presentation of OA, with an estimated prevalence between 12% and 35% in the general population and is considered the leading cause of musculoskeletal disability in the elderly population worldwide. The report by World Health Organization on the global burden of disease indicates that knee OA is likely to become the fourth most important global cause of disability in women and eight most important cause in men. Although commonly seen as a progressive and chronic disorder, the early therapeutic approach can minimize its symptoms.
Objectives: To asses the long-term effects of Low Power Laser Therapy (LPLT) therapy, in combination with a program of exercises on pain, functionality, range of motion, muscular strength and quality of life in patients with osteoarthritis (OA) of the knee.
Methods: All procedures were performed following approval by the Ethics Committee for Research-Project Analysis CAPPesq Hospital Clinic Board of Clinical and Medical School of the University of São Paulo (Process: 0775/08) and National Commission of Ethics in Research- CONEP (Opinion: 838/2009). The clinical trial is registered in www.clinicaltrials.gov- Protocol Registration System under the number: CT01306435. Forty participants with knee OA, 2-4 OA degree according to Kellgren–Lawrence grade, aged between 50 and 75 years and both genders, have knee pain and functional disability for at least three months, and according to the criteria of the American College for Rheumatology. Participants were randomized into one of two groups: Laser Group (Low Power Laser Therapy, dose of 3 Joules and exercises), or Placebo Group (placebo-laser and exercises). The patients were followed up and assessed again six months after entering the study (Clin Rehabil 26. 6. 2012), reapplying the same measures: Pain was assessed using visual analogical scale (VAS), Functionality using the Lequesne questionnaire, Range of motion with the universal goniometer, Muscular strength using a dynamometer, and Activity using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) questionnaire. Data normality was assessed using the Shapiro– Wilk test; homogeneity of data was estimated using the Levene's test. For intergroup analysis were performed using the independent t-test. For intragroup analysis, evaluation times were compared by repeated-measures ANOVA (single effect), followed by the Tukey post-test. Analyses were conducted using the Statistical Package for Social Sciences (SPSS version 17; SPSS Inc., Chicago, IL, USA). An alpha level of 0.05 was set for all comparisons.

Results: In intragroup analysis, improvement in pain (2.53±2.16), range of motion (98.32±15.17), functionality (9.36±5.12), mobility (20.84±14.84) and strong (15.87±6.31) were maintained in three and six months follow-up ($p<0.001$) in group Laser. No significant improvement was seen in the placebo group ($p>0.005$). In intergroup analysis, improvement in pain was significant in the laser group ($p<0.001$).
Conclusion: Our findings suggest that low potency laser therapy when associated with exercises offers promising short- and long-term results in management of patients who have OA of the knees.
Financial Support: Foundation of Research Support of São Paulo State (FAPESP) and Coordination for the Improvement of Higher Level – or Education – Personnel (CAPES).

CO.12

Low Level Laser Therapy and Cryotherapy as Mono- and Adjunctive Therapies for Achilles Tendinopathy in Rats

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Background and Objective: Low-level laser therapy (LLLT) and cryotherapy are widely used treatments in the acute phase of tendon injury. The aim of this study was to investigate the interaction of these two treatments on tendon inflammation and mechanical properties. **Material and methods:** Six groups of six Wistar rats were used in this study. The Achilles tendons of the healthy control group were not subjected to injury or treatment. The tendons of the injured non-treated group were injured, but not treated. The remaining four groups were injured and subjected to LLLT, cryotherapy, LLLT first/cryotherapy, or cryotherapy first/LLLT. All treatments were performed at one hour post-trauma. Inflammatory mediators, tendon histology, and biomechanical properties were assessed at 24 hours post-trauma by comparing the treatment groups to the injured non-treated group. The experimental protocol was submitted and approved by the University of São Paulo Animal Research and Care Committee (No 144/78-2). **Results:** In all treatment groups, the inflammatory process shifted in an anti- inflammatory direction compared to the injured non-treated group. Significant alterations in cytokine expression were only found in the LLLT group (↓IL-1β) and the combined intervention groups (↓IL-1β, ↓TNF-α, ↑IL-6). It was also found that cryotherapy followed by LLLT was the only treatment that significantly ($p<0.05$) improved the biomechanical parameters of force (N) and displacement (mm) at the tendon rupture and corresponded with the best histological scores of all of the treatment groups. **Conclusion:** Our results demonstrate that cryotherapy in combination with LLLT can produce an anti-inflammatory "add-on" effect. The order of therapy administration seems essential, as superior histology and biomechanical results were found in the cryotherapy first/LLLT group.

CO.13

Effects of Low power lasers on in vitro survival and morphology of bacteria from pressure ulcers

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Introduction: In developing countries, chronic ulcers are estimated in 1–2 % of the population. A North American estimate reports that the cost of treating only one chronic ulcer is approximately US$8000 per year, increasing to US$17,000 in infected ulcers. Low power laser therapy (LPLT) has been used to healing process, but the literature is not clear on the use of LPLT on infected pressure ulcers.
Objective: The aim of this work was to evaluate the effects of red and infrared low power lasers on survival and morphology of *Pantoea Agglomerans* cells.
Methods : *P. Agglomerans* cultures were exposed to low power red (660 nm; 100 mW; 1, 2 and 4 J), infrared (830 nm; 100 mW; 1, 2 and 4J) and dichromatic (660 nm and 830 nm; 100 mW, 2 and 4J) lasers in stationary and exponential growth phases to evaluate bacterial survival and cell morphology. Experiments were carried out in triplicate and the results are presented as means and standard deviation of five independent assays. For statistical analysis, data normality was verified by Shapiro-Wilk test, Kruskal-Wallis test was performed to determine possible statistical differences, followed by post hoc Dunn's tests, with $p<0.05$ as the less significant level.
Results: Data from bacterial survival in exponential phase were: 1.0±0.18 (control), 1.1±0.20 (1J, red), 0.9±0.15 (2J, red), 1.4±0.18 (4J, red), 0.9±0.17 (1J, infrared), 0.9±0.19 (2J, infrared), 1.4±0.31 (4J, infrared), 1.2±0.21 (2J, red+infrared) and 0.8±0.26 (4J, red+infrared). In stationary phase: 1.0±0.22 (control), 1.0±0.39 (1J, red), 1.1±0.31 (2J, red), 1.1±0.38 (4J, red), 1.3±0.46 (1J, infrared), 1.1±0.42 (2J, infrared), 1.0±0.40 (4J, infrared), 1.0±0.41 (2J, red+infrared) and 1.1±0.46 (4J, red+infrared). For cell areas in exponential phase were: 6.6±0.56 (control), 7.2±0.54 (1J, red), 7.0±0.21 (2J, red), 6.7±0.65 (4J, red), 6.9±0.60 (1J, infrared), 6.1±0.54(2J, infrared), 7.5±0.14 (4J, infrared), 6.4±0.25 (2J, red+infrared) and 9.8±2.38 (4J, red+infrared). In stationary phase: 6.8±0.05 (control), 5.6±0.57 (1J, red), 5.5±0.48 (2J, red), 6.7±0.65 (4J, red), 6.1±0.30 (1J, infrared), 6.5±0.36(2J, infrared), 6.9±0.56 (4J, infrared), 6.9±0.64 (2J, red+infrared) and 6.2±0.51 (4J, red+infrared).

Conclusion: Data suggest that dichromatic red and infrared low power lasers at 4J decreased bacterial survival, while monochromatic red and infrared lasers at 4J increased bacterial survival in exponential phase. No significant alteration on cell areas was observed in *Pantoea Agglomerans* exposed to lasers in both exponential and stationary growth phases.
Financial Support: CNPq, CAPES, FAPERJ.

CO.14

Effect of low-level laser therapy in biomodulation and genes expression VEGF and IL6 on fibroblast cells (L929)

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Introduction: The low-level laser therapy is a resource frequently used in clinical routine, it presents benefits during the process of tissue repair in injuries.
Objective: Analyze the effect of low-level laser irradiation, with wavelenght of 660nm (AlGaInP), at two energy densities 1 and 5J/cm² on the cells viability and gene expression of vascular endothelial growth (VEGF) and interleukin (IL6) on mouse fibroblast cells.
Methods: This study receives the approval from the ethic committee of Universidade Norte do Paraná (UNOPAR), protocol n° 462.478/2013. A gallium arsenide (GaAs) diode laser (Endophoton - KLD®, Biossistemas Equipamentos Eletrônicos Ltda, São Paulo, modelo LLTO 0107, Brasil) with wavelenght of 660nm, power of 35mW was used for the irradiation of fibroblast cells L929.
Three groups were established, G1: control group (non-irradiated), G2: irradiated at 1J/cm², and G3: irradiated at 5J/cm², the cell cultures were cultivated in a well with 0,3cm² of cross section receiving radiation perpendicularly to the plate at 24-h, 48-h, and 72-h intervals, with laser radiation λ 660 nm. Twenty four hours after each irradiation the cells proliferation were analyzed by the MTT method [3-(4,5-dimetthylthiazol-2-yl)2,5-diphenyltetrazolium bromide], it was selected the best dose and interval time to realize the fluorescence microscopy and gene expression. The experiments were carried out in triplicate. For the fluorescence microscopy it was used fluorescent dyes Rhodamine-phalloidin (cytoskeleton), $DIOC_6$ (endoplasmic reticulum) and DAPI (nucleus), to analyse the gene expression it was performed the qRT-PCR method.
Results: On the analysis of cells proliferation, it was observed that G1 (control group), presents a growth medium of 107.1% (± 0.0) at 24h time, 108.0% (±1.0) at 48h and 106.0 (± 1.0) at 72h, G2 (1 J/cm²) the growth was 108.0% (± 3.4) at 24h, 99.3% (± 7.2) at 48h and 111.3% (± 13.0) at 72h, and on G3 (5 J/cm²) the growth was 114.3% (± 5.5) at 24h, 122.3% (± 6.71) at 48h and 125.3% (± 7.1) at 72h.The analysis were made by the Two-way ANOVA test ($F = 12.55$; $P = 0.16$), and further the Tukey HSD post hoc test where it was found statiscally significant difference on G3 (5 J/cm²) dose that promoted a increase in cells proliferation, when compared with the G2 (1J/cm²) dose at 48h time ($p<0,01$), and still when compared with the G1 control group ($p=0,03$) at 72h time. At the time of 24h, it wasn't observed a statistically significant difference ($p >0,05$).Comparing the numbers of viables cells with MTT test, of the groups G1, G2 and G3 related with the irradiation time (24,48,72h) it wasn't observed a statiscally significant difference ($p= 0,16$). On G3 (5 J/cm²) the fluorescence microscopy, showed an intense reticular activity and biomodulatory effect on the cytoskeleton at the time of 48h and 72h when compared with G1(control group). With the analysis of the gene expression it was possible to identify a statistically significant difference at 72h time of the G3 (5 J/ cm²) when compared with G1 with an increase of 1,98 times ($p<0,05$) on the transcripts of the genes VEGF and a decrease of 4,05 times ($p<0,05$) on the transcripts of the gene IL-6.
Conclusion: Low-level laser therapy stimulates proliferation and metabolism cells as much as gene expression VEGF and inhibits the gene expression of IL6 on cultured mouse fibroblast cells (L929).
Financing sources: CNPQ e FUNADESP.

CO.15

Effects of Low Level Laser Therapy on the prognosis of Split Thickness Skin Graft in Type 3 Burn of Diabetic Patients: A case series

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Abstract:

Objective: Significant populations in burn centers are diabetic burn patients. Healing process in these patients is complicated. Split-thickness skin grafting (STSG) is widely used to treat burn ulcers but in diabetic patient due to impaired tissue perfusion some of this grafts fails and rate of amputation is high. The technique of Low level laser therapy improves tissue perfusion and wound healing. The purpose of this case report is introducing a new therapeutic method for accelerating healing with better prognosis in these patients.

Materials and Methods: Diabetic type 2 patients with grade 3 burn ulcers, candidate for amputation, were enrolled to the study. We used Low Level Laser Therapy (LLLT) red light, 1 J/Cm^2 for bed of the ulcer, 6 J/Cm^2 for the margins along with intravenous laser therapy, before and after Split Thickness Skin Graft (STSG) for treating grade 3 burn ulcers in 13 diabetic ulcers.

Result: All 13 ulcers had complete healing in at last 8 weeks.

Conclusion: In this case series, we present 13 cases of diabetic ulcer with type 3 burn wound, that were healed completely using Low Level Laser Therapy (LLLT) and Split Thickness Skin Graft. This is the first time that these two techniques are combined for treatment of burn ulcer in diabetic patients. Using LLLT with STSG might be a promising treatment for burn victim especially diabetic patients.

CO.16

Effect of laser 904 nm on celular proliferation and gene expression (FA E BMP2) of osteoblastic cells (OFCOL II)

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INTRODUCTION: The low-level laser therapy has been the focus of most of the studies in the process of bone regeneration, and it has been used in this case with the purpose of improvement in the bone mineral density due to the biostimulation effect, allowing an increase in cells proliferation and reducing the repair time in fractures.
OBJECTIVE: Analyse the effect of low-level laser therapy (ƛ=904nm) with different energy densities on osteoblastic cells, evaluating cells proliferation and the relative expression of genes alkaline phosphatase (ALP) and bone morphogenetic protein 2 (BMP2).
METHODS: This study receive the approval from ethic committee of Universidade Norte do Paraná (UNOPAR), protocol n°478/2013. A gallium arsenide (GaAs) diode laser (Endophoton - KLD, Biossistemas Equipamentos Eletrônicos Ltda, São Paulo, modelo LLTO 0107, Brasil) with wavelength of = 904nm, in pulsed mode, with repetition rate of 10 KHz, and power of 50mW, was used for the irradiation of osteoblastic cells sub cultured in 96 well cell culture plates TPP (Switzerland), in a density of $1x10^4$ células/mL. After the plating, the cells stay 24 hours at over night. Four groups were established,Group 1 : non-irradiated (control), Group 2: irradiated with 3J/cm² (18 seconds), Group 3: irradiated with 4J/cm² (25 seconds) and Group 4: irradiated with 5J/cm² (32 seconds). The cell cultures were cultivated in a well with 0.3 cm2 of cross section receiving radiation perpendicularly to the plate at 24-h, 48-h, and 72-h intervals. Control cells (non-irradiated) were submitted to the same condition as the laser-irradiated cells. Twenty four hours after each irradiation the cells proliferation were evaluated with the MTT method [3-(4,5-dimetthylthiazol-2-yl)2,5-diphenyltetrazolium bromide], and the relative expression of the genes ALP and BMP2, with PCR at real time (RT-qPCR), using TaqMan especific for each gene.
RESULTS: The mediated effects on irradiated cells with the laser in different doses was determinated with the aid of the MTT test. It was done the ANOVA test of 2 factors with statistical significance (F=28.62; P<0,001), and further confirmed by the Tukey test. Group 4, irradiated with 5J/cm² presents the better results at 24h time (p< 0.005) and 48 h (p <0.001), when compared to the control group, (0.011) and in the irradiated groups, the group 5J/cm² presents significant statistical at 48h time (p<0.001). In the gene expression, it was a significant difference, were irradiated cells with 5J/cm² presents an increase of 1,7 (p>0,05) the transcripts of the genes BMP2 and a decrease of 5,08 (p>0,05) in transcripts of the gene ALP when compared with the endogenous β-actina gene at the 48h time characterizing the beginning of cells proliferation.
CONCLUSION: The laser diode ƛ = 904nm, promoted a significant response on osteoblastic cells proliferation OFCOL II, highlighting the 5J/cm² doses at 48h, modulating genes ALP and BMP2, important in proliferation and cellular differentiation , estimulating than the bone remodelation process

CO.17

LOW-LEVEL INFRARED LASER MODULATES MUSCLE REPAIR AND CHROMOSOME STABILIZATION GENES IN MYOBLASTS

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Introduction: Infrared laser therapy is used to skeletal muscle repair based on biostimulatory effect on satellite cells. However, shortening of telomere length limits regenerative potential satellite cells, which occurs after each cell division cycle. Also, laser therapy could be more effective on non physiologic tissues.
Objective: This study evaluated effects of low-level infrared laser exposure on mRNA expression from muscle injury repair and telomere stabilization genes in myoblasts in normal and stressful conditions.
Methods: Laser fluences were those used in clinical protocols. C2C12 myoblast cultures were exposed to low level infrared laser (10, 35 and 70J/cm^2) in standard or normal (10%) and reduced (2%) fetal bovine serum concentrations, total RNA was extract for evaluation of mRNA expression from muscle injury repair (MyoD and Pax7) and chromosome stabilization (TRF1 and TRF2) genes by real time quantitative polymerization chain reaction. TRF1 transcript was verified by agarose gel eletrophoresis after the second amplification by RT-qPCR. Data were reported as mean ± standard deviation. The one-way analysis of variance (ANOVA) test was performed to verify possible statistical differences followed by Tukey post-test. Kolmogorov-Smirnov test was performed to verify normality distribution of the data. $p<0.05$ was considered as the less significant level.
Results: Data show that mRNA MyoD expression was significantly increased ($p<0.05$) after laser exposure at the lower fluence evaluated (10J/cm^2) in C2C12 cells in 10% FBS (47.49±18.04) and in 2% FBS (80.38±35.12) when compared to their respective non-irradiated controls (2.91±1.19). Similar to MyoD mRNA, Pax7 mRNA expression was significantly increased ($p<0.05$) after laser exposure at the lower laser fluence in C2C12 cells in 10% FBS (42.26±24.38) and 2% FBS (1055±499.3) when compared to their respective non-irradiated controls (1.07±0.28). TRF2 mRNA expression was significantly increased ($p<0.05$) after laser exposure at the higher fluence (70J/cm^2) in C2C12 cells in 10% FBS (45.52±9.07) and 2% FBS (101.2±48.99) when compared to their respective non-irradiated controls (1.77±0.56). Agarose gel electrophoresis indicated that laser exposure induces expression of mRNA from TRF1 gene in C2C12 cells in both 10% and 2 % FBS at all fluences evaluated.
Conclusion: Low-level infrared laser increases mRNA expression from genes related to muscle repair and telomere stabilization in myoblasts in standard or normal and stressful conditions.
Financial Support: Faperj, Capes and CNPq.

CO.18

Effects of photobiomodulation therapy and diclofenac on functional aspects in an experimental model of skeletal muscle trauma in diabetic rats.

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Introduction: Diabetes mellitus (DM) is associated with delay in tissue repair, closure and contraction of the injury. Aerobics and resistance exercise has been prescribed for the prevention and treatment of patients with DM due to the improvement in glucose control and reduction of other risk factors. Traumatic muscle injuries are directly related to physical activity, which may cause morphological and functional changes on muscle regeneration. Pharmacological and non-pharmacological approaches have been used in the treatment and recovery of musculoskeletal injuries, such as anti-inflammatory drugs and low-level laser therapy (LLLT). However the process of muscle regeneration in a diabetic organism remains unknown. Objective: The aim of this work was to investigate the effects of LLLT on the functional recovery and morphometrical aspects on skeletal muscle trauma induced in diabetic wistar rats, comparing LLLT, diclofenac and both treatments applied together. Methods: This study was approved by the Ethics Committee in the use of animals of Sacred Heart University (nº 34/13). Male Wistar rats were randomized in 6 groups (n=7). Induction of diabetes was performed by intraperitoneal adinistration of streptozotocin (STZ) at concentration of 50 mg/kg body weight. A single trauma was performed employing a mini guillotine, comprising a block weight of 200g that dropped from 20cm on the right posterior limb. Injured groups received treatment 1 hour after injury protocol, diclofenac topically (11.6 mg/g^{-1}), irradiated with LLLT (3J, 810nm, 100mW, 30s), or both treatments applied together. For morphometric analyses were measured 220 muscle fibers per animal on the injury site. The morphometric variables were: area and minimum diameter of the muscle fiber. For functional gait assessment was used the sciatic functional index, where the animals walked by a transparent acrylic hall in which the footprints on the floor were filmed and used for functional analysis. Both analyzes were performed 06, 12 and 24 hours after injury protocol. Results: In the morphometric analysis the treated groups had no difference between them however, all treated groups showed statistically significant difference when compared to the untreated group after 48 hours. Six hours after injury protocol, it was observed that the sciatic functional index showed statistically significant increase in all injured groups when compared to sham group (-6.38±0.78) and diabetic group (-6.52±0.49) with $p<0.01$. Twelve hours after injury protocol, injury+laser group (-10.32±0.80) and injury+laser+diclofenac group (-9.25±0.54) showed less difficulty on functional gait assessment, compared with injury group (-11.48±0.49) and injury+diclofenac group (-12.10±1.10) at $p<0.05$ and $p<0.01$, respectively. Twenty-four hours after the injury protocol, injury+laser+diclofenac group (-8.36±0.49) showed less difficulty on functional gait assessment, compared to other treatment groups and injury group (-12.64±0.62) $p<0.01$. Conclusion: LLLT associated with diclofenac significantly improved functional gait assessment in diabetic animals 12 and 24 hours after muscle injury protocol.

Financial Support: FAPESP

CO.19

Effects of photobiomodulation therapy with combination of super-pulsed lasers and light emitting diodes (LEDs) in experimental model of Duchenne Muscular Dystrophy

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Introduction: Duchenne muscular dystrophy is a lethal disease caused by absence of dystrophin affecting one in every 3500 males. The pre-clinical model widely used to study this disease is the mdx mice. These animals have defficit of protein expression of dystrophin in their organism.Photobiomodulation therapy (PBMT) has been used as a protective therapy to muscular tissue.
Objective: This study aimed to analyze if previously protective effects of photobiomodulation therapy (PBMT) on skeletal muscle tissue could delay dystrophy progression in *mdx* mice.
Methods: For such, mice were randomly divided into 5 different experimental groups: Wild Type (WT), Placebo-control (*mdx* mice), PBMT with doses of 1J (*mdx* mice), 3J (*mdx* mice) and 10J (*mdx* mice). PBMT was performed employing a cluster probe with 12 diodes (4 laser diodes of 905nm, 4 LED diodes of 875nm, and 4 LED diodes of 640nm - manufactured by Multi Radiance Medical™) 3 times a week for 14 weeks. All treatments started with animals at 6 weeks of age. PBMT was applied with direct contact at skin on animals' hindlimbs in a single point (tibialis anterior muscle – bilaterally). It was analyzed muscle morphology, gene and protein expression of dystrophin, and functional performance. Oneway ANOVA test followed by the Bonferroni post-hoc test were performed to verify statistical significance.
Results: PBMT with 10J dose significantly improved ($p<0.001$) functional performance compared to all other experimental groups. Muscle morphology was improved by all PBMT doses, with better outcomes in favor of 3J dose. Gene and protein expression of dystrophin were significantly increased with 3J ($p<0.001$) and 10J ($p<0.01$) doses compared to placebo-control and 1J groups.
Conclusion: We conclude that PBMT mainly can preserve muscle morphology and improve muscular function of *mdx* mice through modulation of gene and protein expression of dystrophin. Furthermore, since PBMT is a non-pharmacological treatment that doesn't present side-effects and it's easy handling, PBMT raises as a promising tool in treatment of Duchenne's Muscular Dystrophy.

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CO.20

Analysis the Effects of Autologous Fibroblast Transplantation alone and Combined with low level laser in Diabetic foot wound healing and comparison of VEGF, FGF, PDGF gene expression in these two methods.

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Back ground: Diabetes Mellitus type 2 is a common disease affecting a wide range of community. Diabetic foot ulcers, being notoriously difficult to cure, are one of the most common health problems in these patients. Low level lasers have been suggested as a promising treatment option for diabetic patients although exact mechanisms are not clearly understood. Aim of this study is to analysis the effects of autologous fibroblast transplantation alone and combined with low level laser in diabetic wound healing and comparison of VEGF, FGF, and PDGF gene expression in these two methods **Materials and methods**: In this study, 7 diabetic and 7 non diabetic male Bulb C mice were used. After extraction the fibroblasts from skin, cells were cultured in 12- well plates with and without gelatin-chitosan scaffold and 6 wells of each plate were laser irradiated and 6 wells were not as control group. After RNA extraction and cDNA synthesis, cells of each mouse containing 4 groups (1-without laser, without scaffold, 2- with laser without scaffold, 3- without laser with scaffold and 4- with laser with scaffold were run in one PCR.
Results: In animal study, after RT PCR on the extracted fibroblasts from skin of diabetic and non diabetic mice, the results showed that expression of *Fgf* increased in both groups. This increase was significant in diabetic mice (P=0.017). Expression of *Egf, Pdgf were* also increased in both groups but it was not statistically significant. Expression of *Vegf* in this study decreased in both groups both it was not statically significant. Using scaffold without laser increased expression of *Vegf* and *Pdgf* in diabetic mice (Vefg p value=0.029 and Pdgf p value= 0.028). Increase of expression of Fgf was very near to significant (0.072).
Discussion: This study showed that using infra red 810 nm laser, 1 J/cm^2 on fibroblasts cultured on Gelatin-Chitosan scaffold can increase the expression of *Fgf, Egf, and Pdgf* and decrease the expression of *Vegf.* Using scaffold alone also increases the expression of *Vefg* and *Pdgf* in diabetic mice. This technique may be useful in treatment of different ulcer including bed sores, Burns and diabetic ulcers.
Key words: Low Level laser Therapy, Scaffold, Wound Healing

CO.21

LASERTERAPHY: AN ADJUVANT TREATMENT IN ACUTE AND CHRONIC WOUNDS REPAIRING

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Introduction Objective: Acute are related to surgery procedures and chronic wounds are related to patients that remain immobile on bed. Nursing assistance is needed in order to prevent the high risk of complications. It's the most common in such situation to find wound ulcer, vascular sore and diabetic neuropathies lesions. Low Level Laser Therapy (LLLT) has being applied to assist the acute and chronics wound repairing process that aid the cellular and tissue resolution through the anti-inflammatory, cicatricial and analgesic effects.

The aim of this study was to evaluate the contribution of lasertherapy in different acute and chronic wounds repairing, as an important adjuvant treatment associate to the hygiene and antisepsis cares in the accomplishment of the lesion dressings.

Methodology: After 13 years of practice in our clinic, against the morbidity prevention, using LLLT for repairing acute wound surgeries and chronic wounds, such as**:** wound pressure, vascular lesion and diabetic neuropathies wound, have been carried out. Is this stdy we consider The basic parameters taking in consideration were**:** the patient general conditions, age, corporal surface and SAEF (Spatial average energy fluency). Diode Lasers with different wavelengths, varies from (630nm to 655nm and Power = 25mW - 30mW) and (808nmm, Power =100mW - 250mW), surrounding the lesions area were applied.

Results: for all treatments there were reductions in sore exudates, the presence of viable granulated tissue with improvement of vascular perfusion, foreseen the edges retraction as same as to control bacterial proliferation, thus preventing skin infection and other complications.

Conclusion: The lasertherapy is the main ally for assisting all types of lesions, which contribute for repairing different acute and chronic wounds with effectiveness, therefore improving the quality of life for all patients treated with LLLT.

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CO.22

Desbridamento de ferida crônica de úlcera por pressão em calcanhar direito utilizando laser de diodo cirúrgico de 980nm – Relato de caso.

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BACKGROUND

Desbridamento é promoção da limpeza da ferida, consistindo na remoção do tecido desvitalizado ou necrosado. Visa acelerar a cicatrização e reparação tecidual. Os métodos atualmente empregados são: cirúrgico, mecânico, autolítico e enzimático.

OBJETIVO

Usar o laser de diodo cirúrgico de 980 nm para desbridamento de ferida de calcâneo.

MATERIAL/MÉTODO

Paciente masculino, 76 anos, cadeirante, diabético, caquético. Lesão grau IV, presença de: tecido necrótico, exsudato exuberante, odor fétido e dor à manipulação.

Limpeza prévia com soro fisiológico. Para o desbridamento foi utilizado laser de diodo cirúrgico de 808 nm, Pmédia: 2,2W, CW, fibra de 600 µm. A ferida recebeu curativo com pomada dermatológica e faixa e semanalmente laserterapia: 100 mW, 4 pontos de 1J / 35J/cm2, 7 sessões) e curativos com soro fisiológico 9% + pomada dermatológica.

Para a avaliação macroscópica, medidas da ferida foram tomadas no sentido antero-posterior e mesio-lateral e fotos foram tomadas.

RESULTADOS

Todo o tecido removido foi recuperado. Não houve: perda de tecido, abaulamento, ou presença de tecido cicatricial. A lesão foi considerada totalmente e satisfatoriamente cicatrizada após 90 dias. O tratamento resultou eficaz na cura da ferida crônica bem como em sua analgesia.

CONCLUSÃO

O desbridamento feito com laser de diodo cirúrgico de 980 nm mostrou ser uma boa opção terapêutica.

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CO.23

Hard tissue bioengineering with photomodulated dental derived mesenchymal stem cells - an in vivo study

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This study evaluated the influence of an rhBMP4-loaded cell carrier (PL; Pluronic® F-127) associated to photobiomodulation (PBM) on dental pulp stem cells (DPSCs) transplantation aiming bone regeneration. Animals were treated according to the approved guidelines from the Animal Research Ethic Committee at the University of São Paulo (Protocol #004/2015). Six-week-old Nude mice were used in this study (n=6). Critical sized calvarial defects were created in the right side of the parietal bone (4.3 mm in diameter) of mice under general anestesia using xylazine (10 mg/kg) and ketamine (100 mg/kg). DPSCs were isolated, characterized and cultured in α-Minimum Essential Medium (α-MEM), supplemented with 15% fetal bovine serum, 2 mM L-glutamine, 100 µM L-ascorbic acid-2-phosphate, 100 U/ml penicillin and 100 µg/ml streptomycin (all from GIBCO/Invitrogen, Grand Island, NY, USA) (Human Ethic Committee protocol #CAE 09731212.2.0000.0075). The defects were fulfilled with PL/rhBMP4 containing 1.25 x 106 cells/ml of hydrogel. PBM was performed in half of the groups trans-operatively using a continuous-wave indium-gallium-aluminum-phosphide (InGaAlP) diode laser (660 nm; DMC, São Carlos, SP, Brazil) with a spot size of 0.028 cm2 (20 mW, 0.71 W/cm2, 5 J/cm2 (7s), 0.14 J). After 4 and 8 weeks post-transplantation, specimens were scanned using a µCT system (SkyScan 1176; Bruker µCT, Kontich, Belgium) for evaluation of ectopic mineralization. After µCT imaging, samples were decalcified in 10% EDTA solution (pH 7.4) for 3 weeks and processed for histology (Hematoxilin/Eosin and Picrosirus Red). Despite the incomplete closure of the calvarial critical sized defects in both treated and untreated groups, it was observed significant increase in bone formation after PBM treated group in relation to the control group, in particular after 8 weeks post-transplantation. PBM treated group presented more mature bone with interconnected bone trabeculae and more packed collagen than the control group. PBM is able to accelerate bone formation and its maturation when the system DPSCs/PL/rhBMP4 is transplantated into critical sized calvarial defects.

CO.24

Effect of laserphototherapy associated or not to Vitamin C in the induction of cell sheets of human dental pulp stem cells

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Cell Sheets, consisting of stem cells (SCs) are self detachable from the cultivation plate, and with no subcultivation can generate large amount of cells. The cell sheets can be transplanted closer to cell physiology environment by keeping the cell connections and the extracellular matrix produced in culture. Ascorbic acid or Vitamin C (VC) has inductive effect on cell sheet formation, increasing the longevity and the stemness of the cell for long period of time. The similarity between biological responses of VC in cell sheets and those of Laserphototherapy (LPT, Laser) on cells and tissues led us to hypothesize that these therapies could improve the prognosis of future clinical application of these cell sheets in regeneration of dental tissues. To test this hypothesis, LPT and VC were applied, associated or not, to induce human dental pulp stem cells (hDPSCs). Therefore, hDPSCs, which expressed typical levels of mesenchymal stem cell surface markers, were plated in 6-well plates ($5x10^4$ cells per well). Twenty-four hours later they were subjected to the treatment of experimental groups: **Control**: hDPSCs in P3 cultured with regular medium; **Senescent**: hDPSCs in P27 cultured with regular medium; **VC:** P3 cultured with regular medium supplemented with VC (20 µg/ml); **Laser**: P3 cultures with regular medium and submitted to LPT (punctual and contact mode-5 points / well, 660 nm, 20 mW, 0.028 cm^2, 0.71 W/cm^2, 7 sec, 5 J/cm^2, 0.14 J per point, 48 hours-intervals) and **Laser+VC**: P3 cultured with regular medium supplemented with VC and submitted to LPT Within 24 hours, 7 and 13 days the hDPSCs of the different experimental groups were observed macroscopically and microscopically, and the telomerase enzyme activity was assessed by PCR-TRAP, complemented by ELISA. To evaluate the expression of genes related to the nature and differentiation (Mitofilina and Oct 4), longevity (catalytic phase of telomerase-hTERT enzyme), and the senescence of the senescent group cells (β-galactosidase), the hDPSCs of all experimental groups were subjected to RT-qPCR. The RT-qPCR data were compared by ANOVA complemented by the Tukey's test ($p \leq 0.05$). The hDPSCs were able to form cell sheets only in the VC and Laser+VC groups (100%). Additionally, the cell sheets of the Laser+VC group presented easier handling. Telomerase activity in hDPSCs was observed only in 24 hours (Control and Laser) and seven days (VC and Laser + VC). The undifferentiating marker (Oct 4) and mesenchymal marker (mitofilin), as well as hTERT were expressed in hDPSCs of all experimental groups. Oct4 and hTERT presented expressions significantly higher at 7 days in VC and Laser+VC groups than in all other groups ($p < 0.0001$, $p = 0.0009$, respectively). The expression of mitofilin was significantly higher in the Laser+VC group, in 7 days ($p = 0.0338$). The technique of obtaining cell sheets of hDPSCs by the methodology here presented was considered appropriate to be further tested in regenerative procedures. The LPT when combined with VC did not interfere with the formation of the cell sheets, neither in the maintenance of longevity and undifferentiating status of hDPSCs. Moreover, LPT improved the handling of the cell sheets. Thus, the association of VC and LPT in the induction of cell sheets seems promising for future use in regenerative dentistry.

CO.25

Low level laser therapy associated with a strength training program on muscle performance in elderly women: a randomized double blind control study

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The aging process leads to a gradual loss of muscle mass and muscle performance, leading to a higher functional dependence. Within this context, many studies have demonstrated the benefits of a combination of physical exercise and low level laser therapy (LLLT) as an intervention that enhances muscle performance in young people and athletes. The aim of this study was to evaluate the effects of combination of LLLT and strength training on muscle performance in elderly women. For this, a hundred elderly women were screened, and 48 met all inclusion criteria to participate in this double-blind placebo-controlled trial. Volunteers were divided in three groups: control (CG = 15), strength training associated with placebo LLLT (TG = 17), and strength training associated with active LLLT (808 nm, 100 mW, 7 J) (TLG = 16). The strength training consisted of knee flexion-extension performed with 80 % of 1-repetition maximum (1-RM) during 8 weeks. Several outcomes related to muscle performance were analyzed through the 6-min walk test (6-MWT), isokinetic dynamometry, surface electromyography (SEMG), lactate concentration, and 1-RM. The results revealed that a higher work ($p = 0.0162$), peak torque ($p = 0.0309$), and power ($p = 0.0223$) were observed in TLG compared to CG. Furthermore, both trained groups increased the 1-RM load (TG vs CG: $p = 0.0067$ and TLG vs CG: $p < 0.0001$) and decreased the lactate concentration in the third minute after isokinetic protocol (CG vs TLG: $p = 0.0289$ and CG vs TG: $p = 0.0085$). No difference in 6-MWT and in fatigue levels were observed among the groups. The present findings suggested that LLLT in combination with strength training was able to improve muscle performance in elderly people.

CO.26

PHOTOBIOMODULATION THERAPY (PBMT) AND/OR CRYOTHERAPY IN SKELETAL MUSCLE RESTITUTION, WHAT IS BETTER? A RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL

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INTRODUCTION: The physiological recovery from fatigue is necessary to prevent overtraining, minimizing injuries. Cryotherapy for post-exercise recovery remains widely used, despite the lack of quality evidence. Photobiomodulation Therapy (PBMT) has demonstrated positive ergogenic effects through use of low-level lasers and light emitting diode (LED), furthermore current scientific evidence supports its use.
OBJECTIVE: The study aims to evaluate PBMT and cryotherapy as a single or combined treatment on skeletal muscle recovery after eccentric contractions of knee extensors.
METHODS: Fifty healthy male volunteers, age between 18 and 25, were recruited and randomized into five groups (n=10): PBMT, Cryotherapy, Cryotherapy+PBMT, PMBT+Cryotherapy or Placebo. A randomized, double-blinded, placebo-controlled trial (approved by institutional ethics committee - process 470.174) was carried out. The exercise performance (maximum voluntary contraction - MVC), delayed onset muscle soreness (DOMS), and muscle damage (Creatine Kinase - CK) were evaluated. Assessments were performed at baseline, immediately after, and at 1, 24, 48, 72 and 96 hours. Comparator treatments were performed 3 minutes after exercise and repeated at 24, 48 and 72 hours. PBMT was applied employing a cordless, portable GameDay™ device (combination of 905nm super-pulsed laser, 875nm and 640nm LEDs - Multi Radiance Medical™) to 6 sites of knee extensors in direct contact with the skin, 39.37J per site. For cryotherapy, the PRICE protocol was employed covering the entire knee extensors, during 20 minutes. After baseline MVC, volunteers performed the eccentric contraction protocol which consisted on 75 eccentric isokinetic contractions of the knee extensor musculature in the non-dominant leg. Data was firstly tested regarding normal distribution using Shapiro-Wilk. Two-way ANOVA test, followed by Bonferroni *post hoc* tests were performed (significance $p<0.05$).
RESULTS: PBMT as single therapy optimized post-exercise recovery with improved MVC, decreased DOMS and CK activity compared to placebo, cryotherapy and Cryotherapy+PBMT. The PBMT group demonstrated significant differences from 24 (112.88%, $p<0.001$ - MVC; 0.91%, $p<0.001$ - DOMS; 54.63%, $p<0.001$ - CK) to 96 (114.59%, $p<0.001$ - MVC; 0.06%, $p<0.05$ - DOMS; 43.66%, $p<0.001$ – CK) hours. In the PBMT+Cryotherapy group, the effect of PBMT was decreased ($p>0.05$), however is still demonstrated significant improvement in MVC, decreased DOMS and CK activity ($p<0.05$). Cryotherapy as single treatment and Cryotherapy+PBMT were similar to placebo ($p>0.05$).
CONCLUSION: We conclude that PBMT used as single treatment is the best modality for enhancement of post-exercise restitution, leading to complete recovery to baseline levels from 24 hours after high-intensity eccentric contractions.
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CO.27

BLUE LIGHT DELAYS MUSCLE FATIGUE

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Introduction: Photobiomodulation (PBM) with red and infrared light can delay the onset of muscle fatigue during activity thereby improving athletic performance *[Leal-Junior, Photomed and Laser Surg, V33, 2015-02, pp1-2]*. Even though low-level light therapy today is mainly applying radiation with wavelengths 600-900nm, the original action spectra for light stimulated cell growth reported by Karu *[Ten Lectures on Basic Science of Laser Phototherapy, 2007, ISBN 978-91-976478-0-92]* include a peak in the blue spectral region. It is therefore interesting to investigate the influence of blue light on exercise-induced muscle fatigue.
Objective: Investigate whether a blue LED light pre-conditioning modulates exercise-induced muscle fatigue.
Method: We integrated seventy blue LEDs, 453nm, into a wearable light patch prototype for the upper leg and tested two irradiation conditions, a continuous wave (CW) and pulsed wave (PW) condition, applied for 15 minutes immediately prior to the exercise. Both conditions had an average irradiance of 13.9 mW/cm^2, a dose of 12.5 J/cm^2 and a treatment area of 273 cm^2. However, the maximum peak irradiance was different: 139 mW/cm^2 for CW and 2780 mW/cm^2 for PW. The study contained three visits: an intake session with an exercise without pre-conditioning and two PBM sessions, which were held once a week at the same time of the day. The study had a within-participant design and was double blinded. The fatigue exercise was isometric: performing a rapid succession of maximal voluntary static leg extensions with the dominant leg in seated position. This leg was kept in position with a pull strap connected to a load cell for force measurement. A video instruction guided the participant to perform the maximal voluntarily contractions (MVC), for 3 seconds on and 2 seconds off, as long as possible. The primary outcome is the decline of muscle force over time. We recruited 14 young, 20-35 year old, male practitioners of regular physical activity, of whom eleven finished the study.
Results: During the fatigue exercise every MVC has its own course with a corresponding maximal value of the force. Typically these maxima show a continuous decrease during the exercise. To compensate for initial force differences between individuals we normalized the values. It is found that the difference in force decline between the intake, without pre-conditioning, and the CW irradiation condition is small and not statistically significant. However, a fatigue delay occurs for the PW condition. A positive difference Δ in normalized force decline CW-PW that increases with the number of MVC's performed is found. The study population Δ was 0.046 ±0.069 (mean, ±SD) at MVC number 50. The delta is statistically significant ($p<0.05$, t-test).
Conclusion: This first of a kind blue LED pre-condition study shows that blue LED irradiation delays exercise-induced muscle fatigue in an irradiation condition dependent manner. The PBM-induced performance increase of about 5% is statistically significant and of relevant size for sports performance.

CO.28

Effect of low level laser associated with activated carbon in bone repair of rats

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Bone diseases such as fractures and bone defects may result from several reasons, resulting in a normally long and painful repair process. The most used therapies are based either on the implantation of a biocompatible prosthesis or through the insertion of a biomaterial in the local of injury. However, those treatments involveextended and costly surgical intervention. Thus, the association of two low cost techniques such as the use of activated carbon (AC) as bone biosubstitute and the application of the low-level laser therapy to assist the bone repair might be an alternative to overcome those problems. The study was performed by induction of a bone defect in rat tibias and subsequent treatment with AC and laser therapy.
Material and methods: male wistar rats, 35 were used between 150 g to 200 g, with 3 months of age. The animals were anesthetized with ketamine and xilasine association. Once anesthetized, the animals were put on surgical table, the skin was dissected and monocortical bone defects were made in the central region of the right tibia of mice. Immediately after surgery the rats were randomized and divided into the following groups: control (CTL), untreated Injury (NT), Lesion treated with activated carbon (CA), Lesion treated with lasertherapy (L6J) and Lesion treated with association of activated carbon and laser 830nm, 6J-100mW (CA+L). After 28 days the animals were euthanized with overdose of the same anesthetic, blood was collected and the tibia was removed for biochemical, histological and biomechanical analysis. Committee of ethics: AN00462014-UNINOVE.
Results: the NT group showed reduction in bending forces and histological changes related to disorganization of the tissue. The groups CA+L and HA showed no improvement in mechanical properties, however, the CA+L group presented histological aspects of improvement. Only the CA group presented better flexion forces, related with improvement of histological aspects and reduced levels of alkaline phosphatase. The NT groups, CA+L and HA presented high alkaline phosphatase levels.
Conclusion: Activated carbon seems to improve the bone repair induced in this study. The laser association with activated carbon showed no improvement of biomechanical properties in spite of the histological results have a better aspect.

CO.29

LOW-LEVEL LASER THERAPY ACCELERATES BONE REPAIR IN RATS: A MICROARRAYS ANALYSIS

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Introduction: Fractures of delayed consolidation and fractures with non-union are commonly found in medical practice and are associated with high morbidity and mortality. Some resources have been studied in order to accelerate the process of bone repair, among these, low-level laser therapy (LLLT) has gained prominence. Several studies suggest that LLLT is able of stimulating osteoblast proliferation and osteogenesis in the fracture site, promoting a greater deposition of bone mass, which is essential for the consolidation process (Photomed Laser Surg. 32.; 618-626, 2014; Lasers Med Sci. 30.; 1599-1607, 2015). Although the positive effects of LLLT on tissue regeneration, the molecular mechanisms by which the LLLT acts on bone are not fully understood (Exp. Gerontol. 47.; 136-142, 2012). **Objective:** In this context, this study aimed to assess the effects of LLLT on consolidation of bone defects induced in the tibiae of rats. **Methods:** This study was conducted in accordance with the Guide for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the Federal University of São Paulo (0808/10). Twenty male Wistar rats (12 weeks old, ± 300 g) were randomly divided into two groups, with 10 animals each: group control bone defect without any treatment and group bone defect irradiated with LLLT. The animals were submitted to laser irradiation (830 nm, 100 mW, 0.028 cm^2, 3.57 W/cm^2, at 120 J/cm^2, energy of 3.4 J, with an irradiation time of 34 s) at a single point on the bone defect for eight sessions, on alternate days. The normality of all variables' distribution was verified using Shapiro–Wilk's W test. The t test was used for variance analysis between groups. Significant difference tests had a statistical significance defined as $p \leq 0.05$. **Results:** Birefringence analysis demonstrated that irradiated bone defects presented higher deposition of collagen fibers (142.60±13.25px) and better organization of these fibers when compared to the control (90.35±11.68px). Morphometric analysis revealed that the irradiated animals showed higher area of newly formed bone (588.24±49.73μm^2) compared to the control group (431.97±42.15μm^2). Moreover, microarray analysis evidenced that LLLT produced a significant increase in the expression of fibroblast growth factor (FGF), transforming growth factor, beta (TGF-β), collagen (COL), bone morphogenetic proteins (BMPs), runt related transcription factor-2 (RUNX-2) and osteocalcin (OC) that could stimulate osteoblast proliferation and differentiation, which may be related to improving the deposition of newly formed bone at the site of the injury. **Conclusion:** This study revealed that LLLT accelerates bone repair process as result of increasing bone formation probably by modulation of genes related to bone healing in a model of tibial bone defect in rats.

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CO.30

On Long Term effects of Low Power Laser Therapy in Bone Repair: a Synchrotron Radiation-based Microtomography study

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The aim of the study was to investigate, using Synchrotron Micro Computer Tomography (MicroCT), the bone volume percentage in the graftin area, after applying 808nm diode laser radiation.
Material and Methods
An experimental study was conducted on Wistar male rats (250-300 grams) which were subjected to transverse osteotomy of the right and left femurs and randomly divided into four experimental groups: animals not grafted with biomaterials and not treated with laser therapy (Group I), animals not grafted with biomaterials but that received laser therapy (Group II), animals grafted with biomaterials and not treated with laser therapy (Group III), animals grafted with biomaterials and that received laser therapy (Group IV). Each animal in the right femur defect was exposed to 808nm laser radiation (D=16 J / cm^2 per exposure) immediately after osteotomy, every 48 hours for the first week and every 72 hours for the next two weeks. Animals were sacrificed after 24 days. Bone regeneration and mineralization degree, with or without biomaterial's grafts, were evaluated by synchrotron-based computed phase-contrast microtomography (SR-microCT).
Results
The values obtained were submitted to t-tests. The significance level adopted was 5%. We demonstrated that, for regenerated bone struts in the dimensional ranges thicker than 200 mm and in absence of any biomaterial graft, the bone volume percentage in the LPLT-treated samples was almost two-fold greater vs. the controls. This effect is magnified in presence of BiOss grafts when the bone volume percentage in the LLLT-treated samples was found to be almost three-fold greater vs. not treated samples.
Conclusion: Based on SR-microCT findings and after 24 days from osteotomy and the beginning of laser treatment, LPLT was able to promote bone thickness growth in presence or absence of BiOss particle mixture used as defect filler.

CO.31

Total mouth Antimicrobial Photodynamic Therapy mediated by blue LED and curcumin in AIDS patients: microbiological analysis and fluorescence spectroscopy

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The occurrence of opportunistic and resistant species that can be found in saliva, buccal mucosa, supragingival plaque and periodontal pockets of patients with AIDS, has been linked to drug therapy, poor immune response and inadequate oral hygiene and are associated with local and systemic infections in these individuals. Besides the mechanical removal of oral biofilms, in HIV / AIDS patients makes often necessary to combine an antifungal and antibiotic drug therapy, as well as a topical therapy with the use of mouthrinses and oral antiseptics which have undesirable side effects and enable the emergence of resistant microorganisms. Thus, the use of alternative techniques and / or adjuncts to conventional therapy in patients with AIDS, such as Antimicrobial Photodynamic Therapy (APDT), is seen as a promising new approach not only to eliminate these infections, and for an effective prophylactic treatment, avoiding the occurrence of infectious lesions and resistant microorganisms. The aim of this study was to evaluate a total mouth therapeutic protocol of APDT mediated by LED associated with curcumin photosensitizer in AIDS individuals and observe the intraoral biofilms with fluorescence spectroscopy in different times. We selected 10 adult patients with AIDS undergoing treatment at a center for sexually transmitted diseases and AIDS in a city of Bahia-Brazil. (CEP IMS UFBA 916.480) APDT was performed Inside the oral cavity, mediated by curcumin 1.5 g / l (pre-irradiation time of 5 minutes) and emitting blue light (450 nm) diode (Prototype, Finep design / Gnatus LED Edixeon, Edison Opto Corporation, New Taipei City, Taiwan) with an intensity of 67 mW / cm2, 5 minutes lighting and an estimated average fluence of 20.1 J / cm2. Before and after APDT, in intervals of 15 days during two months, saliva samples were collected and processed in selective culture medium (Sabouraud dextrose agar with chloramphenicol, McConkey agar, Mitis salivarius agar, mannitol agar and BHI agar) for isolating microorganisms of interest. Patients underwent examination of intraoral biofilms through optical fluorescence before and after APDT in all sessions of treatment and / or monitoring. The data in log10 UFC / mL of APDT were statistically analyzed by ANOVA 5%. The results showed that among the species of interest in the study, APDT with the studied parameters, promoted a larger reduction against Staphylococcus spp. and Candida spp. in the oral cavity of AIDS patients. It was also observed that the fluorescence spectroscopy showed the intraoral biofilms with intense red fluorescence enabling the diagnosis and localization of biofilms on clinical examination. It can be concluded that the APDT with the studied parameters was effective in reducing relevant species related with intraoral lesions in patients with AIDS, being a promising alternative therapy for prevention of pathologies in these individuals. It was also concluded that fluorescence spectroscopy has great potential to be used as a diagnostic tool and verification of results of treatments and techniques that will assist health professionals in the conduct of clinical cases.

CO.32

DOSIMETRIC STUDY OF LOW LEVEL LASER THERAPY IN 5-FU INDUCED ORAL MUCOSITIS IN HAMSTERS: A PILOT STUDY.

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Introduction: Oral mucositis (OM) is a dose-limited debilitating consequence from cancer treatment that could be treated with low level laser therapy (LLLT); however, there is no consensus about its dosimetric parameters for oral mucosa healing.

Objectives: Thus, the aim of this study is to compare different LLLT protocols on the treatment of OM, through clinical and histological analysis.

Methods: Fifteen female *hamsters* (about 150g and eight weeks) were used, in an induced model of OM by 5-Fluorouracil (5-FU) and superficial scratching in oral mucosa, in seven days of follow-up. The animals were divided into 5 groups: “C” which received only anesthesia and chemotherapic vehicle; “Ch” which received anesthesia, 5-FU and scratches; “L1” the same as Ch group + LLLT 6 J/cm²/0.24J (one point); “L2” the same as Ch group + LLLT 25J/cm²/1J (one point); and “L3” the same as Ch group + LLLT 6 J/cm²/0.96 J (4 points of 0.24J). The laser that was used has λ=660 nm, 0.04 cm² of spot area and 40 mW of power. This study was approved by the Ethics Committee on Animal Use of University of Sao Paulo (FOUSP - Process number 2015.010).

Results: The best LLLT protocol to maintain lowest OM levels compared to Ch group was L1, followed by L2 and L3. Histological results demonstrated the same pattern among L1, L2 and C groups, with some blood vessels presence and continuous aspect of a thin epithelium, on day 7.

Conclusion: Our results suggest that the application mode of LLLT and the energy delivered per area could interfere in the oral mucositis healing.

CO.33

Can the photobiostimulation on quadriceps femoral muscle improve the pain, muscle strength and functional performance in patients with knee osteoarthritis? Randomized clinical trial.

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Introduction: Osteoarthritis (OA) is the most prevalent rheumatic diseases in the elderly and is associated with pain, stiffness, deformity and progressive loss of function. Physical therapy through kinesiotherapy, proprioceptive exercises and physical agents can improve symptoms related to OA.
Objective: To evaluate the effect of phototherapy on functional performance, pain and muscle strength in patients with knee osteoarthritis.
Method: Ethical approval: 244,406 (Federal University of sao Paulo); Clinical Trial Registry: RBR-7gkwdj (ReBEC). The study included 16 patients clinically diagnosed with knee OA, unilateral or bilateral, according to the criteria of the American College of Rheumatology through the x -ray image. Patients were randomly divided into Group 1: underwent a treatment program of exercises associated with application of placebo and Group 2: same exercise program associated with phototherapy (7 diodes 660nm; 7 diodes 830nm, power: 100mW each diode; total energy: 168J each member; time: 40 seconds). The groups were treated for 12 weeks with a frequency of 3 times per week. All patients underwent pre and post outcomes with SF-36 (Short Form 36), WOMAC questionnaire, Berg scale, VAS (Visual Analogue Scale for pain), ROM (range of motion), and muscle strength evaluation (hand held dynamometry). To evaluate the difference between the measurements in the Placebo and Phototherapy groups regarding unrelated samples, we used the Student t test. To study the behavior of Placebo and Phototherapy groups over the estimated time, according to the variables of the remaining interest, we used the model ANOVA with repeated measures and the method of multiple comparisons of Bonferroni.
Results: No significant differences for evaluating the quality of life, functionality (placebo group: initial mean 50,5 SD 8,1, final mean 34,1 SD 11,9 vs. phototherapy group: initial mean 65,2 SD 20,2, final mean 36,3 SD 25,5, p=0,156), balance (placebo group: initial mean 41,1 SD 6,5, final mean 53,8 SD 1,7 vs. phototherapy group: initial mean 35,7 SD 6,8, final mean 53,7 SD 2,1, p=0,137), pain (placebo group: initial mean 6,7 SD 1,5, final mean 2,6 SD 1,5 vs. phototherapy group: initial mean 6,5 SD 1,7, final mean 2,1 SD 1,9, p=0,738), flexibility and muscle strength in the intergroup analysis, however, there was a significant improvement in both groups (intragroup) over time were found.
Conclusion: The functional performance, pain and muscle strength showed improvement both in the group submitted to the rehabilitation protocol, as the group associated with phototherapy in the quadriceps muscle.

CO.34

Effectiveness of low-level laser therapy and aerobic exercise training on articular cartilage in an experimental model of osteoarthritis in rats

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Osteoarthritis (OA) is the most common disease of the knee joints in adults throughout the world. Physical exercise and low-level laser therapy (LLLT) has been studied for clinical treatment of OA, even though the effects and action mechanisms have not yet been clarified. Thus, the aim of this study was to evaluate the effects of an aerobic exercise training and low-level laser therapy (LLLT) (associated or not) on degenerative modifications and inflammatory mediators on the articular cartilage using an experimental model of knee OA. This study was approved by the Ethical Committee of the Federal University of São Paulo (2013/814715). Fifty male Wistar rats were randomly divided into 5 groups: control group (CG); knee OA control group (OAC); OA plus exercise training group (OAT); OA plus LLLT group (OAL); OA plus exercise training associated with LLLT group (OATL). The exercise training (treadmill; 16m/min; 50 min/day) and the laser irradiation (2 points- medial and lateral side of the left joint; 24 sessions) started 4 weeks after the surgery, 3 days/week for 8 weeks. The results showed that all treated groups showed (irradiated or not) a lower degenerative process measured by OARSI score and higher thickness values and a reduced expression in IL-1β, iNOS and IL-10 compared to OAC. Moreover, a lower TGF-β expression was observed in OATL. These results suggest that LLLT and aerobic exercise training were effective in preventing cartilage degeneration and modulating inflammatory process in the knees in OA rats.

CO.35

Basic science of different low-level lasers' processing penetration abilities in Achilles at rest and at stretched in healthy individuals

Bordvik DH, Joensen J

Background / introduction data*: Basic science demonstrations of LLLT-penetration abilities over time in human in situ-tissues put in different conditions are lacking.* ***Objective****: The aim of this study was to investigate the penetration-time-profiles for two different lasers used in low-level laser therapy (LLLT), during 150 seconds of exposure both in stretched and rested human Achilles in situ.* ***Material and methods:*** *Both Achilles from seventeen healthy young light-skinned volunteers (16 f, 1 m - avg. age 27.1 years, SD 3.9) were sequent laser irradiated medio-laterally (giving total n=34), in standardized rested and stretched tissue conditions. An 810 nm 200 mW continuous laser, and a 904 nm 60 mW super-pulsed laser were applied. The energy penetrating skin-skin was measured at an optical power meter-system every 30. second. Tissue thicknesses were scored after imaging the tissues by Ultrasonography prior to laser-irradiations. No ethical approval was considered necessary due to the lack of treatment-objectives, and harmless effects of actual irradiation.* ***Results:*** *The 810 nm laser presented a statistical non-significant (95% p>0.05) change in Achilles penetration-ability around 0.17% of mean output-power (MOP) (SEM 0.02), and 0.02% of MOP (SEM 0.004), with the tissues at rest and stretch respectively. The 904 nm laser demonstrated a statistical significant (95% p<0.05) and almost linear increasing penetration-ability from 0.25% (SEM 0.03), to 0.38% of MOP (SEM 0.04) with tissues at rest, and from 0.05% (SEM 0.01) to 0.13% (SEM 0.01) of MOP in stretched tissues. The penetrated energy-amounts proportionally differed between lasers, and tissue-conditions, at all measure-points (95% p<0.05).* ***Conclusions****: Relatively spoken, increasingly more energy penetrates human Achilles tissues from the 904 nm laser compared to the 810 nm laser. Also, stretching the Achilles tissues in LLLT cause a higher energy-attenuation by the tissues.*

Financial support: No additional financial support besides the resources available at the Bergen University College.

CO.36

ASSOCIATION OF PHOTODYNAMIC THERAPY, LASER THERAPY AND CELLULOSE MEMBRANE FOR CALCANEAL PRESSURE ULCER TREATMENT IN DIABETIC PATIENT: A CASE REPORT

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Diabetes mellitus is a metabolic disorder in which the individual has high glucose levels in the blood due to inadequate insulin production by the pancreas accumulating based complex sugar that narrows the blood vessels leading to complications such as retinopathy, nerve damage, limb amputation, stroke and reduced wound healing capacity in these patients. Normally, wounds become chronic taking more time to overcome the inflammatory phase. Infection is a common complication of wounds in diabetics and is associated with increased risk of amputation. The polymicrobial nature of most infections is treated by the use of broad spectrum antibiotics which may contribute to the emergence of multi-resistant bacteria due to their excessive use. Furthermore, the limited penetration of drugs into infected areas (aggravated by ischemia) reduces the effectiveness of such therapy. The aim of this study was to present a pressure ulcer (PU) case report in calcaneal region in diabetic patients treated by the combination techniques of Antimicrobial Photodynamic Therapy (APDT), laser therapy and cellulose membrane. Female patient, 82 years old, caucasian, with type 2 diabetes mellitus, hypertension, Metformin (1700mg / d) and captopril (50 mg / d) user. In the clinical-dermatological examination, the right calcaneal region had pressure ulcer measuring 7.43 cm^2 and edema. After precleaning with 0.85% sterile saline, material for isolation of microorganisms were taken from viable granulation tissue using sterile disposable swab that has been processed in selective media (Mannitol agar, Sabouraud dextrose agar, McConkey agar and blood agar). To perform the APDT, curcumin photosensitive agent emulsion 1.5% was used (PDT Pharma Industry and Trade Pharmaceuticals LTD, Cravinhos, SP, Brazil) applied across the surface of the ulcer. After 30 minutes of application it has been activated using the Lince equipment - Light in Cell (MMOptics, São Carlos, São Paulo, Brazil), composed of 30 LEDs, wavelength of 450 $\pm$ 10 nm (visible blue) . The delivery was light continuously, 12 minutes long, with light intensity of 35 mW/cm^2, fluence of 25.2 J/cm^2. The light was applied from a distance of 5 cm from the tip surface to the surface of ulcers. Two sessions of APDT were carried out with an interval of seven days between them. After APDT, Nanoskin® cellulose biomembrane was positioned over the entire area of ulcers. Laser therapy was performed with 660 nm laser, punctual and continuous manner, spot of 0.04 cm^2, power of 40 mW, 10 seconds irradiation time, fluence of 10 J/cm^2 and irradiance of 1000 mW/cm^2, twice a week. The evaluation of healing was verified by digital photographs taken twice a week and the area of the PU measured with UTHSCA Image Tool, version 1.28 (University of Texas Health Science Center, San Antonio, Texas, USA) software. APDT with the parameters used in this case totally reduce de contamination of the PU. The re-epithelialization occurred after 30 days of treatment with the combination of techniques. We concluded that the association techniques of APDT, low power laser therapy and cellulose membrane was effective in PU healing in a diabetic patient, in a short treatment time. This case report was aproved by Multidisciplinary Health Institute Ethics Commitee (CAAE 36925714.0.0000.5556).

CO.37

Cancer Stem Cell Responses to Photobiomodulation

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Cancer stem cells (CSCs) have been implicated as an important contributory factor in the development of metastasis. CSCs have the same characteristics as normal stem
cells; that is, they can proliferate indefinitely and are capable of both self-renewal and differentiating into specialized cells. The molecular and cellular characteristics of stem cells and CSCs are coded for by cell-specific genes, which can be analyzed using molecular assays setting the standard to work from. Low-intensity laser irradiation (LILI) has been applied in the treatment of numerous diseases and pathological conditions. Photobiomodulation has been shown to stimulate proliferation of cells, capillary growth, and cellular metabolism as observed by adenosine triphosphate activation. It has been shown, by using different fluences and wavelengths, LILI, can either stimulate or inhibit cellular functions. Cancer research is highly focused on improving current cancer treatments. One method of targeted cancer therapy is Photodynamic therapy (PDT), where Low Intensity Laser Irradiation (LILI), along with a photochemical compound, is used. When implementing a mechanism by which CSCs are targeted, LILI might pose as a viable treatment option. Studies have shown that using high fluences of LILI (HF-LILI) cell death may be induced in normal and neoplastic cells. In our work, lung, cervical and breast CSCs were isolated using stem cell markers and irradiated at wavelengths of 636, 825 and 1060 nm at fluences ranging from 5 J/cm^2 to 40 J/cm^2. Post irradiation biochemical assays were conducted to monitor cellular responses including; proliferation and cytotoxicity, after 24 hours incubation. Results indicate that LILI, when treating CSCs, can induce either a bio-stimulatory or bio-inhibitory effect depending on the wavelength and fluence used. This study indicated successful cell damage in CSCs when using HF-LILI, as well as, stimulation of ATP production, when using lower fluences of LILI.

CO.38

Meso-tetrakis-hydroxyphenyl-porphyrin – Polyethylene glycol – Gold Core Shell – Superparamagnetic iron oxide nanoparticles for the possible enhancement of Photodynamic Therapy in Breast Cancer Cells

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Introduction: Breast cancer is the most commonly diagnosed cancer in women and the second leading cause of cancer-related deaths worldwide. Photodynamic therapy (PDT) is an effective treatment modality used for the management of solid tumours, which has few side effects in cancer patients and is minimally invasive. This technique is based on the passive uptake of photosensitizers (PSs) by tumour cells followed by their activation using an appropriate wavelength of laser light. This causes the PSs to produce reactive oxygen species (ROS) which induces tumour cell death. Research has focused on the potential use of porphyrin PSs drugs for the *in vitro* PDT treatment of breast cancer due to their high triplet ROS yield with long triplet lifetimes. However, *in vivo* porphyrins have poor cancer cell uptake, limited tissue penetration and are often ingested by immune system components. As a consequence relatively small amounts of a PS often reach solid tumours and sometimes distribute into healthy tissues, causing side effects, which limit PDT efficiency. Therefore, the development of new PSs agents with enhanced cancer cell uptake, effective tumour homing abilities, which are excitable by red light (better tissue penetration), lower toxic side effects in normal tissues and limited environmental degradation is needed to improve the *in vivo* PDT treatment of solid tumours.

Objective: Chemical synthesis and characterization of an established meso-tetrakis-hydroxyphenyl-porphyrin (MTHPP) PS molecule, which has an enhanced nano-agent magnetic-targeting based tumour drug delivery system, with heightened theranostic abilities. This synthesized drug delivery system should enhance breast tumour MTHHP PS accumulation by physical magnetic field forces that is applied directly to the targeted tumour site, improving PDT treatment efficacy.

Methods: Chemical synthesis of an enhanced PDT drug delivery system; MTHPP PS was conjugated to PEG making it more soluble. The PEG was further functionalized with a gold core shell to stabilize and enhance the final molecules theranostic abilities. Moreover the entire molecular system was linked to superparamagnetic iron oxide nanoparticles (SPIONs) to improve drug tumour accumulation by magnetic field forces. The final structure was chemically characterized using various spectroscopy and microscopy techniques.

Results: UV-Vis Spectroscopy results showed that synthesized MTHPP molecule generated high levels of ROS in when excited with red light at 680nm. FTIR spectroscopy results reported that the MTHPP—gold core shell–SPION was encapsulated in PEG signifying stability of the conjugate system and over 48 hours the molecular structure remained stable producing an average positive to neutral zeta potential of 5.67. Additionally TEM results reported the average size of the final conjugate molecule to be 16.2nm, which is small enough for cellular uptake.

Conclusion: These findings suggest that this MTHPP–PEG–gold core shell–SPION could possibly demonstrate enhanced *in vitro* PDT efficacy in breast cancer cells, through improved MTHPP PS drug delivery, accumulative cellular uptake in tumour cells with deeper tissue penetration activation. Current work involves investigating the *in vitro* cellular localization and PDT cytotoxicity of this enhanced PS drug

delivery system for possible treatment of breast tumours.

Financial Support: University of Johannesburg, SARChI NRF GRANT

CO.39

OROFUNCTIONAL REHABILITATION USING PHOTOKINESIOTHERAPY

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Introduction: The physiological aging leads to dysfunction of the stomatognathic system. Photokinesiotherapy is a program using phototherapy in combination with different kinesiotherapies. Phototherapy can slow down and manage aging process resulting in orofacial dermal-neuro-muscle rehabilitating.
Objective: So, we are searching for a new more efficient therapy to decrease and manage the disfuntion of stomatognathic system.
Method: Under Ethical Comittee approval (CAAE no. 45390715.2.0000.5419), fifteen female patients were selected and divided into 5 groups: G1 - Control group (cosmetics); G2 - Light group (cosmetics + lasers and LEDs systems); G3 - Exercises Group (Cosmetics+ lasers and LEDs systems + orofacial exercises); G4 - Electrotherapy Group (Cosmetics+ lasers and LEDs systems + Electrotherapy - aussie current); and, G5 - Taping Group (Cosmetics+ lasers and LEDs systems + therapy taping). Muscle evaluation was performed using electromyography, ultrasonography and bite force; and, skin evaluation were performed using corneometer, cutometer, ultrasonography and standardized photographies. Measures were done on period's time of initial, after 7 days e after 30 days.
Results: The pilot phase (fifhteen patients) was concluded using ANOVA statistical tests that showed the significant differences between groups. All treatments affected muscle tone and cutaneous elasticity (skin).
Conclusion: We suggest that the combination of cosmetics, mechanical and optical stimulus to all kind of different tissues from stomatognathic system can be the best choice to orofacial functional rehabilitation.
Financial Support: CNPq

CO.40

LOW POWER LASERS AND IMMUNOREGULATORY INTERLEUKINS IN BREAST CANCER CELLS

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Introduction: Lasers (Light amplification by stimulated emission of radiation) devices emit monochromatic, coherent and highly collimated intense beams of light that are useful in several therapeutic protocols. Wavelength, frequency, power, fluence and emission mode properties are determining to the photophysical, photochemical and photobiological responses induced by low power lasers, but molecular mechanisms involving in their biological effects are not well understood yet. These lasers may affect pathways, which could alter expression of important genes, even as immunoregulatory interleukins.
Objective: To evaluate mRNA level from genes involved in immunoregulatory interleukins in breast cancer cells exposed to low power lasers.
Methods: MDA-MB-231 cells were exposed to low power red (660nm) and infrared (808nm) lasers at 25J/cm² in continuous wave emission mode at 100mW. Total RNA was withdrawn and Microarray analysis was performed. Data was found taking into consideration as upregulated genes with fold up to 2, and downregulated genes with fold lower than -2, considered to control group. Results found between these values were considerate statistically not significant.
Results: Interleukin mRNA level found in cells exposed to red laser were between 1.32 and -1.40, as IL18, IL6, IL36A, IL6ST, IL7R, IL7, IL22, IL1A, IL17RD, IRAK4, IL4R, IL1B, IL19, IL11, IL16, IL36B, IL13RA1, IL21, IL2, IL2RA, IL15RA, IL18RAP, IL18R1, IL1RL1,IL1RL2, IL1R1, IL25, IL1R2, IL5RA, IL12RB2, IL23R, IL2RG, IL18BP, IL27RA, IL17RC, IL17RE, IL13RA2, IL1RAPL2, IL12RB1, IRAK3, IL26, IL12A, IL1RAP, IL17C, IL10, IL2RB, ILF3, IL17RB, ILF2, IL10RB,IL1RN, IL1F10,IL36RN, IL37, il4, IL13, IL22RA2, IL20RA, IL3, IL22RA1, IL23A, IL24, IL20, IL9, IL15, IL6R, IL12B, IL10RA, IL21R, IL31RA, IL32, IL5, IL17F, IL1RAPL1, IL11RA, IL17REL, IL17A, IL31, IL36G, IL20RB, IL17D,IL34, IL17B. In cells exposed to infrared laser were between 1.16 and -1.80, as IL19, IL7, IL17A, IL22, IL18, IL11RA, IL4R, IL11, IL17D, IL1B, IL6, IL36A, IL7R, IL1A, IL17RD, IL16, IL36B, IL13RA1, IL21, IL2, IL2RA, IL15RA, IL18RAP, IL18R1, IL1RL1, IL1RL2, IL1R1, IL25, IL1R2, IL5RA, IL12RB2, IL23R, IL2RG, IRAK2, IL18BP, IL27RA, IL17RC, IL17RE, IRAK1, IL13RA2, IL1RAPL2, IL12RB1, IRAK3, IL26, IL12A, IL1RAP, IL17C, IL10, IL2RB, ILF3, IL17RB, ILF2, IL10RB, ILRN, IL1F10, IL36RN, IL37, IL4, IL13, IL22RA2, IL20RA, IL3, IL22RA1, IL23A, IL24, IL20, IL9, IL15, IL6R, IL12B, IL10RA, IL21R, IL31RA, IL32, IL5, IL17F, IL1RAPL1, IL17REL, IL31, IL34, IL6ST, IL36G, IL20RB, IL17B.
Conclusion: Result show that immunoregulatory interleukins mRNA level are not significantly altered in MDA-MB-231 cells exposed to low power lasers at wavelength, fluence, power and emission mode used in therapeutic protocols.
Financial Support: FAPERJ, CNPq, Capes,

CO.41

MATRIX MATABOLISM ALTERATION BY LOW-INTENSITY INFRARED LASER

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Introduction: Arthritis is a musculoskeletal disorder characterized by joint inflammation, considered important cause of chronic disability in worldwide since is associated with motor incapacity. Numerous studies have indicated the positive clinical performance of low-level laser therapy (LLLT) as a non-pharmacological strategy to anti-inflammatory treatments, the mechanisms are still incompletely understood though. **Objective**: Investigated whether LLLT, recommended in device guidelines, could modify the expression of matrix metallopeptidases and their inhibitor, changing the degenerative course of rheumatic disorders. **Methods**: An inflammatory process was induced into the periarticular region of talocrural and subtalar joints (right and left) of male C57BL/6 mice, weighting 24–28g each, by zymosan administration. All experimental procedures were submitted and approved by the Ethical Committee of the Federal University of Juiz de Fora (protocol number 039/2014). The animals were divided: (C) control, (Zy) zymosan induction untreated, (Zy + 3 Jcm^{-2}) zymosan induction treated with infrared laser at 3 Jcm^{-2} energy density and (Zy + 30 Jcm^{-2}) zymosan induction treated with infrared laser at 30 Jcm^{-2} energy density. Laser exposure was performed 5, 29, 53 and 77h after zymosan administration with low-intensity infrared laser: 830 nm (infrared), 10mW at 3 Jcm^{-2} and 30 Jcm^{-2} energy densities, at continuous emission mode. Twenty four hours after the last laser application, the animals were euthanized, their joints dissected and forwarded for analysis: (a) morphological analysis, (b) mRNA expression of matrix metallopeptidase 14 (MMP14), MMP13, and TIMP2 by real time RT-qPCR and (c) immunohistochemistry to matrix metallopeptidase 13 (MMP13) and metallopeptidase inhibitor 2 (TIMP2). **Results**: An inflammatory process was observed in connective tissues for all experimental groups zymosan-treated. This infiltration decreased significantly ($p < 0.05$) after 30 Jcm^{-2} laser exposure. Since RT q-PCR analyses for Zy + 3 Jcm^{-2} group showed a similar MMP14 and TIMP2 and a decrease of MMP13 ($p < 0.01$) mRNA expression, when compared to untreated group (Zy), it expected that the higher MMP13 ($p < 0.001$) and TIMP2 ($p < 0.001$) tissue expression, observed in the infiltrate area by immunohistochemistry could be decreased, suggesting a matrix catabolism reduction in the near future, in consonance with the inflammatory process resolution. Lower MMP13 and MMP14 ($p < 0.01$) and higher TIMP2 mRNA expression ($p < 0.05$) observed in Zy + 30 Jcm^{-2} group could indicated the final stages of inflamatory process, corroborated by inflammatory area decreased. **Conclusion**: Taken together this results demonstrated that the LLLT could alter matrix metallopeptidase and metallopeptidase inhibitor expressions, changing the degenerative progress of rheumatic diseases. **Financial Support**: FAPEMIG / CNPQ.
Keywords: LLLT, matrix metallopeptidase, matrix metallopeptidase inhibitor, zymosan, inflammation.

CO.42

EFFECT OF LOW LEVEL LASER THERAPY IN NERVE REGENERATION AFTER TUBULIZATION TECHNICAL FILLED WITH FAT TISSUE.

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Introduction: Peripheral nerve injuries are common and can trigger the patient a large sensory and functional loss. To minimize this loss, surgical and postsurgical are studied. Thus various tubulization techniques using biological materials (vessels, nerves, vein, etc.) or non-organic (polyethylene tubes, silicon, etc.) with or without padding (adipose tissue, stem cells, etc.) are being tested. As a complementary therapeutic tool, the Low Level Laser Therapy has gained prominence for being a non-invasive treatment and have positive results in regeneration and functional recovery.
Objectives: To evaluate the effect of Low Level Laser Therapy in peripheral nerve injury repair after tubulization technique filled with fat tissue.
Methods: All the procedures adopted were approved by CEUA/USC: 4474300315. Were used 60 Wistar rats, males, with 80 days of life, supplied by the vivarium of University of the Sacred Heart, randomly divided into six groups of 10 animals each: Control group (CG), denervated group (GD), tubulization group (TG), tubulization group with fat tissue (GTG), tubulization and Laser group (GTL) and tubulization group filled with fat tissue and Laser (GTGL). The tubulization groups received jugular vein graft. Groups with fat denomination received filling with fat and the name received low level laser therapy treatment after tubulization surgery. The treatment was performed with Laser AsGaInP (XT Therapy), 808 nm, 100 mW, 0.04 cm^2 beam diameter, energy of 0.09 J (2.25 J/cm^2) with irradiation time 90 seconds during 3x/week for 150 days. Nerve morphometry 220 fibers per animal in each group was performed, and electrophysiological and functional tests (IFC). When comparing the groups, we used analysis of variance (ANOVA) followed by Tukey test, when detected significant difference. In all the significance level analysis was $p < 0.05$.
Results: The values of the functional indices presented by the groups were: GC -7,11 / GD -96.13 / -79.22 GT / GTG -53.23 / GTL -63.87 / -34.12 GTGL. In the morphometric analysis of the nerves average variable area of the fiber, axon area, fiber diameter, the diameter of the axon, the sheath area and thickness of the sheath were respectively: 57.16 / 18.24 / 7.72 / 4, 12 / 39.23 / 3.43 to the GC, 39.84 / 11.82 / 7.01 / 2.84 / 28.03 / 4.10 to GTGL, 32.13 / 7.29 / 6.01 / 2.44 / 24.11 / 3.22 to the GTG, 30.88 / 7.73 / 6.25 / 2.62 / 24.08 / 3.31 for GTL, 15.21 / 5.10 / 3.72 / 2.02 / 9.03 / 1.64 for the GT. In electrophysiological analysis we obtained the values of latency and amplitude respectively: 1.32 2 22.11 for the GC, 1.78 and 19.13 for GTGL, 1.85 and 17.37 for GTG, 1.83 and 17.82 for GTL, 1.94 and 14.64 for the GT, 0 and 10 for GD.
Conclusion: Low Level Laser Therapy acted favorably to enhance nerve regeneration, providing a morphological improvement

CO.43

EFFECT OF 660 NM VISIBLE RED LIGHT ON CELL PROLIFERATION AND VIABILITY IN DIABETIC MODELS IN VITRO UNDER STRESSED CONDITIONS

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Introduction: The treatment plan for non-healing ulcers worldwide remains critical in public health and clinical practice. Photobiomodulation (PBM) is able to improve the quality of life for diabetic patients with chronic ulcers. It is known to reduce pain and inflammation through various biological processes, decreasing numerous secondary complications as well as improve wound healing. This study evaluated the effect of visible red light (660 nm) on cell proliferation and viability in various diabetic models in vitro. Method: Normal (N; unstressed), diabetic wounded (DW; stressed), hypoxic wounded (HW; stressed) and diabetic hypoxic wounded (DHW; stressed) models were created with human skin fibroblast cells (WS1, ATCC CRL-1502; Ethics clearance number, HDC01-13-2014). A diabetic condition was induced by adding 17 mM/L of glucose to the basal concentration, and a hypoxic condition by maintaining the cells under anaerobic condition for 4 h. To achieve a wounded model, a 1 mL sterile pipette was used to create a central scratch and incubated for 30 min before irradiation followed by irradiation with a continuous wave diode laser (wavelength 660 nm (Fremont, California, USA, RGBlase, TECIRL-70G-650SMA); fluence 5 J/cm2; power output 102 mW; spot size 9.1 cm2; power output density 11.23 mW/cm2; duration of irradiation 7 min 25 s). Cells that were not irradiated were used as controls. Irradiated and non-irradiated cells were incubated for 48 h, then analysed using flow cytometry to evaluate the cell proliferation and cycle, and viability with Trypan blue staining. Each experiment was performed four or six times (n=4 or 6) between passage 9 and passage 15. Data was generated and analysed using the BD Accuri C6 software, and paired t-test and one way Anova (Sigma Plot 13). Results: Statistical analysis showed a significant increase in the cell viability in N- ($p= 0.001$); HW- ($p=0.01$) and DHW-cells ($p=0.05$) as compared to their controls. HW-cells showed a significant increase in the S-phase ($p<0.001$), G1/G2 ($p=0.003$) and decrease in G2M phase ($p<0.001$). Also HDW in the S-phase ($P<0.001$) and decrease in G2M phase ($p=0.044$) as compared with non-irradiated control cells. Significant differences were also noticed within groups (ANOVA, $P<0.001$). Significantly low values were especially seen in HW- and HDW-cells. Conclusion:
Although stress affects the cell density, the hypoxic and hypoxic diabetic models responded positively to PBM although the DW-cell model responded better. This study concludes that PBM does not damage stressed cells but have a stimulatory effect on cell viability and proliferation to promote repair and wound healing.

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CO.44

Effects evaluation of Methylene Blue photoactivated on Experimental Model of Walker Tumor 256

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PDT is considered as a new minimally invasive therapeutic modality for the treatment localized and selective destruction of various types of cancers and malignant disorders. This therapy is based on the selective uptake and retention of the photosensitizing agent into target sites which in turn undergoes activation by irradiation of an external source of light leading to the generation of cytotoxic effects by inducing tumor cell death. Methylene blue (MB) is a dye which belongs to the class of phenothiazine dyes and great interest due to their electrochemical properties in the face of NADH4, which is a coenzyme of the group of dehydrogenases Moreover, the same have been shown to be a potent agent photosensitizer with excellent photochemical properties due to having high yield generation of $^1O_2$ and ROS in the presence of reduced agents. Due to its photodynamic - phototoxic properties and show high affinity for mitochondrial membrane, MB have been highlighted as an effective photosensitizer of great potential for medical application as a therapeutic agent in PDT. Main objective of this study was to evaluate the effects of MB-mediated PDT may trigger inflammatory processes interfering with development and tumor progression using an experimental model of the Walker 256 tumor. Thus investigate the regulation and expression of inflammatory pro mediators such as IL-1 β, IL-6, IL-10, TNF- α and prostanoids (PGE_2) as well as gene expression of COX-1 and COX - 2, iNOS, eNOS by RT-PCR, lipid peroxidation by TBARS method and presence of neutrophils by MPO activity in the solid tumor mass. Furthermore, we checked the peri - and intra - tumor morphological changes using histological analysis by HE coloration. Our results showed that treated group with 0.1 % MB + 1J provoked an extremely significant increase, which was statistically different when compared for the different treated groups and the control on the levels of pro inflammatory mediators IL - 1β, IL - 6, IL – 10, TNF – α, PGE_2 as well as the quantification of gene expression of COX - 1, COX - 2, iNOS, lipid peroxidation and MPO activity. Histological analysis also complemented with previous results , indicating that the group treated with 0.1 % MB+ 1J can observe morphological changes represented by large areas of necrosis in the mass tumor solid with presence of lymphocytes. Based on our results we can conclude that treatment with 0.1% MB + 1J was able to generate cytotoxic effects by increasing ROS which consequently increasing the expression of inflammatory mediators, promoting inflammation triggering damage and death cell. Due to this fact we can see that there is evidence of a tumoricidal activity by lymphocyte presence in the mass tumor solid suggesting an anti-tumor immune response and thus promoting the long-term control of growth and progression tumor.

CO.45

The Potential of Photodynamic Therapy to Eradicate Cervical Cancer and Cervical Cancer Stem Cells

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Background: Cervical cancer is the formation of a malignant neoplasm arising from cells originating in the cervix uteri. Current treatments for cervical cancer include; surgery, radiotherapy, chemotherapy, and anti-angiogenic agents. However, despite the various treatments utilized its disease burden remains a global issue. Previous works have demonstrated that cancer stem cells are responsible for the high rate of chemoresistance and tumour relapse, and in the case of cervical cancer patients cancer stem cell markers have been associated with poor prognosis of patients. Cancer stem cells are a population of self-replicating cells within a tumor mass and are thought to be responsible for tumor initiation, progression and recurrence. Cancer stem cells share several characteristics with that of normal stem cells in that they have an unlimited capacity for self-renewal, the ability to differentiate into several cell lineages and intrinsic resistance against cytotoxic therapies. Cancer stem cells have also been linked to the metastatic potential of cancers. The lack of effective treatment for the illumination of cancer stem cells has brought light on the need for new therapeutic approaches that take into account the special properties of cancer stem cells. Photodynamic therapy (PDT) is an encouraging therapeutic procedure that involves a two-step process of administration of a photosensitizing agent, followed by drug activation with non-thermal light of a specific wavelength. The subsequent oxidative burst, results in cytotoxic toxic effects that may kill tumor cells sparing the healthy ones. PDT has been used to successfully treat and manage a variety of tumors, but there is little data on the effects of PDT on the elimination of cervical cancer and cancer stem cells. **Objectives:** This project aimed to determine the effect of PDT using a Sulphonated Zinc phthalocyanine (ZnPcS) on cervical cancer cells (HeLa) and cervical cancer stem cells. **Methods:** Cervical cancer cells were cultured in vitro and treated with ZnPcS at concentrations of 0, 0.25, 0.5, and 1 uM at fluencies of 0, 2, 4, and 8 J/cm^2 using a 680 nm diode laser(spot size 9.1 cm2; power output 52 mW; Power density 5.73 mW/cm2). Subcellular localization was determined by fluorescent microscopy, and the cellular effects were determined by assessing viability (trypan blue staining), proliferation (Adenosine triphosphate, luminescence assay), and toxicity (Lactate Dehydrogenase). **Results:** The preliminary results from this study showed promising effects using a wavelength of 680nm at a fluence of 2 J/cm^2 in the treatment of cervical cancer cells. **Conclusion:** Based on the results from this study PDT using ZnPcS shows promise as a treatment modality for cervical cancer, and the results will be used to further investigate its effects on cervical cancer stem cells. **Financial support:** The project funding provided by the University of Johannesburg and the NRF SARChI fund.

CO.46

Antimicrobial Photodynamic therapy in the treatment of osteoradionecrosis - case report

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Osteoradionecrosis of jaw (ORNJ) is a severe side effect of radiation treatment. ORNJ physiopathology is based on low vascularization of irradiated bone and decrease the bone's ability to resist trauma and infection. Hyperbaric oxygen therapy is current ORNJ's treatment choice but it's expensive and require many sections. Antimicrobial Photodynamic therapy (aPDT) has demonstrated efficacy in situations where conventional antibiotic therapies can be challenged. Further, the anti-inflammatory and healing effects of low level laser therapy (LLLT) can also be observed when aPDT is performed. Thus, the objective of this study is to report a case of osteoradionecrosis treated with PDT. Male patient with 45 years treated oral cancer (localized in retromolar trigone) with 45 gy of radiation therapy and developed ORNJ. The technique of aPDT was performed as follows: application of the photosensitizer methylene blue 0.01% for 5 minutes followed by irradiation with red Diode laser (660nm), 100mW, 0,028cm2 spot size and 9 joules of energy per point (1min and 30 sec of irradiation time). After the aPDT, the area was washed with physiological saline. Necrotic bone was sent off and the tissue healed after 5 sessions of aPDT. The aPDT technique was effective to treatment osteoradionecrosis with good patient acceptance and no pain or discomfort during treatment.

CO.47

Effect of low-level laser therapy on bone metabolism and root resorption during tooth movement in rats

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The purpose of this study was to evaluate the effects of low-level laser therapy (LLLT) on the process of bone remodeling and root resorption, searching to correlate metabolic changes observed at cellular level in the initial days of tooth movement to tissue changes observed microscopically and both architecture and morphology of trabecular and cortical bone. Tooth movement was induced in upper first molars of sixty-eight male Wistar rats and divided into 2 groups: non-irradiated and LLLT (laser parameter of 810 nm wavelength, 100 mW power laser, 0.02cm^2 area, energy of 1.5J/point) and euthanized on days 3, 6, 9, 14 and 21. A negative control group (no movement) was also evaluated. Measurements of tooth movement and histomorphometric analysis were performed at all days. Immunohistochemistry analysis of RANKL, OPG and TRAP markers and scanning electron microscopy (SEM) were made on days 3, 6 and 9. Microcomputed tomography (MicroCT) scanning was performed on days 14 and 21. The results showed significantly greater tooth movement in the irradiated group (increasing 40% in average). Compression side showed higher expression of RANKL and TRAP-positive osteoclasts on days 3, 6 and 9, promoting significant bone resorption and decrease of alveolar bone area on days 6, 9 and 14, and leading to microstructural changes such as reduction of bone volume/total volume and bone mineral density at 14 days. On the tension side, there was an increase in expression of OPG after 9 days, a significant increase in alveolar bone area on days 14 and 21 and increase in bone mineral density and trabecular thickness after 21 days. Results of hyalinized areas at the periodontal ligamento showed significant reduction on days 3, 6 and 9 in irradiated groups, which explains the less odontoclasts on the root surface and a significant reduction of areas of root resorption observed in histology and by SEM images. Irradiated groups also showed less volume of root resorption lacunaes measured by MicroCT especially in the compression side. The study concluded that LLLT had an effect on bone remodeling, increasing osteoclast activity on the compression side, and stimulating bone formation in tension side, resulting in significant tooth movement acceleration and potentially reducing the areas of necrosis in the periodontal ligament and consequently the root resorption process.

CO.48

Hydrogen peroxide followed by methylene blue photodynamic inactivation for endodontic disinfection – a new protocol for use in Endodontics

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Introduction: Antimicrobial photodynamic inactivation (aPDI) is a controversial approach for endodontic disinfection. The aim of this study was to test: photosensitizer (PS) concentration, use of hydrogen peroxide, assessment of optical shielding phenomenon, and minimal energy irradiation to optimize endodontic aPDI in order to suggest a protocol for clinical use. **Methods:** Aqueous solutions of methylene blue (MB) at 50, 100, 150 and 300µM were tested *in vitro* for reactive oxygen species (ROS) production by reduction of RNO absorbance at 440 nm when irradiated with a diode laser (660nm). Ten single-rooted teeth were inoculated with bioluminescent bacteria, *P. aeruginosa* for 72h to form biofilms. Bioluminescence imaging was used to serially evaluate the minimum energy necessary during endodontic aPDI, using MB and a diode laser coupled to an optical fiber, for intracanal microbial reduction. In addition teeth infected with *E. faecalis* were treated with sequential combinations of endodontic aPDI and H_2O_2 and CFU determined. **Results:** ROS production was inversely proportional to MB concentration in solution due to quenching of the MB. Optical shielding limited light penetration at high MB concentrations. The use of H_2O_2 before aPDI achieved higher disinfection than conventional aPDI or when MB was irradiated in a H_2O_2 solution. Energy irradiation of 9.6J achieved a significant reduction and further light delivery did not produce a further reduction. **Conclusions:** In conclusion, PS concentration about 50 µM, biofilm pre-treatment with H_2O_2 for 1 minute and energy irradiation around 10J seems to be an effective protocol for endodontic aPDI.

CO.49

LASER STIMULATED STEM CELLS TREATMENT POST-MYOCARDIAL INFARCTION IN HUMANS: SAFETY AND FEASIBILITY STUDY

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Objective:Determine the long term safety and possible feasibility of low-level laser therapy (LLLT) application to the bone marrow (BM)on patients post acute myocardial infarction (AMI).
Background: The promising field of cell-based therapy offers a supplementary mode of treatment to patients post acute myocardial infarction (AMI). LLLT have been found to have a photobiostimulatory effect on various biological processes.
Methods: Patient suffers acute ST segment elevation MI and candidate to primary percutaneous coronary intervention (PPCI) were included. In the active group, LLLT was applied to the tibia bone for 100 sec non-invasively prior to PPCI, 24 and 72 hrs post-PPCI. The control group had the same protocol, but the LLLT source was powered-off. Blood samples were taken on admission and during first week post MI. Echocardiography performed 1, 20 and 270 days after PPCI post MI.
Results: Twenty two patients were enrolled. Levels of CPK accumulation (area under curve up to 5 days post AMI) were202±75 (arbitrary units) for the laser group with significant statistical trend ($P<0.09$) to be lower than the value(302±53) in the placebo group indicating possible cardioprotection of the ischemic heart by laser treatment to the BM. Leukocytes count were 11200±3200 on admission and 9600±4300 at 72 hours post AMI in the active group and 12300±4300 on admission and 9400±4700 at 72 hours for the placebo group. Platelets count also showed no change during the first week post-MI .The door-to-balloon time was 59±12 min in the laser group compared with 61±9 in the control group. No adverse effects were observed in the laser treated patients.
Conclusion: Applying LLLT to the BM in order to photobiostimulate stem cells for the benefit of the infarcted heart is a safe procedure for application in humans, and offers a novel approach in cell therapy adjunctive to the PPCI.

CO.50

PHOTOBIOMODULATION EFFECT IN SPONTANEOUSLY HYPERTENSIVE RATS AND HUMANS: HEMODYNAMIC RESPONSE AND OXIDATIVE STRESS

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Introduction: Due to the global population growth and senescence, systemic arterial hypertension (SAH) has grown to over 1,2 billion in the last decade. Experimental studies *in vivo* and in humans have shown autonomic dysfunction of the Autonomic Nervous System. Several reports reveal the effective Low Level Laser Therapy (LLLT) response to control inflammation and oxidative stress that are the cause of SAH. **Objective**: It was to evaluate the hemodynamic response in hypertensive and normotensive humans and in spontaneously hypertensive rats (SHR). **Methods**: The study project was approved by The Ethics Committee in The Use of Animals(CEUA) of the University Nove de Julho, protocol 0016/2013. **Phase 1-animals**: 16 SHR were divided into 2 groups, Sham and Laser. It was employed a Diode Laser CW (λ-780nm,Power=40mW,Time=90s,Fluency=30J/cm^2) during 7 weeks, a total of 21 applications. The animals were cannulated for hemodynamic evaluation, further analysis of cardiovascular autonomic modulation. **Phase 2-normotensive and hypertensive individuals**:16 individuals were divided into 2 groups:Hypertension G.(n=8) and Normotensive G.(n=8), were evaluated by HDI/PulseWave Sensor(USA) fitted over the radial artery. Heart Rate (HR), Systolic Blood Pressure (SBP), Cardiac Index(CI) and Systemic Vascular Resistance(SVR) were assessed before and after LLLT application. It was also employed Diode Laser CW (λ-780nm,Power=50mW,Time=30s,Fluency=38J/cm^2,Energy=1.5J/pt) in 6 points on the sublingual vessels in a single application. All individuals were their own placebo. Significance level was established as p<0,05. **Results: Phase 1-** Hemodynamic evaluation, the laser group showed statistically significant differences in Mean Arterial Pressure(MAP -169 ± 4* mmHg vs. 182 ± 4 mmHg Sham Group), Diastolic Blood Pressure(DBP- 143 ± 4 mmHg* vs. 157 ± 3 mmHg Sham Group),HR (312 ± 14 bpm vs. 361 ± 13 bpm Sham Group). Autonomic assessment, the Alpha Index denoted a significant increase in the response in the laser group (1.07 ± 0.23* vs 0.45 ± 0.20 ms/mmHg Sham). Another factor that improved baroreflex sensitivity was VAR-SAP (49.55 ± 15.94* vs 70.51 ± 13.55 mmHg² Sham) and DP-SAP(6.94 ± 1.21* vs 8.68 ± 1.11 mmHg Sham) which laser group was decreased, showing an improvement of the sensitivity of baroreceptor. SHR laser group, it found a reduction of carbonyl by evaluating the oxidative stress, as well as, an increased nitrite and superoxide dismutase. **Phase 2-** Statistically significant changes were observed and substantial improvement in cardiovascular parameters in hypertensive group pre and post LLLT(HR= 71bpm* vs 68,3bpm, SAP=147±15 mmHg* vs 140±18 mmHg, CI= 2,7* vs 2,9 and SVR=1641±143* vs 1552±143 dynes.s.cm-5). It was not identified any hemodynamic changes during all placebo and normotensive. **Conclusion:** In hypertensive individuals the results showed a decrease in HR, SAP, SRV and increasing CI, similar response to SHR (decrease of HR and MAP). Autonomic assessment showed protection of proteins, increased production of nitric oxide, improves the sensitivity of baroreceptor incurring vasodilation, thus reducing sympathetic activity in the heart and blood vessels. LLLT was able to modulate oxidative parameters and the hemodynamic response in both animals and humans.

CO.51

APOPTOSIS IS ONE OF ANTI-INFLAMMATORY MECHANISMS OF LOW-LEVEL LASER IRRADIATION IN INFLAMMATORY CELLS.

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Introduction: Several studies have indicated the positive clinical performance of low-level light therapy (LLLT) as a non-pharmacological strategy to anti-inflammatory, tissue repair and analgesia treatments, including arthritis. The articular inflammatory process begins with polimorphonuclear (PMN) cells influx into the synovial compartment, resulting in hyperplasia of the synovial membrane. PMN cells commonly present a very short half-life, but under inflammatory circumstances, the apoptosis is prevented by inflammatory signals, in which are able to intensify the degenerative processes.
Objective: This study aimed to investigate the anti-inflammatory effects of LLLT, using an inflammatory model zymosan-induced, and to describe whether apoptosis mechanisms could be involved in the inflammatory process resolution.
Methods: All experimental procedures were submitted and approved by the Animal Ethical Committee of the University of Juiz de Fora (protocol number 039/2014). An inflammatory process was induced in the talocrural and subtalar joints of C57BL/6 mice using a zymosan injection in the periarticular region. The animals were divided into 5 groups (n=6): control, zymosan, dexamethasone-treated, laser 3J/cm^2-treated and laser 30J/cm^2-treated. The laser conditions were: 830 nm (infrared), power 10mW, at 3 J/cm^2 and 30J/cm^2 and continuous emission mode. Irradiation was carried out for 4 consecutive days, starting 5 hours after induction. Twenty four hours after the last application of the laser, the animals were euthanized and their joints forwarded for analysis: (a) morphological analysis, (b) DNA fragmentation analysis and (c) expression analysis of genes encoding proteins related to apoptotic.
Results: The results showed an anti-inflammatory effect after LLLT 30 J/cm^2, increased of DNA fragmentation and gene expression of apoptosis proteins in PMN cells. On the other hand, Bcl2 gene expression and Bcl2 protein expression in tissue increased for the LLLT 3 J/cm^2 group more than 30 J/cm^2 group. The results demonstrated that the PMN cells apoptosis process is one of LLLT anti-inflammatory mechanisms, since LLLT could alter the balance between expression of pro-apoptotic and anti-apoptotic proteins. Furthermore, the apoptosis induction was only PMN cells, demonstrating to LLLT selectivity. A possible explanation for the greater and selective effects of LLLT on PMN cells could be associated with the presence of additional mechanism to ROS production, in consequence to NADPH oxidase activation present in the plasmatic membrane of these cells.
Conclusion: The LLLT was able to decrease of PMN cells in the inflammatory infiltrate through the induction of their apoptosis. The use of LLLT may be a non-pharmacological alternative for inflammatory joint disorders treatment.
Financial support: CNPq, FAPEMIG and FAPERJ.

CO.52

NEUROMUSCULAR BEHAVIOR OF BICEPS BRACHIAL SPASTIC MUSCLE IN POST-STROKE CHRONIC INDIVIDUALS AFTER TEN SESSIONS OF LOW-LEVEL LASER THERAPY (LLLT)

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Introduction: The epidemiological profile of the world population, where chronic degenerative diseases, such as the stroke, had a greater impact on people's lives, highlighted the need for inclusion of new clinical approaches in the treatment of those individuals. The stroke results in impairment of motor functions, including muscle weakness in limbs affected by spasticity, leading to peripheral fatigue and to a compromised functionality. Therefore, the search for resources that minimize the muscle damage caused by spasticity grows every day. The clinical use of Low-level laser therapy (LLLT) has provided advances in the treatment of muscle disorders and in the prevention of muscle fatigue. However, there has been a scarcity of research regarding the application of LLLT on the spathic muscle.
Objective: The aim of this study was to analyze the responses of the spastic biceps muscle of patients with chronic hemiparesis after of the application of LLLT.
Methodology: After Ethics Committee approval (CAAE 16381213.2.0000.5503), five healthy subjects composed the control group (CG) and 10 post-stroke chronic individuals participated in this study. The CG performed only one evaluation in isokinetic dynamometer associated with surface electromyography (EMG), in which the subjects carried out a single isometric contraction of elbow flexion for 60 seconds. Meanwhile, the individuals with stroke underwent to the same initial evaluation and then, they were randomized into 2 groups: LLLT group (LLLT-G) and placebo group (PG). LLLT-G (n=5) underwent to 10 sessions of LLLT (Diode laser, 100mW, 808nm, 5J, 50 seconds per point, 16 points) application in biceps brachial muscle. Then, they carried out the final evaluation. The PG (n=5) was submitted to 10 sessions of simulated LLLT and ended with the last evaluation. The overall result provided the RMS and MDF from EMG signal; and the peak of torque, RMS and time to onset fatigue obtained in the isokinetic dynamometer.
Results: The EMG RMS in CG was 63.3±17.3uV, while in LLLT-G it went from 29.9±17.5uV to 60±38.8uV (increased 100.6%) and in PG it went from 25.7±5.1uV to 29.0±11.5uV (increased 12.8%). EMG MDF in CG was 68.2±10.1uV, while LLLT-G went from 73.4±21.5uV to 863.8±19.9uV (increased 14.1%) and PG went from 78.2±10.9uV to 84.0±10.5uV (increased 7.7%). Regarding the torque, the peak in CG was 67.4±23N.m; LLLT-G was 16.5±4.2N.m before the application and 20.3±6.2N.m after ten applications (increased 23%), while PG went from 14.9±5.8N.m to 18.1±9.7N.m (increased 21.5%). Torque RMS was 51.4±16.4N.m in the CG; 10.9±3.5N.m in the LLLT-G before and 14.3±5.4N.m after ten applications (increased 31.2%) and it went from 10.6±5.3N.m to 13.3±9.3N.m in the PG (increased 25.5%). At last, the time to onset fatigue was 27.4±7.6 seconds in the CG; in the LLLT-G it was 15.4±3.8 seconds and 20.8±5.2 seconds after (increased 35.1%); in the PG the time to onset the fatigue went from 11.8±3.3 seconds to 15.4±4.7 seconds (increased 30.5%).
Conclusion: Our results suggest that the application of LLLT was able to increased recruitment of muscle fibers and, hence, to increased onset time of the spastic muscle fatigue, increasing strength and improving spastic muscle performance. Consequently, the inclusion of LLLT in the clinical practice of those individuals may minimize the damage caused by spasticity in chronic post-stroke patients.
Financial support: Coordenação de Aperfeiçoamento Pessoal de Nível Superior - CAPES (PE 030/2008).

CO.53

Different laser wavelengths lesions in intervertebral discs: volumetric evaluation by magnetic resonance.
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Background and Objectives: In a previous report we found out that the 1908nm laser wavelength performs better than the 808, 980 and 1470nm wavelengths for intervertebral disc ablation. However, this experiment was made in defrosted specimens. As the wavelength is an important parameter for water absorption, this study was performed to investigate the action of the laser emission in the near infrared (808nm to 1908nm) region in the context of surgical procedures for percutaneous intervertebral disc decompression (nucleotomy) analyzing the volume of the vaporized tissue through magnetic ressonance.

Study design/Materials and Methods: This study was approved by the Ethics Committee of the Federal University of São Paulo (protocol #4530240314). Twenty intervertebral discs from pigs' lumbar spines were irradiated with a diode laser (= 808, 980, 1470 and 1908nm), one-second on/off time cycles, for 120 cycles and 5W of power with total energy of 1200J, and subjected to magnetic resonance in order to measure the ablation lesions. Five other discs were not irradiated and worked as controls. All evaluations were made in a Magneton Skyra 3T machine (Siemens Medical Solutions, Erlangen, Germany) with a gradient system of 45mT/m, a 16 channels fist coil with 16 combined amplifiers. Volumetric axial slices were taken as follows: T1 measures used a inversion / echo pin turbo recovery: TR/TE/TI = 2,400/15/100, 300, 700, 1.000, 1.400, 1.900 milliseconds, FOV = 113 x 200mm^2; matrix size = 188 x 256; 5 cuts; slice thickness = 3 mm; courteous- interval between two concatenations; = 190Hz bandwidth/pixel. T2 measures used the sequence CPMG TR/TE = 1,400/11, 22, 33, 44, 55, 66, 77, 88, 99, 110, 121, 132, 143, 154, 165, 176, 187, 198, 209, 220 milliseconds; FOV = 112 x 220mm^2; matrix size = 108 x 192; 5 cuts; slice thickness = 4 mm; no interval between cuts; bandwidth = 177Hz/pixel. All data were analyzed by Osirix® software and volumes were calculated semi automatically. All data were taken before and after laser irradiation. Statistical analysis was performed through Kruskal-Wallis, Jonckheere-Terpstra and Mann-Whitney tests.

Results: The difference in the volume of the lesions measured (in mm^3) 0.11±0.515, 3.20±3.439, 5.46±5.501, 6.91±4.690 and 5.58±4.709 for the control, 808, 980, 1470 and 1908 groups respectively. The difference between all groups and the control group was statistically significant ($p<0.05$).

Conclusion: There are no differences in the laser ablation volume at the wavelengths studied, but the 1908nm laser causes less tissue carbonization as seen previously. Therefore, damage caused by the laser at 1908nm is less aggressive than for other wavelengths.

Key words: intervertebral disc, nucleotomy, percutaneous discectomy, laser surgery

CO.54

The Dark Proteome: Illumination by Light

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Introduction: Photobiomodulation (PBM) research has advanced exponentially in the last ten years, with many molecular signalling pathways including signal transduction pathways having been identified. The mechanisms are very complex but despite this there has been a tendency in the past to identify individual pathways such as cytochrome C oxidase, signal transduction by NO and ATP (purigenic signalling) and ion channel conformation as being singularly important. The reality is that PBM is an extremely complex set of interactions, which necessarily requires a systems biology approach to further understand the mechanisms involved. The understanding of the mechanisms of PBM should explain not only the local effect, but also the bystander and indirect/abscopal events and their temporal summation, which results in the systemic and homeostatic effects of PBM.

The importance of these pathways is in the treatment of chronic pain, in particular cervicogenic headache (CGH), which a debilitating problem affecting 19% of the chronic headache population. 25% of CGH sufferers do not respond to traditional treatments, such as medical intervention and physiotherapy and there are no predictors of non-responsiveness. With PBM, 80% of these non-responders show significant improvement, which is similar to other neck pain trials. In a familial study of CGH, the pathophysiology of an ion channelopathy was provisionally identified, suggesting a PBM effect on ion channels. The treatment of the neural sensitivity (channelopathy) of CGH patients, which involves dysregulation of the neural membrane, requires an approach that includes the conformational change of the proteins that make up the small K^+ leak channels, in order to restore neural homeostasis.

The dark proteome is a term coined to describe the increasing numbers of proteins that do not have the traditional well-defined tertiary 3D structure, but instead are intrinsically disordered proteins (IDP) or have intrinsically disordered regions (IDPR) of the protein. These types of proteins appear to be key to cellular regulation, most probably including regulation by PBM, and key components of protein-to-protein interactions, which form an interactome, predicating the functioning of the neural network. These organisational complexes in turn affect the cell membrane, tissue and organism homeostasis (and hormesis).

Objective: To examine the literature to identify proteins modulated by PBM (either directly or indirectly) and to determine whether these proteins were also IDP/IDPR.

Methods: A search of the Database of Protein Disorder (**http://www.disprot.org/)** was conducted in order to assess the degree of intrinsic disorder in proteins that had been identified as being modulated by PBM.

Results: Proteins identified as being modulated by PBM that were also IDP/IDPR included cytoskeleton proteins: actin and □-tubulin, the enzyme tyrosine hydroxylase, genetic transcription factors CREB and p53, and channel proteins TRPV1 and small potassium leak channels (TREK and TRESK).

Conclusion: Many of the known protein targets of PBM were found to be IDP or have IDP regions. These include the channel membrane proteins, which are particularly important in chronic pain caused by channelopathies, which respond to PBM. It is also postulated that other IDPs may also be crucial in the molecular PBM pathways, including SUMO and PrP^C. This presentation will further discuss the effect of light on this Dark Proteome and will discuss its relevance on the treatment effects of PBM and particularly the potential for epigenetic modification in CGH.

CO.55

ACTION OF RED LASER IN PHOTOAGING PREVENTION

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INTRODUCTION: Ultraviolet radiation (UVR), provenient from the sun, is the major etiologic agent of cutaneous photoaging. Cellular and structural alterations arise as consequence of association between UVR and chronologic aging (British Journal of Dermatology UK 157; 874, 2007). Different strategies are used to treat and prevent the photoaging. Low level laser therapy (LLLT) is widely indicated in its treatment and it has been used in different intensities in order to stimulate or inhibit cellular processes (Semin Cutan Med Surg 32; 41, 2013). LLLT cellular photobiomodulation is not yet completely clarified regarding its molecular effects and more studies about it are needed, which will elucidate these mechanisms and the intrinsic beneficial effects of this therapy, including in photoaging process. **OBJECTIVE**: Investigate the red wavelength laser (660nm) action in photoaging prevention. **METHODOLOGY**: The project was approved in UFSCar's ethics committee on animal experimentation (040/2014). Female hairless (HRS/J) mice (n=21), approximately 8 weeks old, were randomly divided in 3 experimental groups: 7 animals that were not photoaged (CS); 7 photoaged animals (CFE) and 7 photoaged animals, which received treatment with red-wavelength laser (L). Groups CFE and L were subjected to photoaging process through administration of ultraviolet (UV) light suberythematosus doses, with chronic and cumulative exposure regime, without causing carcinogenic lesions. Experimental groups photoaging was induced by an incandescent light bulb - *Ultra-Vitalux 300W* (OSRAM). In this study, it was used as standard the following irradiances: 0,1 mW/cm^2 of UVB (280 to 315 nm), 0,8 mW/cm^2 of UVA (315 to 480 nm) and 3,89 mW/cm^2 of total irradiance (280 to 886 nm); there was a 70 cm distance between the light bulb and animal dorsum during irradiation sessions. Irradiated animals were exposed to 100 mJ/cm^2 (1 minimal erithematous dose = 100 mJ/cm^2) seven times a week, during first week, for 16 minutes per session; and then to 200 mJ/cm^2 three times a week, during 5 weeks, for 33 minutes per session, totalizing 6 weeks of photoexposure. Animals of group L were treated three times a week for 4 weeks (starting at third week of photoaging until the last one). Each treatment session with red laser was performed according to following parameters: Photon Lase III, DMC, wavelength 660 nm, 0.028 mm² beam area, 40 mW and 40 J/cm² fluency, for 28 seconds e 1.1 J/cm² per point (6 points). Two days after finishing the treatment, all animals were euthanized and dorsal skin of each animal was collected and subjected to histological processing and paraffin inclusion. Semi-serial slices, 5µm thick, were obtained through rotating microtome and treated with Hematoxylin Eosin (HE), Masson trichrome and Sirius Red techniques for structural analysis. **RESULTS**: In HE staining it was found a difference in thickness between groups; photoaged animals had thicker epidermis, which was preserved by LLLT action. We noticed the absence of skin appendages in photoaged group, which was also avoided by light application. Sirius Red and Masson trichrome staining confirmed collagen hypersecretion reaction in dermis and hypodermis, which was alleviated by phototherapy effects on the skin. **CONCLUSION**: LLLT was effective in prevention the deleterious effects of skin photoaging. **FINANCIAL SUPPORT**: FAPESP.

CO.56

LASERTHERAPY IN ROSACEA: PILOT STUDY

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Introduction: Rosacea is a disease that affects 45 million patients worldwide, with chronic progression, genetically family predisposition and recrudesces periods evolution. It is an incurable auto-immune inflammatory illness involving skin vessels and pilosebaceous unit, affecting mainly the patients' face. The symptoms are basically as reddening, burning, itching and pain, which lead to development of erythema and flushing that should be treated, so avoiding psychological and physical effects. This is a disease without spontaneous healing and with a serious progressive evolution. It is considered a real scourge to patients, many of them develop a situation of high anxiety, frustration, low self-esteem, depression and suicide attempts among other emotional and psychological problems. However, all these cases have been managed with topical and oral drugs, mainly tetracycline, nevertheless with several collateral effects. Not only stimulating factors of crisis should be avoided, although also aggravating outcomes and relapses, since they are caused by extreme physiological stress. **Objective:** To analyze the clinical response to Low Level Laser Therapy (LLLT) in rosacea patients. **Methodology:** Project approval - CAAE 51675115.9.0000.5511. Pilot study, transversal, controlled, with 5 adult rosacea patients with 3,5 y. [2-7 years] diagnosis, constant relapses and severe depression was reported. All medication a week before the Lasertherapy (LLLT) beginning was banned. Followed by questionnaires (illness perception and treatment), so pre and post Laser exams were collected. Patients under went 10 consecutive sessions weekly applying CW Diodo Laser (Twin-MMOptics®, Brazil), through the following parameters: λ= 660nm, P = 15mW, T = 10s, Fluence = 3,8J/cm², ϕ = 0,025cm², $\mathcal{E}_{pt}$= 0,15J/pt and $\mathcal{E}_T$ = 5,8 - 9,4J. The *facis* region pre and post LLLT was evaluated by measuring the cutaneous pH and temperature (digital thermometer). Follow-up examination for the local inflammation signs (erythema, itching and the lesions aspect of cutaneous lesions) were evaluated and photographic record were taken. **Results:** After LLLT 10 sessions all patients reported reduction of flushing, burning, itching and sensitivity of the facial skin, even 2 of them reported an improvement in the burning and itching in their eyes. Also, the temperature and pH on treated area confirmed the anti-inflammatory results encountered. All the larger telangiectasia was drastically faded. The satisfaction degree accessed in the questionnaires was raised by a significant improvement in their life quality reports. **Conclusion:** Lasertherapy in rosacea proved to be efficient technique, safe, without adverse effects and the most of all with cost-effectiveness.

PÔSTERES

PT.01

Low-level laser therapy and bone healing in diabetic rats: evaluation of bone response using a tibial defect experimental model

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Diabetes mellitus (DM) leads to a delay in bone healing. Thus, some therapeutic approaches have been used to accelerate the process of bone repair such as low-level laser therapy (LLLT). Therefore, the present study aimed to evaluate the effects of LLLT, in different fluences, in bone repair in an experimental model of tibial bone defects in diabetic rats. Sixty-four Wistar rats were submitted to a surgical procedure to perform bone defect and distributed in four groups: diabetic control group (DCG), diabetic laser group 30 J/cm^2 (L30), diabetic laser group 60 J/cm^2 (L60), and diabetic laser group 120 J/cm^2 (L120). A 808 nm Ga-Al-As (DMC Equipment, São Carlos, SP, Brazil) laser, 100 mW; 0.028 cm^2; 3.57 W/cm^2 ; 30, 60, and 120 J/cm^2; 0.84, 1.68, and 3.36 J; 8, 16, and 33 s was used. Animals were euthanized 15 and 30 days after the surgery. Histological, morphometric, immunohistochemistry, and biomechanical analyses were performed. In the histological and morphometric evaluation, all laser-treated groups showed a better histological pattern and a higher amount of newly formed bone compared to DCG. An intense RUNX2 immunoexpression was observed in the laser-treated groups, 15 days after the surgery. Receptor activator of nuclear factor κ-β ligand (RANK-L) immunohistochemistry analysis showed a significant decrease in the immunoreactivity for L30 and L120, 30 days after surgery. There was no statistical difference in the biomechanical analysis among the groups. In conclusion, LLLT, in all fluences used, showed an osteogenic potential in bone healing of diabetic rats.

PT.02

Photobiostimulation on elbow: Should we take into consideration the vascularization as a target?

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Background: Lateral epicondylalgia (LE), or tennis elbow, is a common condition, which may cause significant time away from work and sport and it is known as pain over the lateral epicondyle and point tenderness over the extensor muscle origin. Laser photobiomodulation has been used with the aim to improve tissue repair and decrease the pain in patients with LE. **Objective:** Investigate further hypovascularity at the lateral elbow and if the low level laser therapy when delivered to the main vascular points may increase elbow blood flux at the site of application of LLLT. **Methods:** This study was divided in two phases: For the first phase, ten volunteers were recruited with the aim to assess the elbow blood flux at six points, by laser Doppler, selected for their potential pathoanatomical contribution to LE. For the second phase, eight volunteers were recruited with the aim to assess the elbow blood flux assessment during low-level laser photobiostimulation. The laser device used was a 904 nm (GaAs), average power output 90 mW with dose of 4J (42s). We performed the follows measurements periods: pre-placebo and placebo, pre-LLLT and during LLLT. For the first phase analysis was performed using One-way ANOVA and for the second phase using repeated measures ANOVA. The post-hoc Tukey test was used to find the difference. **Results:** For phase one blood flux assessment of the six points at the elbow the highest blood flux means were at P1 (lateral supracondylar ridge) and P5 (lateral epicondyle). For phase two, there was no change in blood flux in the pre-placebo, placebo or pre-LLLT and there was a significant difference between pre-placebo, placebo or pre-LLLT measurement periods when compared to photobiostimulation period ($p<0.05$). **Conclusion:** Selective zones of elbow vascularity are influenced with photobiostimulation. Such zones (in particular P1 as used in this study) may be used for more effective targeting of LLLT application in LE.

PT.03

Effects of Low Intensity Lasertherapy on Miniscrew healing and bone neoformation

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Introdutionc: The success rate of miniscrews (MI) when used as temporary orthodontic anchorage is relatively high, but some factors, such as inflammation could affect its healing process and clinical success. Low Intensity Laser Therapy has been widely used for biostimulation of tissue and wound healing specially for its anti-inflammatory effects.
Objetives: The purpose of this study was to evaluate the effect of low intensity laser therapy over the miniscrew bone healing process.
Methods: 24 wistar rats received mini-screws on palate in maxilla. All the MI were immediately loaded with 50 gf with a NITi coin-spring connected to the first molar. The laser group (n=12) were irradiated with a 808nm diode laser with 100 mWs for 20s (Energy = 2 J) The control group (n=12) received the MI, but no irradiation. The animals were sacrificed at 5, 7, 9 and 14 days after MI installation. Samples were collected from the tissue around MI and histologically evaluated and submitted to Fractal dimension analysis (FD).
Results: The clinical results showed a success rate of 100% for both groups. The histological analysis and FD analysis demonstrated that the laser group had less inflammatory cells than control group and the bone neoformation around mini-screw was more intense.
Conclusion: Low intensity laser therapy increased the success rate of orthodontic miniscrews, probably due to anti-inflammatory effect and bone turnover stimulation.

PT.04

PHOTOTHERAPY IN PAIN, FUNCTIONALITY AND QUALITY OF LIFE ON OSTEOARTHRITIS OF THE KNEE IN OLDER ADULTS: A PILOT STUDY

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Introduction: Population aging is related to the increase of chronic degenerative diseases, including, osteoarthritis (OA). The OA is a degenerative chronic disease characterized by the gradual loss of cartilage affects about 50% of people over 65 and affects approximately 4% of the Brazilian population, its manifestation is more common in the knee and its progression can cause pain, swelling, stiffness, decreased range of motion (ROM) and physical disability. In this context, resources such as phototherapy has shown promising results, stand out as a therapeutic resource known for its anti-inflammatory effects and regeneration of biological tissues.
Objective: To analyze the influence of phototherapy on pain, functionality, mobility and quality of life in older adults with knee osteoarthritis.
Methods: This project was approved by the Ethics Committee in Human Beings of the Federal University of São Paulo / Hospital Sao Paulo (UNIFESP) - Number: 1.368.478. Were evaluated 6 individuals (both sexes) with knee OA that were allocated into 2 groups: control group (CG n= 2) without any intervention and Laser Group (GL, n=4) treatment with irradiation only. The interventions were performed 2x/week for 6 weeks. Phototherapy Cluster type (850nm, 100mW, 4J by point, totaling 28J of 7 diodes) was applied to each session in the lateral and medial side of both knees. For evaluation and re-evaluation, questionnaires were applied as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lequesne, visual analog scale of pain (VAS), Timed Up and Go (TUG), Short Physical Performance Battery (SPPB) and the test six-minute walk (6MWT). For statistical analysis we used t test followed of Mann Whitney test for analysis for intra and inter-group with a significance level of $p < 0.05$.
Results: The results obtained affirm that there was no significant difference in the anthropometric variables: height (1.62m ± 8.76), weight (70kg ± 7.25), age (65.6 years ± 7.22) and BMI (26,8kg / m ± 2.42) between groups. Furthermore, it can be seen that there was no significant intergroup difference VAS (p=0,133); WOMAC - pain (p= 0,2); stiffness (p =0,06); physical function (p= 0,133); Lequesne (p=0,133); TUG (p=0.8); SPPB (p=0,4) and 6MWT (p=0,4) and intragroup compared to VAS (p =0,4); WOMAC - pain (p=0,08); WOMAC - stiffness (p=0,2); WOMAC - physical function (p=0,114); Lequesne (p=0,22); TUG (p=0,48); SPPB (p=0,485); 6MWT (p=0,342).
Conclusion: Therefore, it is concluded that phototherapy was not effective for improving quality of life, functionality and reducing pain in this pilot study. The sample size was relatively small, so more volunteers will be analyzed to try to elucidate the effects of phototherapy older adults with knee OA.

Financial support: National Council of Technological and Scientific Development – CNPq.

PT.05

LOW LEVEL LASER THERAPY IN EXPERIMENTAL MODEL OF STRAIN-INDUCED SKELETAL MUSCLE INJURY IN RATS: EFFECTS ON INFLAMMATORY CYTOKINES AND MUSCLE HISTOLOGY

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Introduction: Muscle strains are among the most prevalent causes for athletes' absence from sports activities. The main objective of this study was to investigate the effects of low level laser therapy (LLLT) on controlled muscle strain in rats. **Method:** Wistar rats were anesthetized for controlled muscle strain induction (n=6). The experimental protocol was approved by the Animal Research and Care Committee of the University of Sao Paulo. A single LLLT session was performed 1 h after muscle trauma with an infrared laser unit (DMC®; Sao Carlos, Brazil). The laser emitted a continuous optical output of 100 mW with a wavelength of 810 nm to a spot size area of 0.028 cm^2, giving a power density of 3.57 W cm^2. The optical output was measured before, halfway through and after the experiment. Laser irradiation was performed in skin contact in the middle of anterior tibialis muscle on the belly with doses of 1 J (35.71 J cm^{-2}), 3 J (107.14 J cm^{-2}), 6 J (214.29 J cm^{-2}) and 9 J (321.43 J cm^{-2}) and corresponding irradiation times of 10, 30, 60, and 90 s, respectively. Outcomes: real time RT-PCR gene expression of COX-1, COX-2, TNF-α, IL-1, IL-6, IL-10 and muscle histology. **Results:** After experimental induction of muscle strain, LLLT was effective reducing pro-inflammatory and increase anti-inflammatory cytokines and improved structural organization of muscle tissue with well-defined cells and a minor amount of fragmented fibers. **Conclusion:** LLLT with 3 J significantly improved the inflammatory markers and muscle histology.

PT.06

LOW LEVEL LASER THERAPY IN MUSCLE REGENERATION AFTER TUBULIZATION TECHNIQUE FILLED WITH FAT TISSUE.

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Introduction: Peripheral nerves are constantly targets of traumatic injuries and rarely present recovery without surgical intervention when they have tissue loss. Thus various techniques tubing using biological materials (vessels, nerves, vein) or non-organic (polyethylene tubes, silicon) with or without padding (adipose tissue stem cells) are being tested. However, even with all technical refinement achieved by microsurgery, still do not get full motor recovery. In the clinical setting, some physical therapy resources have been suggested in an attempt to minimize losses from the denervation framework, highlighting the Low Level Laser Therapy.
Objectives: To evaluate the effect of Low Level Laser Therapy in muscle recovery after tubulization technique filled with fat tissue.
Methods: All the procedures adopted were approved by CEUA/USC: 8853300315. Were used 60 Wistar rats, males, with 80 days of life, supplied by the vivarium of University of the Sacred Heart, randomly divided into six groups of 10 animals each: Control group (CG), denervated group (GD), tubulization group (WG), tubulization group with fat tissue (GTG), tubulization and Laser group (GTL) and tubulization group filled with fat tissue and Laser (GTGL). The tubulization groups received jugular vein graft. Groups with fat denomination received filling with fat and the name received low level laser therapy treatment after tubulization surgery. The treatment was performed with Laser AsGaInP (XT Therapy), 808 nm, 100 mW, 0.04 cm^2 beam diameter, energy of 0.09 J (2.25 J/cm^2) with irradiation time 90 seconds during 3x/week for 150 days. Morphometry 220 muscle fibers per animal in each group was carried out, and electrophysiological and functional tests (IFC). When comparing the groups, we used analysis of variance (ANOVA) followed by Tukey test, when detected significant difference. In all the significance level analysis was $p < 0.05$.
Results: In the morphometric analysis groups presented respectively to the EDL, Soleus and TC the following results: 2998 um², 2872 um² and 4351 um² for GC, 2722 um², 2323 um² and 3852 um² for GTGL, 2377 um², 2086 um² 3184 um² for GTG, 2123 um², 1911 and 3051 um² um² for GTL, 1619 um², 1428 and 1582 um² um² for the GT 154 um², 123 and 172 um² um² for GD. In the strength test the GTGL group got the best rate when compared to GC, reaching to the CT muscle the value of 0.97 against 1.21 of the GC to the EDL muscle 0.71 against 0.87 of the GC and the Soleus 0.68 against 0.86 of the GC. The values of the functional indices presented by the groups were: GC -7,11 / GD -96.13 / -79.22 GT / GTG -53.23 / GTL -63.87 / -34.12 GTGL, where again the GTGL It showed the best result.
Conclusion: Low Level Laser Therapy was presented as a positive protocol for functional recovery of patients with peripheral nerve injury.

PT.07

LLLT actives MMP-2 and increases muscle mechanical resistance after nerve sciatic regeneration.

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Introduction: Traumatic lesions to peripheral nerves are frequent and require specialized care, which not always produce the desired effects, thus leading to functional loss of various degrees, but often incapacitating, with the target skeletal muscles strongly affected. Restoring nerve activity and consequently muscle function is a priority of any therapeutic intervention, including low level laser therapy (LLLT), a physical means with an alleged high level of positive response (over 80%), as indicated by the results of many investigations on this subject**. Objective:** To analyze the LLLT on metalloproteinase expression and the mechanical strength of skeletal muscle regeneration after peripheral nerve injury. **Materials and Methods:** Rats were subjected to crush injury of the right sciatic nerve, followed by LLLT (830nm, 35, 70, 140 and 280 J/cm^2) for 21 consecutive days. Functional gait analysis was performed weekly and the animals were euthanized at day 21 for collection of the gastrocnemius muscles, which were submitted to analysis of resistance, and the anterior tibialis, for evaluation of metalloproteinase-2 (MMP-2). The results were statistically analyzed at a significance level of 5%. **Results:** The sciatic functional index decreased significantly while the mechanical strength increased significantly in all groups during the weeks. For the activity of MMP-2 in the intermediate band it was observed higher values in groups 3 (35J/cm^2), 4 (70J/cm^2) and 5 (140J/cm^2) when compared to groups 1 (normal), 2 (lesion) and 6 (280J/cm^2); while at the activated band, the activity was significantly more intense in group 6 (280J/cm^2) when compared to group 1 (normal). **Conclusion:** These data suggest that LLLT 830nm low (35J/cm^2) moderate (70J/cm^2) and high (140 and 280J/cm^2) energy density accelerates neuromuscular recovery after crush injury of the sciatic nerve in rats.

Key-words: repair of peripheral nerves; laser therapy; denervated muscle; rehabilitation; functional gait analysis.

PT.08

Effects of infrared laser therapy on skeletal muscle repair in diabetic rats

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Diabetes mellitus (DM) leads to an impairment of the capacity of skeletal muscle to repair, representing a challenge for physical rehabilitation.The present study aimed to evaluate the in vivo response of infrared laser wavelength on skeletal muscle repairprocess in diabetic rats. This study was approved by the Ethical Committee of the Federal University of São Paulo (4626280414). Experimental groups: basal control – nondiabetic and muscle injured animals without treatment (BC); diabetic muscle injured without treatment (DC); diabetic muscle injured and infrared laser (DCIR). The injured region was irradiated daily for seven consecutive days, starting immediately after the injury. The histological results demonstrated that in treated group modulation of the inflammatory process and a better tissue organization located in the site of the injury and reduced the injured area and increased myoD and myogenin protein expression. Moreover, infrared light increased the expression of the pro-angiogenic vascular endothelial growth factor (VEGF) and reduced the cycloxygenase (COX-2) protein expression. These results suggest that LLLT was efficient in promoting skeletal muscle repair in diabetic rats by reducing the area of the injury and modulating the expression proteins related to the repair.

PT.09

The Effect of Low Level Laser Irradiation Assessment on the Pharmacokinetics of Topical Diclofenac Gel in Healthy Volunteers

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Introduction: musculoskeletal and joint inflammatory diseases are among the most prevalent causes of physical limitations today. About 60% of the population over 60 has some disease of this type, making continuous use of analgesics and anti-inflammatory drugs. However, the adverse effects of prolonged use of anti-inflammatory drugs are well known, and cause highly relevant and significant impact to the health system, including kidney failure, digestive bleeding, gastritis, ulcers and others. The low power lasers have been used for the treatment of inflammatory diseases, especially articular and tendon conditions. It is believed that it is possible the combination of pharmacological and laser therapies to improve the treatment of musculoskeletal disorders, in order to reduce costs and adverse effects and increasing the effectiveness. In this sense, the application of topical medications seems a viable alternative, with the exception of limitations on drug absorption and bioavailability in the inflammatory site. In view of the reported effects of microcirculation of vasodilation induced by irradiation with low-power laser, we aim to investigate the effects of radiation in the absorption of topical drugs, aimed at increasing plasma drug bioavailability.
Materials and Methods: The present study was approved by the Ethics Committee of the Human UNICAMP. It conducted an open, randomized, crossover, with two treatments, two periods, in which volunteers received in each distinct period, the reference formulation, with or without the application of laser therapy. The study was designed so as to allow to obtain pharmacokinetic parameters (AUC0-All and Cmax) for statistical comparison, aiming to investigate the topical drug plasma levels, with and without the application of laser radiation. Initially low power laser irradiation was performed in a cluster configuration contend 14 issuers, with 07 of wavelength 650 nm and 100 mW of power and 07 810 nm and 100 mW of power. Irradiation was performed for 30 seconds, for a total of 3 Joules of energy per application point. After 5 minutes the gel diclofenac was applied to the dorsal region of each individual. Successive blood samples were collected up to 24 hours after application, to establish a curve pharmacokinetics of diclofenac in plasma of volunteers. The determination of diclofenac concentrations in rat plasma were performed in the Analytical Unit Cartesius - Prof. Laboratory Dr. Gilberto De Nucci in the Department of Pharmacology of the ICB / USP, according to Method Validation of Analytical Protocol METGRU 08-07 - Determination of Diclofenac in human plasma by LC-MS / MS. **Results:** Preliminary results suggest the concept of verification in order that the laser application potentiated about 100% of the pharmacokinetic parameters of diclofenac applied topically to the skin of white healthy male volunteers. The ASCtudo ([ng * hr] / ml) control was 27.23 and passed the laser application 52.53 ([ng * hr] / ml). Cmax achieved was 3.23 (* ng / mL) and 6.1 (* ng / mL) for the laser group. **Conclusions:** Considering the risks inherent in the use of anti-inflammatory steroid (coxibs or nonselective) and the high incidence of osteo-articular diseases in the population, the combination of anti-inflammatory drugs used topically laser offers an interesting therapeutic option.

Keywords: Low power laser, diclofenac, LLLT, Clinical Study.
Funding Agency: CAPES; FAPESP.

PT.10

Structural aspects and oxidative stress on acute induced gouty arthritis in Wistar rats after low-level laser therapy.

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Introduction: Gouty arthritis is characterized to affect especially men of middle age, old-aged and women post menopause. It is responsible for joint degeneration caused by chronic inflammation and its treatment is based on non-steroidal anti-inflammatory drug (NSAIDs) and corticosteroids. Low-level laser therapy (LLLT) can be used like an alternative to medicines for being a non-invasive therapy with analgesic and anti-inflammatory effects and responsible for cell proliferation**. Objective:** The aim of this study was to evaluate the effects of low-level laser therapy on articular tissue structure and in lipid peroxidation by production of reactive species to thiobarbituric acid (TBARs) during the acute phase of experimental gouty arthritis in male Wistar rats. **Methods:** Twenty-four Wistar rats (200g±10g) were assigned into four groups (n=6): A (control), B (induced arthritis-IA), C (IA+laser 830nm) and D (IA+laser 670nm) (Ethics Committee n.048/2015). On time 0, animals from B, C e D were anesthetized with Ketamine/Xilazine/Tramal (10, 0,9 e 5,0 mg/Kg, respectively) and submitted to two intraarticular injections in the right knee by 2mg of $Ca_2P_2O_7$ dissolved in 50µL of saline sterile solution with 24-hour interval. After 48 hours, animals from C and D groups were submitted, respectively, to LLLT with a Gallium-Arsenide (GaAs) laser device (*Bioset*®) with λ=830nm,energy density=13,5J/cm², power=30mW, total energy=0,27J, beam area=0,02cm^2 for 9 seconds and a Indium-Gallium-Aluminum-Phosphorus (InGaAlP, *Bioset*®) λ=670nm, energy density=18J/cm^2, power=30mW, total energy=0,32J, beam area=0,02cm^2 for 12 seconds, both by contact method, point application in patellar region of right knee once a day. After 7 days of laser treatment, serum samples were collected by cardiac puncture for TBARs assays. It was calculate the reading values with 532nm=153.000M^{-1}/cm^{-1} of absorbance in a spectrophotometer. The right knees were histological processed and histopathological analysis realized with the following stain methods: Picrosirius red-Hematoxylin (tissue acidophilia), Toluidine Blue (tissue basophilia) and Hematoxylin and Eosin (qualitative structural analysis). Quantitative data were analyzed by ANOVA and Tukey *post-test* (p<0,05). **Results:** TBARs analysis showed no significant difference (p=0.424) between the mean values of the groups after 7 days: A (0.020, Standard Deviation [SD] ±0.004), B (0.017; SD±0.003), C (0.017; SD±0.003) and D (0.013; SD±0.006). The structural evaluation showed on induced groups when compared to A, independent of the laser therapy, reduction of basophilia in extracellular matrix of cartilage, associated to loss of the articular surface alignment and increased of surface layer thickness. There were no changes in acidophilia matrix, collagen fibers orientation and in morphological characteristics of isogenous groups. In synovial analysis of group A were not detected cellular or fibrous matrix changes. However, induced groups, regardless of the treatment, showed intense inflammatory infiltration, increased thickness of epithelium and dilatation of blood vessels immersed in the matrix. The matrix also shows thicker collagen fibers randomly distributed into the tissue. **Conclusion:** In the acute phase of experimental gouty arthritis, low-level laser therapy (830nm and 670nm) do not change the evolution of the histopathological aspects of the disease and is not able to induce systemic anti-oxidant response.

PT.11

Skin wound healing: effects of frequency and different parameters of Low Level Laser Therapy (LLLT)

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Some studies have shown that Low Level Laser Therapy (LLLT) was able to biomodulate the inflammatory response and to stimulate cellular proliferation, microcirculation and collagen synthesis. However, dosimetric aspects of LLLT, such as appropriate dose, frequency of irradiation and energy delivery mode are still poorly understood. Therefore, the aim of this study was to evaluate these dosimetric parameters of LLLT on the skin wound healing process in surgical wound performed in rats. For this, Wistar rats were divided into seven groups: control group (without irradiation); group L1 (irradiated with one point of 10 J/cm² on day 1 of experiment); L2 (irradiated with 5 points of 10 J/cm² on day 1 of experiment); L3 (irradiated with one point of 50 J/cm² on day 1 of experiment); L4 (irradiated with one point of 10 J/cm² on days 1,2,4,6 and 8 of experiment); L5 (irradiated with 5 points of 10 J/cm² on days 1,2,4,6 and 8 of experiment); L6 (irradiated with one point of 50 J/cm² on days 1,2,4,6 and 8 of experiment). Standardized 6 mm diameter wounds were created on the dorsal skin of rats and biopsied at 7 and 10 days post-wounding, for clinical, histological, immunohistochemical analysis. The laser used was the Photon Lase III, DMC®, λ 660nm, power of 40 mW, spot area of 0.028cm^2 and time irradiation was 10 s/point for the dose of 10 J/cm^2 and 35 s/point for the dose of 50J/cm^2. The ANOVA test showed at 7 days post-wounding that L2 and L5 groups presented a lower percentage of remaining wound, when compared with control group (8.40±6.7 and 6.9±5.7 vs. 22.48±13.7 %, respectively) ($p \leq 0.05$). Regarding histological analysis, at 7 days of the experiment, L1 and L5 groups showed less inflammatory infiltrate and L5 showed higher epithelial migration, when compared with control group (no statistical significance). However, this difference was not observed at 10 days post-wounding. The Kruskal-Wallis test showed at 10 days post-injury that L6 group showed a higher percentage of collagen than the control (52.2±14.0 vs. 25.64±9.0%) ($p \leq 0.05$). Finally, ANOVA test revealed that the percentage of cytokeratin 10 expression for L5 group was higher than the control group, after 10 days of experiment ($p \leq 0.05$). In conclusion, LLLT improved the skin wound healing, decreasing lesion area and increasing collagen formation, however, the frequency of irradiation as well as some laser parameters may interfere with tissue response. FAPESP Grant #2014/21214-1 and FAPESP #2015/24231-7

PT.12

Effects of Two Protocols of application of Low Level Laser Therap In Random Cutaneous Flap Viability in Rats

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Introduction: The skin flap is a technique frequently used in reconstructive plastic or tissue loss surgeries (Plast. Reconstr. Surg. 72, 766, 1983). This technique presents as main complication tissue ischaemia and can lead to flap necrosis (Acta Cir. Bras., 14, 33, 2000), causing longer hospital stays and even an increase in health spending. Researches indicate that low level laser therapy (LLLT) may be used as an effective therapeutic resource to reduce the area of necrosis, however, there is divergence on where LLLT should be applied on random cutaneous flap (Lasers Surg Med. 37, 77.; 2005; Photomedicine and Laser Surgery. 27, 411.; 2009). **Objective:** evaluate the effects of applying the same energy of LLLT (660nm) in two distinct regions of the random skin flap in rats. **Methods:** In this work, 18 (Rattus norvegicus: var. Albinus, Rodentia Mammalia) male Wistar aged about 3 months and body weight 296.39 g ± 26.86 g were used. These animals were randomly divided by draw, which used a sealed envelope, into 3 groups (n = 6): control group (GI), which received LLLT simulation treatment – the equipment was turned off; group that received treatment in 3 points (GII), whose animals received LLLT irradiation in three points, 4 J of energy per point (total of 12 J), for 84 seconds at each point with a 150 J/cm² fluency; and the group 12 points (GIII), whose animals received, in twelve points, LLLT irradiation, which had an energy of 1 J per point (total of 12 J), for 22 seconds at each point, and a fluency of 40 J/cm². The random cranial based skin flap was created with dimensions of 10x4 cm (Plast Reconstr. Surg. 35 .; 177, 1965) and a plastic barrier was placed between the flap and the donor site. The animals were irradiated with an Indium, Gallium, Aluminium, Phosphorus (InGaAlP) laser, 660 nm (Photon Lase III, DMC®, São Carlos, SP, Brazil) with 0.028 cm^2, with a power of 50mW, beam area in continuous emission mode. The treatment started immediately after the surgical procedure and it was reapplied every 24 hours, completing a total of 5 applications. The necrosis area was assessed on the seventh postoperative day with the paper template method (Plast. Reconstr. Surg. 65 .; 152, 1980). For statistical analysis, the Kolmogorof Smirnoff normality test was performed; for comparison between groups, we used the ANOVA-One Way test and for the presence of a significant difference ($p < 0.05$), we used the *post-hoc* Tukey. **Result:** we observed a statistically significant difference ($p = 0.004$) between the GI (63.84% ± 9.33) and GII (38.91% ± 10.75), in which it was observed GII animals showed better results than GI; there was also a statistically significant difference ($p = 0.027$) between GII and GIII (57.82% ± 13:21), in which GII animals showed a decreased necrosis area. There was no significant difference ($p = 0.631$) between the animals of GI and GIII groups. **Conclusion:** there is a difference at application points of LLLT, in order to increase the flap viability. The application of LLLT, with the same irradiation energy and located in the cranial base of the flap on fewer points, was more effective to increase the viability of random skin flap in rats.

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PT.13

Effects of the low level laser therapy associated with Biosilicate®/PLGA composites in tibial bone repair
Fernandes KR, Magri AMP, Kido H, Ueno F, Assis L, Renno ACM

Effects of the low level laser therapy associated with Biosilicate®/PLGA composites in tibial bone repair
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Introduction: Laser therapy has been showing osteogenic effects in *in vitro* and *in vivo* studies. Biosilicate® (BS)is a fully crystallized bioactive glass-ceramic and presenting excellent biocompatibility and osteoconductive properties [1]. To improve the performance of BS, some porogenicmaterials (Poly(D,L-lactic-co-glycolic) acid (PLGA) microparticles) can be introduced into BS to produce microporosity, and allow the passage of cells that are involved in bone repair. Thus the association of both treatments could be more effective to accelerate the process of tissue regeneration and promote bone repair. The aim of this study was to evaluate the *in vivo* biologicalperformance of the phototherapy at 808 nm associated to BS and BS/PLGA.
Material and Methods: For this experiment, 40 mature male Wistar rats were used (12 weeks, weight 300-350 g). All rats received one surgical procedure during the course of this experiment to induce the unilateral noncritical size bone defects (3 mm diameter) in tibia of all animals, which were filled with different composites. The animals were randomly divided into 4 groups (BS; BS + LLLT; BS/PLGA 80/20; BS/PLGA 80/20 + LLLT). Each group was divided into two subgroups, euthanized by carbon dioxide asphyxia after 2 weeks post-surgery (n=10 for each subgroup). Histopathological analyses were performed. This study was approved by the Animal Care Committee guidelines of the Federal University of São Paulo (2013/601498).
Results: Histopathological analysis showed that the introduction of PLGA into BS resulted in a higher material degradation, with the increase of newly formed bone ingrowth when compared to BS. However, the lasertherapy was not able to improve the bone repair in the period analyzed in this study.
Conclusion: Summarizing, the results of this study confirmed our hypothesis that the introduction of PLGA into BS improve the bone formation, constituting an alternative to be used in bone tissue engineering. However, when LLLT and biomaterial were used in association, no positive results were found to accelerate the bone repair.

PT.14

Low-level laser therapy effects on treatment of induced osteoporosis in Wistar rats

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Introduction: Osteoporosis is a disease characterized by decreased of bone mass associated with a bone microarchitecture breakdown, which results in an increased of fragility and risk of bone fracture. The treatment is based in nutritional supplementation, however the loss of bone mass is progressive. Low-level laser therapy has the ability to biostimulate a variety of tissues and cells, like osteoblasts promoting mitosis, DNA replication and protein synthesis in bone tissue, including in osteoporosis. **Objective:** The aim of this study was to evaluate the effects of low-level laser therapy regarding to morphological aspects in an induced model of osteoporosis in Wistar rats. **Methods:** After approval by the Ethics Committee of FHO-UNIARARAS protocol 102/2011, forty-five young females Wistar rats weighing 200g (± 10g) were assigned into three groups: G1 (control; n = 15), G2 (induced osteoporosis; n = 15) and G3 (induced osteoporosis and low-level laser therapy; n = 15). For induction of osteoporosis, groups 2 and 3 were subjected to intramuscular injection of dexamethasone (7mg/kg), once a week, for 5 weeks, while G1 was subjected to sterile saline injections. After induction process, G3 has received low-level laser therapy sessions by a Gallium-Arsenide laser device (Bioset®, Rio Claro, Brazil) with λ=830nm, energy density=13,5J/cm², power=30mW, total energy=0,27J, beam area=0,02cm^2 for 9 seconds in the medial femoral muscle by contact point technique, daily, for up to three weeks. Animals of G2 underwent sessions with the laser device off. Samples were collected 7, 14, 21 days after the start of treatment (n=5, for each period). Euthanasia was performed with anesthetic overdose with Ketamine 10%. The femurs were removed and submitted to histological processing, stained with hematoxylin and eosin for morphometric analysis of the femoral thickness, in micrometer (µM), using Image J software (NIH). Statistical analysis was performed by ANOVA and post-test of Tukey with a significance level of 5% ($p<0.05$) using Prism 5.0 program. **Results:** There were no significant differences in the analysis of femurs thickness between the groups of 7 days ($p=0.3026$). In the 14-day group, significant differences were found in femurs thickness between G2 (median 419,7; standard deviation [SD]±188,2) and G3 (median 752,1; SD±137.7) and p=0,0260. The analysis of the 21-day group showed differences between the groups G1 (median 750,0; SD±128,1) and G3 (median 830,2; SD±50,75) with p=0,0079 and between G2 (median 610,1; SD±70,10) and G3 (mean 830,2; SD±50,75) with p = 0,0031. **Conclusion:** Low-level laser therapy showed to be effective in the treatment of induced osteoporosis particularly in 14 and 21 days of treatment. There was an increase in femoral thickness of animals treated with laser compared to the control and untreated groups, which bring us that low-level laser irradiation is capable to induce osteoblast proliferation and consequent increase of bone mass in osteoporosis. Despite the findings, further studies evaluating other laser parameters are necessary to verify the clinical applicability.

PT.15

Low-level laser therapy: anti-inflammatory and anti-degenerative effects in rat model of knee osteoarthritis

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Osteoarthritis (OA) is the most common arthritis type and also a multifactorial disease, considered inflammatory and degenerative. The clinical syndrome doesn't affect only the cartilage itself, but all the tissues that are part of the joint, as bone, synovial liquid, muscles and ligaments. According to WHO (World Health Organization), OA is the most frequent disease in adults, especially elderly population. Low-level laser therapy (LLLT) is a treatment appliance that demonstrates good clinical results on rehabilitation of OA. At the same time, there still needs more research about the LLLT effects in inflammatory and degenerative process of the cartilage tissue. Therefore, the purpose of this study was to evaluate the effects of LLLT on inflammatory and degenerative markers on the articular cartilage after the anterior cruciate ligament transection (ACLT) in the knee of rats. This study was approved by the Ethical Committee of the Federal University of São Paulo (2015/7792220515). Thirty male Wistar rats were randomly divided into 3 groups: control group (CG); ACLT group (OAG); ACLT plus LLLT (OAL). Laser irradiation (AlGaAs, continuous wave, 808 nm, 50 mW, 28 s, 50 J/cm^2; 0.028 cm^2 1.7 mW/cm^2; 1.4 J, PHOTON LASER II, DMC® equipament Ltda, SP, São Carlos, Brazil) was used. Treatment started 4 weeks after the surgery, at 2 points of left knee joint (medial and lateral side of the joint), for 24 sessions. The results showed that LLLT was able to decrease OARSI score ($P < 0.0001$), increase cartilage thickness ($P = 0.001$) and reduce inflammatory (IL-1β) ($P = 0.008$) and apoptotic markers (caspase-3) ($P < 0.0001$). As a conclusion, these data shows us that LLLT could be an effective therapeutic option to in modulate inflammatory and degenerative process in knee osteoarthritis.

PT.16

ANALYSIS OF CELL PROLIFERATION AND GENE EXPRESSION ON FIBROBLAST CELLS (L929) SUBMITTED TO LOW-LEVEL LASER THERAPY

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Introduction: The low-level laser therapy (LLLT) it's a resource widely used on rehabilitation clinics, it exerts important biomodulation effect on cell and tissue, neovascularization, cell proliferation and synthesis of pro – collagen, contributing directly to the process of tissue rehabilitation.
Objectives: Evaluate the effects of low-level laser therapy with wavelength of 904 nm, doses of 2 and 3 J/cm^2, on cell proliferation and gene expression of COL1α1 and VEGF from fibroblast cells.
Methods: The L929 fibroblast cells were irradiated with Garllium Arsenide diode laser (AsGa). It was divided in 3 groups: G1- Control group (have not received any radiation); G2-irradiated with 2 J/cm^2 and G3- irradiated with 3 J/cm^2). The radiation was performed at 24h, 48h and 72h hours. The cell proliferation was analyzed by the MTT method (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide]) and quantified by microplate reader. The quantification of transcripts of the genes Col1A1 and VEGF was realized by polymerase-chain-reaction using real-time detection (qPCR). The relative expression of the genes was calculated using the reference gene β-Actin and normalized by the gene expression in relation to the control group. To compare and verify the statistically significant difference between the groups, analysis of variance (ANOVA), *One Way* and the *post hoc* of Tukey tests were used. For comparison between the evaluations, was performed the ANOVA test with repeated measures. On the gene expression, the data were analyzed using the Pfaffl method and calculated by the Software Reset 2009, considering standard deviation values and statistic test "Pair wise fixed reallocation", assuming significance of $p<0,05$.
Results: Comparing the number of viable cells of the groups G1 (100%), G2 (131,05±22,9) and G3 (145,64±13,9), it was observed a statistically significant difference 24 hours after the first irradiation ($p= 0,029$). At the times of 48h ($p=0,621$) and 72h ($p=0,084$), any statistically significant difference was found between the groups. On the analyzes of repeated measures of the groups on the different analyzed times, a statistically significant difference was verified ($p=0,01$) only in the radiated G3 cells, where occurred a reduction of viable cells from 24 to 72 hours($p=0,035$). The results of the expression showed an increase of 1,78 times in the RNAm expression of COL1α1 gene ($p=0,036$) on G2, while G3 don't present statistically significant difference ($p=0,138$). On the gene expression of VEGF, regarding to the control group, was observed an increase in the expression of 2,054 ($p=0,037$) on G2 and on G3 the raise in the RNAm levels was 2,562. It was not observed significant difference between G2 and G3 ($p>0,05$).
Conclusion: Low-level laser therapy can stimulate cell proliferation, expression of COL1α1 and VEGF on L929 cell culture.
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PT.17

PHOTOTHERAPY LASER ESTIMULATES COLLAGEN DEPOSITION IN DIABETIC ANIMALS AND MODULATES INFLAMMATION.

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Introduction: Diabetes Mellitus promotes a delay on healing and its main complications are chronic inflammation, a decrease in collagen production and extracellular matrix (Photomed Las Surg USA;26 2008). In diabetes COX-2 expression is up-regulated, which leads us to believe an important role in this prolonged inflammation (Molec Vis 20:1109.2014). Objective: This study investigated Phototherapy Laser effects on healing skin in diabetic animals. Methods: This project it was approved by the Animal Care Committee guidelines at Federal University of Ceará (01/2013). Twenty Wistar rats were randomized into three groups: control group, incision without any treatment (SHAM), diabetic group, incision without any treatment (NID) and diabetic laser group, incision irradiated group (DLASER) with Laser 904 nm. Diabetic groups were submitted one injection with streptozotocin (70 mg/kg). 120 days after diabetes induction glycemic analysis was performed to confirm chronic diabetes status. After that a surgical field with a gap of 2 mm width and 2 cm long in the skin was performed, with reference to the animal's posterior iliac crest and treated by five consecutive days with Laser (904 nm, 40 mW, 1 min, 2,4 J. Histological parameters, collagen with a comparative study between picrosirius and atomic force microscopy (AFM), VEGF and COX2 immunoexpression were analyzed. Results: The healing of a wound requires a well-orchestrated integration of the complex biological and molecular events of extracellular matrix (ECM) deposition and angiogenesis. Collagen is a major component ECM and is synthesized due to the balance between synthesis and degradation a matrix (Front Biosc 9:283.2004). Histological analysis showed in the 5th day discrete inflammatory infiltrates, a moderate amount of fibroblasts and some moderate granulation tissue rich in newly formed blood vessels. An intense amount of fibroblasts fusiform and a moderate deposition of an organized and immature collagen matrix was observed. Data about relative percent of content of collagen on total tissue revealed a statistical difference ($p = 0.01$) between the SHAM group (6,08-$\pm$ 0,80) x DLASER (11,38 $\pm$ 0,1). The comparative study between AFM and picrosirius red stain showed in NID a discrete deposition of the immature collagen matrix. DLASER demonstrated an intense deposition of an organized but immature collagen. The expression of VEGF demonstrated differences only the 5th day between NID versus DLASER ($p < 0,005$). Immunoexpression of COX-2 showed on the 5th day there were differences between NID versus DLASER ($p < 0,005$). In addition, SHAM group demonstrated significantly lower scores than the NID ($p = 0.001$). Conclusion: Phototherapy with Laser (904nm 40 mW, 1 min, 2,4 J) caused an angiogenesis incremented observed by VEGF increased and modulates inflammation by decreased COX-2 expression, effects which resulted in a better collagen deposition in irradiated groups. A possible explanation about this better healing over irradiated tissue can be attributed to a photobiomodulation mechanism. Financial Support: CAPES and FUNCAP

PT.18

EFFECTS OF LOW LEVEL LASER THERAPY ON LUNG INFLAMMATION IN EXPERIMENTAL MODEL OF SEPSIS

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Sepsis is defined as an infection associated with systemic manifestations and it is the major cause of death in hospitalized patients, as it leads to release of large amounts of inflammatory mediators. The acute respiratory distress syndrome (ARDS) is often related to sepsis and is a common cause of death. The low-level laser therapy (LLLT) is a complementary resource and non-invasive mechanism used for therapeutic purposes as a safe and effective treatment. Several studies have shown that LLLT reduces inflammatory cells and enzymes responsible for the release of chemotactic factors during the inflammation's initial phase. Besides that increases the level of antioxidant enzymes in several models of inflammatory diseases. Thus, the aim of this study was to evaluate and compare the effects of LLLT on acute lung inflammation resulting from sepsis in experimental sepsis model (cecal ligation and puncture- CLP). Federal University of São Paulo's Ethical Committee (Number 8948050515) approved this study. Twenty-five male Wistar rats, ±300 g, 3 months old were used. The rats were randomly divided into 3 groups: control (C); sepsis group (S): rats submitted to CLP without LLLT treatment; sepsis laser treated group (SIR). Laser irradiation (AlGaAs, continuous wave, 808 nm, 30 mW, 28 s, 30 J/cm^2; 0.028 cm^2; 0.8 J, PHOTON LASER II, DMC® equipment Ltda, SP, São Carlos, Brazil) was performed immediately after surgery on the anterior portion of the trachea near the carina and anterior portion of the chest near the diaphragm bilaterally, through punctual contact technique. Bronchoalveolar lavage technique was performed as follow: instillation of 3 ml of saline into the trachea (3 times), centrifugation at 1,000 rpm for 10 minutes at 4 ° C, the cell pellet was re-suspended with 1 ml of PBS to count the total number of cells per ml carried out in a Neubauer chamber. Animals were euthanized 24 hours post-surgery. The results showed an increase in the number of cells in the S when compared to C. Meanwhile, no other difference was noticed between the other groups. Histological and immunohistochemical analysis are in progress. The current result does not allow us to conclude the laser effects on lung inflammation induced by sepsis. We believe that further analysis will be important to assess the effects of LLLT in this model, because the CLP technique is the one that is closest to human sepsis due to polymicrobial profile.

PT.19

EVALUTION OF CELL VIABILITY AND THE EXPRESSION OF IL-6 AND VEGF GENES AFTER LOW POWER LASER ON L 929 FIBROBLAST CELLS

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Introduction: The low power laser therapy (TLBP) has been used to accelerate the healing process, reduce pain, to promote the protein synthesis, the organization of collagen fibers, neovascularization, to increase the intracellular levels of ATP (adenosine triphosphate) and calcium, and act also like anti-inflammatory resource in the tissue regeneration process.

Objectives: to analyze the cell viability, cell structure and the gene expression of interleukin -6 (IL-6) gene and vascular endothelial growth factor (VEGF) after TLBP (λ = 660 nm) on fibroblast cells L929

Methodology: fibroblast cells L929 were cultivated in bottles of 25 cm^3 (TPP, Switzerland, Europe) with DMEM/HAM F12 solution supplemented with 5% of FBS (fetal bovine serum) and 1% of antibiotic and antimitotic, were maintained on a 5% CO2 greenhouse at 37 º C; after, the cells were plated on a TPP plate and 24 hours after the sedimentation, cells were irradiated with low power laser (λ = 660 nm) on the time of 24, 48 and 72 hours, according to the groups: G1 – control group (not irradiated); G2 – 4 J/cm^2; G3 – 6 J/cm^2. After each time of 24 incubation hours post radiation, the cells culture were analyzed for the MTT method of cytotoxicity [3-(4,5-dimetthylthiazol-2-yl)2,5-diphenyltetrazolium bromide] for the evaluation of cell viability. The morphology alterations related to the cytoskeleton and endoplasmic reticulum (RE) were analyzed by the Fluorescence microscopy (MF) with the rhodamine-phalloidin markers and $DIOC_6$, respectively. To analyze the IL-6 and VEGF gene expression, the method RT-qPCR was used for the group that presented better growth on the better time.

Results: When analyzing the cells growth, we observed that G1 showed cell proliferation media of 95% (±0,02) on 24 hours, 91% (±0,05) on 48 hours and 99% (±0,00) on 72 hours. While, G2 110% (±0,15) on 24 hours, 91% (±0,19) on 48 hours and 78% (±0,05) on 72 hours. G3 presented media 87% (±0,05) on 24 hours, 121% (±0,32) on 48 hours and 114% (±0,17) on 72 hours. Regarding to cell viability, significant statistic was found in the realization of Anova of two factors (F=40.53; p=0,017), however in the analysis of the posttest related to time and dose no significant difference was found, nevertheless the G3 presented bigger growth curve in time of 48 hours related to the other groups. On the evaluation of MF, in a dose of 6 J/cm^2 , in the times of 24 and 48 hours, positive biomodulation effects were presented regarding to the cytoskeleton with bigger distribution and organization of the actin filaments and also with increase and better distribution of the vesicles of RE in the cytoplasm related to the control group. Concerning the gene expression of VEGF and IL-6 was possible to find a significant difference in G3, on 48 hours on the transcripts genes, being the stimulation of 1,82 to the VEGF and inhibition of 36,75 to IL-6.

Conclusion: the TLBP promotes positive bio modulation effects on cell culture of fibroblast favoring the cell proliferation, increase of the RE activity and morphologic changes in the cytoskeleton; accelerate the healing process through the inhibition of the inflammatory process and stimulation of growth factors, neovascularization and collagen production.

PT.20

Effects of Therapeutic Lasers with Different Wavelengths on the Bone Marrow Mesenchymal Stem Cells (differentiation & proliferation)

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Background: Mesenchymal stem cells (MSCs) are pluripotent undifferentiated cells which can be differentiated to many target tissues like bone, cartilage. They can be guided by tissue engineering to regenerate damaged or impaired tissues or organs. Lasers can be influence proliferation and differentiation processes in this regard. They may have different results when they used solely or combine with other type of laser. Materials and Methods: MSCs was isolated from rabbit bone marrow. They were allocated to one control and 8 experimental laser irradiated groups as: Red (R), Infra-red (IR), Blue (B), Green (G), IR+R, IR+B, R+G, B+G with Energy Density: 4J/cm2. These experimental groups were irradiated during 21 days. Proliferation and differentiation to bone and cartilage were evaluated. SOX9, Aggrecan, COL II, COL X, ALP, COL I, Osteocalcin markers were measured by Real time- PCR in this regard. Results: All of laser groups increased cellular proliferation during 10 days except G group which had adverse effect. IR and IR-B groups significantly increased cartilaginous differentiation by higher SOX9, Aggrecan and COL II expression, while IR increased COL X expression but IR-B group suppressed it. During osseous differentiation, R and IR lasers increased but IR-B, IR-R and G lasers decreased ALP production. R and B-G groups had higher COL I expression however, IR-B, IR-R and G groups had lower expression than control group. Osteocalcin was increased in IR group significantly, while in IR-R and R groups were similar and in B, B-G and G groups were lower than control groups. Conclusion: IR and IR+B lasers had stimulatory effect on cartilagous differentiation; however effect of lasers and their combination on osteo-differentiation may vary on different bone markers. Thus, we could expect stimulatory effect in range of red to IR spectrum and inhibitory effect in green wavelength on the bone.

PT.21

Effect of irradiation with a low-level laser therapy on chondrocyte viability

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INTRODUCTION: Osteoarthritis (OA), the most prevalent form of arthritis, is a chronic and painful disease of synovial joints, most commonly the knees, hips, and hands. It is characterized by gradual degeneration of the joint, progressive destruction of articular cartilage and new bone formation at the joint surface and surrounding areas (Cytokine 70(2):185-93). Due to the very limited cartilage regenerative capacity and consequently the limited efficacy of the standard treatments, the investigation of strategic innovative approaches to prevent the development of the clinical condition of OA is of great interest (World J Orthop 18; 5(3): 351-361). One promising treatment is the use of low-level laser therapy (LLLT) mainly due to its anti-inflammatory and regenerative effects on biological tissues (Lasers Surg Med 2004;35(3):229–35). Therefore, this study aimed to investigate the effects of LLLT on chondrocytes viability. **METHODS:** This study was approved by the Animal Care Committee guidelines at Federal University of São Paulo (CEUA N 2478130315). Chondrocytes were obtained from collagenase-digested femoral growth plate cartilage of 7-week-old rats. Chondrocytes were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco). All tissue culture procedures were performed under strict aseptic conditions in a biological safety cabinet. Chondrocytes were grown in sterile, vented, 175 cm^2 tissue culture flasks (Greiner Bio-one, Utrecht, the Netherlands) in a humidified incubator at 37°C in 5% carbon dioxide (CO_2), 95% air. The cells were expanded for four passages using standard tissue culture techniques. Subsequently, Chondrocytes were seeded in direct contact with the particles at a density of 5.10^4 cells/well. Cells were cultured for 3 days. Cell irradiation was performed using a Photon Laser III - DMC Equipament®, with a wavelength of 808 nm, 50 mW, 30 J/cm^2, 16 seconds, 0,8 J. The cells were irradiated 24 h after seeding, for three consecutive days, once every 24 h, on 48-well culture plates. The action of the laser's biomodulation was evaluated in three experimental groups: G1—control group, which received no irradiation and G2—irradiated at 30 J/cm^2. The irradiation was performed shielded from the light in a darkened room, with the laser pointer tip in direct contact with the plate. The experiment was conducted in triplicate. Cell metabolic activity was evaluated using Alamar Blue® (Invitrogen, Life Technologies), according to the manufacturer's instructions. Subsequently, 200 µl of each sample was transferred to a 96 well plate (in duplicates). Finally, the plate was read in a spectrophotometer (Bio-Tek Instruments, Winooski, USA) at 570 nm. **RESULTS:** The alamarBlue® assay is based on the ability of metabolically active cells to convert the reagent into a fluorescent and colorimetric indicator. Thus, the results revealed that the cell metabolic activity was significantly higher for LLLT compared to control group ($p< 0,0026$) which are evidenced by the appearance a large number of live cells after 3 days. **CONCLUSIONS:** Thus, based on the results of the present study, it has been shown that LLLT within the parameters presented is capable of stimulating the Chondrocytes viability.

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PT.22

LOW-LEVEL LASER THERAPY (660 nm) IMPROVES THE HEALING PROCESS OF THIRD DEGREE BURNS IN RATS

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Introduction: Burns are lesion caused by direct or indirect contact to chemical, physical or biological agents and are associated with tissue damage, infection, pain and even death (Lasers Med Sci 28; 543-54, 2013). Front of the interventions proposed in recent decades low-level laser therapy (LLLT) is considered a promising treatment since it is low-cost, non-invasive, and induces cell proliferation (Lasers Med Sci 29; 313-19, 2014). Laser action is based on absorption of laser light by tissue specific photoreceptor, and after light absorption, important changes in cellular metabolism are observed, resulting in therapeutic effects as the modulation of inflammation, reduction of edema, and stimulating the synthesis of proteins considered essential to due evolution of the healing process. In order to achieve such effects it is necessary that the parameters are compatible with both the pathology and with the depth of the tissue to be treated. However there are discrepancies in the value established in protocols for the treatment of burns and due to this further investigations are necessary.
Objective: To evaluate the effects of low-level laser therapy (660 nm, 25 J/cm^2, 1J) on the healing process of third degree burns in rats.
Methods: The Ethics Committee in Animal Experimentation at the Federal University of São Carlos # 022/2013 approved this study. Twenty male *Wistar* rats (280 g) divided into control group (CG) and LLLT group (LG) were used in the study, and submitted to burn injury through a soldering iron at 150°C, pressed on their back for 10 seconds (Microscopy Res Tech 79, 313-320, 2016). LLLT (660 nm, 100 mW, 25 J/cm^2) with 10 seconds irradiation time per point and energy 1J per point were used. Laser irradiation was initiated immediately after burn induction and on days 2, 4, 6 and 8 after burn induction. The method of application of the laser was point contact in five different points of application, four of which were located on the edge of the injury and the last one was located in the central region. On the tenth day after inducing the injury, the rats were euthanized and the twenty samples were collected. The variance one-way test (ANOVA) was used for statistical analysis and later complemented with Tukey test, where p values ≥0.05 were considered significant.
Results: Histological analysis revealed a decreased inflammatory infiltrate in the LG when compared an intense inflammatory infiltrate found in the CG. The immunostaining of COX-2 was intense (3.6 ± 0.55) in the CG than in the LG that demonstrated a mild immunomarking (2.2 ± 0.45). Conversely, VEGF immunomarking together with the morphometry vessels demonstrated similar results, both being more expressive in the LG (4 ± 0 and 9.17 ± 5.14) than it was in the CG (2.6 ± 0.55 and 3.13 ± 1.17). Collagen birrefringencia analysis also demonstrated statistically significant differences between groups, where LG (54.72 ± 20.28) showed increased deposition of fibers with better tissue organization compared to that found in the GC (49.24 ± 25.57).
Conclusion: Therefore, our findings suggest that the use LLLT (660 nm) of 25 J/cm^2 and 1J of energy was effective in stimulating the cellular processes involved in tissue repair on third-degree burns in rats by reducing the inflammatory phase, improving collagen synthesis and stimulating angiogenesis, thus restoring the local microcirculation which is essential for healing process.

PT.23

Bell's palsy and low level laser therapy - case report.

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Introduction: Bell's palsy is defined as an idiopathic peripheral facial nerve paralysis of sudden onset and is considered the most common cause of facial paralysis. Antiviral therapy, oral corticosteroid and physiotherapy are the methods most widely accepted in the treatment of this pathology. The low-level laser therapy (LLLT) can improve the peripheral nerve regeneration capacity. **Objective**: the aim of this study is to describe the treatment of Bell's palsy which occurred 10 years ago, using the application of LLLT. **Method**: A.C.F.O., female, 42 years old, which presented Bell's palsy 10 years ago. It was held a LLLT protocol with infrared laser (1.0Joule/point; 808nm, 60mW, 40J/cm^2) on the path of the branches of the facial nerve compromised (12 points) in 2 sessions per week for 5 weeks. Clinical (House-Brackmann scale) and electrophysiological (electroneuromyography) evaluation were performed before and after the LLLT protocol. **Results**: The House-Brackmann scale improved from grade V to grade III. Potential pre-existing denervation disappeared. There was an increase in the motor units recruitment of muscles studied (*frontalis*, *orbicularis oculi* and *orbicularis oris*). **Conclusion**: this report demonstrates the efficacy of LLLT in the treatment of Bell's palsy, even after a long time after its installation, and is shown as an additional method in the rehabilitation process if these patients. Prospective clinical studies are needed in order to establish the true efficacy of LLLT in Bell's palsy treatment.

PT.24

Low level laser therapy benefits after bichectomy

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The bichat bags are two clusters of fat found deep in the cheeks. They are located below the buscinador muscle and add volume to the cheeks. These adiposities when removed (bichectomy) can produce a beautiful contour and a more stylized face in patients with rounded faces. However, even being a simple surgery, inflammation caused leads to a large edema that persists for several weeks and generates a great discomfort to the patient. Many studies show the anti-inflammatory and analgesic efficacy of low level laser therapy (LLLT) after oral surgery. The aim of this study was to analyze the benefits of LLLT after bichectomy. 10 patients underwent bichectomy, and half of them received laser therapy after surgery. The laser irradiation was done immediately after the surgery and after 24, 48 and 72h. A Diode laser (DMC Company, São Carlos, SP, Brasil) with 100mW of power, spot size of 0,028cm2, was used. The laser protocol was: 2J of 830nm and 2J of 660nm irradiated together, 20 seconds per point, 6 points around the incision and 4J of 830nm, 40 seconds per point, 4 extra oral points along the mandibular nerve. Furthermore, patients in the laser group received one session of systemic laser therapy consisted of 30 min of continuous 100mW, 660nm, irradiation over the radial artery in the right wrist with total energy of 180 Joules. Postoperative discomfort, pain, swelling and healing time were analyzed. LLLT reduced the postoperative inflammatory symptoms and was well accepted by the patients

PT.25

PHOTODYNAMIC THERAPY, LOW LEVEL LASER THERAPY AND CELLULOSE BIOMEMBRANE FOR PRESSURE ULCER HEALING IN PATIENTS WITH NEUROLOGICAL LESIONS: A CASE REPORT

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Pressure ulcer (PU) is a tissue area of trauma caused by prolonged and continuous pressure exceeding the normal capillary pressure applied to the skin and adjacent tissue, causing ischemia, which may lead to cell death, ulceration of the skin, and other tissues. Data from the National Pressure Ulcer Advisory Panel indicate that 60.000 patients die in the US each year due to complications of this injury closely related to the frequent contamination of the affected tissue. PU is often associated with hospitalized and immobilized patients due to neurological factors having a negative impact on their quality of life. Treatment failures and incorrect use of antimicrobials favor the emergence of resistant microorganisms in these lesions, often prolonging the clinical resolution of the ulcer. Thus, Antimicrobial Photodynamic Therapy (APDT) has been indicated for the treatment of infections associated to UP eliminating the problem of antimicrobial resistance favoring the healing process. The aim of this study was to present the report of 8 cases of patients with neurological injury due to trauma, treated by the combination techniques of photodynamic therapy, low-level laser therapy and cellulose biomembrane for the healing of pressure ulcers. Eight patients were selected from a hospital of Bahia,Brazil, who had a diagnosis of UP. For the realization of APDT, curcumin 1.5% emulsion was applied to the entire surface of the ulcer and after 30 minutes illumination using an active plate with 30 LEDs, wavelength 450 ± 10 nm (blue visible) distributed in 6 rows with five LEDs each. The light delivery was in continuous mode, 12 minutes long, with irradiance of 30m W/cm^2, fluence of 21.6 J/cm^2. The light was applied from a distance of 5 cm from the tip surface to the surface of ulcers. After seven days, the patient received a second PDT session following the same parameters of the first session. Nanoskin® cellulose biomembrane was positioned over the entire area of ulcers. Laser therapy was performed on the biomembrane with 660 nm laser, punctual and continuous manner, spot of 0.04 cm^2, power of 40 mW, 10 seconds irradiation time, generating a fluence of 10 J/cm^2 and irradiance of 1000 W/cm^2, twice a week. The healing progression was evaluated for 6 months, through digital images and had their areas measured in square centimeters, with UTHSCA Image Tool, version 1.28 program. Overall, there was statistic difference between the collections before and after APDT for all microorganisms groups ($p < 0.05$). The greatest reduction in log10 was obtained for *Staphylococcus spp*. (1.19 log10), and for yeasts, *Enterococcus spp*. and total growth of microorganisms reductions were 0.63, 0.41 and 0.92 log10, respectively. Over the 24 weeks of follow-up of the patients, we observed a gradual reduction in the area of ulcers and in one case, there was a complete re-epithelialization. We concluded that the combination of treatments promoted effectively accelerating the healing of PUs treated in the study, and have eliminated the contamination. This case report was approved by Multidisciplinary Health Institute Ethics Committee (CAAE 36925714.0.0000.5556).

PT.26

Effect of activated carbon fiber felt associated with low level laser on the bone healing process in rats tibia.

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Abstract

Bone defects are caused by several factors and the repair process can be slow, were inflammatory and proliferative phases of remodeling of this tissue are extremely important for the quality of the repair. There are several therapies where mainly used is surgery and in this case, the use of bone substitutes can be indicated. However, the repair process may require assistance. Thus, the use of activated charcoal as bone substitute when the implementation of low level laser to aid bone repair can be an alternative to these problems.

Objective: The aim of this study was to verify the use of activated carbon felt as bone substitute and the interaction with the laser in the process of bone repair in rat tibias, assessing biochemical, histological and biomechanical changes.

Material and methods: 35 male wistar rats were used between 150 g to 200 g, with 3 months of age. The animals were anesthetized with ketamine and xilasine association. Once anesthetized, the animals were put on surgical table, the skin was dissected and monocortical bone defects in the central region of the right tibia of mice were made. Immediately after surgery the rats were randomized and divided into the following groups: control (CTL), untreated lesion (NT), Lesion treated with activated carbon felt (AF), Lesion treated with laser therapy (L6J) and Lesion treated with association of activated carbon felt and laser 830nm, 6J-100mW (AF+L). After 28 days the animals were euthanized with overdose of the same anesthetic, blood was collected and the tibia was removed for biochemical, histological and biomechanical analysis. Committee of ethics: AN00462014-UNINOVE.

Results: The NT group presented the lowest values of stress at break, besides histological changes related to disorganization of the tissue. Gradually, the groups L6J, AF and AF+L showed improvement in mechanical properties in comparison to CTL group. The group AF+L presented the highest value of stress at break, organized histological aspects and increase levels of ALP. Thus, the association of two distinct techniques seemed to assist the process of bone healing in rat tibias.

Conclusion: The use of activated carbon felt seems to improve the bone repair induced in this study. The laser association with activated carbon felt showed improvement of biomechanical properties and still the histological results have a better aspect.

Keywords: bone repair, low level laser, activated carbon felt, mechanical properties.

PT.27

ANALYSIS OF THE EFFECTS OF LASER LOW POWER IN SECOND DEGREE BURNS IN PATIENTS HOSPITALIZED

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Introduction: Burns are one of the most common traumas in humans. They are mostly caused by thermal, chemical, electrical or radioactive agents, and fire is the main element to cause skin burn. Laser causes biochemical, bioelectric and bioenergetic changes that lead to a patient's healing process. The use of Low Level Laser Therapy (LLLT) leads to no signs of infection or severe inflammation, reduces swelling and thereby accelerates the healing process of irradiated injuries. **Objetive:** The aim of this study was to analyze the effects of LLLT in second degree burns in hospitalized patients, describe the quality and time to repair injuries and verify reduction of pain. **Method**: After being approved by our Research Ethics Committee, the LLLT was applied in hospitalized patients after the removal of bandages and bathing these patients. The laser used was the type red AlGaInP in the range of 660 nm, continuous mode and dose of 2 J / cm² in the inner part of the burn for 10 consecutive days. The evaluation method was: VAS (Visual Analogue Scale), blind evaluation of the injury through questionnaire, and qualitative evidence from patients and staff. **Result:** The pain and swelling were dimished, analgesic consumption was reduced, the healing quality was satisfactory and the time of injury repair was lower. **Conclusion:** The application of LLLT at a dose of 2 J / cm² in burned patients reduced their healing time and provided better quality of injury healing.

Keywords: Burn, Low Level Light Therapy, tissue repair, cure.

PT.28

INVESTIGATION LED EFFECTS IN THE PROCESS OF HEALING: literature review

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ABSTRACT

The tissue repair is a complex process involving internal and external factors of the tissue site following phases ranging from cell proliferation, angiogenesis passing through to total wound closure by re-epithelialization. Techniques to assist in the tissue repair process has been subject to increasingly frequent studies and the LED is among the promising resources to achieve this goal. The use of LED in tissue repair period increases the healing rate, this because the radiation emitted by the LED affects cellular metabolic processes, promoting biological effects (analgesic, anti -inflammatory and bio-stimulant). The use of low-power light sources such as light emitting diodes, LEDs, provide an optional therapeutic use of conventional due to the low cost advantage and proven in the treatment of wound healing.
Keywords: Wound Healing, Phototherapy, LED.

METHOD

This is a review of literature that sought studies on the application of LED to answer the guiding question "? What is the evidence on the effects of LED on the healing process" to search scientific material were searched electronic databases: Pubmed, Medline, Lilacs, Scielo, Bireme and Google Scholar, adopting the following inclusion criteria: studies that addressed the LED effects on the healing process, in Portuguese or English, who presented as descriptors or keywords: healing, LED, wound healing and Phototherapy, published in the last ten years. The studies found were analyzed and extracted the relevant information the guiding question.

RESULTS

From studies investigated no occurrences of research in humans and animals, are described in most application with LED red wavelength. For many applications the amount was at least one, being adaptable as size and type of injury, and the same occurred with the application time. As for the results in the process of Moura et al healing. (2014) show proliferation of blood vessels and increase in collagen as Meyer et al. (2010) studies in rats reported that granulation tissue was more developed in the irradiated group than in the control group and the amount of chronic inflammatory cells (monocytes, macrophages, lymphocytes and plasma cells) predominated with phototherapy green light . The epithelialization at the wound margins and scarring with better quality occurred with the red LED (620-630nm), which was increased collagen deposition. However, phototherapy not collimated of 620-630nm (red) led to better anti-inflammatory effects.

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effects.

PT.29

Nuclear phenotype evaluation in muscle tissue of Wistar rats exposed to low level laser therapy.

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INTRODUCTION: Low-level laser therapy (LLLT) has been successfully used for therapeutic applications with benefits in soft tissue repair (wound healing), musculoskeletal pain treatment and inflammatory conditions, as well as, improvement to sport performance. The molecular bases of laser action are associated to absorption of photon laser energy by cell chromophores, such as cytochrome c oxidase. Despite the widespread use of LLLT, the molecular mechanisms involved in their biological effects are not fully understood, particularly as regards DNA, which could be targeted by free radicals changing its structure, chromatin organization and ploidy degrees.
OBJECTIVE: We investigated effects of red and infrared laser exposure on nuclear phenotype in muscle cells from *Wistar* rats.
METHODS: Experiments in accordance with the Institutional Committee of Animal Care (CEUA/038/2012). Animals were exposed to LLLT at different fluences (25, 50 and 100 J/cm^2) and wavelengths (660nm and 810nm), using power imput 100 mW. Skeletal muscle samples were collected and processed for histological routine. Geometric (area) and densitometric (integrated optical density) parameters obtained from Feulgen-stained nuclei by image analysis were used to define nuclear phenotypes. DNA fragmentation was evaluated by the TUNEL POD assay, as described by the manufacturer. Normality was verified by Shapiro-Wilk test. Kruskal-Wallis test was performed to determine possible statistical differences, followed by post hoc Dunn's tests, with $p<0.05$ as the less significant level.
RESULTS: All results were expressed in mean ± standard deviation. Area data from muscle tissue exposed to red LLLT were: 28.43±2.30 (control), 36.29±2.20 (25J/cm^2); 37.43±3.57 (50J/cm^2), 33.00±0.70 (100J/cm^2) and to IR LLLT: 38.13±1.38 (25J/cm^2), 40.17±2.13 (50J/cm^2), 40.33±3.78 (100J/cm^2). Integrated Optical Density (IOD) data from muscle tissue exposed to red LLLT were: 9.23±0.77 (control), 11.97±1.05 (25J/cm^2); 12.06±0.73 (50J/cm^2), 24.51±3.32 (100J/cm^2) and to IR LLLT: 12.45±0.83 (25J/cm^2), 12.40±0.93 (50J/cm^2), 12.78±1.78 (100J/cm^2). Data from area to 50 and 100J/cm^2 IR LLLT was significant when compared to control group. Data from IOD shows significance only to red LLLT (100J/cm^2). TUNEL data from muscle tissue exposed to red LLLT were: 2.67±0.88 (control), 2.78±0.51 (25J/cm^2); 4.11±0.84 (50J/cm^2), 5.44±1.84 (100J/cm^2) and to IR LLLT: 2.89±0.51 (25J/cm^2), 2.78±0.69 (50J/cm^2), 4.33±0.67 (100J/cm^2). TUNEL assay shows no significant levels.
CONCLUSION: Our results could indicate that high doses of red (100J/cm^2) and IR LLLT (50 and 100J/cm^2) contribute to changes in nuclear phenotypes. However, these doses could not cause DNA fragmentation in these cells.
FINANCIAL SUPPORT: FAPERJ, FAPEMIG, CNPq.

PT.30

Pre-exercise phototherapy is the best irradiation protocol when applied in association to strength training: a randomized, double-blinded, placebo controlled trial

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Introduction: The effects of phototherapy for performance improvement have been widely studied. However, there are few studies showing its´ effects on muscular training, as well as, there are not studies where different moments of phototherapy irradiations (i.e. before or after training sessions) were tested.
Objective: The aim of this study was to elucidate what is the optimal moment for phototherapy irradiation when used in association with strength training.
Methods: The project received approval from the institutional research ethics committee (protocol number 437.894). Forty-eight volunteers, 26 years old (±5.24), male gender, up to one exercise practice per week completed this study. A strength training protocol was performed applying phototherapy and/or placebo before and/or after each training session. The training protocol lasted 12 weeks and it was assessed peak torque reached in maximum voluntary contraction test (MVC), load in 1-repetition maximum test (1-RM) and thigh circumference (perimetry) at larger cross-sectional area (CSA) at baseline, 4 weeks, 8 weeks and 12 weeks. Data were analyzed both in absolute values and in percentage of change from baseline assessments. Two-way ANOVA test was performed to test between-groups differences (followed by Bonferroni post hoc test). The significance level was set at $p<0.05$.
Results: Volunteers from group irradiated with phototherapy before and placebo after training sessions showed significant ($p<0.05$) changes in MVC for right leg (280.9±38.68) and left leg (311.27±31.36). Significant results were found also for 1-RM tests for both exercises when compared to other groups, leg press 144.83±22.53 and 145.33±18.23 (right and left legs, respectively) and for leg extension 127.83±22.93 and 132.92±16.14 (right and left legs, respectively).
Conclusion: Application of phototherapy combining different wavelengths leads to better results in order to enhance strength gain when is applied before exercise. Moreover, this tool can be used both in sports, during muscular training for sports activities, as well as in prevention and rehabilitation of sports injuries. Furthermore, phototherapy doesn't present side-effects, no damaging thermal effects and it's easy handling.

PT.31

Using Pre-Exercise Photobiomodulation Therapy Combining Super-Pulsed Lasers and Light-Emitting Diodes to Improve Performance in Progressive Cardiopulmonary Exercise Tests

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Context: Skeletal muscle fatigue and exercise performance are novel areas of research and clinical application in the photobiomodulation field, and positive outcomes have been reported in several studies; however, the optimal measures have not been fully established.
Objective: To assess the acute effect of photobiomodulation therapy (PBMT) combining superpulsed lasers (low-level laser therapy) and light-emitting diodes (LEDs) on muscle performance during a progressive cardiopulmonary treadmill exercise test.
Patients or Other Participants: Twenty untrained male volunteers (age 26 ± 6 years, height 175 ± 10 cm, mass 74.8 ± 10.9 kg).
Intervention(s): Participants received PBMT with either combined superpulsed lasers and LED (active PBMT) or placebo at session 1 and the other treatment at session 2. All participants completed a cardiopulmonary test on a treadmill after each treatment. For active PBMT, we performed the irradiation at 17 sites on each lower limb (9 on the quadriceps, 6 on the hamstrings, and 2 on the gastrocnemius muscles), using a cluster with 12 diodes (four 905-nm superpulsed laser diodes with an average power of 0.3125 mW, peak power of 12.5 W for each diode, and frequency of 250 Hz; four 875-nm infrared LED diodes with an average power of 17.5 mW; and four 640-nm red LED diodes with an average power of 15 mW) and delivering a dose of 30 J per site.
Main Outcome Measure(s): Distance covered, time until exhaustion, pulmonary ventilation, and dyspnea score.
Results: The distance covered (1.96 ± 0.30 versus 1.84 ± 0.40 km; $P < .001$) and time until exhaustion on the cardiopulmonary test (780.2 ± 91.0 versus 742.1 ± 94.0 seconds; $P < .001$) was greater after active PBMT than after placebo. Pulmonary ventilation was greater (76.4 ± 21.9 versus 74.3 ± 19.8 L/min; $P < .004$) and the score for dyspnea was lower (3 (0.5–9.0) versus 4.0 (0.0–9.0); $P < .001$) after active PBMT than after placebo.
Conclusions: The combination of lasers and LEDs increased the time, distance, and pulmonary ventilation and decreased the score of dyspnea during a cardiopulmonary test.
Keywords: photobiomodulation therapy, fatigue and progressive-intensity exercise.

PT.32

Lipid peroxidation by malondialdehyde in a muscle submitted to low intensity laser radiation

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Introduction: Low intensity laser acts on the mitochondrial function likely contributing to reduce oxidative damage on the muscle cell membrane. There are reports of increased lipid peroxidation in different stress situations that can provide oxidative damage to cells for the production of Reactive Oxygen Species (ROS) due to leakage of electrons from the electron transport chain in mitochondria. These reports are time specific regarding the permanency of the process and there is no consensus as to the period in which it occurs or even the use of laser in the prevention/modification of oxidative damage period.

Objective: To analyze the behavior of lipid peroxidation on the muscle through malondialdehyde by TBARs technique after low intensity laser action over a period of 12 hours.

Method : This study was approved by the Ethics Committee of the Federal University of São Paulo, #9369060415 . Thirty adult Wistar male rats, weighing around 190g were assigned into groups A (n = 25, 5 animals in each period) and B (n = 5). Group A (laser) was irradiated with low intensity laser of gallium arsenide and aluminum (λ 808nm , power 0.1W , 5J Dose, Fluency 179J/cm2 , spot area 0,028cm2 , time 50s, 1 point for application mode contact). The B group (control) received no procedure. Euthanasia of animals after 1 minute, 3 , 6, 9 and 12 hours of irradiation followed by unilateral excision of the rectus femoris muscle. It was performed with albumin standard curve to determine the concentration values (mg/mL) and dosage of muscle proteins to standardize concentration of 1mg/mL. TBARs (ROS systemic evaluation) was used as an index of lipid peroxidation of the biological membranes. To assess the systemic lipid peroxidation level (serum) the organic phase (with chromogen) was separated, reading the absorbance at 532nm. The concentration of Thiobarbituric Acid Reactive substances (TBARs) was calculated based on the value of 532nm = 153000$M^{-1}cm^{-1}$ (BUEGE; AUST, 1978). Absorbance data were presented using median values and standard deviation analysis between groups (Mann-Whitney test, $p \leq 0.05$) and ANOVA.

Results: There was a very significant difference between the 6 groups analyzed with ANOVA ($p<0.0001$). The median values of TBARs showed variation through the studied time periods: control (0,0210±0,0051), 1minute (0,0295±0,0035), 3h (0,0480±0,0066), 6h (0,0290±0,0040), 9h (0,0470±0,0075) and 12h (0,0290±0,0058). The increase in absorbance was significant when compared the control to the 3h group ($p= 0.0357$) and control to the 9h group ($p=0.0357$). The information is relevant since the increase in absorbance indicates the concentration of TBARS and, consequently, increased oxidative stress.

Conclusion: Lipid lipoperoxidation fluctuates significantly during a 12 hour interval after low-intensity laser irradiation.

PT.33

Effects of 904 nm cluster LASER photobiomodulation on exercise-induced skeletal muscle fatigue in young women.

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Introduction: LASER photobiomodulation has been used to increase muscle performance and to improve recovery when applied before exercise. However, several factors such as parameters of irradiation and mechanisms of action are not yet fully understood.
Objective: The aim of this study was to investigate the effects on indicators of muscle fatigue of 904 nm LASER photobiomodulation applied using a multiple-diode probe on quadriceps muscle performance in young women.
Methods: This study was designed as a randomized, participant and assessor-blinded, within-subject crossover trial with placebo control. All procedures were approved by the Griffith University Human Research Ethics Committee (Approval No.: 2016/026). Eighteen physically active women aged between 20 and 30 years were initially randomly assigned to one of two groups, either: (1) active LASER receiving irradiation with a 12 diode cluster probe (GaAs; 904 nm; 60 mW; 250 Hz; total dose per site 43.2 J, total dose applied 129.6 J) in contact over the rectus femoris muscle of the dominant limb immediately before an isokinetic fatigue protocol; or (2) placebo LASER prior to the isokinetic fatigue protocol. The fatigue protocol consisted of a set of 60 concentric contractions (90º flexion-160º extension) of the quadriceps muscle at 180º/s. Prior to the fatigue protocol, the volunteers undertook a 5-minute cycle ergometer warm-up (speed: 60-70 rpm, no load) and were familiarized with the isokinetic dynamometer (5 submaximal voluntary concentric muscle contractions). Muscle performance was assessed using the isokinetic outcomes of peak torque, time to peak torque, total work, work fatigue index, average power and average peak torque. Muscle fatigue was assessed by the electromyographic fatigue index (EFI) from surface electromyography, blood lactate measures (immediately prior to and after the fatigue protocol, and at 3 and 6 minutes after the fatigue protocol) and ratings of perceived exertion (RPE) before and after the fatigue protocol.
Results: The results showed that LASER photobiomodulation reduced muscle fatigue indicated by a significant increase in EFI (P=0.005) and a significant reduction in RPE (P=0.0139) after LASER compared to the placebo LASER session. No significant difference was observed for lactate concentration between the groups (P>0.05). The isokinetic dynamometer performance analysis demonstrated that LASER resulted in increased muscle performance, observed by the effects on variables of peak torque (P=0.04), time to peak torque (P=0.042), total work (P=0.032), average power (P=0.007) and average peak torque (P=0.019) between the experimental conditions. No significant difference was observed for work fatigue index (P=0.29).
Conclusion: The LASER photobiomodulation (904 nm) was effective in reducing fatigue levels and increasing muscle performance in young active women.
Financial Support: The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for financial support.

PT.34

PHOTOBIOMODULATION THERAPY (PBMT) IMPROVES PERFORMANCE AND ACCELERATES RECOVERY OF HIGH-LEVEL RUGBY PLAYERS IN FIELD TEST

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INTRODUCTION: The rugby consists of intense physical activity with frequent bursts of high-intensity intermingled with short intervals of low-intensity activities. Preparation for play requires training to focus on a combination of muscular strength, power, agility, speed, aerobic and anaerobic endurance. Photobiomodulation therapy (PBMT), with lasers and/or LEDs, has been shown to prevent skeletal muscle fatigue and accelerate recovery. While growing evidence supports the use of PBMT for performance and recovery enhancement, there have only been laboratory-controlled studies.
OBJECTIVE: The aim of this study was to analyze the effects of pre-exercise PBMT (combination of LLLT and LEDT) on performance and recovery of high-level rugby players during an anaerobic field test.
METHODS: Twelve male high-level rugby athletes, with a mean age of 23.50 (±2.32) years old, were recruited in this randomized, crossover, double-blinded, placebo-controlled trial. The study was approved by institutional ethics committee (process 665.347). No interventions were performed before the Bangsbo Sprint Test (BST) at familiarization phase (week 1). At weeks 2 and 3 pre-exercise PBMT or placebo were randomly applied to each athlete in a random way. PBMT irradiation was performed at 17 sites of each lower limb, employing cluster probes with 12 diodes (4 laser diodes of 905nm, 4 LED diodes of 875nm, and 4 LED diodes of 640nm, 30J per site - manufactured by Multi Radiance Medical™). Average time of sprints, best time of sprints, and fatigue index were obtained from BST. Blood lactate levels were assessed at baseline, and at 3, 10, 30 and 60 minutes after BST. Athletes' perceived fatigue was also assessed through a questionnaire. Oneway ANOVA test followed by the Bonferroni post-hoc test were performed to verify statistical significance.

RESULTS: The average time of sprints (ST mean) was significantly different ($p<0.05$) between PBMT (6.55 ± 0.21 sec) and placebo (6.67 ± 0.21 sec). Regarding fatigue index during BST, a significant difference ($p<0.05$) was observed between PBMT (2.66% ± 0.61) and placebo (4.51% ± 0.95). PBMT significantly decreased percentage of change in blood lactate levels ($p<0.05$). The perceived fatigue was significantly lower ($p<0.05$) when compared PBMT (20.16 ± 3.63) to placebo (23.50 ± 2.50).
CONCLUSION: Pre-exercise PBMT with the combination of super-pulsed laser, red and infrared LEDs can enhance performance and accelerate recovery of high-level rugby players in field test. This opens a new avenue for wide use of PBMT in real clinical practice in sports settings.

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PT.35

Strength exercise training associated with low-level laser therapy (LLLT) in muscle strength, functional capacity and balance of older women - randomized placebo- controlled trial

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Aging is associated with structural changes in muscle tissue, which leads to loss of functional independence and increase in the risk of falls. The preservation of the muscle strength through training program has high clinical significance. Moreover, by associating with the low-level laser therapy (LLLT) must be highlighted. The ain was investigate the effects of strength training program associated with LLLT (808nm, 100mW, 35.7 W/cm2 and 7J) on muscle strength, functional capacity and balance in older women. We investigated twenty- seven subjects was divided in Placebo Group (n= 13) and Active Group (n=14) (range 60-70 years), entered a randomized double-blinded placebo- controlled trial. The functionality was evaluated by 6-Minute Walk Test (6MWT) and Short Physical Performance Balance (SPPB), while the Fall Risk Test and the Postural Stability Test were evaluated through Balance System Biodex (BBS). Muscle strength was analyzed by One Maximum Repetition (1- MR). The exercise protocol consisted of knee flexion- extension exercise performed during 8 weeks followed by application of LLLT. The results showed a significant increase in 6MWT (p=0.001), SPPB (p=0.006) and 1-MR (p=0.001) in both groups after strength training. In Postural Stability Test, only the active group presented a significant decrease in stability index (p=0.007) and in Fall Risk (p=0.005). The strength training was significant in the gain muscle strength and functionality, and when combined with the LLLT was significant in the postural stability and risk of falls.

PT.36

AEROBIC EXERCISE AND TRANSCRANIAL LASER THERAPY PRODUCE SIMILAR BENEFITS ON REACTION TIME AND MEMORY PERFORMANCE

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Introduction: Human cognitive performance can be enhanced by low-level laser therapy (LLLT) of the prefrontal cortex (Neuroscience 230: 13-23, 2013) and by an acute bout of aerobic exercise. Since both treatments increase mitochondrial respiration and stimulate prefrontal cortex function, we report here the results of a randomized, controlled study comparing the cognitive effects of acute aerobic exercise and transcranial laser stimulation on the same cognitive tasks.
Objective: We examined whether transcranial infrared laser stimulation of the prefrontal cortex, acute high-intensity aerobic exercise, or their combination may enhance reaction time performance in a sustained attention task and the number of correct responses in a working memory task.
Methods: This study was approved by the Institutional Review Board of the University of Texas at Austin. Healthy young male and female adults (N = 60, 18-30 years old) were randomly assigned to one of the following four treatments: 1) low-level laser therapy (LLLT) with infrared laser to two forehead sites while seated (total 8 min, 1064 nm continuous wave, 250 mW/cm^2, 60 J/cm^2 per site of 13.6 cm^2), 2) acute exercise (EX) of high-intensity (total 20 min, with 10 min treadmill running at 85-90% VO_2max), 3) combined treatment (LLLT+EX), or 4) sham control. Participants were tested for prefrontal measures of sustained attention with the psychomotor vigilance task and working memory with the delayed-match-to-sample task before and after the treatments. Statistical analyses were performed with ANOVA using SPSS (SPSS Inc, Chicago, USA).
Results: Mean (Standard Deviation) reaction times in msec after sham control = 359 (24), EX = 345 (17), LLLT = 351 (26), LLLT+EX = 345 (32). As compared to sham control, both LLLT and EX treatments significantly reduced reaction time in the psychomotor vigilance task [$F(1.56) = 4.134$, $p = .01$, $\eta^2 = .181$]. Mean (Standard Deviation) number of correct responses (30 = maximum score) after sham control = 25 (2), EX = 28 (1), LLLT = 28 (2), LLLT+EX = 28 (1). As compared to sham control, both LLLT and EX treatments significantly increased the number of correct responses in the delayed-match-to-sample memory task [$F(1.56) = 4.690$, $p = .005$, $\eta^2 = .201$], demonstrating a similar enhancing effect of both LLLT and EX on cognitive performance. The cognitive effects of combined LLLT+EX treatments were similar and showed no significantly greater improvement on reaction time or memory performance as compared to LLLT or EX treatments alone.
Conclusion: The transcranial infrared laser stimulation and acute aerobic exercise treatments were similarly effective for enhancing reaction time and memory performance, suggesting that these treatments augment prefrontal cognitive functions similarly.
Financial Support: Supported by institutional research funds from the University of Texas at Austin.

PT.37

The effect of interaction between low-level laser therapy, creatine monohydrate supplementation and swimming training on muscular strength, induced by electrical stimulation in rats.

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Introduction: Physical training can cause muscle fatigue, decreased muscle strength and impairment of motor control. Creatine monohydrate can increase intramuscular creatine levels and facilitate the replacement of phosphocreatine. Low-level laser therapy (LLLT) acts in delaying muscle fatigue, increase strength and improve muscle recovery. However, the effect of interaction of LLLT and creatine monohydrate supplementation in skeletal muscle is unknown.
Objective: The main objective of this study was to evaluate the effect of interaction of LLLT and creatine monohydrate supplementation on muscle strength and endurance (fatigue).
Methods: For analysis of muscle fatigue, was used an experimental model of tetanic contractions induced by indirect electrical stimulation of the tibial muscle of rats. This study was approved by the Ethics Committee in the use of animal of Sacred Heart University (nº 29/14). Male Wistar rats were used, weighing around 300g and randomized into 5 groups of 7 animals: swim group (1), Laser+swim group (2), creatine+swim group (3) Laser+creatine+swim group (4) and sham group (5). The creatine dose was applied via gavage (280mg/kg). Performance analysis was conducted through muscle contraction protocol induced by indirect electrical stimulation and recorded in an electrophysiograph. The swim protocol was performed 30 min/day, three times a week for 8 weeks (J Appl Physiol, 82(2): 711-15, 1997). Irradiation with laser was performed in contact mode, only one point in the middle region of the tibialis anterior muscle of hind limbs of rats 10 minutes before exercise in all sessions during the eight weeks of swimming training protocol. Laser irradiation: Diode lasers with mean output power of 100 mW; spot size of 0.028 cm, continuous mode; and wavelengths of 830 nm (infrared). The total delivered energy for irradiated groups were 3 J per hind limb.
Results: It was found an increase in muscle contraction force in laser+creatine+swim group (35.33±3.2) and laser+swim group (33.33±3.2), when compared to other groups. However, on the time taken for muscle tension decay 50% of its maximum amplitude (fatigue) it was found a statistically significant improvement on laser+creatine+swim group (320±20) when compared to other groups and it was also observed an improvement on fatigue process time on laser+swim (286.7±25.17) and creatine+swim groups (253.3±32.15) when compared to sham and swim groups.
Conclusion: The interaction between LLLT and creatine supplementation improved the muscle strength and slowed the process of fatigue in rats after swim training protocol.

PT.38

Photobiomodulation in management of chemotherapy-induced oral mucositis in oncologic young patients establishing relations with leukocytes levels

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Introduction: Several studies revealed benefits of photobiomodulation (PBM) to reduce the painful symptomatology, severity and duration time of oral mucositis (OM) originated from chemotherapy (CT) and radiotherapy (RT). CT affects cells with high turnover rate, as the cells of the basal layer of the epithelium, and cells from the bone marrow (leukocytes and platelets). Severe leukopenia predisposes the patient to infections and can be an indicative of toxicity by CT. Considering the counting of leukocytes we evaluated PBM performance in the evolution of OM in cases with critical level of white blood cells (WBC).

Objective: This study was conducted to evaluate the effectiveness of PBM in prevention and treatment of OM in young patients who underwent high doses of CT, establishing relations between the OM evolution and the leukocytes counting.

Methods: Were included 15 patients (24 CT cycles) submitted to high doses of methotrexate for the treatment of osteosarcoma and acute lymphoblastic leukemia (informed consent was signed and the study was approved by the Ethical Committee in Research of Santa Marcelina Hospital/TUCCA- protocol 55/05). Patients without OM were included in the preventive group (PG) and received PBM (660nm ±30, 0.028cm^2, 2J, 70J/cm^2, 20s) within the first 24h from the start of CT. Patients with OM (TG) that did not accept to participate in the study initially, were irradiated as soon as they were diagnosed. The evolution of OM was compared with the leukocytes counts. Oral mucosal toxicity was graded according to the NCI-CTC version 2.0. Pain was accessed by visual scale.

Results: waiting for the statistical data

Conclusion: We believe that FBM may decrease the intensity of OM, even in those patients with severe leukopenia, according to the results of our previous study. However we are waiting for the statistical data.

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PT.39

Low-level laser effects on bacterial cultures submitted to heat stress

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INTRODUCTION: Low-level lasers have been used worldwide to treat diseases, pain relief and wound healing. Interest in their therapeutic applications has been demonstrated by the increasing case reports, as well as clinical and experimental studies reporting positive impact on human and animal health. For these purposes, red and infrared lasers are used within so called therapeutic window (600 up to 1100 nm). Despite clinical protocols used, there are doubts about the mechanism of action involved in the biological effects of low-level lasers. As the mechanisms of action of low-level lasers on cells under stressful conditions and cells deficient in DNA repair mechanisms remain disputed, in this work we evaluated the effects of low-level red and infrared lasers on *E. coli* cells deficient in SOS responses submitted to heat stress. **OBJECTIVE:** Evaluate the effects of red and infrared laser on E. coli cells exposed to heat stress in different physiological conditions. **METHODS:** Stationary and exponential *E. coli* AB1157 (wild type), AB2494 (RecA deficient) and AB2363 (LexA deficient) cultures were exposed to red (660nm) and infrared (808nm) laser at different fluences (25, 50 and 100J/cm²) and after, submitted to heat stress (42°C, 20 minutes). Aliquots of bacterial suspensions were spread onto *Petri* dishes, containing nutritive medium and incubated. Colony forming units were counted and the survival fractions were calculated. Controls were bacterial suspensions not exposed to lasers and incubated to 37 or 42ºC. To evaluate bacterial morphology, exponential and stationary *E. coli* suspensions exposed to low-level red or infrared lasers and incubated at 37 °C or 42 °C, as described in the cell survival in bacterial cultures. After that, aliquots were spread onto microscopic slides and stained by the Gram method. Area ratios were calculated by the ratio between the areas of cells in bacterial cultures at 42 °C and the areas of cells in bacterial cultures at 37 °C. **RESULTS:** Survival fractions values in stationary and exponentially *E. coli* AB1157 exposed to red laser were (mean±standard deviation): 2.9±1.54 and 2,1±0.70 (25J/cm² + 42°C), 4.1±0.74 and 1.8±0.48 (50J/cm² + 42/C), 4.8±0.61 and 2.7±0.72 (100J/cm² + 42°C); for infrared laser: 1.2±0.37 and 0.8±0.30 (25J/cm² + 42°C), 1.0±0.23 and 0.9±0.22 (50J/cm² + 42°C), 1.5±0.47 and 0.8±0.23 (100J/cm² + 42°C). Cell area ratios values from stationary and exponentially *E. coli* AB1157 exposed to red laser were (mean±standard deviation): 1.0±0.23 and 0.8±0.14 (25J/cm²), 1.2±0.13 and 0.9±0.06 (50J/cm²), 0.9±0.04 and 0.9±0.06 (100J/cm²); for infrared laser: 1.1±0.08 and 1.0±0.07 (25J/cm²), 0.8±0.14 and 0.9±0.03 (50J/cm²), 1.3±0.21 and 1.1±0.04 (100J/cm²). **CONCLUSION:** Our research suggests that exposure to low-level red and infrared lasers increases cell viability and protects cells from morphological alteration in *E. coli* cultures submitted to heat stress, depending on laser wavelength and SOS responses.

FINANCIAL SUPPORT: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

PT.40

USING AlGaInP *LASER* IN CUTANEOUS RADIONECROSIS HEALING INDUCED BY ^{125}I SEED IN AN ATHYMIC MURINE MODEL

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Introduction: The malignant cancer incidence has increased significantly in recent years due to population growth and aging. The cancer treatment usually consists in individual or combined use of chemotherapy, surgery and radiotherapy depending on the etiology of the tumor. In cases where radiotherapy is used, and considering the therapeutic effects of radiation, specific severe complications can occur, ranging from erythema to radionecrosis. Studies showed that low-level laser therapy (LLLT) can be successfully used for tissue repair, depending on the light parameters and characteristics of the target tissue.
Objective: Evaluate the LLLT influence in radionecrosis guided tissue repair caused by continuous radiation emitted by ^{125}I seed.
Methods: Twelve athymic mice were divided into two groups: group A – 1 week before the implantation of ^{125}I seed (0,98 mCi) subcutaneously into the back of the mouse (n=4) it was submitted a LLLT, wavelength of 660 nm, 40 mW power with 20 s (20 J/cm^2) daily until the radionecrosis emerge and continuous until the wound healing (33 days after the ^{125}I seed implantation); group B – using the same procedure as group A (n=4), but the LLLT was applied only when the radionecrosis emerge and continuous until the wound healing (45 days after the ^{125}I seed implantation). The control group (n=4) using the same procedure as group A but without LLLT treatment. All animals were photographed every 7 days and the wound sized were calculated by the Image J® software.
Results: The animals in group A, showing fast wound healing (in days) and smaller radionecrosis (in area) on comparison between group B and Control. No systemic or lethal sequelae occurred in any animal.
Conclusion: Under the parameters used in this study, LLLT was showing better results when applied before the ^{125}I seed insertion to repair radionecrosis. We are focusing our efforts in using higher energy laser and other parameters, to avoid the laser exposure daily.
Key-Words: ^{125}I seed, LLLT, radionecrosis, radiodermatitis, radiotherapy.
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PT.41

EVALUATION OF INDUCTION OF DNA DAMAGE AND REPAIR BY LOW POWER LASERS

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Introduction: Laser characteristics, such as wavelength, frequency, power, fluence and emission mode properties are determining to the biostimulatory effect. Biochemical reactions after energy absorption increase ATP and RNA synthesis, which constitute the basis of the laser effects on biological tissues. Low power lasers induce free radical generation and alterations in macromolecules (DNA).
Objective: This study evaluated induction of DNA damage in blood cells and mRNA expression from genes related to DNA repair in biological tissues exposed to low power red (660nm) and infrared (808nm) lasers.
Methods: Experiments were approved by Institutional Committee of Animal Care (protocol CEUA/038/2012). *Wistar* rat blood samples were obtained by finger puncture in hind paws and red (R) and infrared (IR) lasers were applied at different fluences (25, 50, and 100 J/cm²), powers (30, 50, and 100mW) and frequencies (continuous wave, 10, 50, and 100 pulses per second). Negative controls were non-irradiated blood samples and positive controls were blood samples incubated with hydrogen peroxide. DNA damage was evaluated by comet assay and damage index (DI) was calculated. *Wistar* rat skin and muscle were exposed to low power lasers at different fluences (25, 50 and 100J/cm²) in continuous wave emission mode at 100mW. From tissue samples, total RNA was extracted, cDNA was synthesized and gene expression was evaluated by real time quantitative polymerase chain reaction. One-way analysis variance (*ANOVA*) test was performed followed by *Tukey* post-test, after Kolmogorov-Smirnov test for normality verification, $p<0.05$ was considered as less significant level.
Results: Mean and standard deviations of DI (in continuous mode, 100mW) were: 26.2±2.79 (control-CTR), 42.5±8.38 (25J/cm², R), 93.0±5.54 (50J/cm², R), 6.0±1.58 (100J/cm², R), 36.0±3.72 (25J/cm², IR), 59.0±1.22 (50J/cm², IR), 16.0±4.41 (100J/cm², IR); for the different powers (continuous mode, 100J/cm²): 48.5±8.76 (30mW, R), 46.0±0.86 (50mW, R), 4.25±1.29 (100mW, R), 36.0±2.82 (30mW, IR), 78.0±3.60 (50mW, IR), 15.8±7.56 (100mW, IR); to the pulse mode (100mW, 100J/cm²): 46.0±0.86 (continuous, R), 99.0±4.20 (10PPS, R), 43.8±2.48 (50PPS, R), 146.0±5.33 (100PPS, R), 78.0±3.60 (continuous, IR), 105.0±2.44 (10PPS, IR), 55.0±2.48 (50PPS, IR), 126.0±9.24 (100PPS, IR). mRNA *ERCC1* relative expression in skin were: 1.94±0.70 (CTR), 1.35±0.70 (25J/cm^2, R), 1.05±0.41 (50J/cm^2, R), 1.10±0.62 (100J/cm^2, R), 0.04±0.01 (25J/cm^2, IR), 0.04±0.01 (50J/cm^2, IR), 0.05±0.04 (100J/cm^2, IR); in muscle: 1.36±0.05 (CTR), 1.87±0.68 (25J/cm^2, R), 1.09±0.38 (50J/cm^2, R), 1.98±0.79 (100J/cm^2, R), 18.97±8.33 (25J/cm^2, IR), 38.42±5,49 (50J/cm^2, IR), 18.65±9.45 (100J/cm^2, IR). For mRNA ERCC2 in skin: 0.86±0.33 (CTR), 0.44±0.24 (25J/cm^2, R), 0.42±0.21 (50J/cm^2, R), 0.99±0.39 (100J/cm^2, R), 0.02±0.00 (25J/cm^2, IR), 0.02±0.00 (50J/cm^2, IR), 0.02±0.01 (100J/cm^2, IR); in muscle: 0.85±0.40 (CTR), 1.09±0.84 (25J/cm^2, R), 5.63±2.4 (50J/cm^2, R), 0.93±0.76 (100J/cm^2, R), 3.04±3.80 (25J/cm^2, IR), 7.97±4.78 (50J/cm^2, IR), 2.04±1.11 (100J/cm^2, IR).
Conclusion: Low power lasers induce DNA damage in blood cells and differently alter *ERCC1* and *ERCC2* mRNA expression in skin and muscle tissue.
Financial Support: FAPERJ and FAPEMIG.

PT.42

Skin changes induced by UV-B radiation (UVR-B)

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Introduction The skin is composed of three tissue structures with different anatomy and physiology. It is a structure rich in cells, which offers resistance, elasticity and protection. Skin damage (physical, chemical, biological) immediately trigger a role of biochemical events. Sunlight exposure provides many photo biological effects (Dermatologia, Azulay RD, 5ed, 2011). The harmful effects on human skin could result by sun exposure, skin type, geographic and atmospheric conditions and thus the injuries may develop from erythema to cancer (An. Bras. Dermatol. 82(1): 7-21 2014). **Objective** The aim of this study was observe the macroscopic and histological changes in rats' skin submitted to UV-B radiation. **Methods** Case-control experimental study approved by Ethics Committee of Federal University of Vale do São Francisco–UNIVASF number 3/100614. Fifty Wistar rats with 3 months, 250g±10g were assigned into two groups: Control (n=10) and Experimental (n=40), the skin on the back was trichotomized and exposed to UVR-B with a light emitting stable wavelength (λ) of 306nm, potency=9J/s (W) installed in a wooden box, 15cm away from the animal's back. Each exposure was realized for 30 seconds three times per week. The exposure time was fixed based on the distance from the lamp to the skin of the animal, the lamp power and minimum time for obtaining twice the minimum erythema dose (Proc Natl Acad Sci U S A. 1977; 74(4):1688–1692). At the end of weeks 5, 10, 15 and 20, the exposure was discontinued in each subgroup (n = 10), the skin was photographed and a biopsy was performed. Control group was submitted to the same conditions with the light device off. Three different blinded pathologists realized histopathological qualitative analysis. Quantitative For statistical analysis to macroscopic (clinical) evaluation Fisher test ($p<0,05$) was realized. **Results** The most significant skin injury at all times was erythema, followed by erythema with desquamation. More severe clinical injury was keratosis, which presented from week 5 with little expressiveness and had its frequency increased in the weeks 15 and 20. Regarding to microscopic findings, five weeks group showed more evident inflammatory characteristics than the other groups, most prominent in the superficial reticular dermis and more moderate in the superficial reticular dermis, moderate atypia of basal keratinocytes and focal hyperkeratosis. The groups 10 and 15 were very similar to each other, with epidermal atrophy and focal hyperkeratosis, atypia of basal keratinocytes and minimal inflammation. In group 20 weeks, the changes were more intense than all other groups with hyperparakeratosis, atypia of basal keratinocytes and architectural skin disorganization, epidermal atrophy, focal hyperkeratosis and moderate interstitial lymphocytic inflammatory infiltrate in superficial reticular dermis. Macroscopic analysis of control group showed no clinical lesion, but microscopic showed mild epidermal atrophy, focal hyperkeratosis and mild inflammatory infiltrate. **Conclusion** The skin lesions are more intense the longer the time of exposure of the animal skin to UVR-B. Apparently, the lesions show changes similar to an inflammatory reaction and the continued exposure have preneoplasic characteristics.

PT.44

Effectiveness of Laser Acupuncture Along With Intensive Short-term Dynamic Psychotherapy on Improvement of Depression: a Case study

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Abstract

Introduction: Based on existing evidence on acupuncture benefits in healing depressed patients, the last decade has witnessed more clinical interest in acupuncture as well as laser acupuncture, as a modality of this treatment, for treatment of depression. Despite heterogeneous results of laser acupuncture effectiveness in depression treatment, it seems to have positive effects on biological aspects of the disorder with possible changes in brain function and neurotransmitters. Given the bio-psychological nature of the disorder, using laser acupuncture along with psychotherapy may lead to more positive outcomes. Therefore, the effectiveness of laser acupuncture along with intensive short-term dynamic psychotherapy - a therapy which work through emotion-focused process-for the treatment of moderate to major depression – was examined.

Objectives: The main aim of the present study was to evaluate the effect of laser acupuncture in combination with psychotherapy on reaching more effective therapeutic results regarding depression symptoms.

Methods: This project was conducted in Iran, and the approval code (L / 95 / 15) was obtained from ethics committee on animal or human experimentation of Iranian medical laser association. In this research, eighteen patients, aged 24–60 years old (M=38.38, SD=12.70; 5 male &13 female) with moderate to major depression were studied. Treatment involved 12 sessions of simulating with Semiconductor Laser Gallium-Aluminum-Arsenide (GaAlAs) with continuous contact on body (LI4-LI11- ST36-ST40-BL18-BL20-BL21-SP4-SP6-LIV3-LIV8-LIV14-GB15-GB20-GB34-REN12-REN17-P6-DU20-HT7) and ear (Heart, Spleen, Stomach, Spleen, Shenmen, Anti-depression, Valium, Master Omega) points which were determined according to TCM diagnosis (Liver Qi congestion- Mucus blocking the Qi flow- Blazing Liver Fire- Blood deficiency), with an average output power of 200 mW, wavelength of 980 nm, Dose of 4j/point and 12 one-hour sessions of ISTDP focused on person triangle (past, present, transference) and conflict triangle (feelings, anxiety and defense). Through Beck Depression Inventory II (BDI II), the change in the severity of depression at baseline and at the end was assessed. Data was analyzed through t-test of paired samples.

Results: The obtained results (M1= 51.00, SD1= 8.45; M2= 6.27, SD2= 1.40; Std. Error Mean= 2.08136; 95% Confidence Interval of the Difference: “Lower= 40.33” & “Upper= 49.11”) indicate a significant improvement in the severity of depression (t = 21.487, df= 17; *P value* = 0.000) with 0.96 effect size (Cohen’s d= 7.37).

Conclusion: Combination of laser acupuncture with ISTDP indicated a clinically and statistically significant benefit in improvement of depression symptoms.

Keywords: Depression, Laser acupuncture, acupuncture, low level laser therapy, intensive short-term dynamic psychotherapy (ISTDP), Beck Depression Inventory II (BDI II)

PT.45

Low level infrared laser effects on bacterial survival

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Introduction: Low-level laser emits monochromatic, coherent and collimated light beams. Wavelength, frequency, power, fluence and emission mode are determining properties of laser bioestimulatory effects in which biochemical reactions occur after absorption of energy by endogenous chromophores. Low level laser in continuous and pulsed emission mode is used in the treatment of many diseases resulting in bioestimulatory effects in the cells. Low level lasers induce free radical generation, increasing the ATP and DNA synthesis. However, there are few data about possible adverse effects on cells, as well the potential risks of laser-induced DNA lesions. DNA lesions, protective effect and adaptive response after laser expoure have been reported on eukaryotic and prokaryotic cells after laser exposure at different powers, wavelenghts and fluences.

Objective: This study evaluated effects of low-level infrared laser on bacterial culture proficient and deficient in repair of oxidative DNA lesions.

Methods: Effects of a low-level laser were assessed in *E.coli* AB1157 (wild type), JW3610 (fpg-mutM protein deficient) and JW1625 (endonuclease III deficient) cultures in stationary and exponential growth phases. Aliquots of bacterial suspensions were exposed to infrared laser (904 nm, spot size of 0.069cm^2) at different fluences (2, 4 and 7J/cm^2) in pulsed mode (power output of 10mW, power density of 144.92W/cm^2 and frequency of 5000Hz) with laser source at 3.0cm from the surface of bacterial suspension aliquots (distance top-botton of a microcenttrifuge flex tube). Exposure time of the aliquots was automatically adjusted by the laser device as a function of fluence. Aliquots not exposed to laser were used as controls. Samples were diluted in saline (0.9% NaCl) spread onto Petri dishes containing nutritive medium. After incubation (37°C, 20 hours) colony forming units were counted and survival fractions were calculated. Data were reported as one-way analysis of variance(ANOVA) test was performed to determine possible statistical differences followed by Dunnet post-test with $p<0.05$ as the less significant level.

Results: Data from bacterial survival in stationary phase were (mean±standard deviation): AB1157: 1.0±0.07 (control), 0.8±0.10 (2J/cm^2), 1.0±0.04 (4J/cm^2), 1.0±0.04 (7J/cm^2); JW3610: 1.0±0.02 (control) 0.7±0.06 (2J/cm^2), 0.8±0.04 (4J/cm^2), 1.1±0.03 (7J/cm^2); JW1625: 1.0±0.02 (control), 0.8±0.06 (2J/cm^2), 0.9±0.05 (4J/cm^2), 1.0±0.07 (7J/cm^2). Data from bacterial survival in exponential phase were: AB1157: 1.0±0.07 (control), 1.0±0.10 (2J/cm^2),1.0±0.09 (4J/cm^2), 1.2±0.09 (7J/cm^2); JW3610: 1.0±0.04 (control), 0,9±0.09 (2J/cm^2), 1.1±0.08 (4J/cm^2), 1.1±0.14 (7J/cm^2); JW1625: 1.0±0.03 (control), 0,8±0.07 (2J/cm^2), 0.9±0.05 (4J/cm^2), 1.0±0.04 (7J/cm^2). Data show that infrared laser exposure alters the survival of AB1157, JW3610 and JW1625 cultures at specific fluences, compared with control group.

Conclusion: Our results suggest that exposure to low-level infrared laser alter the survival of *Escherichia coli* wild type and deficient in repair of oxidative DNA lesions.

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PT.46

TITLE: LOW-LEVEL RED AND INFRARED LASERS MODULATE HOUSEKEEPING GENE EXPRESSIONS IN *Escherichia coli*.

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Introduction: Low-Level Lasers are widely used for treatment of muscle injuries, wound healing and oral diseases, as well for antimicrobial photodynamic therapy. Laser energy absorption is considered occurs at endogenous chromophores, as cytochrome C oxidase in eukaryotes and homologue molecules in prokaryotes. Also, light absorption by exogenous chromophores results in free radicals production and cellular signalization pathways. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is considered a gold standard technique to evaluate mRNA levels and is used to study effects induced by these low-level lasers. However, output data from RT-qPCR are commonly in a relative approach, with the target gene expression is done relative to a housekeeping gene, which is considered do not vary whether the cells are subjected to a treatment.
Objective: The aim of this study was to evaluate housekeeping expression genes from *Escherichia coli* exposed to red or infrared lasers at different fluences.
Methods: Exponential *E. coli* AB1157 cultures were exposed to red (660 nm) and infrared (808 nm) lasers at different fluences (25, 50 and 100 J/cm^2), 100 mW in continuous wave emission mode, beam spot area of 2.75mm^2, and incubated for 20 minutes at 37 ^{o}C. Controls were bacterial cultures not exposed to lasers. After that, total RNA extraction and cDNA synthesis were carried out to evaluate mRNA levels from *araC*, *gyrA* and *rpoA* genes by RT-qPCR. mRNA expression stability was analyzed by geNorm, NormFinder and BestKeeper softwares, providing gene stability factors, which consider values above 1.0 indicative of mRNA expression instability. Data were from three independent experiments.
Results: Data from geNorm for *araC*, *gyrA* and *rpoA* were, respectively: 3.359, 3.359 and 5.345; from NormFinder were: 1.696, 2.899 and 5.880; and from BestKeeper were: 2.958, 3.375 and 3.793.
Conclusion: *E. coli* exponential cells exposed to lasers at different fluences presented *araC*, *gyrA* and *rpoA* gene expression altered. These results suggest that more than one housekeeping gene should be used to evaluate relative mRNA expression in *Escherichia coli* cells exposed to low-level lasers.

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PT.47

EFFECTIVENESS OF PHOTODYNAMIC THERAPY MEDIATED BY CURCUMIN AND BLUE LED TO REDUCE MICROBIAL CONTAMINATION IN PRESSURE ULCERS: A PRELIMINARY STUDY

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Among the wounds with great affections index of patients, pressure ulcers (PU) are a growing concern in clinical practice. PU is an area of tissue trauma caused by prolonged and continuous pressure exceeding the normal capillary pressure applied to the skin and adjacent tissue, causing ischemia which may lead to cell death. Generally occurs when a soft tissue is compressed between a bony prominence and a rigid surface, resulting in poor local blood circulation culminating in necrosis, ulceration of the skin and other tissues, making favorable development of microorganisms due to exudative material, serous or haemorrhagic present on its surface. The bacterial proliferation inside a wound can result in changes in phases of wound healing. Hemostasis can be changed due to the effects of infection on platelets and the complement system. Bacteria can cause agglutination of platelets, thrombocytopenia, prolonged inflammation and tend to alter the function of the leukocytes by the expression of virulence factors and decrease collagen production. The aim of this preliminary study was to evaluate the effectiveness of photodynamic therapy (PDT) mediated by curcumin photosensitive agent and 450 nm LED to reduce the contamination in pressure ulcers. Seven patients were selected assisted by a public hospital in the interior of Bahia, Brazil, with a diagnosis of pressure ulcers. After precleaning with 0.85% sterile saline, material for isolation of microorganisms were taken from viable granulation tissue using sterile disposable swab that has been processed in selective media: Mannitol agar (*Staphylococcus spp*.), Sabouraud dextrose agar (yeasts), MacConkey agar (*Enterococcus spp*.) and blood agar (total count). To perform the PDT, the curcumin photosensitive agent emulsion 1.5% (PDT Pharma Industry and Trade Pharmaceuticals LTDA, Cravinhos, SP, Brazil) was used, applied across the surface of the ulcer. After 30 minutes of application it has been activated using the Lince equipment - Light in Cell (MMOptics, São Carlos, São Paulo, Brazil), composed with 30 LEDs with wavelength of 450 + 10 nm (visible blue) . The delivery of light was continuous, 12 minutes long, with irradiance of 35 mW/cm^2 and fluence of 25.2 J/cm^2. The light was applied from a distance of 5 cm from the tip surface to the surface of ulcers. For each patient were applied different quantities of the proposed treatment in accordance with the presence of exudate during the assessment sessions. Immediately after the performance ofPDT a new microbiological sample was collected and processed under the same conditions of the first. The logarithm of the colony forming units (CFU log10) was calculated and paired t-test with 5% significance level was used for comparison between CFU counts before and after PDT of treated ulcers. In all the culture media used was a significant reduction in log10 CFU ($p<0.05$) indicating effectiveness of the method employed in reducing microbial yeast, *Staphylococcus spp*. and *Enterococcus spp* in pressure ulcers. We conclude that the PDT was effective in reducing contamination of pressure ulcers and can be used as an auxiliary method in the treatment of such injuries.This study was approved by Multidisciplinary Health Institute Ethics Committee (CAAE 36925714.0.0000.5556).

PT.48

Effectiveness of photobiomodulation on activities of daily living and motor examination of Parkinson's disease patients

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Introduction: Recent animal model studies indicated that photobiomodulation using near infrared light (NIR) shows neuroprotective effects on midbrain dopaminergic cells. The beneficial effect of NIR on numerous neurological disorders such as cerebrovascular attack and Parkinson's disease is likely to be related with mitochondrial respiration, where NIR radiation increases ATP synthesis, resulting in enhanced cellular responses of damaged neurons. In spite of the significance of NIR on cellular levels, few clinical studies involving Parkinson's disease patients have been reported. This preclinical trial was designed to evaluate the potential of NIR therapy on improving daily living quality and motor examination of Parkinson's disease (PD) patients.

Materials and Methods: A double-blinded randomized controlled trial was designed. An approval of Institutional Review Board (approval code: WSOH IRB 1606-06) was obtained before the study. PD patients between 40 and 85 years old diagnosed by a neurologist were divided into two comparable groups. Laser group received InGaAlP laser (658 nm) treatment with four probes in each 50 mW (CW mode) on their lower neck and forehead area, while sham group was treated with no light-emitted devices. All patients received the treatment once a day for 30 min, 16-18 times within 6 weeks. The beam size was 0.20 cm2 and the power density was 707.5 mW/cm2. The total energy density of four probes was 1273.5 J/cm² per each session. Laser application procedure was conducted as stationary in skin contact, and sham/real laser apparatus were identical in their external shapes. While performing the trial, enrolled participants who did not receive the allocated treatment more than one time, withdraw their written consents in any reason, and does not meet our criteria for PD were excluded from the analysis.

Expected Results: The on-going study is expected to end at August 2016. The results might be presented in the WALT conference 2016.

Conclusions: The results obtained in this preliminary study indicated that LLLT would be influenced the activities of daily living (ADL) score and motor functions of PD patients.

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PT.49

Exploring the effects of photobiomodulation on the inflammatory process of the adipose tissue of diet-induced obese and hyperglycemic mice

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Introduction: Studies have been reporting obese and hypertrophied white adipose tissue as richly permeated by leukocytes, expressing not only increased TNF-α levels, but also other pro-inflammatory cytokines and chemokines. Inflammatory signaling through activation of Toll-like and TNF receptors (TLRs and TNFRs, respectively) pathways leads to intracellular inhibition of insulin signaling by direct interferences on insulin receptor function and its ability to initiate the insulin signal transduction pathway. If inflammation is a common etiological agent behind all these pathologies, a therapeutic approach that can modulate inflammatory signaling is of noteworthy value. Several studies have been acknowledging photobiomodulation (PBM) as an appealing therapy for inflammatory disorders due to its immunomodulatory properties. Nevertheless, the phototherapeutic approach to manage the chronic inflammatory component of obesity and hyperglycemia has not yet been explored.
Objective: The purpose of this study was to develop a murine model of obesity and hyperglycemia to investigate the effects of PBM on inflammatory infiltrate in adipose tissue of obese and hyperglycemic mice.
Methods: Four week old male adult C57BL/6 mice were submitted to a hypercaloric high-fat diet to induce obesity and hyperglycemia during 8 weeks. After that, animals were treated with PBM during four weeks corresponding to six irradiation sessions using an 843 nm LED (5.7 J cm^{-2} at 19 mW cm^{-2} per session). Animals were irradiated at days 1, 3, 7, 10, 14 and 21 following obesity and hyperglycemia validation. Control animals were submitted to same management but sham-irradiated. All animals received the high-fat diet until the end of experiments. Body mass and blood glucose were assessed regularly during the entire experimental course. Twenty-four-h after the last irradiation, animals were euthanized and subcutaneous samples from abdominal tissue were carefully collected, fixed at formalin 10% and routinely processed for hematoxilin-eosin staining and histological analysis. Standardized areas were selected from each slide and Image J software was used to quantify the areas of inflammatory infiltrate in adipose tissue. Data were submitted to paired sample t-test to attest changes in body mass and blood glucose levels after obesity and hyperglycemia induction. Mann-Whitney test was performed to verify differences between groups regarding inflammatory infiltrate in adipose tissue. Significance was established at $p<0.05$.
Results: Obesity and hyperglycemia were successfully induced after 8 weeks. The body mass and glucose blood significantly increased about 32.2% ($p<0.001$) and 13.7% ($p=0.034$), respectively. Regarding the inflammatory infiltrate in the adipose tissue, non-irradiated control animals displayed areas almost five times higher than the treated group ($p<0.001$). In fact, control group presented 23.3% of area populated with inflammatory cells while for irradiated animal the area corresponded to 4.3%. Considering that the abdominal adipose tissue from treated animals presented diminished areas of inflammatory infiltrates, we assumed that their adipocytes were less exposed to pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6.
Conclusion: Our results suggest that PBM could be used to improve insulin response in hyperglycemic subjects.
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PT.50

Blood sugar levels of analysis in diabetic patients type II after application of systemic laser

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Type II diabetes mellitus is chiefly characterized by the increase of blood glucose levels, this hyperglycemia triggers the release of free radicals and oxidative stress in the body, the role of ILIB (Intravascular Laser Irradiation of Blood) is the antioxidant form, causing an increase in the levels of the superoxide enzyme dismutase (SOD), with such an increase is a partial restoration of the insulin synthesis and minimizes the intensity of the attack from free radicals to the pancreatic cells. To investigate the blood glucose levels in type II diabetic patients using the treatment with modified ILIB (Intravascular Laser Irradiation of Blood) by analyzing the results through the right handed, before, during and after the intervention in addition to the analysis of sleep quality before and after the intervention by the Pittsburgh Questionnaire, pain assessment by Visual Analogue Scale (VAS) and the ankle-brachial index (ABI) using the Doppler as an evaluation tool before and after intervention. This is a randomized blind controlled study. It has been selected six with Diabetes Mellitus Type II patients, being three male and three female. They were randomized between the CG (control group) and GI (Intervention Group) through a raffle software were selected and from this we have obtained three patients in GI and three in G. Questionnaire Pittsburgh, EVA, ABI of each carrier and then were subjected to the use of the Modified ILIB, where the GC did use the GI ILIB off and made use of was evaluated ILIB on five consecutive days over 30 minutes positioned on the right radial artery. Data were analyzed by GC and average GI, comparing them from beginning to end of the intervention. The use of Modified ILIB promoted a decrease of blood glucose levels, increasing quality of sleep, decreased VAS and normalization of ITB in GI, while the GC showed the same items without significant changes. Although few studies about the ILIB Modified, it follows that the same benefits afforded to the GI compared to the GC in relation to the items evaluated, demonstrating the effectiveness of the technique for patients with diabetes type II.

Keywords: Laser therapy. ILIB. Diabetes Mellitus. Free Radicals.

PT.51

Photobiomodulation with Low Level Laser Therapy in temporomandibular joint tenderness associated with initiation of mandibular advancement device use in sleep apnea - A single subject design

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Introduction: Mandibular advancement devices (MAD) are being increasingly used in the treatment of sleep apnea. MADs are designed to protrude the lower jaw to open upper airways during sleep, and consequently they stretch the synovia, ligaments and surrounding muscles of the temporomandibular joint (TMJ). Some patients experience discomfort and pain when initiating MAD use, and this may negatively affect MAD compliance.

Objective: Can Low Level Laser Therapy (LLLT) reduce TMJ-related tenderness associated with initiation of MAD use?

Methods: Single subject design (n=1). Material: Sleep apnea patients who experienced discomfort with pain increase (> 30 mm) and lowered pain pressure threshold (PPT > - 0.5 kg/cm^2) 4 weeks after inititation of MAD treatment. Outcome measures: Pain on a 100mm visual analogue scale (VAS), pain pressure threshold (PPT) measured algometer, and mouth opening in mm. Intervention: LLLT (λ=810nm/200mW) applied for 3 consecutive days to two points overlying the TMJ, and two points each in pterygoid and masseter muscles, respectively (6 points in total). Each point received 6 joules of energy during an irradiation time per point of 30 seconds. Pain was immediately before LLLT and then daily during the LLLT intervention period.

Results: Pain increased during initiation of MAD use (+34mm on VAS), and PPT was lowered (-1.2 kg/cm^2), while mouth opening became slightly impaired (-3mm). All outcome measures returned to near normal after 3 days of LLLT

Conclusion: LLLT seemed to reduce pain and discomfort associated with MAD use, and may have potential for increasing MAD compliance.

PT.52

LOW-LEVEL LASER THERAPY (LLLT) 670nm IN THE TIME COURSE ON WOUND HEALING IN RATS

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INTRODUCTION: The use of resources and treatments to restore the epithelial tissue damaged by injury is growing exponentially, and in many cases in a disorganized way and without scientific basis to verify and prove their effectiveness. One of the treatments that have good results in tissue repair, because it has good re-epithelialization is the low-level laser therapy (LLLT), which has been intensifying in recent decades, either as sole therapy or in combination with biological and technological resourcesGUIRRO, ECO et al. Photom Laser Surgery; 28(5): 629-635; 2010. The LLLT is indicated in wound healing by stimulating fibroblasts proliferation and angiogenesis AVCI, P.; Semin Cutan Med Surg 32:41-52, 2013. **OBJECTIVE:** was evaluate the effect of laser GaAlAs in cutaneous wounds in the time course. **METHODS:** This study was approved by the Ethics Committee on Animal Use UFSCar, No. 2-007 / 2014. 20 male rats were submetted a surgical incision by punch (10mm) and divided into 2 groups (n=10): Control (CG) with 0.9% saline; Laser Group (LG) GaAlAs, 670nm, 39mW of power output, energy per point of 1,17J, radiating by 1 point in 30s. The treatment took 16 consecutive days. Biopsy time points were made on the days 4, 11, 16. H&E analysis were performed in addition to immunohistocchemistry (IHC). The results was compared intra-groups by one-away ANOVA with post hoc Tukey ($p<0,05$). **RESULTS:** Inflammatory infiltrate decreased significantly to LaserG on 11th day ($p<0,001$), and extremely significant to LaserG, on the 16th day ($p<0,001$) compared to CG. In the analysis of VEGF-A expression factor had increased from the 4th and 11th day to LaserG ($p<0,01$). **DISCUSSION:** The results showed that LaserG induce tissue granulation and accelerate epithelization in acute wound were effective in the healing process over the 16 days of treatment. **CONCLUSION**: Shown that Laser potentiates healing by reduced wound and may have aplication for clinical management.

PT.53

THE USE OF LOW POWER LASER IN THE TREATMENT OF COMPLICATIONS ASSOCIATED WITH TOOTH EXTRACTION OF THIRD MOLARS.

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Introduction: The dental extraction of third molars is a minor oral surgical procedure perfomed quite often in dentistry and it presents specific/characteristic indications. The pacient, in the postoperative period, frequently shows pain, edema and reduction of buccal opening in the early days. However, it is important to consider other possible complications associated with the surgery, such as bleeding, alveolitis, infection, root fracture, maxillary tuberosity fracture, oral/buccal-sinus communication and adjacent teeth injury Subconjunctival or periorbital bleeding are considered rare. An alternative and effective treatment that helps in healing and in reducing pain, inflammation and swelling after surgery is low power laser therapy, as it increases cell production and the microcirculation in the affected area. **Objective:** to report a case of fracture of maxillary tuberosity and trismus and the use of low power laser (LLLT) to promote reduction of edema, periorbital and intraoral welt (palate) and aid in healing. **Case Report:** Male patient, 25 years, sought the extension project "Laser in dentistry" at Federal University of Maranhão 5 days after dental extraction of teeth 18 and 48 with complaints of swelling and reduction of buccal opening. During the clinical examination it became clear that the mucous membrane of the tuber was with a whitish aspect and with bruises on the palate and periorbital, as well as the presence of trismus, formation of granulation tissue in the palate and welts deriving from the fracture of the tuber. It was used then the low power diode laser in this region following the protocol of 100 mW, 3 J of energy per point, wavelength of 660 nm, 30 seconds 10 intraoral points to promote healing. With the 808 nm wavelength and 3J of energy, 12 extraoral points were irradiated for trismus, with a total of 10 sessions. There was decrease of edema, improvement in the oral opening from 12 mm to 26 mm. **Conclusion:** The LLLT in the parameters used was effective in the treatment of trismus, in reducing edema and postoperative wound healing. **Financial Suport:** FAPEMA.

Keywords: Lasers, Surgery oral, Trimus.

LISTA DE AUTORES

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Alchorne MMA	CO.56
Alchorne OA	CO.56
Aleixo DC	CO.52
Alfredo PP	CO.11
Almeida JS	CO.14, CO.16, PT.16, PT.19
Almeida LG	PT.29
Almeida P	CO.28, PT.26
Almeida-Lopes L	PT.22, PT.38
Alonso H	PT.10
Amaral-Labat G	CO.28
Amirkhani A	CO.15, CO.20
Andrade ALM	CO.09, CO.55, PT.52
Andraus RAC	PT.07
Anjos LMJ	CO.41
Antunes HKM	PT.35
Arana-Chavez VE	CO.32, PT.11
Araujo DR	CO.23
Araújo TSD	CO.31
Araújo-Moreira FM	PT.22
Arisawa EAL	CO.52
Arnold G	PT.26
Asefi S	PT.20
Assis L	CO.10, CO.34, PT.01, PT.08, PT.13, PT.15, PT.18, PT.21
Assunção FFO	PT.28
Avo L	PT.17
Ayuk SM	CO.43
Bagnato VS	CO.31, CO.36, CO.39, PT.25, PT.47
Baldo Jr D	PT.23
Baptista IMC	CO.21
Barbieri CH	PT.07
Belotto R	CO.56
Bernardes-Filho R	PT.17
Bernardo GC	CO.29, CO.42, PT.06
Bertin LD	CO.14, CO.16, PT.16, PT.19
Bicknell B	CO.54
Bisset L	PT.02
Bjordal JM	CO.11, CO.12, PT.51
Blatt A	CO.49
Bomfim FRC	CO.53, PT.10, PT.14, PT.32

Bordbar S	PT.20
Bordvik DH	CO.35
Bortoluci CHF	CO.42, PT.37
Bossini PS	CO.09, CO.29, CO.42, PT.12, PT.22, PT.52
Bovo JL	PT.10, PT.32
Brassolatti P	PT.22, PT.52
Bravo MP	PT.16
Buchaim RL	CO.42
Bueno AP	PT.14
Bueno CRS	CO.42
Cabral D	PT.08
Camillo de Carvalho PT	PT.30
Campos L	CO.32, PT.11
Canto-de-Souza ALM	CO.09
Canuto KC	CO.40
Carbonaro CC	PT.28
Cardili L	PT.42
Carreira AC	CO.23
Carvalho RLP	CO.18, PT.05, PT.06, PT.26, PT.37
Casalechi HL	CO.26, PT.34
Casarotto RA	CO.11
Castelli DM	PT.36
Castro FS	CO.46
Castro JR	PT.11
Chavantes MC	CO.21, CO.50, CO.56
Chiquie S	PT.50
Claudino V	CO.10, CO.34
Coelho IS	CO.02
Cominetti MR	CO.55
Consolim-Colombo F	CO.50
Costa FC	PT.40
Cotomacio CC	CO.32
Criniti C	PT.15
Crocco J H	PT.28
Cury V	PT.18
da Silva F	CO.56
Dahmardei M	CO.15
Dale CS	CO.01, CO.03
Dalmaso RBM	CO.28, PT.26
Dantas EO	PT.01
Dantas HP	PT.34
D'Arienzo F	CO.53
De Angelis K	CO.50
de Avó LRS	PT.22, PT.52
de Carvalho MHC	CO.44
de Moraes APG	PT.06
De Nucci G	PT.09
de Souza DN	CO.32
Demange LK	CO.53, PT.32
Diniz IMA	CO.23, CO.24

Domingos H	CO.10
Domingues H	CO.34
dos Anjos LMJ	CO.51
Elbaz-Greener G	CO.49
Epelman S	PT.38
Ervolino E	CO.47
Eslaminejad B M	PT.20
Esquisatto MAM	PT.10
Evans K	PT.02
Fakayode O	CO.38
Fekrazad R	PT.20
Feldmann C	PT.24
Fernandes D	PT.15
Fernandes K	PT.15
Fernandes KBP	PT.07
Fernandes KR	PT.01, PT.08, PT.13
Fernandes TOM	PT.42
Ferrara Jr JI	CO.01
Ferreira C	CO.53
Ferreira OC	PT.53
Ferreira WDR	PT.53
Folha RAC	CO.05
Fonseca AS	CO.13, CO.17, CO.40, CO.41, CO.51, PT.29, PT.39, PT.41, PT.45, PT.46
Foresi N	PT.27
Freire L	CO.23
Freixedas EMR	CO.56
Frigo L	CO.12, CO.28, CO.44
Gaiato M	PT.06
Gameiro J	CO.41, CO.51
Garcez AS	CO.47, CO.48
Geller M	PT.39
Gjerde K	PT.51
Gonçalves EM	PT.39
Gonçalves J	PT.10, PT.32
Gonçalves JML	PT.06
Gonçalves LH	PT.04
Gonzalez-Lima F	PT.36
Grandinetti VS	PT.31
Guimarães OR	PT.39
Guirro RRJ	PT.07
Gusmão AGS	CO.36, PT.25, PT.47
Hamblin MR	CO.48
Hameetman CW	CO.27
Hanashiro KH	PT.28
Haslerud S	CO.12
Hodgkinson N	CO.45
Houreld NN	CO.43
Hudinik AF	PT.37
Hwang J	PT.36

Jafarian NF	PT.44
Janeiro JG	PT.18
Jang I	CO.07, PT.48
Jeong M	PT.48
Joensen J	CO.04, CO.12, CO.35
Johansson AA	PT.51
Kazemikhoo N	CO.15, CO.20
Kido H	PT.13, PT.18
Kido HW	CO.29, PT.22
Korinfsky JP	PT.42
Kress MRVZ	CO.29
Kruger CA	CO.38
Laakso EL	PT.02, PT.33
Labat GA	PT.26
Labat RM	CO.28
Lago ADN	PT.53
Langella LG	CO.06
Largura LZ	PT.24
Leal-Junior ECP	CO.06, CO.19, CO.26, PT.30, PT.31, PT.34
Leitman M	CO.49
Lenz e Silva GFB	CO.28
Liebano RE	PT.12
Liebert A	CO.54
Lim B	CO.07
Lim DJ	CO.07, PT.48
Lima FPS	CO.52
Lima LJ	PT.40
Lima MO	CO.52
Lima MPO	CO.14, CO.16, PT.16, PT.19
Lino ADS	PT.07, PT.52
Lizarelli RFZ	CO.39
Lobo FS	PT.24
Lograd E	PT.28
Lopes LA	CO.22
Lopes-Martins RAB	CO.12, CO.28, CO.44, PT.05, PT.09
Luca R	CO.30
Luri R	CO.25
Machado CSM	PT.30
Machado YLRC	PT.46
Magliano GC	PT.11
Magnussen LH	CO.12
Magri A	PT.08, PT.15
Magri AMP	PT.01, PT.13
Maia Campos PMBG	CO.39
Maia LP	PT.07
Malavasi P	PT.27
Malavazi I	CO.29
Mancini MW	CO.53
Manescu A	CO.30
Manis C	PT.08

Marcos RL	CO.12, CO.18, CO.44, PT.05, PT.26
Marcuzzo JS	PT.26
Maria DA	CO.44
Marques AP	CO.11
Marques ERMC	CO.56
Marques MM	CO.23, CO.24
Marques RH	PT.09
Martignago CCS	CO.55, PT.12, PT.16, PT.21
Martinez BR	CO.05
Martins PSLL	PT.09
Martins RABL	CO.11, PT.26
Mauricio Junior N	CO.39
Melo MO	CO.39
Mencalha AL	CO.17, CO.40, PT.41, PT.45, PT.46
Mendes IS	CO.52
Mendes JPM	CO.13
Mendes NA	PT.01
Meneguzzo DT	CO.46, PT.24
Menezes VG	PT.42
Miguel NA	PT.37
Miranda EF	CO.06, CO.26, PT.30, PT.31, PT.34
Mituyama LY	PT.18
Monteiro KKDS	CO.06, PT.31
Moon W	CO.47
Morais T	CO.50
Moreira MS	CO.23
Mori E	PT.03
Morini Junior N	CO.39
Moriyama JH	PT.18
Mosca RC	PT.40
Muscará MN	CO.44
Nakagawa IT	PT.10
Nakashima CF	CO.46
Napolitano AAT	CO.18, PT.37
Naterstad IF	CO.12
Natividade V	PT.50
Natividade VSS	PT.27, PT.28
Negrutiu ML	CO.30
Neto PN	CO.40
Neves LF	PT.42
Neves LMG	CO.55, PT.12, PT.21
Neves MF	CO.52
Nilforoushzadeh MA	CO.15, CO.20
Nilzen MS	PT.49
Nobre JS	PT.09
Nunes FRS	PT.53
Oancea R	CO.30
Oliveira CAB	PT.12
Oliveira CAM	CO.55
Oliveira JM	CO.36, PT.25, PT.47

Oliveira MCD	PT.22
Oliveira MX	PT.33
Oliveira PD	CO.14, CO.16, PT.16, PT.19
Oliveira RF	CO.14, CO.16, PT.16, PT.19
Oliveira VRS	CO.03
Oliveria C	PT.21
Oluwafemi SO	CO.38
Oron U	CO.49
Otterço AN	PT.52
Oversluizen G	CO.27
Paiva PRV	CO.26, PT.30, PT.31
Pallotta RC	CO.28, CO.44
Paoli F	CO.40, CO.41, CO.51, PT.29, PT.39, PT.41, PT.45
Pareta LCO	PT.50
Parizotto NA	CO.02, CO.09, CO.29, CO.55, PT.12, PT.17, PT.21, PT.22, PT.23, PT.52
Pazim L	PT.28
Pedroni ACF	CO.24
Pelissari C	CO.23
Pereira F	PT.11
Pereira NTC	CO.14, CO.16, PT.16, PT.19
Petrellis MC	CO.28, CO.44
Piccirillo S	CO.28
Pigatto GR	CO.02, PT.23
Pigoso AA	PT.10, PT.32
Pinfildi CE	CO.05, CO.33, PT.02
Pinheiro AFS	PT.53
Pinto AMFF	CO.22
Pinto LAP	CO.13
Pinto NC	CO.50, CO.56
Pires FB	CO.14, CO.16, PT.16, PT.19
Pires-Oliveira DA	CO.14, CO.16, PT.16, PT.19
Plapler H	CO.53, PT.32, PT.42
Poli-Frederico RC	CO.14, CO.16, PT.16, PT.19
Prado CM	PT.18
Rahouadj R	CO.28
Ramos L	PT.05
Ramos TAM	PT.05
Regalo SCH	CO.39
Reis MCR	CO.52
Renno AC	CO.25
Renno ACM	CO.10, CO.29, CO.34, PT.01, PT.04, PT.08, PT.13, PT.15, PT.18, PT.21, PT.33, PT.35
Ribeiro MS	CO.47, PT.40, PT.49
Rodrigues JA	CO.40
Rodrigues PLF	CO.31
Rodrigues VO	PT.14
Rosa Júnior GM	CO.42, PT.06, PT.37

Rosa LP	CO.31, CO.36, PT.25, PT.47
Sabino CP	PT.49
Sales TDB	PT.03
Saltorato JC	CO.18
Sampaio FCJL	PT.50
Sanches IC	CO.50
Sanches M	PT.15
Santana IC	CO.42
Santos AF	PT.19
Santos ALYSS	PT.01
Santos ARS	CO.02
Santos I	PT.42
Santos LS	CO.18
Santos MS	CO.31
Santos MYO	CO.05
Santos NAC	CO.36, PT.25, PT.47
Santos TCP	CO.42, PT.06
Sardim AC	CO.05, CO.33
Scalize PH	CO.39
Scheffelmeier ALA	CO.42
Seabra AGL	PT.42
Seelaender MCL	CO.44
Segundo ASG	PT.03
Sella VRG	CO.53, PT.32
Semprini M	CO.39
Sergio LPS	PT.29, PT.41, PT.45
Shojaeddin A	PT.44
Silva ACPP	CO.52
Silva AFS	CO.16, PT.19
Silva BPA	CO.50
Silva CL	CO.17
Silva FC	CO.31, CO.36, PT.25, PT.47
Silva GFBL	PT.26
Silva JL	CO.16
Silva LP	PT.50
Silva TR	PT.26
Simionato LH	PT.06, PT.37
Simões A	CO.32, PT.11
Sinescu C	CO.30
Sipert CR	CO.24
Siqueira A	CO.10, CO.34
Soares GP	PT.16
Soares LC	CO.13
Sogayar MC	CO.23
Songca SP	CO.38
Souza AC	PT.14
Souza BP	CO.13
Souza MV	CO.14
Spadaccini AF	CO.14, PT.16
Spoorendonk WHC	CO.27

Stumbo AC	CO.17
Suzuki H	CO.47, PT.03
Suzuki SS	CO.47, PT.03
Szezerbaty SKF	CO.14, CO.16, PT.16, PT.19
Taghiyar L	PT.20
Tanajura BR	CO.36, PT.25, PT.47
Tatmatsu JC	PT.21
Tatmatsu-Rocha JC	PT.17
Teixeira AF	PT.46
Thomé AM	CO.13
Tim C	CO.34
Tim CR	CO.29, CO.55, PT.12, PT.17, PT.21
Todea DC	CO.30
Toma RL	PT.33, PT.35
Tomimura S	CO.50
Toniolo EF	CO.03
Trajano ETL	CO.13, CO.17
Trajano LASN	CO.17
Trivelin MLOAA	CO.50, CO.56
Tzuman O	CO.49
Ueno F	PT.13
van Abeelen F	CO.27
Vanin AA	CO.26, PT.30, PT.31, PT.34
Vasconcelos PB	CO.39
Vassão PG	CO.25, PT.04, PT.35
Ventura MCA	CO.05
Vered Z	CO.49
Veronez S	PT.08
Vicentini JC	PT.45
Vicentini SC	PT.29, PT.41, PT.45
Vieira RL	CO.36, PT.25, PT.47
Vines JB	CO.07
Xavier MO	PT.02
Yamashita LB	CO.03
Yamauchi LY	PT.18
Yoshimura TM	PT.49
Zeituni CA	PT.40