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.

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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 cm^2 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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Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo

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.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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FINANCIAL SUPPORT: Fundação de Amparo à Pesquisa do Estado de São Paulo

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.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.

Cotomacio CC, Campos L, de Souza DN, Arana-Chavez VE, Simões A

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.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.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.
Financial support: We acknowledge the University of Johannesburg, National Laser Centre (NLC), African Laser Centre (ALC) and the University of Johannesburg research council (URC) for funding this project.

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.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.

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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.

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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).

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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.

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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.

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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, ø = 0,025cm², E_{pt}= 0,15J/pt and 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.