

PT.01

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

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Diabetes mellitus (DM) leads to a delay in bone healing. Thus, some therapeutic approaches have been used to accelerate the process of bone repair such as low-level laser therapy (LLLT). Therefore, the present study aimed to evaluate the effects of LLLT, in different fluences, in bone repair in an experimental model of tibial bone defects in diabetic rats. Sixty-four Wistar rats were submitted to a surgical procedure to perform bone defect and distributed in four groups: diabetic control group (DCG), diabetic laser group 30 J/cm² (L30), diabetic laser group 60 J/cm² (L60), and diabetic laser group 120 J/cm² (L120). A 808 nm Ga-Al-As (DMC Equipment, São Carlos, SP, Brazil) laser, 100 mW; 0.028 cm²; 3.57 W/cm²; 30, 60, and 120 J/cm²; 0.84, 1.68, and 3.36 J; 8, 16, and 33 s was used. Animals were euthanized 15 and 30 days after the surgery. Histological, morphometric, immunohistochemistry, and biomechanical analyses were performed. In the histological and morphometric evaluation, all laser-treated groups showed a better histological pattern and a higher amount of newly formed bone compared to DCG. An intense RUNX2 immunoexpression was observed in the laser-treated groups, 15 days after the surgery. Receptor activator of nuclear factor κ - β ligand (RANK-L) immunohistochemistry analysis showed a significant decrease in the immunoreactivity for L30 and L120, 30 days after surgery. There was no statistical difference in the biomechanical analysis among the groups. In conclusion, LLLT, in all fluences used, showed an osteogenic potential in bone healing of diabetic rats.

PT.02

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

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Background: Lateral epicondylalgia (LE), or tennis elbow, is a common condition, which may cause significant time away from work and sport and it is known as pain over the lateral epicondyle and point tenderness over the extensor muscle origin. Laser photobiomodulation has been used with the aim to improve tissue repair and decrease the pain in patients with LE.

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

PT.03

Effects of Low Intensity Lasertherapy on Miniscrew healing and bone neoformation

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Introdutionc: The success rate of miniscrews (MI) when used as temporary orthodontic anchorage is relatively high, but some factors, such as inflammation could affect its healing process and clinical success. Low Intensity Laser Therapy has been widely used for biostimulation of tissue and wound healing specially for its anti-inflammatory effects.

Objetives: The purpose of this study was to evaluate the effect of low intensity laser therapy over the miniscrew bone healing process.

Methods: 24 wistar rats received mini-screws on palate in maxilla. All the MI were immediately loaded with 50 gf with a NITi coin-spring connected to the first molar. The laser group (n=12) were irradiated with a 808nm diode laser with 100 mWs for 20s (Energy = 2 J) The control group (n=12) received the MI, but no irradiation. The animals were sacrificed at 5, 7, 9 and 14 days after MI installation. Samples were collected from the tissue around MI and histologically evaluated and submitted to Fractal dimension analysis (FD). **Results:** The clinical results showed a success rate of 100% for both groups. The histological analysis and FD analysis demonstrated that the laser group had less inflammatory cells than control group and the bone neoformation around mini-screw was more intense.

Conclusion: Low intensity laser therapy increased the success rate of orthodontic miniscrews, probably due to anti-inflammatory effect and bone turnover stimulation.

PT.04

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

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Introduction: Population aging is related to the increase of chronic degenerative diseases, including, osteoarthritis (OA). The OA is a degenerative chronic disease characterized by the gradual loss of cartilage affects about 50% of people over 65 and affects approximately 4% of the Brazilian population, its manifestation is more common in the knee and its progression can cause pain, swelling, stiffness, decreased range of motion (ROM) and physical disability. In this context, resources such as phototherapy has shown promising results, stand out as a therapeutic resource known for its anti-inflammatory effects and regeneration of biological tissues.

Objective: To analyze the influence of phototherapy on pain, functionality, mobility and quality of life in older adults with knee osteoarthritis.

Methods: This project was approved by the Ethics Committee in Human Beings of the Federal University of São Paulo / Hospital Sao Paulo (UNIFESP) - Number: 1.368.478. Were evaluated 6 individuals (both sexes) with knee OA that were allocated into 2 groups: control group (CG n= 2) without any intervention and Laser Group (GL, n=4) treatment with irradiation only. The interventions were performed 2x/week for 6 weeks. Phototherapy Cluster type (850nm, 100mW, 4J by point, totaling 28J of 7 diodes) was applied to each session in the lateral and medial side of both knees. For evaluation and re-evaluation, questionnaires were applied as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Lequesne, visual analog scale of pain (VAS), Timed Up and Go (TUG), Short Physical Performance Battery (SPPB) and the test six-minute walk (6MWT). For statistical analysis we used t test followed of Mann Whitney test for analysis for intra and inter-group with a significance level of $p < 0.05$.

Results: The results obtained affirm that there was no significant difference in the anthropometric variables: height ($1.62\text{m} \pm 8.76$), weight ($70\text{kg} \pm 7.25$), age ($65.6\text{ years} \pm 7.22$) and BMI ($26,8\text{kg} / \text{m} \pm 2.42$) between groups. Furthermore, it can be seen that there was no significant intergroup difference VAS ($p=0,133$); WOMAC - pain ($p= 0,2$); stiffness ($p =0,06$); physical function ($p= 0,133$); Lequesne ($p=0,133$); TUG ($p=0.8$); SPPB ($p=0,4$) and 6MWT ($p=0,4$) and intragroup compared to VAS ($p =0,4$); WOMAC - pain ($p=0,08$); WOMAC - stiffness ($p=0,2$); WOMAC - physical function ($p=0,114$); Lequesne ($p=0,22$); TUG ($p=0,48$); SPPB ($p=0,485$); 6MWT ($p=0,342$).

Conclusion: Therefore, it is concluded that phototherapy was not effective for improving quality of life, functionality and reducing pain in this pilot study. The sample size was relatively small, so more volunteers will be analyzed to try to elucidate the effects of phototherapy older adults with knee OA.

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

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

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Introduction: Muscle strains are among the most prevalent causes for athletes' absence from sports activities. The main objective of this study was to investigate the effects of low level laser therapy (LLLT) on controlled muscle strain in rats. **Method:** Wistar rats were anesthetized for controlled muscle strain induction (n=6). The experimental protocol was approved by the Animal Research and Care Committee of the University of Sao Paulo. A single LLLT session was performed 1 h after muscle trauma with an infrared laser unit (DMC®; Sao Carlos, Brazil). The laser emitted a continuous optical output of 100 mW with a wavelength of 810 nm to a spot size area of 0.028 cm², giving a power density of 3.57 W cm². The optical output was measured before, halfway through and after the experiment. Laser irradiation was performed in skin contact in the middle of anterior tibialis muscle on the belly with doses of 1 J (35.71 J cm⁻²), 3 J (107.14 J cm⁻²), 6 J (214.29 J cm⁻²) and 9 J (321.43 J cm⁻²) and corresponding irradiation times of 10, 30, 60, and 90 s, respectively. Outcomes: real time RT-PCR gene expression of COX-1, COX-2, TNF- α , IL-1, IL-6, IL-10 and muscle histology. **Results:** After experimental induction of muscle strain, LLLT was effective reducing pro-inflammatory and increase anti-inflammatory cytokines and improved structural organization of muscle tissue with well-defined cells and a minor amount of fragmented fibers. **Conclusion:** LLLT with 3 J significantly improved the inflammatory markers and muscle histology.

PT.06

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

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Introduction: Peripheral nerves are constantly targets of traumatic injuries and rarely present recovery without surgical intervention when they have tissue loss. Thus various techniques tubing using biological materials (vessels, nerves, vein) or non-organic (polyethylene tubes, silicon) with or without padding (adipose tissue stem cells) are being tested. However, even with all technical refinement achieved by microsurgery, still do not get full motor recovery. In the clinical setting, some physical therapy resources have been suggested in an attempt to minimize losses from the denervation framework, highlighting the Low Level Laser Therapy.

Objectives: To evaluate the effect of Low Level Laser Therapy in muscle recovery after tubulization technique filled with fat tissue.

Methods: All the procedures adopted were approved by CEUA/USC: 8853300315. Were used 60 Wistar rats, males, with 80 days of life, supplied by the vivarium of University of the Sacred Heart, randomly divided into six groups of 10 animals each: Control group (CG), denervated group (GD), tubulization group (WG), tubulization group with fat tissue (GTG), tubulization and Laser group (GTL) and tubulization group filled with fat tissue and Laser (GTGL). The tubulization groups received jugular vein graft. Groups with fat denomination received filling with fat and the name received low level laser therapy treatment after tubulization surgery. The treatment was performed with Laser AsGaInP (XT Therapy), 808 nm, 100 mW, 0.04 cm² beam diameter, energy of 0.09 J (2.25 J/cm²) with irradiation time 90 seconds during 3x/week for 150 days. Morphometry 220 muscle fibers per animal in each group was carried out, and electrophysiological and functional tests (IFC). When comparing the groups, we used analysis of variance (ANOVA) followed by Tukey test, when detected significant difference. In all the significance level analysis was $p < 0.05$.

Results: In the morphometric analysis groups presented respectively to the EDL, Soleus and TC the following results: 2998 μm^2 , 2872 μm^2 and 4351 μm^2 for GC, 2722 μm^2 , 2323 μm^2 and 3852 μm^2 for GTGL, 2377 μm^2 , 2086 μm^2 3184 μm^2 for GTG, 2123 μm^2 , 1911 and 3051 μm^2 μm^2 for GTL, 1619 μm^2 , 1428 and 1582 μm^2 μm^2 for the GT 154 μm^2 , 123 and 172 μm^2 μm^2 for GD. In the strength test the GTGL group got the best rate when compared to GC, reaching to the CT muscle the value of 0.97 against 1.21 of the GC to the EDL muscle 0.71 against 0.87 of the GC and the Soleus 0.68 against 0.86 of the GC. The values of the functional indices presented by the groups were: GC -7,11 / GD -96.13 / -79.22 GT / GTG -53.23 / GTL -63.87 / -34.12 GTGL, where again the GTGL It showed the best result.

Conclusion: Low Level Laser Therapy was presented as a positive protocol for functional recovery of patients with peripheral nerve injury.

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

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Introduction: Traumatic lesions to peripheral nerves are frequent and require specialized care, which not always produce the desired effects, thus leading to functional loss of various degrees, but often incapacitating, with the target skeletal muscles strongly affected. Restoring nerve activity and consequently muscle function is a priority of any therapeutic intervention, including low level laser therapy (LLLT), a physical means with an alleged high level of positive response (over 80%), as indicated by the results of many investigations on this subject. **Objective:** To analyze the LLLT on metalloproteinase expression and the mechanical strength of skeletal muscle regeneration after peripheral nerve injury. **Materials and Methods:** Rats were subjected to crush injury of the right sciatic nerve, followed by LLLT (830nm, 35, 70, 140 and 280 J/cm²) for 21 consecutive days. Functional gait analysis was performed weekly and the animals were euthanized at day 21 for collection of the gastrocnemius muscles, which were submitted to analysis of resistance, and the anterior tibialis, for evaluation of metalloproteinase-2 (MMP-2). The results were statistically analyzed at a significance level of 5%. **Results:** The sciatic functional index decreased significantly while the mechanical strength increased significantly in all groups during the weeks. For the activity of MMP-2 in the intermediate band it was observed higher values in groups 3 (35J/cm²), 4 (70J/cm²) and 5 (140J/cm²) when compared to groups 1 (normal), 2 (lesion) and 6 (280J/cm²); while at the activated band, the activity was significantly more intense in group 6 (280J/cm²) when compared to group 1 (normal). **Conclusion:** These data suggest that LLLT 830nm low (35J/cm²) moderate (70J/cm²) and high (140 and 280J/cm²) energy density accelerates neuromuscular recovery after crush injury of the sciatic nerve in rats.

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

PT.08

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

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

PT.09

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

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Introduction: musculoskeletal and joint inflammatory diseases are among the most prevalent causes of physical limitations today. About 60% of the population over 60 has some disease of this type, making continuous use of analgesics and anti-inflammatory drugs. However, the adverse effects of prolonged use of anti-inflammatory drugs are well known, and cause highly relevant and significant impact to the health system, including kidney failure, digestive bleeding, gastritis, ulcers and others. The low power lasers have been used for the treatment of inflammatory diseases, especially articular and tendon conditions. It is believed that it is possible the combination of pharmacological and laser therapies to improve the treatment of musculoskeletal disorders, in order to reduce costs and adverse effects and increasing the effectiveness. In this sense, the application of topical medications seems a viable alternative, with the exception of limitations on drug absorption and bioavailability in the inflammatory site. In view of the reported effects of microcirculation of vasodilation induced by irradiation with low-power laser, we aim to investigate the effects of radiation in the absorption of topical drugs, aimed at increasing plasma drug bioavailability.

Materials and Methods: The present study was approved by the Ethics Committee of the Human UNICAMP. It conducted an open, randomized, crossover, with two treatments, two periods, in which volunteers received in each distinct period, the reference formulation, with or without the application of laser therapy. The study was designed so as to allow to obtain pharmacokinetic parameters (AUC_{0-All} and C_{max}) for statistical comparison, aiming to investigate the topical drug plasma levels, with and without the application of laser radiation. Initially low power laser irradiation was performed in a cluster configuration contend 14 issuers, with 07 of wavelength 650 nm and 100 mW of power and 07 810 nm and 100 mW of power. Irradiation was performed for 30 seconds, for a total of 3 Joules of energy per application point. After 5 minutes the gel diclofenac was applied to the dorsal region of each individual. Successive blood samples were collected up to 24 hours after application, to establish a curve pharmacokinetics of diclofenac in plasma of volunteers. The determination of diclofenac concentrations in rat plasma were performed in the Analytical Unit Cartesius - Prof. Laboratory Dr. Gilberto De Nucci in the Department of Pharmacology of the ICB / USP, according to Method Validation of Analytical Protocol METGRU 08-07 - Determination of Diclofenac in human plasma by LC-MS / MS. **Results:** Preliminary results suggest the concept of verification in order that the laser application potentiated about 100% of the pharmacokinetic parameters of diclofenac applied topically to the skin of white healthy male volunteers. The ASC_{tudo} ([ng * hr] / ml) control was 27.23 and passed the laser application 52.53 ([ng * hr] / ml). C_{max} achieved was 3.23 (* ng / mL) and 6.1 (* ng / mL) for the laser group. **Conclusions:** Considering the risks inherent in the use of anti-inflammatory steroid (coxibs or nonselective) and the high incidence of osteo-articular diseases in the

population, the combination of anti-inflammatory drugs used topically laser offers an interesting therapeutic option.

Keywords: Low power laser, diclofenac, LLLT, Clinical Study.

Funding Agency: CAPES; FAPESP.

PT.10

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

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Introduction: Gouty arthritis is characterized to affect especially men of middle age, old-aged and women post menopause. It is responsible for joint degeneration caused by chronic inflammation and its treatment is based on non-steroidal anti-inflammatory drug (NSAIDs) and corticosteroids. Low-level laser therapy (LLLT) can be used like an alternative to medicines for being a non-invasive therapy with analgesic and anti-inflammatory effects and responsible for cell proliferation. **Objective:** The aim of this study was to evaluate the effects of low-level laser therapy on articular tissue structure and in lipid peroxidation by production of reactive species to thiobarbituric acid (TBARs) during the acute phase of experimental gouty arthritis in male Wistar rats. **Methods:** Twenty-four Wistar rats (200g±10g) were assigned into four groups (n=6): A (control), B (induced arthritis-IA), C (IA+laser 830nm) and D (IA+laser 670nm) (Ethics Committee n.048/2015). On time 0, animals from B, C e D were anesthetized with Ketamine/Xilazine/Tramal (10, 0,9 e 5,0 mg/Kg, respectively) and submitted to two intraarticular injections in the right knee by 2mg of Ca₂P₂O₇ dissolved in 50µL of saline sterile solution with 24-hour interval. After 48 hours, animals from C and D groups were submitted, respectively, to LLLT with a Gallium-Arsenide (GaAs) laser device (*Bioiset*®) with λ=830nm, energy density=13,5J/cm², power=30mW, total energy=0,27J, beam area=0,02cm² for 9 seconds and a Indium-Gallium-Aluminum-Phosphorus (InGaAlP, *Bioiset*®) λ=670nm, energy density=18J/cm², power=30mW, total energy=0,32J, beam area=0,02cm² for 12 seconds, both by contact method, point application in patellar region of right knee once a day. After 7 days of laser treatment, serum samples were collected by cardiac puncture for TBARs assays. It was calculate the reading values with 532nm=153.000M⁻¹/cm⁻¹ of absorbance in a spectrophotometer. The right knees were histological processed and histopathological analysis realized with the following stain methods: Picrosirius red-Hematoxylin (tissue acidophilia), Toluidine Blue (tissue basophilia) and Hematoxylin and Eosin (qualitative structural analysis). Quantitative data were analyzed by ANOVA and Tukey *post-test* (p<0,05). **Results:** TBARs analysis showed no significant difference (p=0.424) between the mean values of the groups after 7 days: A (0.020, Standard Deviation [SD] ±0.004), B (0.017; SD±0.003), C (0.017; SD±0.003) and D (0.013; SD±0.006). The structural evaluation showed on induced groups when compared to A, independent of the laser therapy, reduction of basophilia in extracellular matrix of cartilage, associated to loss of the articular surface alignment and increased of surface layer thickness. There were no changes in acidophilia matrix, collagen fibers orientation and in morphological characteristics of isogenous groups. In synovial analysis of group A were not detected cellular or fibrous matrix changes. However, induced groups, regardless of the treatment, showed intense inflammatory infiltration, increased thickness of epithelium and dilatation of blood vessels

immersed in the matrix. The matrix also shows thicker collagen fibers randomly distributed into the tissue. **Conclusion:** In the acute phase of experimental gouty arthritis, low-level laser therapy (830nm and 670nm) do not change the evolution of the histopathological aspects of the disease and is not able to induce systemic anti-oxidant response.

PT.11

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

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

PT.12

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

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Introduction: The skin flap is a technique frequently used in reconstructive plastic or tissue loss surgeries (Plast. Reconstr. Surg. 72, 766, 1983). This technique presents as main complication tissue ischaemia and can lead to flap necrosis (Acta Cir. Bras., 14, 33, 2000), causing longer hospital stays and even an increase in health spending. Researches indicate that low level laser therapy (LLLT) may be used as an effective therapeutic resource to reduce the area of necrosis, however, there is divergence on where LLLT should be applied on random cutaneous flap (Lasers Surg Med. 37, 77.; 2005; Photomedicine and Laser Surgery. 27, 411.; 2009). **Objective:** evaluate the effects of applying the same energy of LLLT (660nm) in two distinct regions of the random skin flap in rats. **Methods:** In this work, 18 (*Rattus norvegicus*: var. *Albinus*, Rodentia Mammalia) male Wistar aged about 3 months and body weight $296.39 \text{ g} \pm 26.86 \text{ g}$ were used. These animals were randomly divided by draw, which used a sealed envelope, into 3 groups (n = 6): control group (GI), which received LLLT simulation treatment - the equipment was turned off; group that received treatment in 3 points (GII), whose animals received LLLT irradiation in three points, 4 J of energy per point (total of 12 J), for 84 seconds at each point with a 150 J/cm^2 fluency; and the group 12 points (GIII), whose animals received, in twelve points, LLLT irradiation, which had an energy of 1 J per point (total of 12 J), for 22 seconds at each point, and a fluency of 40 J/cm^2 . The random cranial based skin flap was created with dimensions of 10x4 cm (Plast Reconstr. Surg. 35 .; 177, 1965) and a plastic barrier was placed between the flap and the donor site. The animals were irradiated with an Indium, Gallium, Aluminium, Phosphorus (InGaAlP) laser, 660 nm (Photon Lase III, DMC®, São Carlos, SP, Brazil) with 0.028 cm^2 , with a power of 50mW, beam area in continuous emission mode. The treatment started immediately after the surgical procedure and it was reapplied every 24 hours, completing a total of 5 applications. The necrosis area was assessed on the seventh postoperative day with the paper template method (Plast. Reconstr. Surg. 65 .; 152, 1980). For statistical analysis, the Kolmogorof Smirnof normality test was performed; for comparison between groups, we used the ANOVA-One Way test and for the presence of a significant difference ($p < 0.05$), we used the *post-hoc* Tukey. **Result:** we observed a statistically significant difference ($p = 0.004$) between the GI ($63.84\% \pm 9.33$) and GII ($38.91\% \pm 10.75$), in which it was observed GII animals showed better results than GI; there was also a statistically significant difference ($p = 0.027$) between GII and GIII ($57.82\% \pm 13.21$), in which GII animals showed a decreased necrosis area. There was no significant difference ($p = 0.631$) between the animals of GI and GIII groups. **Conclusion:** there is a difference at application points of LLLT, in order to increase the flap viability. The application of LLLT, with the same irradiation energy and located in the cranial base of the flap on fewer points, was more effective to increase the viability of random skin flap in rats.

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PT.13

Effects of the low level laser therapy associated with Biosilicate®/PLGA composites in tibial bone repair

Fernandes KR, Magri AMP, Kido H, Ueno F, Assis L, Renno ACM

Effects of the low level laser therapy associated with Biosilicate®/PLGA composites in tibial bone repair

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Introduction: Laser therapy has been showing osteogenic effects in *in vitro* and *in vivo* studies. Biosilicate® (BS) is a fully crystallized bioactive glass-ceramic and presenting excellent biocompatibility and osteoconductive properties [1]. To improve the performance of BS, some porogenic materials (Poly(D,L-lactic-co-glycolic) acid (PLGA) microparticles) can be introduced into BS to produce microporosity, and allow the passage of cells that are involved in bone repair. Thus the association of both treatments could be more effective to accelerate the process of tissue regeneration and promote bone repair. The aim of this study was to evaluate the *in vivo* biological performance of the phototherapy at 808 nm associated to BS and BS/PLGA.

Material and Methods: For this experiment, 40 mature male Wistar rats were used (12 weeks, weight 300-350 g). All rats received one surgical procedure during the course of this experiment to induce the unilateral noncritical size bone defects (3 mm diameter) in tibia of all animals, which were filled with different composites. The animals were randomly divided into 4 groups (BS; BS + LLLT; BS/PLGA 80/20; BS/PLGA 80/20 + LLLT). Each group was divided into two subgroups, euthanized by carbon dioxide asphyxia after 2 weeks post-surgery (n=10 for each subgroup). Histopathological analyses were performed. This study was approved by the Animal Care Committee guidelines of the Federal University of São Paulo (2013/601498).

Results: Histopathological analysis showed that the introduction of PLGA into BS resulted in a higher material degradation, with the increase of newly formed bone ingrowth when compared to BS. However, the laser therapy was not able to improve the bone repair in the period analyzed in this study.

Conclusion: Summarizing, the results of this study confirmed our hypothesis that the introduction of PLGA into BS improve the bone formation, constituting an alternative to be used in bone tissue engineering. However, when LLLT and biomaterial were used in association, no positive results were found to accelerate the bone repair.

PT.14

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

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Introduction: Osteoporosis is a disease characterized by decreased of bone mass associated with a bone microarchitecture breakdown, which results in an increased of fragility and risk of bone fracture. The treatment is based in nutritional supplementation, however the loss of bone mass is progressive. Low-level laser therapy has the ability to biostimulate a variety of tissues and cells, like osteoblasts promoting mitosis, DNA replication and protein synthesis in bone tissue, including in osteoporosis. **Objective:** The aim of this study was to evaluate the effects of low-level laser therapy regarding to morphological aspects in an induced model of osteoporosis in Wistar rats. **Methods:** After approval by the Ethics Committee of FHO-UNIARARAS protocol 102/2011, forty-five young females Wistar rats weighing 200g (\pm 10g) were assigned into three groups: G1 (control; n = 15), G2 (induced osteoporosis; n = 15) and G3 (induced osteoporosis and low-level laser therapy; n = 15). For induction of osteoporosis, groups 2 and 3 were subjected to intramuscular injection of dexamethasone (7mg/kg), once a week, for 5 weeks, while G1 was subjected to sterile saline injections. After induction process, G3 has received low-level laser therapy sessions by a Gallium-Arsenide laser device (BioSet®, Rio Claro, Brazil) with $\lambda=830\text{nm}$, energy density= $13,5\text{J}/\text{cm}^2$, power= 30mW , total energy= $0,27\text{J}$, beam area= $0,02\text{cm}^2$ for 9 seconds in the medial femoral muscle by contact point technique, daily, for up to three weeks. Animals of G2 underwent sessions with the laser device off. Samples were collected 7, 14, 21 days after the start of treatment (n=5, for each period). Euthanasia was performed with anesthetic overdose with Ketamine 10%. The femurs were removed and submitted to histological processing, stained with hematoxylin and eosin for morphometric analysis of the femoral thickness, in micrometer (μM), using Image J software (NIH). Statistical analysis was performed by ANOVA and post-test of Tukey with a significance level of 5% ($p<0.05$) using Prism 5.0 program. **Results:** There were no significant differences in the analysis of femurs thickness between the groups of 7 days ($p=0.3026$). In the 14-day group, significant differences were found in femurs thickness between G2 (median 419,7; standard deviation [SD] $\pm 188,2$) and G3 (median 752,1; SD $\pm 137,7$) and $p=0,0260$. The analysis of the 21-day group showed differences between the groups G1 (median 750,0; SD $\pm 128,1$) and G3 (median 830,2; SD $\pm 50,75$) with $p=0,0079$ and between G2 (median 610,1; SD $\pm 70,10$) and G3 (mean 830,2; SD $\pm 50,75$) with $p = 0,0031$. **Conclusion:** Low-level laser therapy showed to be effective in the treatment of induced osteoporosis particularly in 14 and 21 days of treatment. There was an increase in femoral thickness of animals treated with laser compared to the control and untreated groups, which bring us that low-level laser irradiation is capable to induce osteoblast proliferation and consequent increase of bone mass in osteoporosis. Despite the findings, further studies evaluating other laser parameters are necessary to verify the clinical applicability.

PT.15

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

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Osteoarthritis (OA) is the most common arthritis type and also a multifactorial disease, considered inflammatory and degenerative. The clinical syndrome doesn't affect only the cartilage itself, but all the tissues that are part of the joint, as bone, synovial liquid, muscles and ligaments. According to WHO (World Health Organization), OA is the most frequent disease in adults, especially elderly population. Low-level laser therapy (LLLT) is a treatment appliance that demonstrates good clinical results on rehabilitation of OA. At the same time, there still needs more research about the LLLT effects in inflammatory and degenerative process of the cartilage tissue. Therefore, the purpose of this study was to evaluate the effects of LLLT on inflammatory and degenerative markers on the articular cartilage after the anterior cruciate ligament transection (ACLT) in the knee of rats. This study was approved by the Ethical Committee of the Federal University of São Paulo (2015/7792220515). Thirty male Wistar rats were randomly divided into 3 groups: control group (CG); ACLT group (OAG); ACLT plus LLLT (OAL). Laser irradiation (AlGaAs, continuous wave, 808 nm, 50 mW, 28 s, 50 J/cm²; 0.028 cm² 1.7 mW/cm²; 1.4 J, PHOTON LASER II, DMC[®] equipament Ltda, SP, São Carlos, Brazil) was used. Treatment started 4 weeks after the surgery, at 2 points of left knee joint (medial and lateral side of the joint), for 24 sessions. The results showed that LLLT was able to decrease OARSI score ($P < 0.0001$), increase cartilage thickness ($P = 0.001$) and reduce inflammatory (IL-1 β) ($P = 0.008$) and apoptotic markers (caspase-3) ($P < 0.0001$). As a conclusion, these data shows us that LLLT could be an effective therapeutic option to in modulate inflammatory and degenerative process in knee osteoarthritis.

PT.16

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

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Introduction: The low-level laser therapy (LLLT) it's a resource widely used on rehabilitation clinics, it exerts important biomodulation effect on cell and tissue, neovascularization, cell proliferation and synthesis of pro - collagen, contributing directly to the process of tissue rehabilitation.

Objectives: Evaluate the effects of low-level laser therapy with wavelength of 904 nm, doses of 2 and 3 J/cm², on cell proliferation and gene expression of COL1 α 1 and VEGF from fibroblast cells.

Methods: The L929 fibroblast cells were irradiated with Gallium Arsenide diode laser (AsGa). It was divided in 3 groups: G1- Control group (have not received any radiation); G2-irradiated with 2 J/cm² and G3- irradiated with 3 J/cm²). The radiation was performed at 24h, 48h and 72h hours. The cell proliferation was analyzed by the MTT method (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide] and quantified by microplate reader. The quantification of transcripts of the genes Col1A1 and VEGF was realized by polymerase-chain-reaction using real-time detection (qPCR). The relative expression of the genes was calculated using the reference gene β -Actin and normalized by the gene expression in relation to the control group. To compare and verify the statistically significant difference between the groups, analysis of variance (ANOVA), *One Way* and the *post hoc* of Tukey tests were used. For comparison between the evaluations, was performed the ANOVA test with repeated measures. On the gene expression, the data were analyzed using the Pfaffl method and calculated by the Software Reset 2009, considering standard deviation values and statistic test "Pair wise fixed reallocation", assuming significance of $p < 0,05$.

Results: Comparing the number of viable cells of the groups G1 (100%), G2 (131,05 \pm 22,9) and G3 (145,64 \pm 13,9), it was observed a statistically significant difference 24 hours after the first irradiation ($p = 0,029$). At the times of 48h ($p = 0,621$) and 72h ($p = 0,084$), any statistically significant difference was found between the groups. On the analyzes of repeated measures of the groups on the different analyzed times, a statistically significant difference was verified ($p = 0,01$) only in the radiated G3 cells, where occurred a reduction of viable cells from 24 to 72 hours ($p = 0,035$). The results of the expression showed an increase of 1,78 times in the RNAm expression of COL1 α 1 gene ($p = 0,036$) on G2, while G3 don't present statistically significant difference ($p = 0,138$). On the gene expression of VEGF, regarding to the control group, was observed an increase in the expression of 2,054 ($p = 0,037$) on G2 and on G3 the raise in the RNAm levels was 2,562. It was not observed significant difference between G2 and G3 ($p > 0,05$).

Conclusion: Low-level laser therapy can stimulate cell proliferation, expression of COL1 α 1 and VEGF on L929 cell culture.

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PT.17

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

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

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

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Sepsis is defined as an infection associated with systemic manifestations and it is the major cause of death in hospitalized patients, as it leads to release of large amounts of inflammatory mediators. The acute respiratory distress syndrome (ARDS) is often related to sepsis and is a common cause of death. The low-level laser therapy (LLLT) is a complementary resource and non-invasive mechanism used for therapeutic purposes as a safe and effective treatment. Several studies have shown that LLLT reduces inflammatory cells and enzymes responsible for the release of chemotactic factors during the inflammation's initial phase. Besides that increases the level of antioxidant enzymes in several models of inflammatory diseases. Thus, the aim of this study was to evaluate and compare the effects of LLLT on acute lung inflammation resulting from sepsis in experimental sepsis model (cecal ligation and puncture- CLP). Federal University of São Paulo's Ethical Committee (Number 8948050515) approved this study. Twenty-five male Wistar rats, ± 300 g, 3 months old were used. The rats were randomly divided into 3 groups: control (C); sepsis group (S): rats submitted to CLP without LLLT treatment; sepsis laser treated group (SIR). Laser irradiation (AlGaAs, continuous wave, 808 nm, 30 mW, 28 s, 30 J/cm²; 0.028 cm²; 0.8 J, PHOTON LASER II, DMC[®] equipment Ltda, SP, São Carlos, Brazil) was performed immediately after surgery on the anterior portion of the trachea near the carina and anterior portion of the chest near the diaphragm bilaterally, through punctual contact technique. Bronchoalveolar lavage technique was performed as follow: instillation of 3 ml of saline into the trachea (3 times), centrifugation at 1,000 rpm for 10 minutes at 4 ° C, the cell pellet was re-suspended with 1 ml of PBS to count the total number of cells per ml carried out in a Neubauer chamber. Animals were euthanized 24 hours post-surgery. The results showed an increase in the number of cells in the S when compared to C. Meanwhile, no other difference was noticed between the other groups. Histological and immunohistochemical analysis are in progress. The current result does not allow us to conclude the laser effects on lung inflammation induced by sepsis. We believe that further analysis will be important to assess the effects of LLLT in this model, because the CLP technique is the one that is closest to human sepsis due to polymicrobial profile.

PT.19

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

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

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

Methodology: fibroblast cells L929 were cultivated in bottles of 25 cm^3 (TPP, Switzerland, Europe) with DMEM/HAM F12 solution supplemented with 5% of FBS (fetal bovine serum) and 1% of antibiotic and antimetabolic, were maintained on a 5% CO₂ greenhouse at 37 ° C; after, the cells were plated on a TPP plate and 24 hours after the sedimentation, cells were irradiated with low power laser ($\lambda = 660 \text{ nm}$) on the time of 24, 48 and 72 hours, according to the groups: G1 - control group (not irradiated); G2 - 4 J/cm²; G3 - 6 J/cm². After each time of 24 incubation hours post radiation, the cells culture were analyzed for the MTT method of cytotoxicity [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] for the evaluation of cell viability. The morphology alterations related to the cytoskeleton and endoplasmic reticulum (RE) were analyzed by the Fluorescence microscopy (MF) with the rhodamine-phalloidin markers and DIOC₆, respectively. To analyze the IL-6 and VEGF gene expression, the method RT-qPCR was used for the group that presented better growth on the better time.

Results: When analyzing the cells growth, we observed that G1 showed cell proliferation media of 95% ($\pm 0,02$) on 24 hours, 91% ($\pm 0,05$) on 48 hours and 99% ($\pm 0,00$) on 72 hours. While, G2 110% ($\pm 0,15$) on 24 hours, 91% ($\pm 0,19$) on 48 hours and 78% ($\pm 0,05$) on 72 hours. G3 presented media 87% ($\pm 0,05$) on 24 hours, 121% ($\pm 0,32$) on 48 hours and 114% ($\pm 0,17$) on 72 hours. Regarding to cell viability, significant statistic was found in the realization of Anova of two factors ($F=40.53$; $p=0,017$), however in the analysis of the posttest related to time and dose no significant difference was found, nevertheless the G3 presented bigger growth curve in time of 48 hours related to the other groups. On the evaluation of MF, in a dose of 6 J/cm², in the times of 24 and 48 hours, positive biomodulation effects were presented regarding to the cytoskeleton with bigger distribution and organization of the actin filaments and also with increase and better distribution of the vesicles of RE in the cytoplasm related to the control group. Concerning the gene expression of VEGF and IL-6 was possible to find a significant difference in G3, on 48 hours on the transcripts genes, being the stimulation of 1,82 to the VEGF and inhibition of 36,75 to IL-6.

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

PT.20

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

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

PT.21

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

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INTRODUCTION: Osteoarthritis (OA), the most prevalent form of arthritis, is a chronic and painful disease of synovial joints, most commonly the knees, hips, and hands. It is characterized by gradual degeneration of the joint, progressive destruction of articular cartilage and new bone formation at the joint surface and surrounding areas (Cytokine 70(2):185-93). Due to the very limited cartilage regenerative capacity and consequently the limited efficacy of the standard treatments, the investigation of strategic innovative approaches to prevent the development of the clinical condition of OA is of great interest (World J Orthop 18; 5(3): 351-361). One promising treatment is the use of low-level laser therapy (LLLT) mainly due to its anti-inflammatory and regenerative effects on biological tissues (Lasers Surg Med 2004;35(3):229-35). Therefore, this study aimed to investigate the effects of LLLT on chondrocytes viability. **METHODS:** This study was approved by the Animal Care Committee guidelines at Federal University of São Paulo (CEUA N 2478130315). Chondrocytes were obtained from collagenase-digested femoral growth plate cartilage of 7-week-old rats. Chondrocytes were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco). All tissue culture procedures were performed under strict aseptic conditions in a biological safety cabinet. Chondrocytes were grown in sterile, vented, 175 cm² tissue culture flasks (Greiner Bio-one, Utrecht, the Netherlands) in a humidified incubator at 37°C in 5% carbon dioxide (CO₂), 95% air. The cells were expanded for four passages using standard tissue culture techniques. Subsequently, Chondrocytes were seeded in direct contact with the particles at a density of 5.10⁴ cells/well. Cells were cultured for 3 days. Cell irradiation was performed using a Photon Laser III - DMC Equipament®, with a wavelength of 808 nm, 50 mW, 30 J/cm², 16 seconds, 0,8 J. The cells were irradiated 24 h after seeding, for three consecutive days, once every 24 h, on 48-well culture plates. The action of the laser's biomodulation was evaluated in three experimental groups: G1—control group, which received no irradiation and G2—irradiated at 30 J/cm². The irradiation was performed shielded from the light in a darkened room, with the laser pointer tip in direct contact with the plate. The experiment was conducted in triplicate. Cell metabolic activity was evaluated using Alamar Blue® (Invitrogen, Life Technologies), according to the manufacturer's instructions. Subsequently, 200 µl of each sample was transferred to a 96 well plate (in duplicates). Finally, the plate was read in a spectrophotometer (Bio-Tek Instruments, Winooski, USA) at 570 nm. **RESULTS:** The alamarBlue® assay is based on the ability of metabolically active cells to convert the reagent into a fluorescent and colorimetric indicator. Thus, the results revealed that the cell metabolic activity was significantly higher for LLLT compared to control group (p< 0,0026) which are evidenced by the appearance a large number of live cells after 3 days. **CONCLUSIONS:** Thus, based on the results of the present study, it has been shown that LLLT within the parameters presented is capable of stimulating the Chondrocytes viability.

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

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Introduction: Burns are lesion caused by direct or indirect contact to chemical, physical or biological agents and are associated with tissue damage, infection, pain and even death (Lasers Med Sci 28; 543-54, 2013). Front of the interventions proposed in recent decades low-level laser therapy (LLLT) is considered a promising treatment since it is low-cost, non-invasive, and induces cell proliferation (Lasers Med Sci 29; 313-19, 2014). Laser action is based on absorption of laser light by tissue specific photoreceptor, and after light absorption, important changes in cellular metabolism are observed, resulting in therapeutic effects as the modulation of inflammation, reduction of edema, and stimulating the synthesis of proteins considered essential to due evolution of the healing process. In order to achieve such effects it is necessary that the parameters are compatible with both the pathology and with the depth of the tissue to be treated. However there are discrepancies in the value established in protocols for the treatment of burns and due to this further investigations are necessary.

Objective: To evaluate the effects of low-level laser therapy (660 nm, 25 J/cm², 1J) on the healing process of third degree burns in rats.

Methods: The Ethics Committee in Animal Experimentation at the Federal University of São Carlos # 022/2013 approved this study. Twenty male *Wistar* rats (280 g) divided into control group (CG) and LLLT group (LG) were used in the study, and submitted to burn injury through a soldering iron at 150°C, pressed on their back for 10 seconds (Microscopy Res Tech 79, 313-320, 2016). LLLT (660 nm, 100 mW, 25 J/cm²) with 10 seconds irradiation time per point and energy 1J per point were used. Laser irradiation was initiated immediately after burn induction and on days 2, 4, 6 and 8 after burn induction. The method of application of the laser was point contact in five different points of application, four of which were located on the edge of the injury and the last one was located in the central region. On the tenth day after inducing the injury, the rats were euthanized and the twenty samples were collected. The variance one-way test (ANOVA) was used for statistical analysis and later complemented with Tukey test, where p values ≥ 0.05 were considered significant.

Results: Histological analysis revealed a decreased inflammatory infiltrate in the LG when compared an intense inflammatory infiltrate found in the CG. The immunostaining of COX-2 was intense (3.6 ± 0.55) in the CG than in the LG that demonstrated a mild immunomarking (2.2 ± 0.45). Conversely, VEGF immunomarking together with the morphometry vessels demonstrated similar results, both being more expressive in the LG (4 ± 0 and 9.17 ± 5.14) than it was in the CG (2.6 ± 0.55 and 3.13 ± 1.17). Collagen birrefringencia analysis also demonstrated statistically significant differences between groups, where LG (54.72 ± 20.28) showed increased

deposition of fibers with better tissue organization compared to that found in the GC (49.24 ± 25.57).

Conclusion: Therefore, our findings suggest that the use LLLT (660 nm) of 25 J/cm^2 and 1J of energy was effective in stimulating the cellular processes involved in tissue repair on third-degree burns in rats by reducing the inflammatory phase, improving collagen synthesis and stimulating angiogenesis, thus restoring the local microcirculation which is essential for healing process.

PT.23

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

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Introduction: Bell's palsy is defined as an idiopathic peripheral facial nerve paralysis of sudden onset and is considered the most common cause of facial paralysis. Antiviral therapy, oral corticosteroid and physiotherapy are the methods most widely accepted in the treatment of this pathology. The low-level laser therapy (LLLT) can improve the peripheral nerve regeneration capacity. **Objective:** the aim of this study is to describe the treatment of Bell's palsy which occurred 10 years ago, using the application of LLLT.

Method: A.C.F.O., female, 42 years old, which presented Bell's palsy 10 years ago. It was held a LLLT protocol with infrared laser (1.0Joule/point; 808nm, 60mW, 40J/cm²) on the path of the branches of the facial nerve compromised (12 points) in 2 sessions per week for 5 weeks. Clinical (House-Brackmann scale) and electrophysiological (electroneuromyography) evaluation were performed before and after the LLLT protocol. **Results:** The House-Brackmann scale improved from grade V to grade III. Potential pre-existing denervation disappeared. There was an increase in the motor units recruitment of muscles studied (*frontalis*, *orbicularis oculi* and *orbicularis oris*). **Conclusion:** this report demonstrates the efficacy of LLLT in the treatment of Bell's palsy, even after a long time after its installation, and is shown as an additional method in the rehabilitation process if these patients. Prospective clinical studies are needed in order to establish the true efficacy of LLLT in Bell's palsy treatment.

PT.24

Low level laser therapy benefits after bichectomy

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

PT.26

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

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Abstract

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

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

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

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

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

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

PT.27

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

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

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

**INVESTIGATION LED EFFECTS IN THE PROCESS OF HEALING:
literature review**

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ABSTRACT

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

Keywords: Wound Healing, Phototherapy, LED.

METHOD

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

RESULTS

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

results in the process of Moura et al healing. (2014) show proliferation of blood vessels and increase in collagen as Meyer et al. (2010) studies in rats reported that granulation tissue was more developed in the irradiated group than in the control group and the amount of chronic inflammatory cells (monocytes, macrophages, lymphocytes and plasma cells) predominated with phototherapy green light . The epithelialization at the wound margins and scarring with better quality occurred with the red LED (620-630nm), which was increased collagen deposition. However, phototherapy not collimated of 620-630nm (red) led to better anti-inflammatory effects.

PT.29

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

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INTRODUCTION: Low-level laser therapy (LLLT) has been successfully used for therapeutic applications with benefits in soft tissue repair (wound healing), musculoskeletal pain treatment and inflammatory conditions, as well as, improvement to sport performance. The molecular bases of laser action are associated to absorption of photon laser energy by cell chromophores, such as cytochrome c oxidase. Despite the widespread use of LLLT, the molecular mechanisms involved in their biological effects are not fully understood, particularly as regards DNA, which could be targeted by free radicals changing its structure, chromatin organization and ploidy degrees.

OBJECTIVE: We investigated effects of red and infrared laser exposure on nuclear phenotype in muscle cells from *Wistar* rats.

METHODS: Experiments in accordance with the Institutional Committee of Animal Care (CEUA/038/2012). Animals were exposed to LLLT at different fluences (25, 50 and 100 J/cm²) and wavelengths (660nm and 810nm), using power input 100 mW. Skeletal muscle samples were collected and processed for histological routine. Geometric (area) and densitometric (integrated optical density) parameters obtained from Feulgen-stained nuclei by image analysis were used to define nuclear phenotypes. DNA fragmentation was evaluated by the TUNEL POD assay, as described by the manufacturer. Normality was verified by Shapiro-Wilk test. Kruskal-Wallis test was performed to determine possible statistical differences, followed by post hoc Dunn's tests, with p<0.05 as the less significant level.

RESULTS: All results were expressed in mean ± standard deviation. Area data from muscle tissue exposed to red LLLT were: 28.43±2.30 (control), 36.29±2.20 (25J/cm²); 37.43±3.57 (50J/cm²), 33.00±0.70 (100J/cm²) and to IR LLLT: 38.13±1.38 (25J/cm²), 40.17±2.13 (50J/cm²), 40.33±3.78 (100J/cm²). Integrated Optical Density (IOD) data from muscle tissue exposed to red LLLT were: 9.23±0.77 (control), 11.97±1.05 (25J/cm²); 12.06±0.73 (50J/cm²), 24.51±3.32 (100J/cm²) and to IR LLLT: 12.45±0.83 (25J/cm²), 12.40±0.93 (50J/cm²), 12.78±1.78 (100J/cm²). Data from area to 50 and 100J/cm² IR LLLT was significant when compared to control group. Data from IOD shows significance only to red LLLT (100J/cm²). TUNEL data from muscle tissue exposed to red LLLT were: 2.67±0.88 (control), 2.78±0.51 (25J/cm²); 4.11±0.84 (50J/cm²), 5.44±1.84 (100J/cm²) and to IR LLLT: 2.89±0.51 (25J/cm²), 2.78±0.69 (50J/cm²), 4.33±0.67 (100J/cm²).

TUNEL assay shows no significant levels.

CONCLUSION: Our results could indicate that high doses of red (100J/cm²) and IR LLLT (50 and 100J/cm²) contribute to changes in nuclear phenotypes. However, these doses could not cause DNA fragmentation in these cells.

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PT.30

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

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Introduction: The effects of phototherapy for performance improvement have been widely studied. However, there are few studies showing its effects on muscular training, as well as, there are not studies where different moments of phototherapy irradiations (i.e. before or after training sessions) were tested.

Objective: The aim of this study was to elucidate what is the optimal moment for phototherapy irradiation when used in association with strength training.

Methods: The project received approval from the institutional research ethics committee (protocol number 437.894). Forty-eight volunteers, 26 years old (± 5.24), male gender, up to one exercise practice per week completed this study. A strength training protocol was performed applying phototherapy and/or placebo before and/or after each training session. The training protocol lasted 12 weeks and it was assessed peak torque reached in maximum voluntary contraction test (MVC), load in 1-repetition maximum test (1-RM) and thigh circumference (perimetry) at larger cross-sectional area (CSA) at baseline, 4 weeks, 8 weeks and 12 weeks. Data were analyzed both in absolute values and in percentage of change from baseline assessments. Two-way ANOVA test was performed to test between-groups differences (followed by Bonferroni post hoc test). The significance level was set at $p < 0.05$.

Results: Volunteers from group irradiated with phototherapy before and placebo after training sessions showed significant ($p < 0.05$) changes in MVC for right leg (280.9 ± 38.68) and left leg (311.27 ± 31.36). Significant results were found also for 1-RM tests for both exercises when compared to other groups, leg press 144.83 ± 22.53 and 145.33 ± 18.23 (right and left legs, respectively) and for leg extension 127.83 ± 22.93 and 132.92 ± 16.14 (right and left legs, respectively).

Conclusion: Application of phototherapy combining different wavelengths leads to better results in order to enhance strength gain when is applied before exercise. Moreover, this tool can be used both in sports, during muscular training for sports activities, as well as in prevention and rehabilitation of sports injuries. Furthermore, phototherapy doesn't present side-effects, no damaging thermal effects and it's easy handling.

PT.31

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

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Context: Skeletal muscle fatigue and exercise performance are novel areas of research and clinical application in the photobiomodulation field, and positive outcomes have been reported in several studies; however, the optimal measures have not been fully established.

Objective: To assess the acute effect of photobiomodulation therapy (PBMT) combining superpulsed lasers (low-level laser therapy) and light-emitting diodes (LEDs) on muscle performance during a progressive cardiopulmonary treadmill exercise test.

Patients or Other Participants: Twenty untrained male volunteers (age 26 ± 6 years, height 175 ± 10 cm, mass 74.8 ± 10.9 kg).

Intervention(s): Participants received PBMT with either combined superpulsed lasers and LED (active PBMT) or placebo at session 1 and the other treatment at session 2. All participants completed a cardiopulmonary test on a treadmill after each treatment. For active PBMT, we performed the irradiation at 17 sites on each lower limb (9 on the quadriceps, 6 on the hamstrings, and 2 on the gastrocnemius muscles), using a cluster with 12 diodes (four 905-nm superpulsed laser diodes with an average power of 0.3125 mW, peak power of 12.5 W for each diode, and frequency of 250 Hz; four 875-nm infrared LED diodes with an average power of 17.5 mW; and four 640-nm red LED diodes with an average power of 15 mW) and delivering a dose of 30 J per site.

Main Outcome Measure(s): Distance covered, time until exhaustion, pulmonary ventilation, and dyspnea score.

Results: The distance covered (1.96 ± 0.30 versus 1.84 ± 0.40 km; $P < .001$) and time until exhaustion on the cardiopulmonary test (780.2 ± 91.0 versus 742.1 ± 94.0 seconds; $P < .001$) was greater after active PBMT than after placebo. Pulmonary ventilation was greater (76.4 ± 21.9 versus 74.3 ± 19.8 L/min; $P < .004$) and the score for dyspnea was lower ($3 (0.5-9.0)$ versus $4.0 (0.0-9.0)$; $P < .001$) after active PBMT than after placebo.

Conclusions: The combination of lasers and LEDs increased the time, distance, and pulmonary ventilation and decreased the score of dyspnea during a cardiopulmonary test.

Keywords: photobiomodulation therapy, fatigue and progressive-intensity exercise.

PT.32

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

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

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

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

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

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

PT.33

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

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Introduction: LASER photobiomodulation has been used to increase muscle performance and to improve recovery when applied before exercise. However, several factors such as parameters of irradiation and mechanisms of action are not yet fully understood.

Objective: The aim of this study was to investigate the effects on indicators of muscle fatigue of 904 nm LASER photobiomodulation applied using a multiple-diode probe on quadriceps muscle performance in young women.

Methods: This study was designed as a randomized, participant and assessor-blinded, within-subject crossover trial with placebo control. All procedures were approved by the Griffith University Human Research Ethics Committee (Approval No.: 2016/026). Eighteen physically active women aged between 20 and 30 years were initially randomly assigned to one of two groups, either: (1) active LASER receiving irradiation with a 12 diode cluster probe (GaAs; 904 nm; 60 mW; 250 Hz; total dose per site 43.2 J, total dose applied 129.6 J) in contact over the rectus femoris muscle of the dominant limb immediately before an isokinetic fatigue protocol; or (2) placebo LASER prior to the isokinetic fatigue protocol. The fatigue protocol consisted of a set of 60 concentric contractions (90° flexion-160° extension) of the quadriceps muscle at 180°/s. Prior to the fatigue protocol, the volunteers undertook a 5-minute cycle ergometer warm-up (speed: 60-70 rpm, no load) and were familiarized with the isokinetic dynamometer (5 submaximal voluntary concentric muscle contractions). Muscle performance was assessed using the isokinetic outcomes of peak torque, time to peak torque, total work, work fatigue index, average power and average peak torque. Muscle fatigue was assessed by the electromyographic fatigue index (EFI) from surface electromyography, blood lactate measures (immediately prior to and after the fatigue protocol, and at 3 and 6 minutes after the fatigue protocol) and ratings of perceived exertion (RPE) before and after the fatigue protocol.

Results: The results showed that LASER photobiomodulation reduced muscle fatigue indicated by a significant increase in EFI ($P=0.005$) and a significant reduction in RPE ($P=0.0139$) after LASER compared to the placebo LASER session. No significant difference was observed for lactate concentration between the groups ($P>0.05$). The isokinetic dynamometer performance analysis demonstrated that LASER resulted in increased muscle performance, observed by the effects on variables of peak torque ($P=0.04$), time to peak torque ($P=0.042$), total work ($P=0.032$), average power ($P=0.007$) and average peak torque ($P=0.019$) between the experimental conditions. No significant difference was observed for work fatigue index ($P=0.29$).

Conclusion: The LASER photobiomodulation (904 nm) was effective in reducing fatigue levels and increasing muscle performance in young active women.

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PT.34

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

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INTRODUCTION: The rugby consists of intense physical activity with frequent bursts of high-intensity intermingled with short intervals of low-intensity activities. Preparation for play requires training to focus on a combination of muscular strength, power, agility, speed, aerobic and anaerobic endurance. Photobiomodulation therapy (PBMT), with lasers and/or LEDs, has been shown to prevent skeletal muscle fatigue and accelerate recovery. While growing evidence supports the use of PBMT for performance and recovery enhancement, there have only been laboratory-controlled studies.

OBJECTIVE: The aim of this study was to analyze the effects of pre-exercise PBMT (combination of LLLT and LEDT) on performance and recovery of high-level rugby players during an anaerobic field test.

METHODS: Twelve male high-level rugby athletes, with a mean age of 23.50 (± 2.32)

years old, were recruited in this randomized, crossover, double-blinded, placebo-controlled trial. The study was approved by institutional ethics committee (process 665.347). No interventions were performed before the Bangsbo Sprint Test (BST) at familiarization phase (week 1). At weeks 2 and 3 pre-exercise PBMT or placebo were randomly applied to each athlete in a random way. PBMT irradiation was performed at 17 sites of each lower limb, employing cluster probes with 12 diodes (4 laser diodes of 905nm, 4 LED diodes of 875nm, and 4 LED diodes of 640nm, 30J per site - manufactured by Multi Radiance Medical™). Average time of sprints, best time of sprints, and fatigue index were obtained from BST. Blood lactate levels were assessed at baseline, and at 3, 10, 30 and 60 minutes after BST. Athletes' perceived fatigue was also assessed through a questionnaire. Oneway ANOVA test followed by the Bonferroni post-hoc test were performed to verify statistical significance.

RESULTS: The average time of sprints (ST mean) was significantly different ($p < 0.05$) between PBMT (6.55 ± 0.21 sec) and placebo (6.67 ± 0.21 sec). Regarding fatigue index during BST, a significant difference ($p < 0.05$) was observed between PBMT ($2.66\% \pm 0.61$) and placebo ($4.51\% \pm 0.95$). PBMT significantly decreased percentage of change in blood lactate levels ($p < 0.05$). The perceived fatigue was significantly lower ($p < 0.05$) when compared PBMT (20.16 ± 3.63) to placebo (23.50 ± 2.50).

CONCLUSION: Pre-exercise PBMT with the combination of super-pulsed laser, red and infrared LEDs can enhance performance and accelerate recovery of high-level rugby players in field test. This opens a new avenue for wide use of PBMT in real clinical practice in sports settings.

REFERENCES:

Albuquerque-Pontes GM, Vieira RP, Tomazoni SS, et al. (2015). Lasers Med Sci, 30:59-66.

Allen DG, Lamb GD, Westerblad H. (2008). Physiol Rev, 88:287-332.

Girard O, Mendez-Villanueva A, Bishop D. (2011). Sports Med, 41:673-94.

Halsen, SL. (2014). Sports Med, 44:139-47.

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PT.35

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

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

PT.36

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

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Introduction: Human cognitive performance can be enhanced by low-level laser therapy (LLLT) of the prefrontal cortex (Neuroscience 230: 13-23, 2013) and by an acute bout of aerobic exercise. Since both treatments increase mitochondrial respiration and stimulate prefrontal cortex function, we report here the results of a randomized, controlled study comparing the cognitive effects of acute aerobic exercise and transcranial laser stimulation on the same cognitive tasks.

Objective: We examined whether transcranial infrared laser stimulation of the prefrontal cortex, acute high-intensity aerobic exercise, or their combination may enhance reaction time performance in a sustained attention task and the number of correct responses in a working memory task.

Methods: This study was approved by the Institutional Review Board of the University of Texas at Austin. Healthy young male and female adults (N = 60, 18-30 years old) were randomly assigned to one of the following four treatments: 1) low-level laser therapy (LLLT) with infrared laser to two forehead sites while seated (total 8 min, 1064 nm continuous wave, 250 mW/cm², 60 J/cm² per site of 13.6 cm²), 2) acute exercise (EX) of high-intensity (total 20 min, with 10 min treadmill running at 85-90% VO₂max), 3) combined treatment (LLLT+EX), or 4) sham control. Participants were tested for prefrontal measures of sustained attention with the psychomotor vigilance task and working memory with the delayed-match-to-sample task before and after the treatments. Statistical analyses were performed with ANOVA using SPSS (SPSS Inc, Chicago, USA).

Results: Mean (Standard Deviation) reaction times in msec after sham control = 359 (24), EX = 345 (17), LLLT = 351 (26), LLLT+EX = 345 (32). As compared to sham control, both LLLT and EX treatments significantly reduced reaction time in the psychomotor vigilance task [$F(1,56) = 4.134, p = .01, \eta^2 = .181$]. Mean (Standard Deviation) number of correct responses (30 = maximum score) after sham control = 25 (2), EX = 28 (1), LLLT = 28 (2), LLLT+EX = 28 (1). As compared to sham control, both LLLT and EX treatments significantly increased the number of correct responses in the delayed-match-to-sample memory task [$F(1,56) = 4.690, p = .005, \eta^2 = .201$], demonstrating a similar enhancing effect of both LLLT and EX on cognitive performance. The cognitive effects of combined LLLT+EX treatments were similar and showed no significantly greater improvement on reaction time or memory performance as compared to LLLT or EX treatments alone.

Conclusion: The transcranial infrared laser stimulation and acute aerobic exercise treatments were similarly effective for enhancing reaction time and memory performance, suggesting that these treatments augment prefrontal cognitive functions similarly.

Financial Support: Supported by institutional research funds from the University of Texas at Austin.

PT.37

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

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Introduction: Physical training can cause muscle fatigue, decreased muscle strength and impairment of motor control. Creatine monohydrate can increase intramuscular creatine levels and facilitate the replacement of phosphocreatine. Low-level laser therapy (LLLT) acts in delaying muscle fatigue, increase strength and improve muscle recovery. However, the effect of interaction of LLLT and creatine monohydrate supplementation in skeletal muscle is unknown.

Objective: The main objective of this study was to evaluate the effect of interaction of LLLT and creatine monohydrate supplementation on muscle strength and endurance (fatigue).

Methods: For analysis of muscle fatigue, was used an experimental model of tetanic contractions induced by indirect electrical stimulation of the tibial muscle of rats. This study was approved by the Ethics Committee in the use of animal of Sacred Heart University (nº 29/14). Male Wistar rats were used, weighing around 300g and randomized into 5 groups of 7 animals: swim group (1), Laser+swim group (2), creatine+swim group (3)

Laser+creatine+swim group (4) and sham group (5). The creatine dose was applied via gavage (280mg/kg). Performance analysis was conducted through muscle contraction protocol induced by indirect electrical stimulation and recorded in an electrophysiograph. The swim protocol was performed 30 min/day, three times a week for 8 weeks (J Appl Physiol, 82(2): 711-15, 1997). Irradiation with laser was performed in contact mode, only one point in the middle region of the tibialis anterior muscle of hind limbs of rats 10 minutes before exercise in all sessions during the eight weeks of swimming training protocol. Laser irradiation: Diode lasers with mean output power of 100 mW; spot size of 0.028 cm, continuous mode; and wavelengths of 830 nm (infrared). The total delivered energy for irradiated groups were 3 J per hind limb.

Results: It was found an increase in muscle contraction force in laser+creatine+swim group (35.33 ± 3.2) and laser+swim group (33.33 ± 3.2), when compared to other groups. However, on the time taken for muscle tension decay 50% of its maximum amplitude (fatigue) it was found a statistically significant improvement on laser+creatine+swim group (320 ± 20) when compared to other groups and it was also observed an improvement on fatigue process time on laser+swim (286.7 ± 25.17) and creatine+swim groups (253.3 ± 32.15) when compared to sham and swim groups.

Conclusion: The interaction between LLLT and creatine supplementation improved the muscle strength and slowed the process of fatigue in rats after swim training protocol.

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

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Introduction: Several studies revealed benefits of photobiomodulation (PBM) to alleviate pain, reduce severity and duration time of oral mucositis (OM) originated from chemotherapy (CT) and radiotherapy (RT). Some specific CT regimen affect cells with high turnover rate, as the cells of the basal layer of the epithelium, and cells from the bone marrow (leukocytes and platelets). We hypothesized that this oscillation into the white blood cells (WBC) may become an important parameter to identify patients at risk of developing OM. The aim of this study is to assess the efficacy of PBM in patients who present severe leukopenia.

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

Methods: A total of 15 patients (mean age 11.3 ± 1.3) were submitted to high doses of methotrexate (MTX) (25 CT cycles) for the treatment of osteosarcoma ($12\text{g}/\text{m}^2$) and acute lymphoblastic leukemia ($5\text{g}/\text{m}^2$) (informed consent was signed and the study was approved by the Ethical Committee in Research of Santa Marcelina Hospital - protocol 55/05). Patients without OM were included in the preventive group (PG, n=15 cycles) and received three sessions of PBM ($660\text{nm} \pm 30$, 100mW , 0.028cm^2 , 2J, $70\text{J}/\text{cm}^2$, 20s). The first irradiation was performed within 24 hours of beginning of CT. Patients who had already presented OM were placed in therapeutic group (TG, n=10 cycles). The leukocytes counts were classified in >3000 ; between $2000-3000$; between $1000-2000$ and $<1000/\text{mm}^3$.

Oral mucosal toxicity was graded according to the NCI-CTC version 2.0. Pain was accessed by visual scale.

Results: In the third evaluation of PG, we observed a significant decrease of leucocytes counts ($p=0.008$). There were 8 cases of OM grade 0, and 5 cases grade 1, what represents 86.6% of the patients with absence of pain and normal nourishing.

In the TG, in the second evaluation, there was a significant pain reduction ($p<0.05$) and significant leucocytes count reduction ($p<0.02$) ($<1000/\text{mm}^3$). In the third evaluation, 70% of the patients presented OM grades between 0 and 1, whereas all patients maintained leukopenia ($<2000/\text{mm}^3$).

Conclusion: In this pilot study, besides the reduced size of the sample, it was possible to notice that leukocytes are affected by high doses of MTX, and, as soon as the serum levels of the drug diminish, there is an increase in

the WBC counting. This relation may be considered an important indicator of the drug's toxicity.

We observed that PBM preserved oral mucosa integrity in most patients, relieved pain, and reduced OM severity, even in cases with severe leukopenia, demonstrating its preventive and therapeutic supportive role in young patients submitted to high doses of MTX.

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

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INTRODUCTION: Low-level lasers have been used worldwide to treat diseases, pain relief and wound healing. Interest in their therapeutic applications has been demonstrated by the increasing case reports, as well as clinical and experimental studies reporting positive impact on human and animal health. For these purposes, red and infrared lasers are used within so called therapeutic window (600 up to 1100 nm). Despite clinical protocols used, there are doubts about the mechanism of action involved in the biological effects of low-level lasers. As the mechanisms of action of low-level lasers on cells under stressful conditions and cells deficient in DNA repair mechanisms remain disputed, in this work we evaluated the effects of low-level red and infrared lasers on *E. coli* cells deficient in SOS responses submitted to heat stress. **OBJECTIVE:** Evaluate the effects of red and infrared laser on *E. coli* cells exposed to heat stress in different physiological conditions. **METHODS:** Stationary and exponential *E. coli* AB1157 (wild type), AB2494 (RecA deficient) and AB2363 (LexA deficient) cultures were exposed to red (660nm) and infrared (808nm) laser at different fluences (25, 50 and 100J/cm²) and after, submitted to heat stress (42°C, 20 minutes). Aliquots of bacterial suspensions were spread onto *Petri* dishes, containing nutritive medium and incubated. Colony forming units were counted and the survival fractions were calculated. Controls were bacterial suspensions not exposed to lasers and incubated to 37 or 42°C. To evaluate bacterial morphology, exponential and stationary *E. coli* suspensions exposed to low-level red or infrared lasers and incubated at 37 °C or 42 °C, as described in the cell survival in bacterial cultures. After that, aliquots were spread onto microscopic slides and stained by the Gram method. Area ratios were calculated by the ratio between the areas of cells in bacterial cultures at 42 °C and the areas of cells in bacterial cultures at 37 °C. **RESULTS:** Survival fractions values in stationary and exponentially *E. coli* AB1157 exposed to red laser were (mean±standard deviation): 2.9±1.54 and 2.1±0.70 (25J/cm² + 42°C), 4.1±0.74 and 1.8±0.48 (50J/cm² + 42/C), 4.8±0.61 and 2.7±0.72 (100J/cm² + 42°C); for infrared laser: 1.2±0.37 and 0.8±0.30 (25J/cm² + 42°C), 1.0±0.23 and 0.9±0.22 (50J/cm² + 42°C), 1.5±0.47 and 0.8±0.23 (100J/cm² + 42°C). Cell area ratios values from stationary and exponentially *E. coli* AB1157 exposed to red laser were (mean±standard deviation): 1.0±0.23 and 0.8±0.14 (25J/cm²), 1.2±0.13 and 0.9±0.06 (50J/cm²), 0.9±0.04 and 0.9±0.06 (100J/cm²); for infrared laser: 1.1±0.08 and 1.0±0.07 (25J/cm²), 0.8±0.14 and 0.9±0.03 (50J/cm²), 1.3±0.21 and 1.1±0.04 (100J/cm²). **CONCLUSION:** Our research suggests that exposure to low-level red and infrared lasers increases cell viability and protects cells

from morphological alteration in *E. coli* cultures submitted to heat stress, depending on laser wavelength and SOS responses.

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

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Introduction: The malignant cancer incidence has increased significantly in recent years due to population growth and aging. The cancer treatment usually consists in individual or combined use of chemotherapy, surgery and radiotherapy depending on the etiology of the tumor. In cases where radiotherapy is used, and considering the therapeutic effects of radiation, specific severe complications can occur, ranging from erythema to radionecrosis. Studies showed that low-level laser therapy (LLLT) can be successfully used for tissue repair, depending on the light parameters and characteristics of the target tissue.

Objective: Evaluate the LLLT influence in radionecrosis guided tissue repair caused by continuous radiation emitted by ¹²⁵I seed.

Methods: Twelve athymic mice were divided into two groups: group A - 1 week before the implantation of ¹²⁵I seed (0,98 mCi) subcutaneously into the back of the mouse (n=4) it was submitted a LLLT, wavelength of 660 nm, 40 mW power with 20 s (20 J/cm²) daily until the radionecrosis emerge and continuous until the wound healing (33 days after the ¹²⁵I seed implantation); group B - using the same procedure as group A (n=4), but the LLLT was applied only when the radionecrosis emerge and continuous until the wound healing (45 days after the ¹²⁵I seed implantation). The control group (n=4) using the same procedure as group A but without LLLT treatment. All animals were photographed every 7 days and the wound sized were calculated by the Image J® software.

Results: The animals in group A, showing fast wound healing (in days) and smaller radionecrosis (in area) on comparison between group B and Control. No systemic or lethal sequelae occurred in any animal.

Conclusion: Under the parameters used in this study, LLLT was showing better results when applied before the ¹²⁵I seed insertion to repair radionecrosis. We are focusing our efforts in using higher energy laser and other parameters, to avoid the laser exposure daily.

Key-Words: ¹²⁵I seed, LLLT, radionecrosis, radiodermatitis, radiotherapy.

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

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Introduction: Laser characteristics, such as wavelength, frequency, power, fluence and emission mode properties are determining to the biostimulatory effect. Biochemical reactions after energy absorption increase ATP and RNA synthesis, which constitute the basis of the laser effects on biological tissues. Low power lasers induce free radical generation and alterations in macromolecules (DNA).

Objective: This study evaluated induction of DNA damage in blood cells and mRNA expression from genes related to DNA repair in biological tissues exposed to low power red (660nm) and infrared (808nm) lasers.

Methods: Experiments were approved by Institutional Committee of Animal Care (protocol CEUA/038/2012). *Wistar* rat blood samples were obtained by finger puncture in hind paws and red (R) and infrared (IR) lasers were applied at different fluences (25, 50, and 100 J/cm²), powers (30, 50, and 100mW) and frequencies (continuous wave, 10, 50, and 100 pulses per second). Negative controls were non-irradiated blood samples and positive controls were blood samples incubated with hydrogen peroxide. DNA damage was evaluated by comet assay and damage index (DI) was calculated. *Wistar* rat skin and muscle were exposed to low power lasers at different fluences (25, 50 and 100J/cm²) in continuous wave emission mode at 100mW. From tissue samples, total RNA was extracted, cDNA was synthesized and gene expression was evaluated by real time quantitative polymerase chain reaction. One-way analysis variance (ANOVA) test was performed followed by *Tukey* post-test, after Kolmogorov-Smirnov test for normality verification, p<0.05 was considered as less significant level.

Results: Mean and standard deviations of DI (in continuous mode, 100mW) were: 26.2±2.79 (control-CTR), 42.5±8.38 (25J/cm², R), 93.0±5.54 (50J/cm², R), 6.0±1.58 (100J/cm², R), 36.0±3.72 (25J/cm², IR), 59.0±1.22 (50J/cm², IR), 16.0±4.41 (100J/cm², IR); for the different powers (continuous mode, 100J/cm²): 48.5±8.76 (30mW, R), 46.0±0.86 (50mW, R), 4.25±1.29 (100mW, R), 36.0±2.82 (30mW, IR), 78.0±3.60 (50mW, IR), 15.8±7.56 (100mW, IR); to the pulse mode (100mW, 100J/cm²): 46.0±0.86 (continuous, R), 99.0±4.20 (10PPS, R), 43.8±2.48 (50PPS, R), 146.0±5.33 (100PPS, R), 78.0±3.60 (continuous, IR), 105.0±2.44 (10PPS, IR), 55.0±2.48 (50PPS, IR), 126.0±9.24 (100PPS, IR). mRNA *ERCC1* relative expression in skin were: 1.94±0.70 (CTR), 1.35±0.70 (25J/cm², R), 1.05±0.41 (50J/cm², R), 1.10±0.62 (100J/cm², R), 0.04±0.01 (25J/cm², IR), 0.04±0.01 (50J/cm², IR), 0.05±0.04 (100J/cm², IR); in muscle: 1.36±0.05 (CTR), 1.87±0.68 (25J/cm², R), 1.09±0.38 (50J/cm², R), 1.98±0.79 (100J/cm², R), 18.97±8.33 (25J/cm², IR), 38.42±5.49 (50J/cm², IR), 18.65±9.45 (100J/cm², IR). For mRNA *ERCC2* in skin: 0.86±0.33 (CTR), 0.44±0.24 (25J/cm², R), 0.42±0.21 (50J/cm², R), 0.99±0.39 (100J/cm², R), 0.02±0.00 (25J/cm², IR), 0.02±0.00 (50J/cm², IR), 0.02±0.01 (100J/cm², IR); in muscle: 0.85±0.40 (CTR), 1.09±0.84 (25J/cm², R), 5.63±2.4 (50J/cm², R), 0.93±0.76 (100J/cm², R), 3.04±3.80 (25J/cm², IR), 7.97±4.78 (50J/cm², IR), 2.04±1.11 (100J/cm², IR).

Conclusion: Low power lasers induce DNA damage in blood cells and

differently alter *ERCC1* and *ERCC2* mRNA expression in skin and muscle tissue.

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

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Introduction The skin is composed of three tissue structures with different anatomy and physiology. It is a structure rich in cells, which offers resistance, elasticity and protection. Skin damage (physical, chemical, biological) immediately trigger a role of biochemical events. Sunlight exposure provides many photo biological effects (Dermatologia, Azulay RD, 5ed, 2011). The harmful effects on human skin could result by sun exposure, skin type, geographic and atmospheric conditions and thus the injuries may develop from erythema to cancer (An. Bras. Dermatol. 82(1): 7-21 2014).

Objective The aim of this study was observe the macroscopic and histological changes in rats' skin submitted to UV-B radiation. **Methods** Case-control experimental study approved by Ethics Committee of Federal University of Vale do São Francisco-UNIVASF number 3/100614. Fifty Wistar rats with 3 months, 250g±10g were assigned into two groups: Control (n=10) and Experimental (n=40), the skin on the back was trichotomized and exposed to UVR-B with a light emitting stable wavelength (λ) of 306nm, potency=9J/s (W) installed in a wooden box, 15cm away from the animal's back. Each exposure was realized for 30 seconds three times per week. The exposure time was fixed based on the distance from the lamp to the skin of the animal, the lamp power and minimum time for obtaining twice the minimum erythema dose (Proc Natl Acad Sci U S A. 1977; 74(4):1688-1692). At the end of weeks 5, 10, 15 and 20, the exposure was discontinued in each subgroup (n = 10), the skin was photographed and a biopsy was performed. Control group was submitted to the same conditions with the light device off. Three different blinded pathologists realized histopathological qualitative analysis. Quantitative For statistical analysis to macroscopic (clinical) evaluation Fisher test ($p < 0,05$) was realized. **Results** The most significant skin injury at all times was erythema, followed by erythema with desquamation. More severe clinical injury was keratosis, which presented from week 5 with little expressiveness and had its frequency increased in the weeks 15 and 20. Regarding to microscopic findings, five weeks group showed more evident inflammatory characteristics than the other groups, most prominent in the superficial reticular dermis and more moderate in the superficial reticular dermis, moderate atypia of basal keratinocytes and focal hyperkeratosis. The groups 10 and 15 were very similar to each other, with epidermal atrophy and focal hyperkeratosis, atypia of basal keratinocytes and minimal inflammation. In group 20 weeks, the changes were more intense than all other groups with hyperparakeratosis, atypia of basal keratinocytes and architectural skin disorganization, epidermal atrophy, focal hyperkeratosis and moderate interstitial lymphocytic inflammatory infiltrate in superficial reticular dermis. Macroscopic analysis of control group showed no clinical lesion, but microscopic showed mild epidermal atrophy, focal hyperkeratosis and mild inflammatory infiltrate. **Conclusion** The skin lesions are more intense the longer the time of exposure of the animal skin to UVR-B. Apparently, the lesions show changes similar to an inflammatory reaction and the continued

exposure have preneoplastic characteristics.

PT.44

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

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Abstract

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

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

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

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

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

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

PT.45

Low level infrared laser effects on bacterial survival

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

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

Methods: Effects of a low-level laser were assessed in *E.coli* AB1157 (wild type), JW3610 (fpg-mutM protein deficient) and JW1625 (endonuclease III deficient) cultures in stationary and exponential growth phases. Aliquots of bacterial suspensions were exposed to infrared laser (904 nm, spot size of 0.069cm²) at different fluences (2, 4 and 7J/cm²) in pulsed mode (power output of 10mW, power density of 144.92W/cm² and frequency of 5000Hz) with laser source at 3.0cm from the surface of bacterial suspension aliquots (distance top-bottom of a microcentrifuge flex tube). Exposure time of the aliquots was automatically adjusted by the laser device as a function of fluence. Aliquots not exposed to laser were used as controls. Samples were diluted in saline (0.9% NaCl) spread onto Petri dishes containing nutritive medium. After incubation (37°C, 20 hours) colony forming units were counted and survival fractions were calculated. Data were reported as one-way analysis of variance(ANOVA) test was performed to determine possible statistical differences followed by Dunnet post-test with p<0.05 as the less significant level.

Results: Data from bacterial survival in stationary phase were (mean±standard deviation): AB1157: 1.0±0.07 (control), 0.8±0.10 (2J/cm²), 1.0±0.04 (4J/cm²), 1.0±0.04 (7J/cm²); JW3610: 1.0±0.02 (control) 0.7±0.06 (2J/cm²), 0.8±0.04 (4J/cm²), 1.1±0.03 (7J/cm²); JW1625: 1.0±0.02 (control), 0.8±0.06 (2J/cm²), 0.9±0.05 (4J/cm²), 1.0±0.07 (7J/cm²). Data from bacterial survival in exponential phase were: AB1157: 1.0±0.07 (control), 1.0±0.10 (2J/cm²), 1.0±0.09 (4J/cm²), 1.2±0.09 (7J/cm²); JW3610: 1.0±0.04 (control), 0.9±0.09 (2J/cm²), 1.1±0.08 (4J/cm²), 1.1±0.14 (7J/cm²); JW1625: 1.0±0.03 (control), 0.8±0.07 (2J/cm²), 0.9±0.05 (4J/cm²), 1.0±0.04 (7J/cm²). Data show that infrared laser exposure alters the survival of AB1157, JW3610 and JW1625 cultures at specific fluences, compared with control group.

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

Financial Support: FAPERJ and FAPEMIG

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

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Introduction: Low-Level Lasers are widely used for treatment of muscle injuries, wound healing and oral diseases, as well for antimicrobial photodynamic therapy. Laser energy absorption is considered occurs at endogenous chromophores, as cytochrome C oxidase in eukaryotes and homologue molecules in prokaryotes. Also, light absorption by exogenous chromophores results in free radicals production and cellular signalization pathways. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is considered a gold standard technique to evaluate mRNA levels and is used to study effects induced by these low-level lasers. However, output data from RT-qPCR are commonly in a relative approach, with the target gene expression is done relative to a housekeeping gene, which is considered do not vary whether the cells are subjected to a treatment.

Objective: The aim of this study was to evaluate housekeeping expression genes from *Escherichia coli* exposed to red or infrared lasers at different fluences.

Methods: Exponential *E. coli* AB1157 cultures were exposed to red (660 nm) and infrared (808 nm) lasers at different fluences (25, 50 and 100 J/cm²), 100 mW in continuous wave emission mode, beam spot area of 2.75mm², and incubated for 20 minutes at 37 °C. Controls were bacterial cultures not exposed to lasers. After that, total RNA extraction and cDNA synthesis were carried out to evaluate mRNA levels from *araC*, *gyrA* and *rpoA* genes by RT-qPCR. mRNA expression stability was analyzed by geNorm, NormFinder and BestKeeper softwares, providing gene stability factors, which consider values above 1.0 indicative of mRNA expression instability. Data were from three independent experiments.

Results: Data from geNorm for *araC*, *gyrA* and *rpoA* were, respectively: 3.359, 3.359 and 5.345; from NormFinder were: 1.696, 2.899 and 5.880; and from BestKeeper were: 2.958, 3.375 and 3.793.

Conclusion: *E. coli* exponential cells exposed to lasers at different fluences presented *araC*, *gyrA* and *rpoA* gene expression altered. These results suggest that more than one housekeeping gene should be used to evaluate relative mRNA expression in *Escherichia coli* cells exposed to low-level lasers.

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

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Among the wounds with great affections index of patients, pressure ulcers (PU) are a growing concern in clinical practice. PU is an area of tissue trauma caused by prolonged and continuous pressure exceeding the normal capillary pressure applied to the skin and adjacent tissue, causing ischemia which may lead to cell death. Generally occurs when a soft tissue is compressed between a bony prominence and a rigid surface, resulting in poor local blood circulation culminating in necrosis, ulceration of the skin and other tissues, making favorable development of microorganisms due to exudative material, serous or haemorrhagic present on its surface. The bacterial proliferation inside a wound can result in changes in phases of wound healing. Hemostasis can be changed due to the effects of infection on platelets and the complement system. Bacteria can cause agglutination of platelets, thrombocytopenia, prolonged inflammation and tend to alter the function of the leukocytes by the expression of virulence factors and decrease collagen production. The aim of this preliminary study was to evaluate the effectiveness of photodynamic therapy (PDT) mediated by curcumin photosensitive agent and 450 nm LED to reduce the contamination in pressure ulcers. Seven patients were selected assisted by a public hospital in the interior of Bahia, Brazil, with a diagnosis of pressure ulcers. After precleaning with 0.85% sterile saline, material for isolation of microorganisms were taken from viable granulation tissue using sterile disposable swab that has been processed in selective media: Mannitol agar (*Staphylococcus spp.*), Sabouraud dextrose agar (yeasts), MacConkey agar (*Enterococcus spp.*) and blood agar (total count). To perform the PDT, the curcumin photosensitive agent emulsion 1.5% (PDT Pharma Industry and Trade Pharmaceuticals LTDA, Cravinhos, SP, Brazil) was used, applied across the surface of the ulcer. After 30 minutes of application it has been activated using the Lince equipment - Light in Cell (MMOptics, São Carlos, São Paulo, Brazil), composed with 30 LEDs with wavelength of 450 + 10 nm (visible blue) . The delivery of light was continuous, 12 minutes long, with irradiance of 35 mW/cm² and fluence of 25.2 J/cm². The light was applied from a distance of 5 cm from the tip surface to the surface of ulcers. For each patient were applied different quantities of the proposed treatment in accordance with the presence of exudate during the assessment sessions. Immediately after the performance of PDT a new microbiological sample was collected and processed under the same conditions of the first. The logarithm of the colony forming units (CFU log₁₀) was calculated and paired t-test with 5% significance level was used for comparison between CFU counts before and after PDT of treated ulcers. In all the culture media used was a significant reduction in log₁₀ CFU (p<0.05) indicating effectiveness of the method employed in reducing microbial yeast, *Staphylococcus spp.* and *Enterococcus spp* in pressure ulcers. We conclude that the PDT was effective in reducing contamination of pressure ulcers and can be used as an auxiliary method in the treatment of such injuries. This study was approved by

Multidisciplinary Health Institute Ethics Committee (CAAE
36925714.0.0000.5556).

PT.48

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

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

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

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

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

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PT.49

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

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Introduction: Studies have been reporting obese and hypertrophied white adipose tissue as richly permeated by leukocytes, expressing not only increased TNF- α levels, but also other pro-inflammatory cytokines and chemokines. Inflammatory signaling through activation of Toll-like and TNF receptors (TLRs and TNFRs, respectively) pathways leads to intracellular inhibition of insulin signaling by direct interferences on insulin receptor function and its ability to initiate the insulin signal transduction pathway. If inflammation is a common etiological agent behind all these pathologies, a therapeutic approach that can modulate inflammatory signaling is of noteworthy value. Several studies have been acknowledging photobiomodulation (PBM) as an appealing therapy for inflammatory disorders due to its immunomodulatory properties. Nevertheless, the phototherapeutic approach to manage the chronic inflammatory component of obesity and hyperglycemia has not yet been explored.

Objective: The purpose of this study was to develop a murine model of obesity and hyperglycemia to investigate the effects of PBM on inflammatory infiltrate in adipose tissue of obese and hyperglycemic mice.

Methods: Four week old male adult C57BL/6 mice were submitted to a hypercaloric high-fat diet to induce obesity and hyperglycemia during 8 weeks. After that, animals were treated with PBM during four weeks corresponding to six irradiation sessions using an 843 nm LED (5.7 J cm⁻² at 19 mW cm⁻² per session). Animals were irradiated at days 1, 3, 7, 10, 14 and 21 following obesity and hyperglycemia validation. Control animals were submitted to same management but sham-irradiated. All animals received the high-fat diet until the end of experiments. Body mass and blood glucose were assessed regularly during the entire experimental course. Twenty-four h after the last irradiation, animals were euthanized and subcutaneous samples from abdominal tissue were carefully collected, fixed at formalin 10% and routinely processed for hematoxylin-eosin staining and histological analysis. Standardized areas were selected from each slide and Image J software was used to quantify the areas of inflammatory infiltrate in adipose tissue. Data were submitted to paired sample t-test to attest changes in body mass and blood glucose levels after obesity and hyperglycemia induction. Mann-Whitney test was performed to verify differences between groups regarding inflammatory infiltrate in adipose tissue. Significance was established at $p < 0.05$.

Results: Obesity and hyperglycemia were successfully induced after 8 weeks. The body mass and glucose blood significantly increased about 32.2% ($p < 0.001$) and 13.7% ($p = 0.034$), respectively. Regarding the inflammatory infiltrate in the adipose tissue, non-irradiated control animals displayed areas almost five times higher than the treated group ($p < 0.001$). In fact, control group presented 23.3% of area populated with inflammatory cells while for irradiated animal the area corresponded to 4.3%. Considering that the abdominal adipose tissue from treated animals presented diminished areas of inflammatory infiltrates, we assumed that their

adipocytes were less exposed to pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6.

Conclusion: Our results suggest that PBM could be used to improve insulin response in hyperglycemic subjects.

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

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Type II diabetes mellitus is chiefly characterized by the increase of blood glucose levels, this hyperglycemia triggers the release of free radicals and oxidative stress in the body, the role of ILIB (Intravascular Laser Irradiation of Blood) is the antioxidant form, causing an increase in the levels of the superoxide enzyme dismutase (SOD), with such an increase is a partial restoration of the insulin synthesis and minimizes the intensity of the attack from free radicals to the pancreatic cells. To investigate the blood glucose levels in type II diabetic patients using the treatment with modified ILIB (Intravascular Laser Irradiation of Blood) by analyzing the results through the right handed, before, during and after the intervention in addition to the analysis of sleep quality before and after the intervention by the Pittsburgh Questionnaire, pain assessment by Visual Analogue Scale (VAS) and the ankle-brachial index (ABI) using the Doppler as an evaluation tool before and after intervention. This is a randomized blind controlled study. It has been selected six with Diabetes Mellitus Type II patients, being three male and three female. They were randomized between the CG (control group) and GI (Intervention Group) through a raffle software were selected and from this we have obtained three patients in GI and three in G.

Questionnaire Pittsburgh, EVA, ABI of each carrier and then were subjected to the use of the Modified ILIB, where the GC did use the GI ILIB off and made use of was evaluated ILIB on five consecutive days over 30 minutes positioned on the right radial artery. Data were analyzed by GC and average GI, comparing them from beginning to end of the intervention. The use of Modified ILIB promoted a decrease of blood glucose levels, increasing quality of sleep, decreased VAS and normalization of ITB in GI, while the GC showed the same items without significant changes. Although few studies about the ILIB Modified, it follows that the same benefits afforded to the GI compared to the GC in relation to the items evaluated, demonstrating the effectiveness of the technique for patients with diabetes type II.

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

PT.51

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

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

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

Methods: Single subject design (n=1). Material: Sleep apnea patients who experienced discomfort with pain increase (> 30 mm) and lowered pain pressure threshold (PPT > - 0.5 kg/cm²) 4 weeks after initiation of MAD treatment. Outcome measures: Pain on a 100mm visual analogue scale (VAS), pain pressure threshold (PPT) measured algometer, and mouth opening in mm. Intervention: LLLT ($\lambda=810\text{nm}/200\text{mW}$) applied for 3 consecutive days to two points overlying the TMJ, and two points each in pterygoid and masseter muscles, respectively (6 points in total). Each point received 6 joules of energy during an irradiation time per point of 30 seconds. Pain was immediately before LLLT and then daily during the LLLT intervention period.

Results: Pain increased during initiation of MAD use (+34mm on VAS), and PPT was lowered (-1.2 kg/cm²), while mouth opening became slightly impaired (-3mm). All outcome measures returned to near normal after 3 days of LLLT

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

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

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

PT.53

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

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

Keywords: Lasers, Surgery oral, Trismus.

PT.54

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

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The aging process leads to a gradual loss of muscle mass and muscle performance, leading to a higher functional dependence. Within this context, many studies have demonstrated the benefits of a combination of physical exercise and low level laser therapy (LLLT) as an intervention that enhances muscle performance in young people and athletes. The aim of this study was to evaluate the effects of combination of LLLT and strength

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

CO.55

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

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