III FORUM INTERNACIONAL DE LASERTERAPIA e
III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

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BIOFOTÔNICA APLICADA
ÀS CIÊNCIAS DA SAÚDE

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Prefácio:

O III FÓRUM INTERNACIONAL DE LASERTERAPIA se constituiu novamente num fórum de discussão de temas relacionados à Biofotônica e suas aplicações em Ciências da Saúde, voltado a divulgar as amplas possibilidades de pesquisa resultantes de uma visão interdisciplinar aplicada à essa temática. O evento teve como público os pesquisadores, docentes e discentes, bem como os profissionais da área tanto no sistema privado como no Sistema Único de Saúde (SUS). Ao aproximar estes dois perfis, busca-se maior integração entre a teoria desenvolvida na Academia e a prática verificada no contexto dos serviços de saúde na área de Biofotônica. Esta escola privilegia as discussões de caráter científico e tecnológico, proporcionando assim melhoria da formação dos pesquisadores e profissionais envolvidos nas pesquisas dessa área de conhecimento.

Foram 1502 inscritos fazendo deste o maior evento de Biofotônica do Brasil em número de inscritos e abrangência de temas.

Esta edição do evento contou com a participação dos palestrantes: Tayyaba Hasan (Harvard Medical School); Imran Rizvi (Harvard Medical School), Lotharl Lilge (University of Toronto), Antônio Pinheiro (Universidade Federal da Bahia); Martha Ribeiro (Instituto de Pesquisas Energéticas e Nucleares); Carlos Eduardo Pinfild (Universidade Federal de São Paulo), Marcia Martins Marques (Universidade de São Paulo) além dos docentes do PPG em Biofotônica aplicada às Ciências da Saúde da UNINOVE e diversos de seus alunos e ex-alunos.

Os 104 trabalhos submetidos ao III Fórum Internacional de Laserterapia, passaram pela seleção e revisão dos editores e do Comitê Científico do evento e 99 foram aceitos para publicação neste *Proceedings.*

Gostaríamos de agradecer imensamente a todos que se dedicaram para a realização deste evento; aos voluntários que se empenharam na organização e logística; à Priscila e à Camila (Secretárias acadêmicas), ao departamento de TI e de Infraestrutura da UNINOVE; aos membros do Comitê Cientifico; aos membros da Comissão Organizadora; à Profa. Luciana L. Ginezi; e de maneira muito especial à Profa. Renata Malva por sua dedicação e cuidado com o cerimonial do evento. Também agradecemos imensamente à UNINOVE, FAPESP (2017/08096-8) e ao CNPq pelo apoio e às empresas patrocinadoras:

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- Profa. Dra. Tayyaba Hasan (Harvard Medical School);
- Prof. Dr. Imran Rizvi (Harvard Medical School),
- Prof. Dr. Lotharl Lilge (University of Toronto),
- Prof. Dr. Antônio Pinheiro (Universidade Federal da Bahia);
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Chemical and histomorphometric analysis of dentin treatments after erosion

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OBJECTIVE

The purpose of this study was to evaluate dentin treatment after erosion by means of Er:YAG laser, Sensodyne Repair & Protect (Novamin/bioglass) and their associations.

MATERIAL AND METHODS

This study was approved for the Ethics Committee on Human Research of Unopar (Protocol 1.209.605). The occlusal surfaces of twenty five third molars were sectioned to obtain 1.5mm thick dentin discs that were stored into ultrapure water for 3 days, the erosion cycle was applied: 5min in DES solution + 3h in RE solution (6 cycles a day during 8 days). The specimens were divided into five groups (n=5): G1: Erosion- control (E); G2: Erosion + Sensodyne Repair&Protect (ES); G3: Erosion + Er:YAG laser (EL) (40mJ, 10Hz, 50 microseconds); G4: Erosion + Er:YAG laser+ Sensodyne (ELS); G5: Erosion + Sensodyne + Er:YAG laser (ESL). After treatments and storage into ultrapure water (37°C/14days), the chemical composition of dentin was analysed by X-Ray Dispersive Spectroscopy, the Ca/P ratio evaluated, the dentin surfaces were observed in a scanning electron microscope and the percentage of openned dentin tubules calculated. The data of Ca/P ratio and the percentage of dentin tublules were treated by One-Way Analysis of Variance and Tukey's tests at 5%.

RESULTS

Table 1 shows the mean and standard deviations of G1 to G5 experimental conditions. There was difference to the Ca/P ratio ($p<0.0001$).

G1	G2	G3	G4	G5
27.53A	12.69B	9.29C	9.97C	7.52D
1.59	1.03	0.97	1.67	0.13

Table 1. Mean and Standard Deviation of Ca/P ratio for G1 to G5. Similar letters indicate no significant difference.

The area of exposed dentin tubules varied among G1 to G5 ($p<0.0001$). When associated the higher occluded dentin tubules were observed after Er:YAG laser + Sensodyne in this sequence.

G1	G2	G3	G4	G5
11.55A	0.13B	5.05C	5.02C	3.30D
1.38	0.10	1.00	0.95	0.95

Table 2. Mean and Standard Deviation of the percentgage of oppened dentin tubules for G1 to G5. Similar letters indicate no significant difference.

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Unrevealing the Relevance and Role of the Photosensitive System of the Skin and its Deregulation in Skin Cancer

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The concept that light can be captured by a complex biological system, leading, for example, to image formation has long been known. At the core of this system lie the opsins – a biological class of light receptors. An important breakthrough in the field was that a retinal ganglion cell opsin, melanopsin, could also detect light and participate in non-forming image photoperception such as entrainment of biological rhythms, melatonin synthesis, and pupillary reflex. Nowadays, it is widely acknowledged the relevance of this photosensitive system of the eye. It is interesting to observe that the same system of light detection is found in the skin; however, our knowledge regarding its biological significance is still poorly comprehended. In fact, only a handful of studies have demonstrated that the photosensitive system of the skin participates in melanin synthesis and proliferative process in melanocytes[1] and keratinocytes[2], respectively. Within this line, our lab has extensively evaluated the skin biological processes modulated by this complex light detection system. We have shown that murine melanocytes and melanoma cells express melanopsin (OPN4), rhodopsin (OPN2), and short-wave-sensitive opsin (OPN1SW)[3]. Pulses of visible white light (650 lx, 95.18 μW/cm2, during 15 minutes) [3] and UVA radiation (4.4 kJ/m2)[4] lead to a marked response of the clock gene machinery of malignant cells when compared to melanocytes. Interestingly, only in malignant melanocytes visible light induces OPN4 migration from a region capping the nucleus to the cytoplasm and membrane[3]. These findings suggest that melanoma cells bear a more responsive photosensitive system when compared to melanocytes. In addition, we have shown that UVA radiation promotes immediate pigment darkening (IPD) in both cell lines, which is lost when this stimulus is associated with heat[4]. Surprisingly, we have demonstrated that OPN2 and OPN4 act as UVA sensor, since upon knockdown of either OPN2 or OPN4, the IPD process is lost. In Drosophila, opsins may detect not only light but also thermal energy[5]; thus, another line of investigation we are pursuing is whether the light detection system in mammalian skin can also detect and feed thermal information to the cutaneous biological clock. In fact, we have just shown that in both cell lines heat stimulus increases the transcription of some members of the clock machinery, increase that is lost when OPN4 is either silenced or pharmacologically blocked. Taken altogether, our lab has provided data regarding the functional role of this photo- and more recently demonstrated thermo-detection system in melanocytes, and how it is deregulated in melanoma cells.

Funding. FAPESP and CNPq

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Study protocol to compare the efficacy of phototherapy with red versus amber LED to treat facial aging

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A Randomized, split face controlled trial to compare the efficacy of phototherapy with red versus amber LED to treat facial aging. The treatment will be conducted in 10 sessions, each participant receiving simultaneously both treatments. The primary variable of the study is the thickness and depth of periocular wrikles (crow´s feet), the secondary variables are elasticity/flaccidity, hydration, transepidermal water loss, quality of life and self-assessment of participants.

The skin undergoes morphological and physiological changes with advancing age. Intrinsic and extrinsic factors contribute to cellular aging, including skin aging. Exposure of the skin to extrinsic factors promotes the formation of excess free radicals, called oxidative stress. This phenomenon is responsible for innumerable cellular damages such as DNA alteration and oxidation of proteins and lipids. In addition, matrix metalloproteinases (MMPs) degrade collagen and elastin fibers causing structural damages to the dermis, forming wrinkles and favoring tissue flaccidity. (1) Some findings suggest that if the correct therapeutic parameters are used, LEDs act on skin regeneration by modulating cellular activity. Thus, phototherapy seems to increase collagen content by decreasing the expression of MMPs that promote degradation of the supporting fibers (collagen). (2) In terms of wavelength, literature suggests the use of red and infrared for the treatment of cutaneous flaccidity and scarring. On the other hand, the use of the amber led is suggested to maintain the integrity of the skin barrier. (3) Due to this, the objective of this clinical protocol is to compare the effects of the f red and amber LEDs for the treatment of facial aging.

Methods and analysis: The treatment will be conducted in 10 sessions, each patient receiving simultaneously both the amber and red LED treatments, one in each hemiface. The application of each LED will last 14min (5.4J/cm ^{2}). After an interval of 180 days, patients will receive cross-treatment. The primary variable of the study is the thickness and depth of periocular wrinkles (crow's feet). The measurement of this parameter will be performed through Visioface (CK Eletronic) equipment. The secondary measurements include: skin viscoelasticity measured through the Cutometer - dual MPA 580 (CK Eletronic); hydration measured through the Corneometer - CM 825 (CK Eletronic) probe; the transepidermal water loss evaluated by the Tewameter - TM 300 (CK Eletronic). Besides, quality of life and self-assessment of patients will be evaluated by questionnaires. This protocol was approved by the Research Ethics Committee of the Nove de Julho University.

Acknowledgment. We thank Tecnotests Produtos e Serviços Ltda for gently provide the equipaments for skin measurements.

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Photomodulation in the treatment of pain in patients with TMD: Protocol for cost-effectiveness

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Introduction. Epidemiological data show that the signs and symptoms of TMD start to become apparent from six years of age, and in adolescence these signs and symptoms are similar to those of adults. The present study aims: to estimate the direct costs of treatment of muscle pain in patients with TMD with low-intensity laser and with occlusal splint and a placebo group; to evaluate the effectiveness of the treatments with low-intensity laser and occlusal splint for muscle pain in patients with TMD; to analyze the cost-effectiveness of the two proposed treatments for pain; and to describe and compare the results of analyses of treatments for pain in patients with TMD [1,2].

Methods and analysis. It is a prospective trial of clinical and economic analysis. It will include 45 patients aged between 15 and 25 years with TMD, randomly assigned to a treatment group: G1 (low level laser), G2 (occlusal splint) and G3 (placebo). The analysis will be based on the costs of each treatment during the 12-month period. The outcome for the analysis of the effectiveness will be the pain, measured periodically by means of the clinical examination of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). The cost-effectiveness ratio will be calculated using, as endpoints, pain and the calculation of the ratio between the difference in costs between the groups studied. The evaluation of the impact of the treatment on quality of life will be determined by applying the adapted EuroQol-5D [3,4].

Ethics approval and consent to participate: This study was approved the Nove de Julho University Ethics Committee - Protocol Number: 2.014.339. All participants will provide informed consent before participating in this study.
Trial registration: NCT01331031.
Competing interest: The authors have no conflict of interest, financial or otherwise to declare.

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Preliminary data for the role of low level laser therapy (LLLT) compared to carvedilol therapy in cardiac remodeling after myocardial infarction

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Introduction: LLLT is a promising tool to attenuate cardiac remodeling resulting from myocardial infarction (MI). Previous studies point the repercussion of LLLT, in which its action added or compared to standard pharmacological therapy of heart failure was not investigated. The aimed of the study was to compare the benefits of LLLT with carvedilol therapy in rats submitted to MI. The carvedilol was used because of their therapeutic actions like broad beta-blocking, anti-apoptotic and antioxidant action in the infarcted myocardium.

Methods: 40 female rats were randomized to four experimental groups (Unifesp ethics committee: 8806150615): SHAM; Infarcted untreated (MI); Infarcted + caverdilol (MIC); Infarcted + LLLT (MIL). The LLLT (λ 830 nm, 100 mW, 200 s, 20 J) was applied transthoracic three times weekly. Animals receiving caverdilol daily doses of 10 mg / kg. Biometric data were collected. Heart and left ventricle (LV) and the pulmonary water content (H2O) analyzed. The hemodynamic parameters of the LV were evaluated at baseline. The following variables were obtained: systolic pressure (PS); Final diastolic pressure (PD2); derivative of positive pressure (+ dP / dt) and negative (-dP / dt); Cardiac output; Systolic work. Only animals with infarctions ≥37% were included in the study. Data are expressed as mean ± standard deviation and ANOVA (post-hoc: Bonferroni).

Results: LLLT did not affect the ratio of heart / body weight (SHAM: 3.9 ± 1.3, MI: 4.9 ± 0.4, MIC: 3.9 ± 0.7, IML: 4.7 ± 0.7, mg / g), but attenuated the increase in LV / body weight ratio (SHAM: 2.5 ± 0.06, MI: 3.2 ± 0.2, IMC: 2.6 ± 0.3, IML: 2.5 ± 0.3; mg / g) induced by MI, a situation that also prevailed in the IMC group. H 2 O was attenuated by LLLT and carvedilol (SHAM: 78.4 ± 0.8, MI: 80.4 ± 0.9, IMC: 79.4 ± 0.7, IML: 78.7 ± 0.57,%). PS was significantly reduced in all infarcted groups (SHAM: 130 ± 12, MI: 102 ± 7.8, IMC: 107.7 ± 9, IML: 113.8 ± 7.8, mm / Hg). The increase in post-MI PD2 was reduced only with pharmacological treatment (SHAM: 7 ± 2, IM: 16 ± 3, IMC: 6 ± 3, IML: 12 ± 2; mm / Hg). Both + dP / dt (SHAM: 9530 ± 2311; MI: 5241 ± 639; IMC: 5756 ± 639; IML: 6908 ± 586; mmHg / s) and -dP / dt (SHAM:7984±1579; MI:4575±687; IMC: 4419±656; IML:5376±459; mmHg/s) were reduced with MI and unaffected by therapies. CD was similar between groups (SHAM: 68.9 ± 7, MI: 74.5 ± 9, MIC: 62.4 ± 11, IML: 63.6 ± 7 mL / min), but TS was lower (SHAM: 0.4091 ± 0.075; IM: 0.26657 ± 0.0183; MIC: 0.2749 ± 0.047; IML: 0.2950 ± 0.041; g·m/beat).

Conclusion: Our results are the first to demonstrate the effects of LLLT as continuation application in post-MI treatment. The characteristic diastolic dysfunction in post-MI seems to be attenuated by LBI.

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EFFECTS OF LOW INTENSITY LASER ON BONE HEALING AFTER MOLAR EXTRACTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: There is an association between hypertensive individuals and reduced bone mineral density. Low intensity laser (LBI) has anti-inflammatory activity, accelerates the healing of bone defects *in vivo* and *in vitro*. It stimulates blood flow, recruitment and activation of osteoblasts, osteosynthesis, and decreased osteoclastic activity. **Objective**: The aim of this study was evaluate if photobiomodulation with LBI improves bone repair after extration in spontaneously hypertensive rats (SHR). **Methods:** After approval by the CEUA-Uninove (AN0032.2015**),** twenty-four female SHR (200-250 g) were used in which the right and left lower molars were extracted. The left alveolus was the control and the right one received the laser therapy according to the following parameters: ƛ: 808 (± 10) nm, P = 100 mW, beam area: 0.002826 cm^2, time = 40 seconds, total energy = 4J, power density = 138 mW/cm^2. Two applications were performed, one in the immediate postoperative period and the other on 7^{th} day. On days 7^{th}, 14^{th}, 30^{th} and 60^{th} the animals were euthanized and the mandible fragments, staining with hematoxylin & eosin and Mallory's trichrome. The samples were evaluated in order to analyze the presence and type of inflammation, necrosis, bacteria, epithelialization, neoformed bone area and number of osteocytes. The parameters of the random repair evaluated were: amount of necrotic bone, amount of bone matrix, presence or absence of ulceration, inflammation, osteoblasts in activity and osteoclasts, granulation. The data were evaluated using Student's t-test. **Results:** None of the animals presented oral ulcer, edema, or fistula. All animals presented epithelization; no necrosis or empty osteocytic lacunae in all experimental days. **Conclusion**: Under the conditions of this study, photobiomodulation did not interfere in the bone repair of spontaneously hypertensive rats.

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Phenothiazine photodynamic efficiency is aggregation dependent

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The efficacy increases in the series Azure A (AA) <Azure B (AB) <Methylene Blue (MB). The evaluation of the dimer to monomer ratio showed that the aggregation increases in the order MB <AB <AA. Thus, the photodynamic efficiency of these compounds is dependent on aggregation.

Background. Photodynamic therapy (PDT) is a therapeutic aid for the treatment of cancer, infections and some benign diseases, which consists in the use of a photosensitizer (PS) associated with light. The mechanism of action is based upon the production of reactive oxygen species (ROS), among them, singlet oxygen ($^1O_2$), which react with the lipids and proteins of organelles, leading to cell death. Methylene blue (MB) is a phenothiazine that have been studied in several clinical trials and experimental models for PDT studies.[1,2] Other phenothiazines, which presents similar structure, such as Azure A (AA) and Azure B (AB) may also present interesting activity for PDT, but there is lack of information regarding their photophysical, photochemical and photobiological properties. The objective of this work is to compare the photodynamic efficiency of the phenothiazines MB, AA and AB, in an *in vitro* model using a neoplastic cell line.

Results. In order to evaluate the photodynamic efficiency of the PSs, the IC_{50} values were determined in the dark and with irradiation by MTT colorimetric method. In the dark, IC_{50} values for MB, AA and AB are 182, 141 and 78 µmol/L respectively. Note that AB is more cytotoxic than the other PSs in this condition. The IC_{50} values, with irradiation, are 1.1, 6.3, 1.8 µmol/L for MB, AA and AB, respectively. The phototoxicity of MB and AB are similar, whereas AA shown to be less phototoxic. Since these PSs have similar structures, a huge difference in efficacy was not expected. The cell uptake and singlet oxygen production quantum yield do not explain these differences in efficacy. Junqueira showed aggregation properties of MB and their effects on ROS production.[1] Due to the structural similarity, azures also may present aggregation. Thus, the dimer to monomer ratios (D/M) of the PSs were evaluated. It was observed that the higher the concentration evaluated the higher the D/M value, regardless the PS and the medium used. The presence of methyl groups in the periphery of the chromophore reduces the tendency of phenothiazines to aggregate due to a steric effect. Therefore, MB with 4 peripheral methyl groups presents the lowest aggregation tendency; AB, with 3 methyl groups, presents intermediate aggregation and AA, which presents only 2 peripheral methyl groups, presents the highest tendency of aggregation among the compounds studied.

In view of the obtained results, it is possible to conclude that MB is the most effective PS in this series due to the presence of four peripheral groups that reduce aggregation. Aggregation is a parameter dependent on the molecular structure of the PS and that shown to be a determinant factor for PDT effectiveness.

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Assessment of the quantity of microorganisms associated with bronchiectasis in saliva, sputum and nasal lavage after periodontal treatment: a study protocol of a randomised controlled trial

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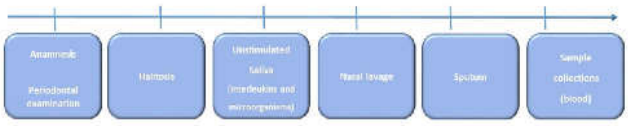
Introduction: The association between periodontal disease (PD) and chronic obstructive pulmonary disease (COPD) has been widely studied, with aspiration of periodontal pathogens being one of the most accepted causal mechanisms for pulmonary exacerbation. Periodontal treatment (PT) was associated with a decrease in these exacerbations. Bronquiectasis is a pulmonary disease that has many similarties to COPD, however, there are no studies correlating this condition to PD is far.This study will evaluate if PT reduces proinflammatory cytokines in serum and saliva, as well as halitosis and the amount of microorganisms associated with exacerbation of bronchiectasis in saliva, sputum and nasal lavage 3 months after PT.

Methods and analysis: A total of 182 patients with PD and bronchiectasis will be randomly allocated to group 1 (positive control,;scaling and root planing (SRP)+oral hygiene (OH) or group 2 (experimental; SRP+photodynamic therapy+OH). After 3 mouths, samples of saliva, nasal lavage and sputum will be collected to determinate the level of *Pseudomonas aeruginosa, Staphilococcus aureus* and *Porphyromonas gingivalis* by quantitative PCR. Ths protocol will determinate the efficacy of PT in reducing the most likely niches of bronchiectasis exacerbation by comparing pre- and post-treatment microbiology samples. Furthermore, there will be assessment of oral halitosis and verification of inflammatory cytokines in serum and saliva.

Figure 1. Timeline showing the sequence of procedure



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Acute and chronic effects of a single low-level laser application (LLLT) on cardiac remodeling after myocardial infarction (MI)

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Introduction: A single LLLT application post-MI can generate benefits for heart failure (HF), even with treatment discontinuation. Unfortunately, these findings are limited because there is no control for confounding variables, e.g. infarct size. The aimed of the study was to analyze whether the benefits of LLLT application post-infarction persist in HF stage.

Methods: Female rats were allocated for SHAM, infarcted (MIN) or infarcted plus LLLT (MIL) group. A single LLLT (λ 660 mm, energy 1.1J) was applied post-injury. Echocardiography was performed after three days (acute MI stage) and five weeks (chronic MI stage) of injury to determine MI size, left ventricular shortening fraction (LVSF, %) and LV diameter (LVD, mm) in rats with MI ≥40%. LV hemodynamic was evaluated in baseline and afterload stress to measure: +dP/dt (mmHg/s); −dP/dt (mmHg/s); cardiac output (CO, mL/min); stroke work (SW, g·m/beat). VEGF, Akt_1 and $phoAkt_1$ tissue protein were evaluated. Data are shown as mean±SD.

Results: LLLT not affect LVD on the acute (SHAM:7.3±0.7; MIN:7.8±0.5; MIL:8.2±0.7) and chronic (SHAM:7.1±0.2; MIN:9.4±1; MIL:9.7±0.4) MI stage. LLLT attenuated LV dysfunction on the acute MI stage (LVSF-SHAM:46±4; MIN:18±7; MIL:27±6) without chronic stage effect (LVSF- SHAM:49±5; MIN:19±6; MIL:13±6). LLLT did not affect +dP/dt (SHAM:8425±1117; MIN:6058±957; MIL: 6035±1041) and −dP/dt (SHAM:5062±600; MIN:4338±347; MIL:3716±485) reduction in baseline. There were no differences in CO (SHAM:35±7; MIN:30±9; MIL:35±11) and SW (SHAM:0.138±0.037; MIN:0.115±0.035; MIL:0.1702±0.065). SHAM response to afterload stress included increment on +dP/dt (39±6%), -dP/dt (43±16%), SW (26±15%) and reduction on CO (-21±16%). The infarct induced attenuation in +dP/dt (Δ%:22±6) and -dP/dt (Δ%:8±25), associated with reduction on CO (-103±28%) and SW (-18±20%). LLLT did not change these findings (+dP/dt:20±11; -dP/dt:-7±20; CO:-108±50; SW:-29±43; %). There were no differences between groups for VEGF (SHAM:109±34, MIN:104±1, MIL:102±5, a.u.), Akt_1 (SHAM:100±52, MIN:157±107, MIL:154±71, a.u.), $phoAkt_1$ (SHAM:100±54, MIN:139±122, MIL:112±73, a.u.).

Conclusion: A single LLLT application can improve cardiac function in acute MI stage without impact on HF stage.

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The effects of papain-based and Bixa Orellana gel on collagen and fibroblasts: A spectroscopic and cytotoxicity analysis.

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Background: Papacarie Duo™ is clinically used and has a proven effectiveness; however, it is necessary to improve its antimicrobial action. The combined treatment of Papacarie Duo™ with urucum (Bixa Orellana) could create a potential tool for dental caries treatment. Annatto, the extract contained in the pericarp of the seeds that consists of the pigment in the plant was used in a water-soluble form in dilute alkaline solution. The primary pigment of the seed is cis-bixin with smaller amounts of other carotenoids. The dicarboxylic acid salts of cis-norbixin and trans-norbixin occur in heated alkaline solutions. **Objective**: An in vitro study was performed to analyze the absorption spectra, cytotoxicity and with human dermal fibroblasts in different concentrations of urucum, associated or not with PapacarieDuo™. The effects of pure urucum, PapacarieDuo™, and PapaUrucum on the microstructure of collagen were also analyzed. **Methods:** An in vitro study was performed that analyzed: the absorption spectra, cytotoxicity with human dermal fibroblasts of the pure urucum, PapacarieDuo™ and PapaUrucum; the effects of pure urucum, PapacarieDuo™, and PapaUrucum on the microstructure of collagen.**Results:** The application of a papain-based gel with urucum did not present cytotoxicity, and did not alter the chemical structure of collagen. **Conclusion**: Indicating that PapaUrucum is a potential tool for the treatment of carious lesions, combining the action of Papacarie Duo™ with a possible antimicrobial action, as well as being a potential product for Antimicrobial Photodynamic Therapy (aPDT) application.

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EVALUATION OF PERIODONTAL TREATMENT WITH PHOTODYNAMIC THERAPY IN LUNG INFLAMMATION PARAMETERS IN EXPERIMENTAL MODEL OF PERIODONTAL DISEASE

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Background: Different mechanisms have been proposed to explain the relationship between periodontal disease (DP) and lung inflammations. Studies have shown that oral cavity is a potential reservoir for respiratory pathogens. There seems to be a positive dose-response effect between both disease and possibly an improvement in the pulmonary exacerbation against when periodontal treatment (TP) is realized in humans [1]. **Objectives:** To evaluate the TP associated with PDT in parameters of lung inflammation in experimental model of DP. **Methods:** After CEP-Uninove approval (CEUA 020/2015**),** twenty-four Balb / c male mice, 2 months years-old and 25 g were divided into 4 groups (n = 6): C-Control, DP, DP + TP; DP + TP + PDT. Periodontitis was induced with ligation technique. After 15 days, TP was performed. For the PDT was used methylene blue (0.005%) and irradiated with red diode laser (100 mW, λ = 660 nm, energy density of 6,369 J / cm², two irradiation points, 9J per point, for 90s). After 21 days all mice were euthanized. Total and differential cell counts were performed, and the level of inflammatory cytokines in bronchoalveolar lavage (BAL). For the statistical analysis was used one-way ANOVA followed by the Student-Newman-Keuls test. **Results:** For characterization of DP there was an increase of alkaline phosphatase 52 ± 7U / L and bone resorption 880 pixels. DP caused release of IL-5 in the lung 162 ± 10 pg / ml ($p < 0.05$), as well as PDT-associated TP, was able to decrease the total amount of inflammatory cells in BAL by 6.7 ± 1x104 ($p<0.05$). In addition, there was an increase in the amount of mucus in the inner part of the airway in the DP group 204 ± 15% ($p < 0.01$). **Conclusion**: These data suggest that PD can influence the parameters of pulmonary inflammation, and its treatment, in the remission of these factors, regardless of the periodontal therapy chosen

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Analysis of the inflammatory profile and apoptotic factors in the myocardium remote to infarction induced by coronary artery occlusion after photobiomodulation

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Introduction: Large infarcts induce the process of cardiac remodeling in the infarcted area and the myocardium remote from the infarction. After infarction, tissue injury and death of cardiomyocytes by necrosis and apoptosis activate the acute inflammatory response. These post-MI events can have adverse consequences (cardiac rupture or dilatation). The aimed of the study was to analyze the inflammatory profile and apoptotic factors in a model of coronary occlusion submitted to irradiation with low intensity laser.

Methods: 21 rats were randomized into three experimental groups (ethics committee of the University Nove de Julho: 0015-2012): control (SHAM); untreated infarct (IM); infarcted + laser (IML). Irradiation (λ 660 mm, 15 mW, 60 s, 1.1 J) was applied to the infarcted area 60s after MI induction. Echocardiography was performed on 3 day. Only animals with MI size ≥37% were included. Protein and gene expression of Bax and Bcl-2 and cleaved caspase 3 protein expression were analyzed. Pro-inflammatory cytokines (IL-6, CINC-1 and TNF-α) and anti-inflammatory cytokines (IL-10) were analyzed by ELISA in the remote and ischemic area after MI. Data are expressed as mean ± SD and ANOVA (post-hoc: Newman Keuls).

Results: Photobiomodulation did not affect left ventricular ejection fraction (LV) after 3 days of MI (Sham, 0,8143±0,04; IM, 0,5296±0,14; IML, 0,5773± 0,10, %). However, photobiomodulation decreases the expression of IL-6 proinflammatory cytokines (Sham, 57,08±18,35; IM, 5650±1924; IML, 3758±466, pg/mg), CINC-1 (Sham, 67,57±17,28; IM, 141,2±48,10; IML, 80,02±6,93, pg/mg) and TNF-α (Sham, 180,6±206,7; IM, 824,7±243; IML, 322,4±79,6, pg/mg). Photobiomodulation did not modulate the ischemic area of IM, IL-6 (Sham, 57,08±18,35; IM, 80,05±37,75; IML, 50,94±20,45, pg/mg), CINC-1 (Sham, 68,09±20,72; IM, 60,06±27,15; IML, 48,93±9,08, pg/mg) e TNF-α (Sham, 7,13±7,03; IM, 3,71±3,76; IML, 1,29±1,19, pg/mg). There was no significant change in IL-10 expression in the remote area (Sham, 155.1 ± 100, IM, 353.2 ± 132.3, IML, 432.4 ± 68.35, pg / mg) and in the ischemic area (Sham, 87.83 ± 44.23, IM, 242.2 ± 102.6, IML, 154.4 ± 19.8, pg / mg) by photobiomodulation. Treatment with photobiomodulation significantly increased the gene expression (Sham, 0.914 ± 0.07, IM 0.324 ± 0.11, IML, 0.742 ± 0.09, a.u.) and protein (Sham, 100 ± 12.20, IM 43, 78 ± 2.8, IML, 59.54 ± 7.3, a.u.) of Bcl-2. We observed no significant difference in gene expression (Sham, 0.254 ± 0.04, IM 0.4988 ± 0.27, IML, 0.304 ± 0.09, a.u.) and protein (Sham, 100 ± 8.8, IM 319.4 Â ± 155.4; IML, 183.8± 89.71, a.u.) Bax. Photobiolulation modulated the cleaved caspase 3 protein expression (Sham, 0.0 ± 0.0; IM, 100 ± 9.6, IML, 15.4 ± 6.5, u.a.).

Conclusion: The effects of photobiomodulation on inflammation and apoptotic factors could augment cellular survival of the viable area of the heart after infarction, contributing to the consequences of pathological cardiac remodeling.

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THE EFFECT OF ANTIMICROBIAL PDT AS A CO-ADJUVANT IN THE ENDODONTICAL TREATMENT ON PRIMARY TEETH - CASE SERIES

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Abstract

The main causes of pulpal necrosis and inflammation of deciduous teeth are carious lesions and traumatic lesions. Studies indicate that 75% of the teeth with deep caries have their pulp compromised, without possibility of reversion, leading to the need of radical root canal treatment. In order for root canal treatment to be successful, the root canal must be completely decontaminated. Thus, some methods have been researched in an attempt to effectively eliminate it from the interior of the root canal system (SCR); Among them, is conventional endodontic therapy in conjunction with photodynamic therapy (aPDT), used in this study.

After the endodontic treatments, the microbiological analysis of the samples collected before and after the treatments was performed. Observing the results obtained in this case series and the noticed difficulty in the success of endodontic treatment of deciduous teeth, aPDT can be considered an alternative of easy application, and that does not generate microbial resistance to act as a coadjuvant in the decontamination of the root conduits. However, in order to verify the effectiveness and long-term success of this therapy, more clinical studies will be required.

Keywords: Endodontic Treatment, Primary Teeth, Photodynamic Therapy, Photobiomodulation

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Effect of Photodynamic Therapy in the Reduction of Halitosis in Patients with Multiple Sclerosis: Clinical Trial

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Smell and odours play a vital role in social interaction. Halitosis is a social problem that affects one-third of the population causing a negative impact in the quality of life[1]. There is little knowledge on the prevalence and management of halitosis in Multiple Sclerosis (MS) patients. The present study aimed to evaluate the presence of halitosis in patients with MS when compared to a control group and the treatment with antimicrobial photodynamic therapy (aPDT) against halitosis. This is a case-control clinical study where 60 patients were evaluated: 30 MS patients in treatment at the Specialties Clinic School of Medicine, and 30 healthy patients, matched in age and gender, for the control group. Data was collected on duration of disease, degree of disability and medication use in the MS group, and for all patients, halitosis was assessed with Oralchroma™. Individuals with halitosis underwent treatment with tongue scraping and aPDT. The technique described by Lopes *et al*[2] for photodynamic therapy was used. The photosensitizer was methylene blue (0.005%) with the laser THERAPY XT-EC® (660nm, 9J, 100mW for 90 seconds per point, 320J/cm^2, 3537mW/cm^2). Six points in the tongue dorsum were irradiated with a distance of 1cm between them. There was a positive correlation between disability and disease duration. No parameter was correlated with halitosis. Patients with MS have higher levels of SH_2 compounds when compared to the control group (p=0.003, Mann-Whitney) (Fig. 1.a), but after the aPDT both groups significantly reduced the levels to under the halitosis threshold (Fig. 1.b). The treatment with scraping an aPDT was effective in the immediate reduction of halitosis in both groups.

Clinical Trials Number: NCT02007993.

Figures.

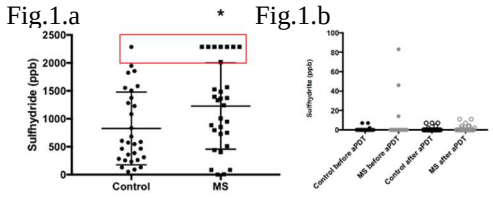

Fig. 1. (a) Comparison of the initial SH2 levels between MS and control groups, (b) SH2 levels before and after treatment in both groups.

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Systemic photobiomodulation with low-level laser/ ILIB: Review the literature

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Background: Systemic photobiomodulation (PBM) initially called as intravascular laser irradiation of blood, was developed by the Soviet scientists over 30 years ago. PBM into the blood has been associated with biostimulating, analgesic, antiallergic, immunocorreactive, vasodilating, antiarrhythmic, antitoxic, spasmolytic and anti-inflammatory properties. Thus, it has been used for pain treatment, inflammatory processes, tissue repair, restenosis, chronic spinal cord injury, stroke, rheumatoid arthritis, atherosclerosis, systemic arterial hypertension, angiogenesis, acute myocardial infarction, vasospasm, diabetes mellitus, and immune disorders.

Objective: This study aimed to review the literature published from 2012 to August 2017 regarding the effect of systemic PBM, the dosimetric parameters as well as different methods to deliver energy.

Methods: The study was carried out in PubMed/ MEDLINE®, Scopus and Web of Science databases. Original articles were used to study the effects of systemic PMB, published from 2012 to August 2017, according to the following inclusion criteria: English language, systemic PBM in clinical studies, animal model and tissue regeneration. The descriptors used was intravascular laser irradiation (ILIB) OR intravascular laser therapy (IRLT) OR non-invasive laser irradiation of blood (NLIB) OR transdermal illumination of radial artery OR systemic photobiomodulation.

Results: Of the 524 potentially relevant articles, 22 were excluded because they were duplicated, 25 were excluded due to other languages (most of which were Russian, Polish, Ukrainian and Chinese), and 463 were excluded because they did not meet the purpose of the study. Thus, 14 articles were kept for a detailed analysis (Figure 1). Of these, 7 used PBM between 632.8 and 670 nm, 4 used between 780 and 808 nm, 1 used between 405 and 532 nm, 1 used between 532 and 632,8, and 1 used 405 nm. The irradiation time of each session ranged from 90 seconds to 1 hour. The number of sessions ranged from 1 to 120 applications. There are 10 articles using intravenous/ intravascular, and 4 using transdermal, and/ or transdermal, and/ or over venous application. No adverse effects have been reported with the use of systemic photobiomodulation. The studies verified a hemodynamics changes, decrease in the incidence of restenosis, reduction of oxidative stress, and glucose levels, greater tissue repair, increases NO production, vasodilation, decreasing EGFR expression which reduce neuroinflammation.

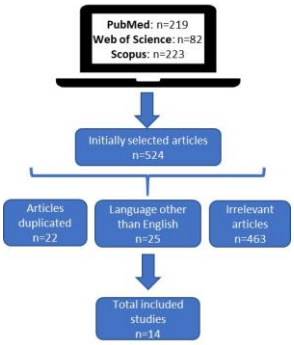

Fig. 1. Schematic flow chart representing the studies included in this review.

Conclusions: No clear conclusion can be obtained with the use of systemic PBM due to the scarce number of publications and the non-standardization methods of the dosimetric parameters. The studies showed encouraging results for the use of systemic PBM to treat diverse disorders and pathologies. Therefore, more studies are necessary to fill this gap through the development of optimal dosimetric parameters, understanding its biological effects as well as less invasive approaches, such as those used transdermally, transcutaneously or over venous.

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Effect of phototherapy in the cross-section area of muscle fibers of the tibialis anterior muscle after peripheral nerve injury of sciatic nerve

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Introduction

The low level laser therapy (LLLT) has been used to accelerate the regenerative processes, to achieve an early return of the patient's functionality and has demonstrated positive results in the treatment of peripheral nerve injuries[1]. However, the focus of the research has been the functional analysis[2] and findings of the histological evaluation remain unclear. Thus, the current study aimed to evaluate, through the histological analysis, the effects of photobiomodulation on neuromuscular repair of peripheral nerve injuries.

Methods

Eighty-five Wistar rats were randomly divided into 5 groups: Control, Injury, Injury+LLLTn (LLLT in the area corresponding to the nerve injury), Injury+ LLLTnm (LLLT in the area of nerve injury and in left tibialis anterior - TA muscle) and Injury+LLLTm (LLLT in left tibialis anterior muscle) with 20 animals per group. The lesion groups were analyzed after 1, 2, 3 and 4 weeks. The lesion procedure of the left sciatic nerve was performed by the crushing technique using a hemostatic clamp (6.3 MPa) through a compression of 30 s. The laser treatment was started after 2h with LLLT (780 nm, 0.04 cm2, 1 W/cm2, 3.2 J) in the nerve damage area (20 J/cm², 0.8 J per point in 4 points), and/or in TA muscle (10 J/cm², 0.4 J per point in 8 points).

For histological analysis TA muscles were carefully removed and submitted to histological analysis using hematoxylin and eosin staining for cross section area analysis.

Results and Conclusions

The findings revealed significant differences between groups in the cross-section area (figure 1) of the muscle fibers after peripheral nerve injury at 1, 2, 3 and 4 weeks.

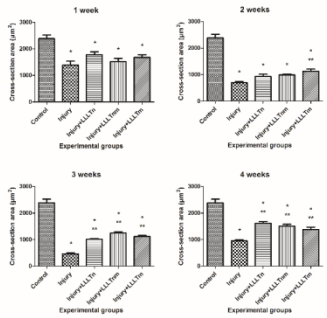

Figure 1. Effects of photobiomodulation with LLLT on the diameter of muscle fibers post peripheral nerve injury at 1, 2, 3 and 4 weeks. *p<0.05 vs Control, **p<0.05 vs Injury.

LLLT promoted an increase of muscle aspect through the cross-section area analysis with similar results intergroups.

Funding sources and acknowledgments. This study was supported by UNINOVE, FAPESP and CAPES.

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CO_2 laser for dental alumina ceramic welding: SEM and diffractometry.

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This work tested the CO_2 laser (λ = 10.6 µm) as a heating source for a dental ceramic material welding. Alumina sticks were weld and characterized by scanning electron microscopy (SEM) and diffraction.

Aim. Metal framework of dental prosthesis may be welded when a failure or a maladaptation occurs. Due to its high strength, some polycrystalline ceramics (zirconia, alumina) may also be used as a framework in extensive dental prosthesis. However, there is no evidence that these ceramic materials may be welded, if needed. This study aimed to test the possibility of welding a ceramic structure using CO_2 laser. The second objective of the study was to analyze the microstructure of the material in the weld zone.

Materials and methods. Alumina blocks (AL20 VITA Zahnfabrik) were sectioned and then sintered to sticks with the final dimension of 10.0 x 1.2 x 1.2mm. The sticks were adapted in pairs to a LHPG (Laser Heated Pedestal Growth) chamber device. CO_2 laser beam achieved the extremity of two sticks with a ring format of 1.0mm diameter (300µm thickness) creating a cylindrical molten zone. The LHPG apparatus is described elsewhere (Fejer MM et al. 1984).

The laser was applied in a continuous mode with 40W nominal power (5 seconds). The fused specimens were then analyzed in stereomicroscope up to 45X magnification and SEM. A diffraction analysis was conducted in one sample.

Results. The ceramic sticks were successfully fused. The aspect of the fusion zone differed in color and translucency from the original sintered alumina. SEM showed the presence of porosity and voids in the center of the fusion zone, suggesting a reduction in material resistance at that level.

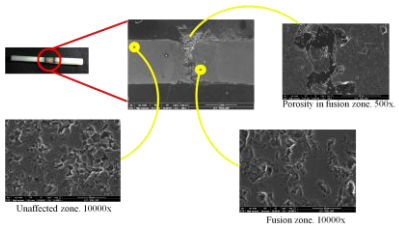

Fig 1. SEM images from different regions of the fused specimens.

Diffraction pointed to a reduction in crystallite size by two to four times in the welded region of specimens (fig. 2).

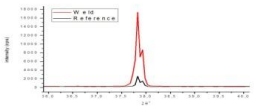

Fig 2. Diffractometry graph (2θ=38) showing the decrease in crystallite size in weld zone.

Conclusions. CO_2 laser was effective welding a dental ceramic structure. There was a change in crystallite size when the material was submitted to irradiation. Porosity observed in molten zone gives cause for concern regarding weld resistance.

Acknowledgment. We thank to Prof. Márcia Carvalho de Abreu Fantini (IF-USP) for the diffraction analysis.

Reference

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Effects of low level laser on the distribution and organization of collagen in the tibialis anterior muscle of elderly rats

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Introduction: The use of low level laser therapy (LLLT) is well grounded in the literature due to its effects on inflammatory disorders, tissue regeneration and skeletal muscle repair. LLLT has positive modulatory effects on muscle precursor cells and collagen remodeling [1]. However, the few studies that associate LLLT with skeletal muscle regeneration in older animals differ with regard to the dosimetric parameters employed [2]. Therefore, the purpose of the current study was to evaluate the effects of LLLT on the distribution and organization of collagen in the tibialis anterior (TA) muscle of elderly rats submitted to cryoinjury.

Methods: Thirty legs of male Wistar rats were distributed into three groups: control animals not injured or treated with LLLT (n=4); cryoinjury without LLLT treatment (n=13); and cryoinjury treated with infrared LLLT (n=13). LLLT was applied to the TA 2h after of the injury induction and consisted of daily applications until the sacrifice (1, 3 or 7 days). The following parameters were used: λ=780nm, power density: 1000mW/cm^2; power: 40mW; total energy: 3.2J; exposure time: 10s. Additional cuts were stained with picrosirius red and examined with the aid of a polarized light microscope.

Results and Conclusions: At Day 7, the treated injury group exhibited better collagen organization compared to the untreated injury group, which exhibited disorganized collagen. No differences were found in the amount of collagen in the untreated and treated groups at days 1, 3 and 7, but the group submitted to LLLT exhibited better collagen organization at Day 7 compared to the other treated and untreated injury groups. Moreover, an increase in the concentration of collagen was found in the untreated and treated injury groups compared to the control group at day 7 ($p < 0.05$). In conclusion, LLLT demonstrated a modulatory effect on the muscle repair process in elderly animals with regard to collagen remodeling of muscle tissue.

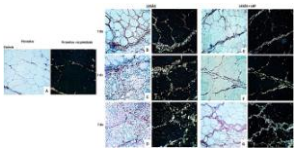

Fig. 1. Photomicrograph of histological section of the muscle stained with Picrosirius Red with or without polarized light (original magnification X 400). The figures illustrate images used to quantify the percentage of collagen fibers per area, made under a polarized light microscope. (A) Control; (B) Injury group after 1 day; (C) Injury + LLLT group after 1 day; (D) Injury group after 3 days; (E) Injury + LLLT group after 3 days; (F) Injury group after 7 days; (G) Injury + LLLT group after 7 days.

Funding sources and acknowledgments: This study was supported by UNINOVE and FAPESP (2016/ 21204-1).

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DIAGNOSIS OF OCCLUSAL CARIES LESIONS IN DECIDUOUS MOLARS BY COHERENT LIGHT SCATTERING PATTERN SPECKLE

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Introducion

Detecting incipient lesions of caries is a clinical challenger due to the anatomy of the tissue, especially on occlusal surface that have fissures and grooves. Visual inspection is subjective[1] and almost always sensitive for older lesions, as it is more efficient in the diagnosis of cavitated lesions than non-cavitated and incipient lesions[2].

When a coherent light falls onto a rough surface, it causes a diffuse reflection, which generates an optical phenomenon called speckle. By capturing the light intensity of each point through a digital image, it is possible to quantify this pattern using statistical theory[3]. The use of speckle patterns for qualifying lesions in dental tissues is a promising technique that may improve caries diagnosis in the spatial domain of the optical scatter pattern. Speckle method for observing the contrast between the pattern of granulated tissue of healthy dental tissue and that of tissue from dental caries was demonstrated[4]. This method is non-invasive, non-destructive, works in real-time, and is effective and capable of increasing the contrast between healthy dental tissue and a caries lesion in the initial stage.

Materials and Methods

We used 30 healthy deciduous molar teeth collected from the Biobank Human Teeth, Faculty of Dentistry, University of São Paulo who had carious lesions induced by the pH cycling method. The samples were evaluated for the diagnosis of caries by two methods: ICDAS and speckle pattern of coherent light scattering after 5, 10 and 15 days and the results were statistically analyzed using $\alpha = 0.05$ significance level.

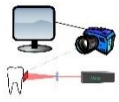

Fig.1-Photo of the experimental setup used in this project. Source: author

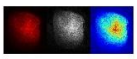

Fig 2-A) Photo no standard treatment speckle scattering, B) average photo image size 4x4, C) average photo with false colors for easier viewing, where Blue is the healthy tissue, yellow intermediate phase and Red is the carious tissue. Source: author

Results and Conclusion

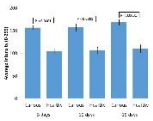

Fig 3- Difference of the speckle in carious tissue and healthy tissue in different time intervals. Error bars represent standard error

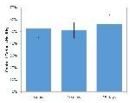

Fig 4. Graph contrast between the speckle in carious tissue and healthy tissue groups after 5, 10 and 15 days. Error bars represent standard error

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EFFECT OF PHOTODYNAMIC THERAPY FOR THE TREATMENT OF HALITOSIS IN ADOLESCENTS – A CONTROLLED, MICROBIOLOGICAL, CLINICAL TRIAL

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ABSTRACT

Halitosis is the term used to describe an unpleasant odor that emanates from the oral cavity or breath. It main cause is in the oral cavity, being 51% of cases related to the presence of tongue coating, resulting from the degradation of organic substrates for anaerobic bacteria and the production of volatile sulfur compounds (VSC). It is believed that the primary source of exhalation of these compounds is in the back of the tongue. The conventional treatment of halitosis is the chemical and mechanical removal of microorganisms with rinses and scrapers, but there is much disagreement in studies on the effectiveness of these methods. Photodynamic therapy involves the use of a dye and a light source of a specific wavelength which has an antimicrobial action. The objective of this study was to evaluate the clinical and microbiological effect of photodynamic therapy for the treatment of halitosis in adolescents between 12 and 19 years. 46 adolescents were randomly divided into three groups: Group 1 treatment with photodynamic therapy, group 2 with tongue scraper and group 3 associations of treatments. Clinical evaluation of halitosis were made by means of gas chromatography and microbiological testing by counting colony forming units per ml (CFU / ml). It was used the Kruskal-Wallis tests for comparison followed by the Student-Newman-Keuls test. To check colony forming units per ml (CFU / ml) before and after treatment the ANOVA test were used. For all analyzes were considered a level significance of $\alpha = 0.05$. After treatment there was a statistically significant decrease in all groups ($p < 0.001$), but the combination of photodynamic therapy to the tongue scraper proved more effective at all hydrates of sulfur reduction (median = 0). Microbiologically it was observed a statistically significant difference before the treatment for the groups 1 and 2 ($p<0,001$). This study provides a new treatment option for halitosis in adolescents with immediate effect without mechanical aggression common in lingual papillae when the conventional treatment is used.

Key words: Halitosis, Photodynamic Therapy, Adolescents, Tongue, laser.

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Nipple trauma and photobiomodulation: a bibliometric analysis of scientific production

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Introduction

Nipple trauma represents the second main cause of interruption of breastfeeding[1], and can be classified as circular or longitudinal, presenting many sizes. When circular, the lesion locates at the nipple-areolar junction, while the longitudinal lesion may be located throughout the nipple, vertically or horizontally, even dividing it into two.[2]

One of the treatment options for nipple trauma is phototherapy, where electromagnetic waves within the spectral range of red and infrared are applied to the tissue by means of low intensity light devices such as LED (Light Emitting Diode) and LASER, since studies have already shown its anti-inflammatory properties, contributing in the repair of the wounds[3,4] as well as in the control of the pain.[5]

Objective

The presente study aims to perform a bibliometric analysis of the studies published from 2006 to 2017.

Justification:

Studies like this are important to guide the help of new research and raise new questions that have not yet been answered, but are very relevant in the field of Health Sciences.

Methods:

With the selection of the PubMed database, the search terms "nipple trauma" AND "phototherapy" were used, as well as "nipple trauma" AND "photobiomodulation". The period of search of the article interval was defined from 2006-2017, and the article type as a randomized clinical study.

Results:

Before the initial search, a previous analysis of the found articles was carried out, verifying title and publication vehicle. We selected from that reading those previous, those studies that contemplated the criteria proposed in this research. After the search, only 4 academic papers were selected, all published in the period between 2012 and 2016.

We see the need for new studies on the subject to contribute to and enrich the scientific bibliographic collection.

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III FORUM INTERNACIONAL DE LASERTERAPIA e
III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

Nipple Trauma and photobiomodulation: a clinical study protocol

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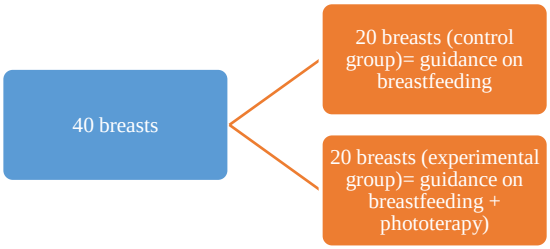
Introduction.

Nipple trauma is the second leading cause of breastfeeding discontinuation, with inadequate milk sensation in the first place, leading to the habit of the baby bottle. [1] Among the causes of nipple trauma are the wrong position of the child in relation to the breast, the number of suckles and their inadequate durations, in addition to incorrect sucking.[2] Discontinuation of breastfeeding does not provide important nutrients for the baby, in addition to immunological components. For the mother, it hinders uterine involution, increases the risk of hemorrhage, cancer of the breast and ovary, not to mention the affective bond.[3] One of the treatment options for breast fissures has been the treatment with LED (Light Emitting Diode), for its anti-inflammatory properties [4,5].

Methods and analysis.

This is a randomized clinical study involving 20 puerperal women attending the Mandaqui Hospital, who must present a medical diagnosis of bilateral nipple lesions, characterized as cracks of the nipple. 40 breasts will participate in the study. The breasts will be randomized by means of a lottery made by the participant for one of the two treatment groups: control and experimental, which will be followed up for 6 weeks.
LED application will be done 3 times a week. The cost of treatment will be raised in both groups, and a quality of life questionnaire will be applied to verify the impact of phototherapy.



Ethics approval and consent to participate: The study is awaiting approval by the Ethics and Research Committee.

Competing interest: The authors have no conflict of interest, financial or otherwise to declare.

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LIGHT-EMITTING DIODE TREATMENT AMELIORATES ACUTE LUNG INJURY INDUCED BY SEPSIS

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Abstract: Sepsis is a severe disease with high mortality index and responsible to development of acute lung injury (ALI), which is not efficient treated by current drugs. Light-emitting diode (LED) treatment displays anti-inflammatory effects, and here we studied the effects of LED on ALI induced by sepsis.

Introduction: Sepsis is a severe disease with high mortality index[1]. The acute lung injury (ALI), characterized by alveolar damage, lung inflammation, and impaired gas exchange, is a serious manifestation of sepsis, which is not efficient treated by current drugs[2,3]. Therefore, novelty on ALI treatments is substantial. It is known that light-emitting diode (LED) treatment displays anti-inflammatory effects, and here we studied the effects of LED treatment on ALI induced by sepsis.

Methods: Balb-c mice were injected with lipopolysaccharide (LPS) or saline (i.p.) and, irradiated or not with LED on trachea and lungs, for 150 s, 2 and 6 h after the injections. Twenty four hours after LPS or saline injections, local and systemic effects of LED treatment were investigated.

Results: We showed that LED treatment reduced LPS-induced neutrophils influx, decreased levels of IL-1β, TNF-α, IL-17A and enhanced levels of IFN-gamma in the bronchoalveolar fluid (BAL). LED treatment enhanced RNAm levels of IL-10 and IFN-gamma and partially reduced the elevated oxidative burst. In addition, LED treatment did not alter the activation of NF-kappa B and TLR4 in the lung tissue, and did not also reduced oedema and mucus production. LED treatment impaired the infiltration of inflammatory cells into peribronchiolar and perivascular areas.

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Acknowledgment. We thank Alessandro Melo De Ana by LED device.

Conclusions: Our data show the beneficial effect of short treatment with LED on ALI caused by sepsis, and suggest that LED application as an inexpensive and non-invasive additional treatment to sepsis.

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Biofotônica aplicada às Ciências da Saúde

Effects of photobiomodulation in the cellular viability of oral squamous cell carcinoma

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This *in vitro* study aimed to investigate the effects of photobiomodulation (PBM) in the cellular viability of oral squamous cell carcinoma cell lines.

Introduction. The most common malignant tumor of the oral cavity is the oral squamous cell carcinoma (OSCC). The treatment of this tumor involves surgery, chemotherapy and/or radiotherapy[1]. These therapies may cause mucositis and PBM has been reported to reduce oral mucositis successfully, although some controversy exist towards the effects of PBM in malignant cells that could be remained in the tissue [2]. Some studies have demonstrated that PBM in OSCC can promote cell proliferation by modulating signaling pathways involved with the malignant behaviour. However, inhibitory effects of PBM was also observed *in vitro* and *in vivo* in OSCC[3].

Material and methods.

Cell culture: The SCC9 (ATCC), Luc4 and CA1 cell lines were cultivated in DMEM/F12 (Invitrogen, E.U.A.) with 10% serum (FBS, Invitrogen), 1% antibiotic (Invitrogen), 400 ng/ml hydrocortisone (Sigma) and RM^+ supplement. Cells were maintained at 37˚C in a humidified atmosphere with 5% CO^2.

PBM Irradiation: The parameters used were 660nm and 780nm, 4J/cm^2, 70 mW.

Cellular viability assay: 5x10^3 and 10^4 SCC9, CA1 and Luc4 cells were seeded per well in a 96 well plate immediately after PBM. After 24h and 72h, cells were fixed and stained with Neutral Red (NR) and MTT.

Statistical Analysis: All assays were independently repeated three times and significance difference was calculated using one-way ANOVA and Tukey's post test. P value < 0.05 was considered as significant.

Results. Luc4 cell line treated with PBM 780nm showed significant decrease in cellular viability (NR and MTT) after 24h and 72h when compared to control cells (p=0.001 and p=0.05, respectively). However, Luc4 cells treated with PBM 660nm demonstrated a significant increase in cellular viability in relation to control cells (p=0.001). No significant difference was observed in cellular viability in CA1 and SCC9 treated with PBM.

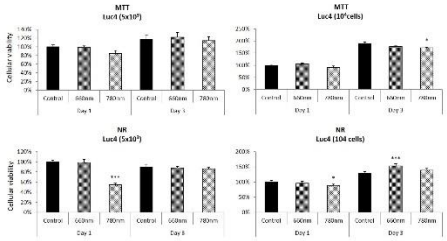

Fig. 1. Cellular viability assay (MTT and NR) in Luc4 cell line. *p=0.05; *** p=0.001

Conclusion. PBM with 780nm decreased cellular viability in Luc4 cell line and showed no effect in CA1 and SCC9 cell lines. On the other hand, PBM with 660nm increased cellular viability in Luc4, highlighting that distinct PMB parameters can promote different cellular behavior in OSCC.

Funding. UNINOVE.

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The effect of photobiomodulation on the macrophages present in the skeletal muscle of senile rats after acute injury

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National estimates (IBGE) and global (OMS) indicate a continuous growth of the elderly population (> 60 years) and this brings new challenges to health services, since aging is accompanied by a decline in muscle mass and consequently a greater number of falls and eventual lesions, being those of musculoskeletal order the most common in this population. On the other hand, the repair of the skeletal muscles in senile individuals is slow and often incomplete. As macrophages have a prominent role in the muscle repair process and photobiomodulation (FBM) has demonstrated good results in the treatment of muscular lesions, it is important to evaluate its effect on senile muscle, especially with regard to the role of macrophages in this context .

Objective: to evaluate the effect of FBM on the presence of total macrophages after acute injury in muscle of elderly rats. Methodology: 35 Wistar rats at 24 months of age were divided into three groups: control (n = 5); cryoinjury; cryoinjury and treated with FBM (n = 15). The evaluation took place after 1, 3 and 7 days. The cryoinjury mimics a muscle injury, which is a very frequent injury. Treatment was daily and irradiation (780 nm, 40 mW, 10 seconds and 0.4 J / point, total energy of 3.2J) was performed at 8 points around the lesion. At the end of the experimental periods the animals were euthanized and the TA muscles removed for analysis of the macrophage infiltrates by means of immunoblotting (CD68 +). The images were analyzed using the Image J. software. This study was approved by the UNINOVE animal ethics committee (AN 0002/2014).

Results: In the periods 1 and 7 days after injury there was no difference between the injured group (16 ± 6; 51 ± 1) and the group injured and treated with FBM (16 ± 6; 42 ± 12) in relation to the number of macrophages found in the lesion area. On the third day after the occurrence of the lesion, there was an increase in the number of macrophages in the animals treated with FBM (92 ± 25) in relation to the untreated animals (75 ± 35).

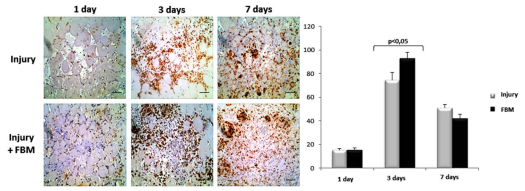

Fig. 1. Mean number of positive cells (CD68 +). Results presented in mean and standard deviation.

Conclusion: The FBM was able to increase the presence of macrophages after 3 days of injury (considered the peak of its presence in the tissue after injury). This modulation can be considered positive, since the elderly present the reduced inflammatory immune response when compared to the young ones.

Acknowledgment. Grant Fapesp number: 2013 075021; Grant Fapesp number: 2014 123811

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Evaluation of photobiomodulation on major salivary glands on sialometry and salivary biochemistry of patients with Diabetes Mellitus.

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Introduction

Patients with diabetes mellitus usually develop several chronic systemic complications and may be related to oral manifestations such as gingivitis, dental caries, periodontitis, opportunistic infections and xerostomia. The glycemic decompensation of these patients can aggravate such oral manifestations. Besides saliva being widely used in the diagnosis of a variety of oral and systemic diseases, it is an essential fluid in oral health, thus, success in the dental treatment is closely connected to the quantity and quality of the saliva each individual produces. Regarding the treatment for salivary alterations, there is still a need for investigations and/or studies, mainly those related to chronic systemic diseases, however, low intensity laser has shown satisfactory results in the improvement of salivary flow and consequently in the quality of life of diabetic patients, with Sjoegrem's Syndrome and undergoing chemotherapy and radiotherapy.
This work aims to understand the effect of photobiomodulation on the salivary changes in patients with Diabetes Mellitus.

Material and method

A pilot study was carried out on 12 patients with the disease and with a diagnosis of xerostomia.
The protocol established for this study was sialometries prior to and after therapy, being 3 extra oral applications in the regions of the parotid, submandibular and sublingual glands; and 3 intra oral, using a low intensity laser in the following parameters: Wavelength of 660 nm, 100 mW of power and 4 J per point (48 J total energy per application), to compare the volume, pH and biochemistry of the collected saliva samples. Table 1 show the spectroscopic and irradiation parameters

Table 1: Spectroscopic and irradiation parameters.

Parameter	***Value***
Wavelength (nm)	660
Spectral bandwidth (FWHM) (nm)	2
Operation mode	Continuous
Radiant power (mW)	100
Polarization	*Random*
Beam profile	Multimod
Beam area (cm^2)	0.0177
Irradiance at target (mW/cm^2)	5650
N. of sessions of treatments	1
Exposure duration (s)	40
Radiant energy (J)	4
Radiant exposure (J/cm^2)	226
Aplication technique	Close contact

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Photobiomodulation leads to reduced oxidative stress in rats submitted to high intensity resistive exercise

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Introduction: The aim of this study was to determine whether oxidative stress markers are influenced by low-intensity laser therapy (LLLT) in rats subjected to a high-intensity resistive exercise session (RE).

Method: 40 Wistar rats divided into three-experimental groups (Ctr: control; 4J: LLLT and RE), and subdivided based on the sampling times (instantly or 24 h post-exercise) underwent to irradiation with LLLT used three-point transcutaneous method on the hind legs, in which the gastrocnemius muscle at the distal, medial and proximal points. Laser (830 nm; 100 mW; 3,57 W/cm^2; 0,028 (spot, cm^2); 142,84 J/cm^2; 4J) or placebo (device off) were carried out 60 sec prior RE that consisted of four climbs bearing the maximum load with a 2-min time interval between each climb. Lipoperoxidation, carboxylation and Western blot assays were carried out to evaluate oxidative status in muscle.

Results: There was no significant differences in fitness performance (Fig.1) Lipoperoxidation levels were increased (4HNE and CL markers) main instantly after RE (Fig.2). LLLT prior to RE avoided the increase of the lipid peroxidation levels. Singular results also were notified for oxidation protein assays (Fig.3). SOD increased 24h after RE, while CAT activity did not change with RE or LLLT (Fig. 4). The GPx and FRAP activities did not reduce instantly or 24h after RE (Fig.5).

Conclusion: LLLT previously RE reduced the oxidative stress markers, as well as, avoided reduce and still increased the antioxidant capacity.

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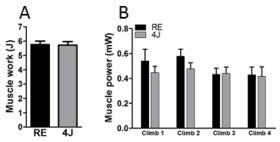

Fi. 1. (A) Muscle work developed by rats that only underwent a resistance exercise session (RE). (B) Muscle power.

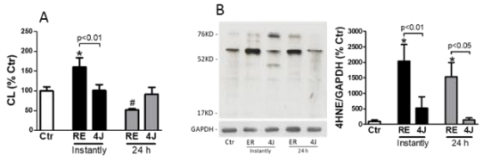

Fig. 2. Lipid peroxidation levels. (A) Chemiluminescence initiated by tert-butil (CL; n= 8-9 per group). (B) 4-hydroxynonenal (4HNE) expression *p<0.05 vs. Ctr group; #p<0.05 vs. instantly time.

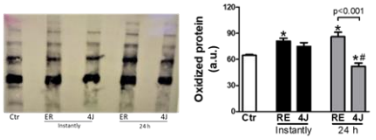

Fig. 3. Carbonyl protein expression by Western blot. *p<0.05 vs. Ctr group; #p<0.05 vs. instantly time.

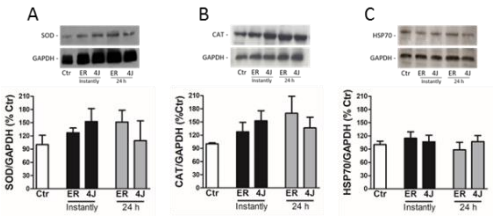

Fig. 4. Protein expression of superoxide dismutase (A, SOD), catalase (B, CAT) and HSP70 (C).

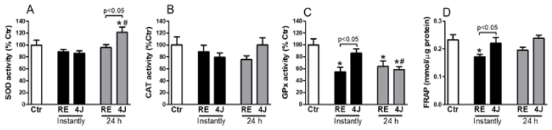

Fig. 5. Enzymatic activity of superoxide dismutase (A, SOD), catalase (B, CAT) and glutathione peroxidase (C, GPx) in gastrocnemius muscle *p<0.05 vs. Ctr group; #p<0.05 vs. instantly time.

Photobiomodulation for prevention and management of Oral Mucositis

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Oral Mucositis (OM) is a mouth and throat inflammation that can lead to painful and sore ulcers in these regions. It occurs in up to 40% of patients receiving chemotherapy and radiotherapy, affecting the quality of life and may extend hospitalization time with increasing the costs of medical treatment.

Poor oral hygiene, age, nutritional deficit, alcoholism, smoking, maladaptive prostheses, xerostomic conditions and opportunistic infections associated with treatments for myelosuppression are main risk factors for OM.
The aim of this study was to compare the treatment of OM by traditional methods such as palifermin, oral cryotherapy, mouthwashes, antiviral and antifungal prophylaxis, topical anesthetics, corticosteroids, systemic opioids with Photobiomodulation at λ = 632nm; 650nm and 780nm.
We evaluated 80 individuals in São Paulo Hospital – Federal University of São Paulo 2014-2016 with:

Acute Myeloid Leukemia (AML) -25; Multiple Myeloma (MM) -16; Acute Lymphocytic Leukemia (ALL) -7; Chronic Myeloid Leukemia (CML) -1; Hodgkin's Lymphoma (HL) -8; Non-Hodgkin's Lymphoma (NHL) -14; Myelodysplasia (MD) -3; Aplastic Anemia (AA) -2; Chronic Myeloproliferative Disease (CMD) -1; Syndrome Myelodysplastic (SMD) – 1; Acute Myelocytic Pro Leukemia (AMPL) - 1 and Mantle Lymphoma (ML) - 1. (Fig. 1)

Conventional treatment involves the use of several products, including systemic ones that alter the patient's waking state, in addition to hyperglycaemic effect, dyslipidemia, and osteoporosis.

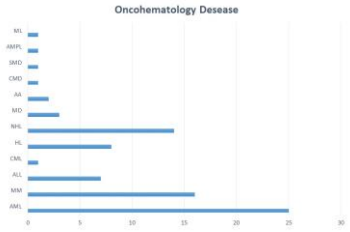

Fig. 1. Patients distribution with oncohematology diseases.

Photobiomodulation is a simple and atraumatic technique that may be used for the treatment of oral mucositis, showing significant improvement on the inflammatory response, analgesine and increased tissue repair. It is a safe, efficient and low cost method for prevention of OM.

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Photobiomodulation reverts the effects of UVA in keratinocytes in culture

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UVA radiation (13.5 J / cm²) causes a decrease in the viability of keratinocytes and Fig extract does not exhibit recuperative action on these cells, but rather decreases the viability even further. On the other hand, red LED photobiomodulation (1,0 J / cm²) was able to increase the viability of cells previously exposed to UVA.

Introduction: In Brazil and worldwide there is a trend in the use of plant extracts in cosmetic products of all kinds. These extracts present antioxidant activity, mainly due to the presence of polyphenols. For this reason, these compounds are used to stimulate cell renewal or inhibit deleterious processes induced by ultraviolet radiation (UV) in the skin. On the other hand, photobiomodulation has been shown to be an efficient tool to induce cell renewal.[1] The combination of antioxidant therapy with extracts and photobiomodulation seems to be an interesting possibility to reduce the harmful effects of UV radiation.

Objective: The objective of this work is to evaluate the therapeutic effect of Fig extract in human keratinocytes in culture combined with red LED, to minimize the effects of UV-A radiation.

Method: Human normal keratinocytes (HaCaT - CLS CM1) were seeded in 48 well plates (60,000 cells/well). The cells were exposed to UV-A (366 ± 10 nm, 2.5 mW / cm², 90 minutes), then treated with fig extract (0.3% for 24 hours) and, finally, red LED photobiomodulation (640 nm ± 12.5 nm, 2.6 mW/cm², 7 minutes). Untreated controls were also performed. At the end of the treatments, cells were washed with PBS and 10% FBS DMEM was added, keeping the cells in the incubator for 48 hours. Finally, the MTT colorimetric assay was performed.[2]

Results: It was observed that 13.5 J / cm² of UV-A causes damage in keratinocytes, reducing the amount of living cells to 80%. However, the use of photobiomodulation (2.6 mW / cm²) after UV-A damage promoted recovery (to approx. 92%), but did not reach the baseline levels. The application of LED without previous damages has no effect on the keratinocytes, as well as the application of Fig extract. On the other hand, treatment of keratinocytes with Fig extract after UV-A damage caused a 28% reduction in cell amount. Also in this case, the photobiomodulation promoted recovery (to approx. 85%) without reaching basal levels.

Conclusion: After oxidative damage caused by UV-A radiation, keratinocytes were sensitive to Fig extract, presenting toxicity. On the other hand, photobiomodulation could recover cells after UV-A damage, as well as after the toxicity presented by the Fig extract. Further studies are necessary to understand clearly this effect, since MTT evaluates living cells in terms of mitochondrial activity and the observed effect can be related to increase in mitochondrial activity.

Acknowledgment. We thank Bioextract Farma Service for kindly provide the Fig extract.

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Phenothiazinium dyes for Candida albicans inactivation by aPDT

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Oral candidiasis is an opportunistic fungal infection usually caused by *Candida albicans*. Due to the disadvantages of antifungal treatment, such as hepatotoxicity without prolonged use and development of microbial resistance, other therapies have been proposed. Antimicrobial photodynamic therapy (aPDT) is a promising treatment to inactivate microorganisms. The aPDT technique is based upon the use of a photosensitizer (PS), in the presence of oxygen and light at a specific wavelength. Methylene blue (MB), a phenothiazinium dye, has been a widespread PS due to its efficacy and low cost.[1] Other phenothiazinium dyes may present interesting properties and should be investigated in details, such as Azure A (AA) and Azure B (AB).

The objective of this study is to compare the AA and AB photosensitizers for inactivation of Candida albicans in planktonic culture.

The *C albicans* strain was grown on Sabouraud Agar Dextrose 48 hours prior to the experiment. The cells were harvested, a water suspension was prepared and counted in the Neubauer Chamber. In microplates, were added 200µL of Candida albicans suspension ($1x10^8$ cels/mL) and 200µL of FS solution (0-100 µg/mL) and incubated in the dark for 5 minutes. Thereafter, one plate was irradiated for 30 minutes (640 ± 12.5nm, 2.6mW/cm^2), while another plate was held for the same time in the dark. At the end, an aliquot of each sample underwent successive dilutions (up to 10^{-5}) that were spread in Sabouraud Dextrose Agar.[2] Plates were held for 24h at 37° C and the number of CFU/mL counted. The experiments were performed in triplicate and three independent experiments.

The dose-response curves were obtained in the dark and with irradiation for both PSs. The IC_{90} values were calculated from each dose-response curve. Note that AA exhibits very similar values of IC_{90} both in dark and irradiated, showing small photodynamic effect (Table 1). On the other hand, AB showed small toxicity in the dark (IC_{90} = 607 µg/mL) and higher phototoxicity (IC_{90} = 405 µg/mL).

As a way of comparing the efficacy of the PSs studied, the IC_{90} values for each of the treatments are presented in Table 1, as well as the efficacy of the PDT (IC_{90} irradiated / IC_{90} dark) for both were calculated.

Table 1: IC_{90} values (µg/mL).

	Dark	Irradiated	PDTe
AA	286	275	1.0
AB	607	405	1.4

Note that AB may be considered a more effective PS. The search for PSs that does not have action without irradiation is desirable so that it does not have effect alone. In this way, the therapy is controlled and this prevents injury to adjacent tissues, acting only in the area of interest for aPDT control.

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Evaluation of the frequency and efficacy of the type of treatment used after burns

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The aim of this study was identify, among the clinical research published, in the last ten years, in Brazil, the most frequent types of treatment in the wound caused by burns. Integrative research, with studies published in the last ten years on products used in burns. Including the clinical trials that have described the use of stablished or innovative therapies in burns and published in national online journals in the last ten years. Excluding the studies stablished before 2007, that didn´t focus on the treatments of the lesions secondary to burns and those that portrayed therapy used outside Brazil. Six articles were selected. Hialuronic acid was used in two studies; in one of them, alone and in another, was compared with the Essencial Fatty Acid (EFA). Nanocrystalline Silver Sulfadiazine 1% was used in a study, also in isolation. Heparin and collagenase were compared in another study. Soft silicone foam and porous cellulose membrane were used, in isolation, in two studies.

Researches	Title	Type of study
Gonçalves N, Franzolin RA, PG de Oliveira, Castilho JC	Comparison of the effects of hyaluronic acid 0.2% and essential fatty acids in burn victim due to fertilizer exposure: case report	Case report
Moser HH, Pereima MJL, Soares FF, Feijó R.	Use of silver preparations on the treatment of children's burns at Joana de Gusmão Children's Hospital	Epidemiological study of cross-sectional design.
Teles GGA do, Bastos JAV, Amary A, Rufatto LA, Ritty RS, Broglio LAP et al	Treatment of superficial second degree burn of face and neck with topical heparin: a comparative, prospective and randomized study	Comparative, prospective and randomized study
Proto RS, Gozzano RN, Brasileiro F, Moreira SS, Gonella HA	Dressings and soft silicone foam: an alternative for the treatment of burns in hand	Case report
Vieira JC et al.	Porous cellulose membrane in burn's treatment	Prospective study
Silva MN et al	Effects of topical treatment with hyaluronic acid 0.2% in second-degree burn: an experience report	Case report

Fig. 1. Terapias para queimaduras

A Silver Sulfadiazine 1% is the oldest of the therapies used for burns, but it was described in only one study. Hialuronic Acid, as well as soft silicone foam and the cellulosic membrane are more moderns. Collagenase has been used for a long time in various types of wounds, such as ulcerations, necrosis and severely burned areas. Heparin is commonly used, in the prevention of thromboembolic disorders; however, in the study found, the authors reported that research suggests its use in burns with good results, however, they also emphasized that since these studies are of poor quality, there isn`t consistent evidence. EFA is widely used to moisturize the skin and prevent wounds. This review of the literature showed that this is a large divegence regarding the choice of the best treatment in cases of burns. Studies with controlled methodology are necessary for the stablishment of a therapy that provides a better repair, in a shorter time and with lower risk of complications.

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III FORUM INTERNACIONAL DE LASERTERAPIA e
III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

Motor response to photobiomodulation in the treatment of patients with spinal cord injury

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Photobiomodulation is a noninvasive treatment that has been widely used in neurotrauma and neurodegenerative diseases. In the present study, low-level laser therapy was administered to patients with spinal cord injury.

The study involved 25 patients. The volunteers were randomly allocated to either the control group or treatment group. Evaluations were conducted before and after the intervention using electromyography to assess the motor response.The treatment group received phototherapy and irradiation was administered to the injury site transcutaneously at a wavelength of 808 nm using a Quantum diode laser (Ecco Fibras e Dispositivos, Brazil).

Twelve sessions were held (three per week over four weeks).The EMG signals were captured using a four-channel acquisition system and the electrodes were placed on the right brachial biceps muscle in quadriplegic patients and the right femoral quadriceps muscle in paraplegic patients.

Table 1 – Laser parameters

Parameter	Infrared laser
Center wavelength [nm]	808
Average radiant power [mW]	120
Aperture diameter [cm]	0.18
Irradiance at aperture [W/cm^2]	4.72
Irradiance at target [W/cm^2]	4.72
Exposure duration [s]	208 (per point)
Radiant exposure [J/cm^2]	983
Energy density at aperture [J/cm^2]	983
Radiant energy [J]	25
Number of points irradiated	5
Number and frequency of treatment sessions	Three per week over four weeks (12 sessions)

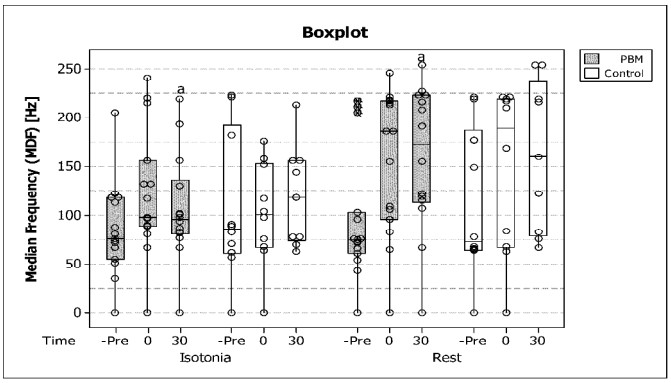

Fig. 1. Median (25%, 75% quartile) of median frequency recorded at rest and during isotonic contraction. (p = 0.0258).

This is the first randomized controlled study in which the role of phototherapy was evaluated in patients with spinal cord injury. The results demonstrate that phototherapy stimulated the injured tissue, achieving an improved motor response. The EMG data demonstrate a difference in comparison to the pre-intervention evaluation, with higher MDF values at rest and during isotonic contraction 30 days after the end of treatment. MDF is related to the firing and synchronism of motor units. An increase in MDF indicates either an improvement in the firing synchronism of the motor units or an increase in the firing frequency of the motor units. Thus, LLLT may contribute to an improvement in motor recruitment.

Acknowledgment. We thank University Nove de Julho (São Paulo, Brazil) for the use of their equipment.

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Effect of Nd:YAG laser on the penetration of a bioceramic sealer into dentinal surfaces of the root canal: an analysis by confocal microscopy

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The success of endodontic treatment is dependent on the removal of smear layer and the obturation technique used to seal the treated root canal[1]. The correlation between the success of endodontic treatment and root canal sealing is well known; however, the effect of Nd:YAG laser on the penetration of bioceramic sealers in apical root dentin is not known[2]. The objective of this study was to analyze the effects of Nd:YAG laser on the penetration of a bioceramic sealer into dentinal surfaces of the root canal. **Methods:** After approval by the CEP-Uninove (#1.358.755**),** the morphology of radicular dentin surface irradiated with Nd:YAG laser was evaluated qualitatively with the use of scanning electron microscopy (SEM). Twenty extracted human inferior premolars were prepared with Reciproc®, and randomly divided into 4 groups, receiving different treatments in the dentin: Group A - EDTA 17%; Group B- Laser Nd:YAG; Group C- EDTA 17% + Laser Nd:YAG and Group C - control (without treatment). As a second part of the study, the penetration of a bioceramic sealer into apical root dentin of endodontically treated teeth was analyzed quantitatively after treatment of this surface with Nd:YAG laser. Another forty extracted human inferior premolars were prepared with Reciproc®, irrigated with 17% EDTA and randomly divided into 4 groups: Group 1 - AH Plus® sealer, Group 2- sealer Endosequence BC Sealer®, Group 3 laser Nd:YAG + sealer AH Plus®, Group 4 laser Nd:YAG + sealer Endosequence BC Sealer®. The samples were sectioned at 3mm and 5mm from the foraminal apex and evaluated by confocal microscopy for sealer penetration into dentinal tubules of root canal surface. It was also evaluated the percentage of sealer penetrated into intracanal perimeter at 3 and 5mm from the foraminal apex. Two-way ANOVA with post hoc Bonferroni was used for the variables sealer penetration and sealer perimeter into root canals. The significance in all tests was $\alpha = 0.05$. **Results:** When evaluating sealer penetration at 3mm and 5mm from the apex, we observed that BC Sealer cement had higher penetration than AH Plus cement ($p<0,05$), regardless of the use of the laser. When evaluating the perimeter of the cement penetration line at 3mm from the apex, we observed that there was no difference between the analyzed groups. At 5mm from the apex, the BC Sealer cement showed greater perimeter penetration, when associated to the laser ($p<0,05$). **Conclusion**: Under the conditions of this study, Nd:YAG laser did not interfere on the penetration of bioceramic sealer into dentinal surfaces of root canal.

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EFFECT OF PHOTOBIOMODULATION AT DIFFERENT TIMES ON THE MRNA EXPRESSION OF MYOSIN HEAVY CHAINS DURING THE SKELETAL MUSCLE REPAIR PROCESS.

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Background: Muscle injuries are a common occurrence and can have a major impact on the performance of athletes and amateur sports enthusiasts. The determining of the type of myosin heavy chain (MyHC) expressed is a very important aspect in the muscle repair process, since it reflects as the progress of differentiation process as to the capacity and type of muscle fiber contraction during the repair process (1). Low-level laser therapy (LLLT) has shown positive effects on the modulation of muscle repair (2). Thus, the aim of the present study was to analyze the effects of LLLT on the mRNA expression of MyHC isoforms (1 and 2b) during the muscle repair process**.**

Methods: Wistar rats were divided into five groups: Control (n = 7); Injury (n = 14); LLLT + Injury (n = 14); Injury + LLLT (n = 14) and LLLT + Injury + LLLT (n = 14). Cryoinjury was performed on the tibialis anterior (TA) muscle. The injured groups were euthanized at seven and 14 days after injury. LLLT was performed with a diode laser (wavelength: 780 nm; output power: 40 mW and total energy: 3.2 J). After euthanasia, the tibialis anterior muscle was removed for the isolation of total RNA and analysis of MyHC-1 and MyHC-2b using real-time quantitative PCR. The data were analyzed statistically by Kruskal-wallis, followed by Dunns test's ($p < 0.05$).

Results: A significant decrease in MyHC-1 mRNA was found in the injury + LLLT group in comparison to the and injury group ($p < 0.01$) after seven and 14 days. A significant decrease in MyHC-2b mRNA was found at seven days in the injury + LLLT group in comparison to the injury group ($p < 0.01$) (fig. 1).

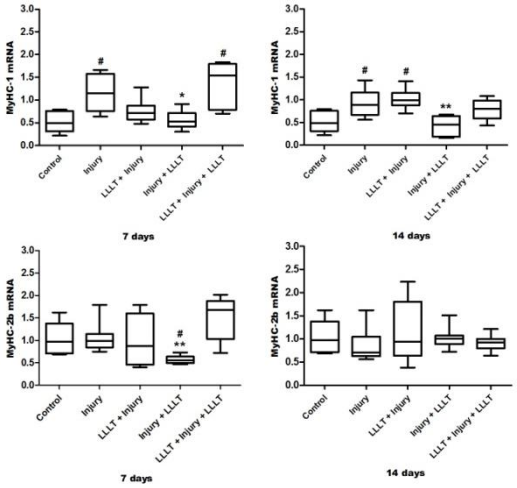

Figure 1. mRNA expression of MyHC-1 and MyHC-2b; Data expressed as box spot (Kruskal-wallis/Dunns). #$p < 0.05$ vs. control group; *$p < 0.05$ vs. injury group;**$p < 0.01$ vs. injury group;

Conclusion: LLLT administered after skeletal muscle injury modulates the mRNA expression of MyHC-1 and MyHC-2b during the repair process.

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Evaluation of photobiomodulation in oral lichen planus: study protocol for a randomized controlled trial

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Introduction: Oral Lichen Planus (OLP) is a T-cell mediated autoimmune disease in which the cytotoxic $CD8^+$ T cells promote apoptosis of the basal cells of the oral epithelium. Oral lesions comprise different clinical aspects including white reticular patches, erosive and atrophic lesions, both associated with intense symptomatology. Conventional treatment consists in administration of topic and systemic corticosteroids, which are associated with side effects. Moreover, some patients are irresponsive to conventional treatment. Some studies have demonstrated that Photobiomodulation (PBM) can be used as a therapeutic tool to treat OLPS with beneficial effects, reducing pain, recurrence and improving the clinical aspects[1].

Materials and Methods: This is a randomized controlled clinical trial comparing the efficacy of PBM and corticosteroids in patients with OLP.
Inclusion Criteria: It will involve patients ≥ 20 years with a diagnosis of symptomatic OLP based on WHO criteria.
Exclusion Criteria: Pregnant, lactating, those with history of previous treatment in the last months, uncontrolled systemic disease, use of drugs, lesions adjacent to amalgam fillings, and use of drugs associated with lichenoid reactions will be excluded.
After recruitment, patients will be randomly allocated in one of two groups:

Control Group (G1=40). Participants will be treated with clobetasol propionate 0.05%, daily during 4 consecutive weeks. To prevent oral candidiasis, patients will use micostatin solution during treatment.
Experimental Group (G2 n=40). Participants will be treated with PBM (660nm), 40mW, power density of 1000 mW/cm^2, and total energy of 1J each point, twice a week during 4 consecutive weeks.
The trial will last 4 weeks and the following outcomes will be evaluated once a week during treatment and 30, 60 and 90 days after treatment. Clinical OLP scores, according to Thongprasom et al.[2] Pain intensity will be analyzed evaluation by Visual Analogue Scale (VAS). Quality of life will be accessed using the OHIP-14 Questionnaire. Functional scores will analyze the chewing function, swallowing, fluid intake, and altered sense of taste as described by Lilleby et al.[3] Clinical resolution and recurrence rate will be evaluated as recommended by Carozzo et al.[4] IL-6, IL-8 and TNF-α levels will be evaluated before and after the last week of treatment in salivary and blood samples using a competitive enzyme-linked immunosorbent assay.

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Efficacy of photobiomodulation in the treatment of oral lichen planus: literature review

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Introduction: Oral lichen planus (OLP) is a T-lymphocyte- mediated autoimmune disease that affects approximately 0.3 to 2.3 % of adults. Clinically, OLP is divided in three major aspects: reticular, atrophic and erosive which are associated with symptoms ranging from burning sensation to severe pain. Conventional treatment consists of topical or systemic use of corticosteroids, both associated with adverse effects. As a therapeutic alternative, photobiomodulation (PBM) has shown promising results reducing pain and recurrence as well as favoring the acquisition of the reticular clinical aspect in relation to the atrophic and/or erosive.[1,2]

Materials and Methods: A bibliographic survey was carried out using the database PubMed, Scopus e Web of Science. Published studies from 2003 to 2017 were included according to the following criteria: publication in English, clinical studies, control group, corticosteroids and evaluation of PBM efficacy by clinical score. The following terms were used in English: (lichen planus) OR (oral lichen planus) OR (erosive lichen planus) OR (wickham striae) AND (photodynamic therapy) OR (pdt) OR (low level laser therapy) OR (laser).

Results: Of the 91 articles identified, 85 were excluded because they did not show the necessary inclusion criteria. Thus six articles were included for detailed analysis of the effects of FBM on the treatment of OLP. The wavelength used for PBM ranged from 633nm to 970nm, Power out-put 40mW to 3W and 0.3-0.5 J/cm² to 6J/cm² of radiant exposure. The number of sessions ranged from 2 to 12 sessions with a minimum of two sessions per week. PBM was able to promote improvement of the clinical aspect of OLP compared to conventional treatment in 2 articles while 4 articles showed better results with corticosteroids.

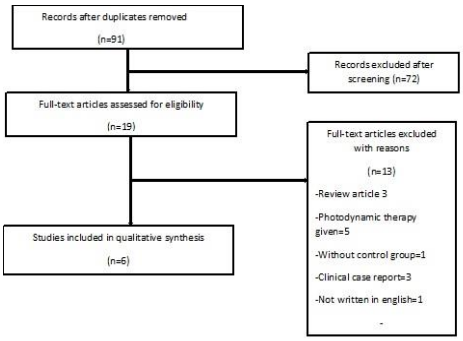

Figure 1: Schematic flow chart representing the studies included in the literature review.

Conclusion: The scientific evidences regarding the effects of FBM on OLP are still scarce and there are doubts about its effectiveness in relation to conventional treatment. Randomized clinical studies are still necessary to obtain a consistent conclusion of the effects of FBM in OLP.

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Evaluation of the effect of photobiostimulation on the salivary flow of patients with chronic renal failure.

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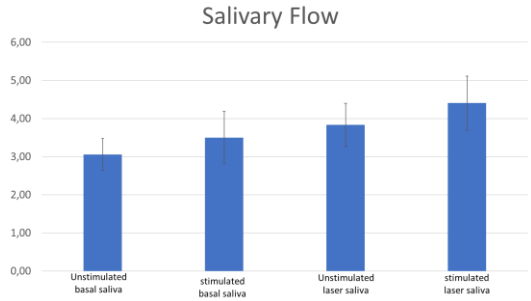
Chronic kidney disease is due an injury or decreased kidney function for a period less than three months and the classification comprises different stages indicating the progression of severity. In those advanced stages the patients need to the hemodialysis or kidney transplantation.

The aim of this study was to evaluate the effectiveness of low-level laser therapy in the salivary flow and biochemistry, we performed this study with 28 patients with chronic renal failure. Of these 21 patients (treated group) were submitted to photobiomodulation and in 17 patients (placebo group), where the laser application simulation was performed.

Saliva sample was collected Saliva was collected before and after three laser applications 808nm, 500mW, 4J/cm² in three points out of the mouth in the parotid glands, submandibular and sublingual sites as well as in three inside the mouth points. The amount of saliva in the initial and final period was measured.

In our results we noticed that a significant increase of the final salivary volume occurred when compared to the initial one of the treated patients. When comparing the final and initial volume of the placebo group there was no statistical difference.



Tab. 1. Salivary Flow

We conclude that, in this group of patients, the photobiostimulation of the salivary glands, in the protocol used, increased the salivary flow significantly.

Funding University Nove de Julho

Acknowledgment. We thank the São Paulo Clinical Hospital (HCSP)

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OPTICAL CHARACTERIZATION OF CUTANEOUS DRESSINGS

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The materials chosen for the treatment of wounds have evolved since the prehistory, where the main raw material were the extracts of plants for the manufacture of dressing.

Over time, during the war, there was a need to combat infections chemically using phenol solutions in compresses and sutures. Then, from the 20th century, the sterile dressings arise.

There are currently many curative options and methods for the treatment of cutaneous wounds: silver sulfadiazine, collagenases + chloramphenicol, essential fatty acids, hydrocolloids, amorphous hydrogel, calcium alginate, activated charcoal, hydrophobic adhesive, vacuum dressing and film semi-permeable polyurethane, among others[3].

Since the 1960s, another method has been highlightedin wound treatment: photobiomodulation (PBM), whose photons are capable of accelerating tissue healing through cell proliferation, increased vascularization, and improved collagen organization.

Currently, for the application of the PBM in the wound it is necessary that the primary and secondary dressing must be removed. In this way, a dressing that could stay up to 7 days acting on the wound needs to be removed, according to the frequency of application of the FBM, causing beyond the discomfort to the patient, the increased cost in treating this wound.

Therefore, the objective of this work is to determine the absorption coefficient, depth of penetration, reflection and optical transmission of the most commonly used dressings, in order to verify the real necessity of the removal of these bandage during the FBM.

Perform the search for the main dressingscurrently used in the treatment of cutaneous wounds.

An Ocean Optics® (USA) spectrophotometer, USB 2000 model, will be used to measure the absorbance, transmittance and reflectance of the dressings between 400 and 1000 nm. Thereafter, the absorption coefficients and depth of penetration of each dressing will be calculated. The reflection and the transmission will be represented in percentage. The results will be presented through charts and tables.

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The use of photobiomodulation on pain control in temporomandibular joint disorder: a bibliometric analysis of scientific production

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Introduction. Temporomandibular disorder (TMD) is a term used to define a number of clinical signs and symptoms that affect the masticatory muscles, the temporomandibular joint (TMJ), and associated structures. The most common signs and symptoms are sensitivity of the masticatory muscles, pain in one or both of TMJs, limited mandibular movement, articular noises, headache associated dizziness, hearing loss, and tinnitus may also occur. The changes caused by the TMD, especially pain, can interfere in the quality of life of these patients [1].

Various treatment features have been proposed, mainly for pain control, such as occlusal splints, acupuncture, kinesiotherapy, massage therapy, postural training, psychotherapy, joint mobilizations, drug therapy, and laser therapy. Low -level laser (LLL) therapy is a non-invasive, non-pharmacological treatment that, according to various studies, has shown beneficial results in the treatment of pain associated with TMD [2].

Bibliometry allows to map to analyze the scientific production in specific subject, contributing to the decision-making and showing the main and emerging issues to guide new research [3].

Objective. The main goal of this study is to analyze the scientific production about the effectiveness of LLI in the treatment of TMJ in the last 10 years of international literature. The authors searched publications between 2007 and 2017 using the PubMed database and following a bibliometric analysis.

Methods and analysis. After selecting the PubMed database, we searched for the term "muscle pain" then added the term "laser therapy", delimiting the search using AND ("muscle pain" AND "laser therapy"), the following searches were also made: ("muscle pain" AND "phototherapy") and ("muscle pain" AND "photobiomodulation"). To be included in the analysis the publication had to be a clinical study and treated TMD. Then the initial search, a previous analysis of the articles was made, by reading the title and summary; and duplicities were eliminated. The initial search resulted in 82 studies, 51 were excluded after the first and second analyzes, and 31 articles were selected for the complete analyses and then are focused in the period from 2009 to 2017.

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EFFECT OF PERIODONTAL TREATMENT ASSOCIATED WITH PHOTODYNAMIC THERAPY IN EXPERIMENTAL MODEL OFSMA.

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Background: Asthma and periodontal disease (PD) present high economic and social relevance[1]. There are some evidence that PD can exert systemic immunomodulatory effects. Recently, conventional periodontal treatment (PD) has been associated with photodynamic therapy (PDT), with promissing results especially in regions with restricted access[2]. **Objectives:** To evaluate the influence of TP associated with PDT on the modulation of pulmonary inflammation in experimental model of acute asthma. **Methods:** After approval by the CEP-Uninove (CEUA 020/2015**),** fourthy-five Balb/ c male mice, 2 months, 25g were divided into 5 groups (n = 9): G1. Basal, G2. Asthma (A), G3. A+PD, G4. A+PD+TP, G5. A+PD+TP+PDT. Periodontitis was induced with ligature-induced technique (15 days) and the asthma by administration of ovalbumin (OVA) subcutaneously (days 0 and 7) and nebulization with OVA (3 x / week, for 2 weeks). TP was performed with curettes and PDT with methylene blue (0.005%) irradiated with red diode laser - λ = 660 nm, energy density 6,369 J /-cm², with 9 J per point, delivered in 90 s, 2 points. After 21 days, euthanasia was performed for morphological analysis of the lung and mandible. Cytokines IL-4, IL-5, IL-10, IFN-γ, TNF-α, IL-1β and IL-6 and mucus production were evaluated. Total and differential counts of inflammatory cells were performed in the Broncho Alveolar Lavage (BAL) and serum. The frequency of inflammatory cells was assessed by flow cytometry. The statistical analysis was used one-way ANOVA followed by the Student-Newman-Keuls test. **Results:** Group 2 presented an increase in the total number of cells 31,25 (±5,41) in BAL ($p < 0.05$), corresponding to ± 8% of total leukocytes. In group 3, it was observed that the presence of PD in asthmatic mice decreased 81 (± 41) ($p < 0.001$) the release of IL-5 when compared to the control group. Group 2 had reduced IFN-γ values 230,5 (±67,17) 250 pg / ml ($p < 0.05$). There was an increase in mucus production in groups 2 342,99 (±79) and 5 295,22(±65,44) ($p < 0.001$). **Conclusion**: PD can exacerbate lung inflammation in experimental model of Asthma. However, in G3 there was an improvement in the parameters of pulmonary inflammation, that returned to present levels near the basal level after PD associated with PDT treatment.

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Low-level laser reduces oxidative stress and apoptosis in mesenchymal stem cells derived from adipose tissue subjected to doxorubicin toxicity

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Doxorubicin is a chemotherapy agent used for the treatment of hematologic diseases and neoplasia. However, doxorubicin causes cell damage in non-neoplastic cells (i.e. cardiomyocytes), a condition in which is persistent after the end of drug administration. We highlight the use of stem cell therapy. In which cardioprotective effects have been reported. However, doxorubicin also imposes cytotoxicity on transplanted stem cells, reducing its therapeutic potential.

Objective: to analyze whether low level laser (LLL) induces cell protection in mesenchymal stem cells derived from adipose tissue (MSCA) submitted to doxorubicin toxicity.

Material and Methods: MSCA at the concentration of 1×10^5/0.1 mL of DMEM were plated in a 96-well plate on experimental sets: Control - MSCA untreated with doxorubicin and/or LLL; D25 and D50 - MSCA incubated with doxorubicin (25 µg/mL and 50 µg/mL, respectively); D25 + LLL/D50 + LLL - MSCA irradiated with 0.2 J, 0.4 J and 0.7 J LLL (660 nm) and incubated with doxorubicin (25 µg/mL and 50 µg/mL, respectively). Cell viability and apoptosis were determined by flow cytometry with 7AAD and Annexin V, respectively. Cell permeabilization with DCFH was used as a marker of reactive oxygen species (ROS), and intracellular ATP content was determined by luminescence assay.

Results: Irradiations of 0.4 and 0.7 J resulted in a higher concentration of viable MSCA independent of doxorubicin dose. Similar results were observed for the apoptosis assays in cells incubated with 50 µg of doxorubicin and irradiated with 0.2 and 0.4 J. Considering that 0.4 J was effective in increasing the viability and reducing apoptosis of the MSCA at the concentration of 50 µg, we directed to continue the experiments only with this dose of energy and concentration of the drug. Thus, 0.4 J inhibited ROS increases, however, it did not result in a higher concentration of intracellular ATP.

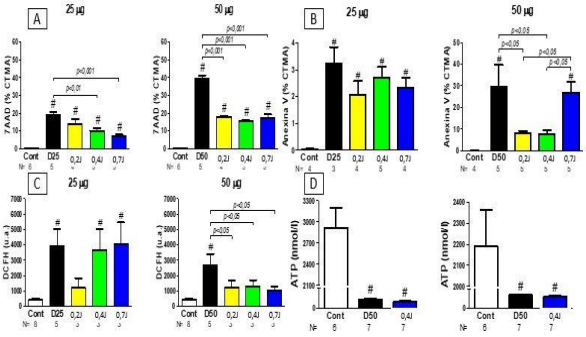

Figura 1. A – Cell viability; B – Apoptosis; C – Oxadative stress; D – ATP

Conclusion: Preliminary data indicate that LLL reduces apoptosis in MSCA, a situation that prevails with the prevention of oxidative stress.

Funding. Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP

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PHOTOBIOMODULATION EFFECTS ON GENE EXPRESSION OF *CCL2, CCL4 AND TNFα* BY J774 MACROPHAGES POLARIZED TO M2C PHENOTYPE

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Macrophages are essential cells during the tissue repair process. Besides responding to biological pathogens, they are responsible for the production of important mediators that regulate this process. Macrophages can differentiate into M1 (proinflammatory) and M2a, M2b or M2c (anti-inflammatory/ repairer) phenotypes according to the signaling pathways of the lesion microenvironment. Photobiomodulation (PBM) has been extensively studied in clinical and experimental settings because of its beneficial effects on tissue repair, but little is known about its effects on the different macrophages profiles.

Objective. To compare the effects of PBM (2 dosimetric combinations of parameters) on the gene expression of the proinflammatory mediators *CCL2*, *CCL4* and *TNFα* by macrophages polarized to M2c phenotype.

Methodology. J774 macrophages were grown in DMEM medium and supplemented with 10% FSB and 2mM L-Glutamine. To induce the M2c phenotype, $1x10^6$ J774 cells were treated with IL-10 and dexamethasone for 24h. Following this, macrophages were washed, centrifuged and irradiated with PBM of 660 nm and 780 nm (1J, 70 mW, 17.5 J/cm^2, 15 s). After 24h of irradiation, cell cultures were collected to assess gene expression of *CCL2*, *CCL4* e *TNFα* using custom qPCR array plates. Non-irradiated and non-activated cells served as control. The experiments were performed in biological duplicates. *GAPDH* gene was used for normalization.

Results. M2c activated macrophages irradiated with PBM of 660 nm and 780 nm showed a significant decrease in *TNFα* gene expression when compared to non-irradiated M2c macrophages. However, only the irradiation with PBM of 780 nm was able to reduce the gene expression of *CCL2* and *CCL4* by M2c macrophages.

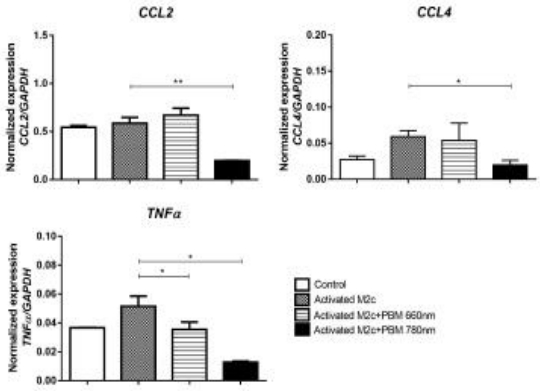

Fig. 1. Gene expression of *CCL2*, *CCL4* and *TNFα* by J774 macrophages polarized to M2c phenotype and irradiated for 24 h with PBM of 660 nm and 780 nm.

Conclusion. Photobiomodulation was able to reduce the gene expression of proinflammatory mediators by J774 macrophages polarized in M2c/repairer phenotype. Moreover, the irradiation with 780 nm laser provided more pronounced effects in these cells. This study is important because it improves understanding of PBM and how it affects modulations in the patterns of chemokines and cytokines involved in the repair process after an injury.

Acknowledgment. Grant Fapesp number: 2013/07502-1; Grant Fapesp number: 2015/21219-6.

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Biofotônica aplicada às Ciências da Saúde

Effects of treatment using LED on the gait functionality after peripheral nerve injury of Wistar rats

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Introduction

The treatment with irradiation using Light Emitting Diode (LED) has demonstrated positive effects on the nervous tissue repair after peripheral nerve injury (PNI), however there is little description in the literature regarding the effect on muscle tissue.

Objective

To analyze the effects of LED irradiation on nervous and muscle repair after crush injury of the sciatic nerve (NI).

Methods

The study used 85 Wistar rats (200-250g) were randomly assigned to 5 groups. Left sciatic nerve injury: performed by the crushing technique using flat hemostatic forceps with latch (6.3MPa) by means of a compression for 30s. Treatment: started 2 hours after injury End of experimental periods: functional gait analysis through the sciatic functional index. The LED irradiation was performed using the following parameters: LEDn consisted of 4 points during 20s each one using an energy density of 0,8J/cm^2 and LEDmm consisted on 8 points during 10s each one using an energy density of 0,4J/cm^2. Data submitted to statistical analysis (ANOVA / Tukey, $p < 0.05$).

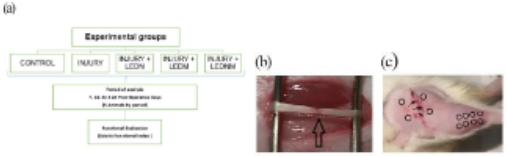
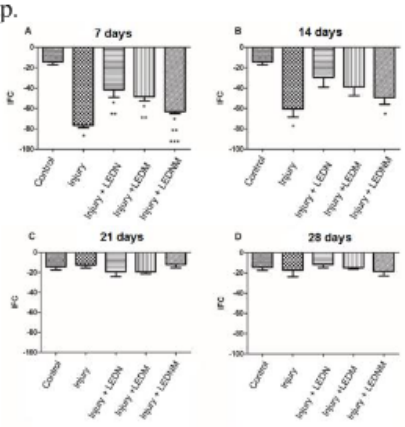


Fig.1: (a) study flowchart; (b) sciatic nerve injury; (c) application points LED.

Results

At the end of the experimental period, the gait analysis was performed after 1 week using the Sciatic Functional Index (SFI) showed that all the injured groups showed a decrease in functionality compared to the control group, however, all LED groups showed an increase in functionality over the injury group. After 2 weeks, the Injury+LEDn and Injury+LEDnm presented an increase in SFI with values similar to control group while the Injury and Injury+LEDmm groups showed a decrease in gait functionality when compared to control group.



Conclusion

It can be concluded that LED increased gait functionality assessed by the IFC after 1 and 2 weeks post peripheral nerve injury, especially when it is used in the nerve region associated or not with the muscle region.

Funding: PROSUP - CAPES

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Evaluation of Bleaching in-office with Violet Led Light (405 nm): a study protocol of a randomised controlled trial

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Introduction

Dental bleaching, has already been much studied, and it is still a wish for most patients. However, the techniques already studied present a limiting factor that is the development of dentin hipersensitivity. The violet LED 405 nm is capable of interacting physically with the organic molecules in the dentin (that causes its darkening) resulting in the whitening process. Although there is little clinical evidence, Violet LED does not causes dentin sensitivity, increasing the amount of referred patients. This protocol will evaluate colorimetric changes after supervised whitening treatment, in-office, using Violet LED light of 405 nm associated or not to the use of carbamide peroxide 35% gel.

Material and Method

After approval by the CEP-UNINOVE (#2.034.518**)** and www.clinicaltrial.gov registration **(**NCT03192852**).** Sixty patients will be divided into three groups. Group 1 (n=20) Violet LED light (405 nm). Group 2 (n=20) Violet LED light + carbamide peroxide 35% gel. Group 3 (n=20) hydrogen peroxide 35%. The colorimetric changes will be measured through the Vitapan Classical (Vita) color scale, and by digital spectrophotometer (Vita Easyshade, VITA Zanhnfabrik, Bad Säckingrn, Germany). The measures will be done before and right after each whitening session. The follow-up will be 3 months after whitening period. The sensibility degree will be measured by the Visual Analogue Scale (VAS) in every sessions in all groups. The degree of satisfaction of the patient and the life quality will be evaluated before and after aesthetic result achieved by Psychosocial Impacto of Dental Aesthetics Questionnaire Life Quality related to Dental Aesthetics. The results will be presented by means and standard deviation (±sd). After checking the normality of the data, a one-way ANOVA will be performed. In case the data do not follow a normal distribution, it will be utilized the Kruskal-Wallis test and a value of $p < 0.05$.

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Photodynamic Therapy with Methylene blue in Porphyromonas gingivalis in vitro: Literature review of experimental parameters

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Introduction and Contextualization

Photodynamic Therapy (PDT) is based upon the use of a photosensitizer (PS) and light at an appropriate wavelength in the presence of oxygen. The absorption of light induces PS to generate oxidative species capable of inducing cell death, so that PDT can be used with bactericidal action. Porphyromonas gingivalis is a species of gram-negative anaerobic bacteria, the primary etiological agent of periodontal disease. Colonization by this microorganism results in tissue injury, through the production of a variety of virulence factors and deregulation of the immune and inflammatory systems of the host. An increasing number of studies investigate the clinical potential of PDT as adjuvant to clinical treatment. In this sense, the standardization of application parameters for an effective therapy is necessary.

Object and Objective (s)

The objective of the present study is to compare data from the literature in terms of the *in vitro* antimicrobial efficacy of photodynamic therapy with methylene blue (MB) in the *Porphyromonas gingivalis* evaluating the experimental parameters used.

Development (methodology, materials, data collection)

For a bibliographic survey, a search was made in the Pubmed database, using the following keywords: photodynamic therapy, methylene blue; *Porphyromonas gingivalis* and *in vitro*, from June 1 to September 7, 2017. During the research, the key words were submitted to associated search. The research results mentioned seven articles, but only 2 articles used low power laser and Porphyromonas gingivalis ATCC 33277 studied in suspension; the article by Braham et al., and the article by Street and collaborators.

Search results

Comparing the two papers selected in terms of the parameters used, both were found to use 0.01% w/v MB in the Periowave™ and 670nm diode laser. None of the studies reported whether there was pre-irradiation time and both performed MB dilution in buffer. While the Street study used 2 times diluted MB, Braham studied several different dilutions. The study of Street verified 4, 4, 5 and 7 log of bacterial reduction with 2,3; 4.7; 7.0; and 9.4 J/cm^2 radiant exposure, respectively, which equaled to 15, 30, 45 and 60s irradiation. Braham et al., at the same dilution of MB used by Street, found 7 log of bacterial reduction radiating for 60s with 150mW laser, despite not reporting radiant exposure.

Conclusion and final considerations

In summary, both studies showed that MB 0.005% w/v diluted in buffer and photoactivated by 60s with diode laser emitting at 670nm were able to inactivate *Porphyromonas gingivalis* (standard strain ATCC 33277), presenting a reduction of 7 log. It is noteworthy that although these parameters are effective *in vitro*, clinical efficacy may be reduced. The Street study itself showed that the bacterial reduction is lower (5 log) when this bacterial strain is organized in biofilms. In addition, bacteria from clinical strains are usually more resistant than standard strains.

Keywords: Photodynamic Therapy, Methylene Blue, Porphyromonas gingivalis, in vitro, Periodontitis.

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PHOTOBIOMODULATION THERAPY AMELIORATES LUNG INFLAMMATION AND ELASTANCE IN THE PULMONARY FIBROSIS INDUCED BY BLEOMYCIN

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Abstract: Pulmonary fibrosis (PF) is a chronic and progressive lung disease characterized by pulmonary parenchyma progressive lesion, inflammatory infiltration, and interstitial fibrosis. It is developed by excessive collagen deposition and other cellular matrix components, resulting in severe changes in the alveolar architecture. Considering the absence of effective treatment, the aim of this study was to investigate the effect of photobiomodulation therapy (PBMT) on the development of PF.

Introduction: Pulmonary fibrosis is a chronic and progressive lung disease whose etiology is unknown[1]. Its incidence is high presenting considerable morbidity and mortality. PF is characterized by progressive damage to the lung parenchyma, inflammatory infiltrate and fibrosis in the interstitial tissue[2]. The treatment of PF is not effective in prolonging survival, disease stabilization or improvement in quality of life. Thus, studies that investigate new therapies are relevant. Phototherapy emerges as an alternative of treatment, with good results for various diseases with inflammatory character, including lung diseases[3]. The photo stimulation of LED light acts on cells by interfering with their permeability, acts on mitochondria, the ATP synthesis and proteins like collagen and elastin. Also acts as anti-inflammatory. So our objective was to investigate the effect of treatment with LED on PF.

Methods and results: Adult male mice (C57BL/6) were subjected to the PF induction by administration of bleomycin by orotracheal route (1.5 U/kg). In parallel, group of mice was treated with PBMT 14 days after the induction of PF (1x/day for 8 days; 660 nm, 100mW, 5J/cm2, 150 s). The application of PBMT was performed on respiratory tract by direct contact with skin. After 24h the parameters were evaluated. Our results showed that LED significantly reduced the number of inflammatory cells in the alveolar space as well as static and dynamic lung elastance with $P<0.05$. No differences were observed in collagen production.

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Acknowledgment. We thank Alessandro Melo De Ana by LED device.

Conclusions: PBMT had anti-inflammatory and anti-fibrotic effects pointed it as a promising therapy to treat PF.

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EVALUATION OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN THE TREATMENT OF PERI-IMPLANTITIS. CONTROLLED CLINICAL TRIAL

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The elimination of pathogenic microorganisms from the peri-implant system is one of the main success points in the treatment of peri-implantitis.

The purpose of this study is to conduct a randomized, controlled clinical trial to evaluate the effectiveness of photodynamic therapy in the treatment of peri-implantitis. Twenty implants with peri-implantitis will be selected. Implants will be randomly divided into two groups (n = 10), group I control - conventional treatment and group II - conventional treatment and antimicrobial photodynamic therapy (aPDT).

The treatment will consist of oral hygiene orientation, with brushing technique instructions and daily flossing recommendation. The calculus deposits on the teeth will be removed with ultrasound equipment and curettes for root scaling and planing. In implants, calculus will be removed with specific curettes for use on the implant surface. The aPDT will be performed at the end of each periodontal treatment session, in sites with pockets greater than or equal to 5 mm. The PapaMblue® photosensitizer will be deposited in the periodontal pockets with a syringe and a pre-irradiation time of 1 min will be adopted. Then the laser emitting wavelength at 660 nm, with output radiant power of 100 mW, for 2 min, radiant exposure 30 J/cm^2 and power density I = 250 mW/cm^2 will be applied. Patients will undergo to clinical evaluations at 15, 30, 60 and 90 days after the end of treatment; and microbiological tests before and 30 days after the treatments. The data will be analyzed statistically through the test and a comparison of the treatment performed in the two groups will be performed. We will verify the distribution of the data within each group and the homogeneity of the variances for the choice of a variance analysis or not. With this information, the most appropriate statistical test in each experiment will be used. The sample calculation is based on the literature and the significance level of 5% will be adopted.

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Biofotônica aplicada às Ciências da Saúde

Technological and minimally invasive approach of dental caries in a pediatric patient: a case report

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Background: New materials and alternative methods to traditional caries management bring the promise of patient comfort increase, such as reducing the use of handpieces, resulting in less noise and vibration, and a minimally invasive approach to sound dental tissues. Alternative techniques for caries removal include chemical-mechanical methods like Papacarie Duo and, more recently, the use of antimicrobial photodynamic therapy (APT) powering the cavity disinfection. Thus, the concomitant use of both technologies is encouraged due to investigate its clinical performance.

Case report: Papacarie Duo® followed by APT were used in a 9-year-old patient with a deep dental caries lesion in the lower right first permanent molar. After 4 minutes of papain gel application, the decayed tissue and gel were carefully removed with water spray. Then, the deepest portion of the cavity was stained for 1 minute with a solution of rose bengal (20μM) and irradiated by a high intensity LED, an output power of 0.6 W, energy of 240 J at 6 minutes of irradiation. After the photodynamic application, it was definitively restored with composite resin. Despite the cavity decontamination and atraumatic approach, the time spent using both alternative approaches was too long.

Conclusions: Thus, due to parameters used, the technologies are recommended to be singly applied and further investigations aimed to optimize the clinical performance are necessary. To use this template, you will need to apply the embedded styles to each paragraph-level item in your manuscript, or simply use this template as a visual guide.

Comparison between reflection spectrum of skin diagnosed with lichen sclerosus vulvar *in vivo* when treated with corticosteroid therapy, photobiomodulation or PDT

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Introduction: Reflective spectroscopy is a noninvasive diagnostic method sensitive to small changes in the sample. The area under curve of the spectrum transmits information about the intensity of light reflected by the sample. Lichen sclerosus vulvar (LSV) is a skin disease in which some symptoms are related to changes in the skin, especially whitening. Since white is the reflection of all wavelengths, the study hypothesis is that the optical reflectance is changed as the skin is treated.
Objective: The aim of this study was to verify if skin changes its reflectance comparing with three different treatments.
Methods: Fifteen patients with LSV in the outpatient clinic of Hospital Pérola Byington, in São Paulo, BR, were studied after approval by the Research Ethics Committee. The patients were randomly divided into three groups of five patients each. The first group was treated during one month with topical corticosteroid once daily, as recommended by the International Society for the Study of Vulvovaginal Disease. The second group was treated fro one month once a week with photobiomodulation (PBM). The last one was treated for one month, once a week with PDT with methylene blue as photosensitizer. Measurements were taken in the begining and one month after treatment start. The spectra were obtained from two regions, one LSV-free and other with LSV. An Ocean Optics spectrophotometer (USB2000, USA) was used to obtain the reflective spectrum in the range between 300 and 1040 nm. The areas under the curves were integrated with the aid of Origin Pro software 8. A significance of $\alpha < 0.05$ was used for all the statistical analysis tests. After confirmation of normality with the Kolmogorov-Smirnov test, and homogeneity by Levene test. The areas were compared with ANOVA two-way and post hoc Fisher.
Results: There was significant difference ($p = 0.0133$) in the LS region before and after PBM treatment. The PBM group presented a reduction by 50.8% in reflection. Comparing the groups after the treatments, the PBM had the lowest reflection in relation to the PDT ($p = 0.0063$) and the corticosteroids therapy ($p = 0.0457$).
Conclusion: The PBM caused significant decrease in the optical reflection of the vulvar skin in the LS region when compared with corticosteroid therapy or PDT. It can be inferred that optical changes in the skin with LSV were caused by the PBM. Parallel studies are being conducted to correlate this finding to the symptoms and also histologically after treatments.

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Blue light for decontamination of pressure injuries - Case Report.

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Pressure injury can be defined as "*localized damage to the skin and/or underlying soft tissue usually over a bony prominence or related to a medical or other device. The injury can present as intact skin or an open ulcer and may be painful(...)*"[(1)] Currently, phototherapy, by coherent (laser) and non-coherent (LED) lights, stands out as a treatment option for pressure injuries. Several studies have demonstrated the effectiveness of blue light as an antimicrobial agent[(2–4)]. This study reports the application of phototherapy in PI using light blue (465 + 10nm) without the use of photosensitizers, to analyze the decontamination of the lesion.

Case report: M.M.N., 70 years old, female, hospitalized for pneumonia evolving to sepsis, sequelae of previous ischemic stroke. Carrier of sacral PI, stage IV, with a diameter of 3cm^2 and depth around 4cm (Figure 1A).

A **B**

Figure 1. A e B, first day and third day of application of Blue LED, respectively.

Methods: The research was approved by the Research Ethics Committee by the number: 53876416.2.0000.5551. By signing the Informed Consent Term, phototherapy was applied for three consecutive days, for 15 minutes, using the cluster for phototherapy of the Multi Radiance Medical, MR4 ACTIV®, the frequency used was 5000 Hz; total power 250 mW; wave-lenght 465 (±) 10 nm; spot size 0,9 cm^2 per LED (2,7 cm^2 total); power density 117,9 mW/cm^2 and density of energy 106,10 J/cm^2. The material for the microbiological examination was collected using sterile Swab in the three consecutive sessions of phototherapy, before and after each application, with aseptic technique. The collected material was cultivated in Nutrient Agar plates and incubated in a greenhouse for 24 hours at 35ºC ± 2ºC. After 24h, the plates were read and the CFU counted (Figure 2). The data obtained were tabulated in Microsoft Excel Software.

A **B** **C**

Figure 2. A, B e C plates for counting UFCs - D1, D2 and D3, respectively.

Results: A reduction was found in the CFU count of 65% between the first and the last day of application. (Means before and after, 5.1 and 4.3, 5.3 and 4.7, 4.3 and 3.8 in D1, D2 and D3, respectively). Although the literature demonstrates there is a reduction in PI microbiota in vitro [(2-4)], in the conditions presented in our study, sacral PI, with microclimate favorable to moisture contamination due to fecal and urinary incontinence, phototherapy with blue light, without the use of photosensitizers proved to be ineffective at the dose of 106.10 J / cm2.

Conclusions: As the first case studied, phototherapy using the blue light for PI decontamination has proved to be a practical alternative, but it must still be improved to obtain a significant reduction in the number of microorganisms, comparing different doses, to evaluate the effectiveness of this PI decontamination alternative.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Radiometric Parameters in the Efficiency of aPDT with PpIX

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Conventional antimicrobial strategies are becoming ineffective due to the resistance of pathogenic microorganisms to the drugs, triggering the need for the exploration of alternative treatments and approaches for the control of these infections.

Introduction: Candidiasis is a serious public health problem, entails unavoidable consequences and imposes high economic costs on health services.

Thus, the inactivation of microorganisms by aPDT is promising to the use of conventional fungicides and can be used both to control localized fungal infections and to eliminate pathogenic species from the environment.

For the clinical application of PDT, it is essential to ensure adequate radiometric parameters and to confirm the effectiveness of its action by the absorption and emission of photons. Thus, determining the ideal photodynamic parameters for this therapy to occur successfully is of paramount importance.

Objectives: To evaluate the efficacy of the PDT with the photosensitizer Protoporphyrin IX for inactivation of C. albicans as a function of radiant photon energy, evaluating the influence of photon energy, the influence of photon number and the influence of total energy on the efficiency of the photon. PDT.

Method: This work is a controlled laboratory test, which aims to evaluate the efficacy of aPDT with the photosensitizer PpIX for inactivation of C. albicans as a function of the radiometric parameters. The experiments were carried out in two steps: in the first step, the same total energy of the LEDs were maintained at the wavelengths: 630 nm, 570 nm, 520 nm and 440 nm during the 60 s, 120 s, 240 s and 480 s (the number of photons emitted by each wavelength was different due to its energy). In the second step, the same photon flux was maintained at all wavelengths: 630 nm, 570 nm, 520 nm and 440 nm during the 240 s, 480 s and 960 s times. (different total energy).

Results: PpIX showed to be a very efficient photosensitizer capable of generating sufficient reactive oxygen species and inducing the death of C. albicans in a significant way. The evaluation of the results showed that the higher the photon flux, the greater the antimicrobial action of PDT with PpIX, since it generates a greater amount of ROS, resulting in a greater efficacy in the elimination of C. albicans. In this way, when the wavelength is greater, photon energy will be smaller, however different total energies with the same number of photons produce similar results.

Conclusion: Inactivation of microorganisms by PDT is a promising alternative to the use of conventional fungicides and can be used both to control localized fungal infections and to eliminate pathogenic species from the environment. In conclusion, the results showed that the higher the number of photons, the greater the antimicrobial action of PDT with PpIX.

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Light Scattering in Soft and Hard Tissues in RED spectrum region - A comparative Study

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Samples of hard (dentine) and soft (muscle) bovine tissues were illuminated with laser beam. Scattered light of red laser beam was measured and its profile was fitted with Gaussian function. Hard tissue presented a characteristic parameter ω=32 mm, and soft tissue presented ω=4 mm. Surface light distribution plots were presented from both tissues.

keyWords: light scatter, biological tissues, phototherapy, biophotonics.

Light have been used to several purposes in human health. Application of light beam can be useful to detect some diseases, as well as to promote therapies (1, 2). On the other hand, these applications can be modulated through optical properties of the different biological tissues such as absorption, scattering and transmission. To promote therapeutic benefits, light can reach the site of injury in adequate quantities. Light properties, like wavelength, power and collimation can influence significantly. In this way, we studied the scattering of visible light on two different tissues: hard (bovine dentine) and soft (bovine muscle) in different thickness.

Materials/Methods: visible laser light beam (λ=650 nm, P=10 mW, Coherent Inc.) was used. Samples of hard tissue (thickness L=0.5 and 1.0 mm) were prepared from bovine dentine. Soft tissues (L=1.0-10.0 mm) were prepared by cutting parallels pieces of bovine muscle. Light beam was focused perpendicularly on tissues and scattered light was captured with a digital camera. The profiles of scattered light were treated with Gaussian function:

$$I(x) = I_0 + A\left(\frac{e^{-\left(2x^2/\omega^2\right)}}{\omega\sqrt{\pi/2}}\right) \quad (1)$$

Results & Discussion. Figure 1 shows the digital images of hard (L=0.5 mm) and soft (L=1.0 mm) tissues where we can view the scattered light.

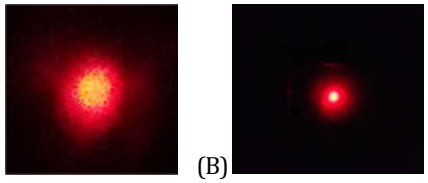

Fig. 1. Distribution of laser light beam (λ=650 nm) in hard (A, dentine) and soft (B, muscle) tissues.

Figure 2 shows the Profiles of scattered light fitted with Eq. 1. We can show that the hard tissue scattered significantly the visible light when compared with soft tissue, ω=31mm, and ω=4mm, respectively.

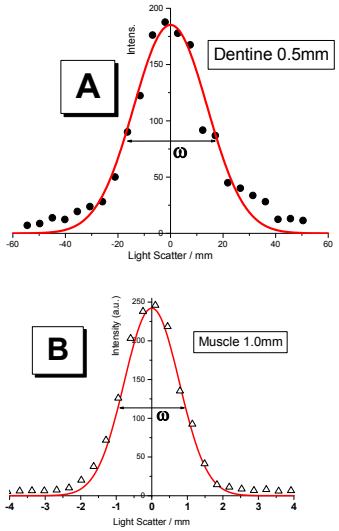

Fig. 2. Plot Profiles of scattered light beam (λ=650 nm) in hard (A, dentine) and soft (B, muscle) tissues.

APPLICATION of PHOTODYNAMIC THERAPY WITH LED AND METHYLENE BLUE IN *Streptococcus mutans* IN THE PRESENCE OF GLUCOSE- *in vitro* STUDY.

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Introduction

Streptococcus mutans is a microorganism associated with tooth decay; many genes that express adhesion, biofilm formation, extracellular polysaccharide, carbohydrate uptake and acid tolerance regulate its cariogenic properties. *S. mutans* inhabits a complex biofilm and it produces a large amount of exopolysaccharides to promote adhesion and enabling transport pumps. Photodynamic therapy involves the use of a photosensitizer (PS), which is absorbed by specific cells followed by irradiation with visible light, resulting in cell death. The aim of this study was to investigate antimicrobial photodynamic therapy on *Streptococcus mutans* in the presence of glucose.

Methods

Streptococcus mutans ATCC 25175 was grown in brain heart infusion (BHI) at 37ºC for 48h. Inocula were prepared with pure colonies, which were suspended in phosphate buffered saline (PBS) with and without 50 mM glucose. One-hundred micromolar methylene blue was used as photosensitizer and the experiments were performed with groups (control, irradiated with LED, FS without irradiation, and PDT 30, 60, and 120s). Colony form units were counted and statistically analyzed (one-way ANOVA and Tukey 5%).

Results

The irradiation as well as the photosensitizer in the dark did not cause cell death. In contrast, in experiments without glucose, PDT caused cell death proportional the amount of light used. The microbial inactivation increased in function of the irradiation and after 2 min a reduction of 7 logs (100%) was observed. In experiments with glucose cell death was observed even increasing the radiant exposure.

Conclusion

We can conclude that PDT is a viable solution for inactivation of *S. mutans*, and that in the presence of glucose delays the inactivation of bacteria. The greater the radiant exposure, the greater the inactivation of *S. mutans*.

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EFFECTS OF PHOTOBIOMODULATION IN GENE EXPRESSION OF TGF-β IN THE MUSCLE REPAIR PROCESS AFTER ACUTE LESION

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Muscle lesions are frequent in professional and recreational sports and in daily activities, though a higher incidence is observed in those who practice sports, resulting in direct or indirect trauma and in a local inflammatory response. TGF-β is mostly produced by immune cells, such as macrophages, and is considered an important growth factor involved in hematopoiesis, embryogenesis, tissue regeneration and cell differentiation. Also, it decreases the inflammatory effects of mediators such as TNF, IL-1β and IL-12. Several therapeutic modalities have been researched to provide adequate modulation in the tissue repair, and photobiomodulation (PBM) has shown promising results in the treatment of muscle lesions. However, few studies have evaluated the effect of PBM in TGF-β expression during muscle injury repair.

Objective. To evaluate the effects of photobiomodulation (660nm and 780nm laser) on TGFβ1 gene expression in muscle tissue that has sustained an acute lesion.

Methodology. Wistar rats, divided in 4 groups: control (without lesion, without treatment), muscle lesion without treatment, muscle lesion treated with PBM (660 nm - 70mW; 1 J/point, 8 points) and muscle lesion treated with PBM (780 nm - 70mW; 1J/point, 8 points). After 2, 4 and 7 days of irradiation, the animals were euthanatized and the tibialis anterior muscle was removed and processed for analysis. TGFβ1 gene expression was evaluated with qPCR.

Results. There were no significant differences in gene expression of TGFβ1 between control and lesion group without treatment in the three periods evaluated. In contrast, groups treated with 780 nm PBM showed an increase in TGFβ1 gene expression compared to lesion group without treatment and the group treated with 660nm PBM in the three periods. The group treated with 660nm PBM presented no significant differences in TGFβ1 expression when compared to the lesion group without treatment in all experimental periods.

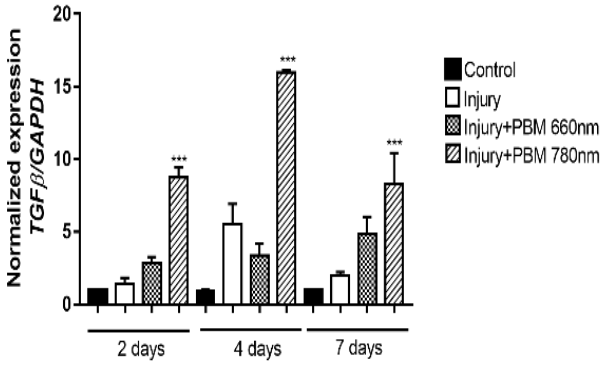

Figure 1: TGFβ1 gene expression after a period of 2, 4 and 7 days of PBM treatment. p<0.001***.

Conclusion. PBM with infrared laser with 780 nm was able to increase gene expression of the growth factor TGFβ1 in the three different periods, evidencing one of the action mechanisms of this therapeutic tool, since TGFβ1 has an immunomodulatory effect and plays an important role in regeneration, proliferation and cell differentiation.

Acknowledgment. Grant Fapesp number: 2013/07502-1; Grant Fapesp number: 2015/21219-6.

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Effect of CO_2 laser on resin/dentin bond strength of primary teeth

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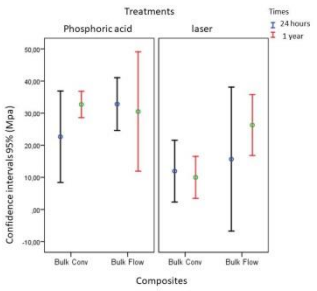
Background: CO_2 laser has been found to achieve adequate sealing and obliteration of dentinal tubules, so as to resolve dentinal sensitivity (1). Dentinal hypersensitivity is a clinical condition characterized by short sharp pain arising from exposed dentin (2). Dentine caries lesion requires restorative treatment, thus, it is important to take into account the adhesion performance of composites in such substrate, so that there is not post-operative sensitivity.

Objective: the aim of this study was to evaluate, *in vitro,* the bond strength of "bulk fill" composites on treated surfaces with CO_2 laser and conventional acid etching after 24 hours and after 1 year of storage.

Methods: 24 sound primary molars were selected. The occlusal surfaces were removed up to expose the dentin. Teeth were randomly divided into 8 groups (n-=3), half of the groups was analyzed after 24 h, and the other half, after 1 year: 1) Gio+etch: etching with phosphoric acid 37% (SDI) and Giomer Beautiful Bulk (Shofu Inc) restoration; 2) SDR+etch: etching with phosphoric acid 37% (SDI) and SDR Bulk Fill flow restoration (Dentsply); 3) Gio+CO_2: pretreating with CO_2: laser and Giomer Beautiful Bulk (Shofu Inc) restoration; 4) SDR+ CO_2:: pretreating with CO_2: laser and SDR Bulk Fill flow restoration (Dentsply). Phosphoric acid and CO_2 laser were applied previously the application of the adhesive (Ambar Universal, FGM). After the application of the adhesive, the restorative materials were inserted in increments to build up resin blocks with an adapted matrix (4mm height x 5 mm diameter), followed by light-curing for 40 seconds (Radii-Cal, SDI, 1200 mW/cm²). The samples were stored in distilled water for 24 h and 1 year. After, samples were sectioned to produce sticks of 1 mm of transversal section area. The sticks were attached to the device of the microtensile machine (EZ Test, Shimazu, Kyoto, Japão). Each stick received charge tension up to the fracture at a speed of 1 mm/min. Data were analyzed by three-way ANOVA and Tukey's test (p=0.05).

Results: After 24 hours, the groups SDR+etch showed the highest values, however, GIO+CO_2 showed the lowest values. After 1 year of storage, Gio+etch presented the highest values and Gio+ CO_2 showed the lowest values, and the groups did not show statistical significant difference among them when compared to the groups after 24 hours. (Graphic 1).



Graphic 1. Graphic shows the bond strength of the treated surfaces with the different materials.

Conclusion: It could be concluded that there was no statistical significant difference on bond strength of the materials after 1 year of storage and previous acid etching showed better results than the pretreatment with CO_2 laser.

Acknowledgment. We thank the Dental Materials Department of the Piracicaba Dental School – University of Campinas for the use of their equipment.

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USE OF ULTRAPULSED CO2 LASER FOR REPAIR IN COMPOSITE RESIN RESTORATION

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The CO2 lasers are currently being used to prevent tooth decay by making the enamel more acid resistant, however the doses for its use are not well established in the literature and the use of laser for treatment of incipient injury has been poorly reported

The objective of the present study was to evaluate the CO2 laser surface treatment in bond strength (BS) in composite resin (BS) repairs. Fifty blocks of composite resin (Opalis) measuring 5X5X5mm3 were prepared. They were divided into 5 groups (n = 10) according to the surface treatment, then a composite resin repair was performed and stored in distilled water for 7 days. After the repair, each test specimen was fixed to a precision metallographic section (ISOMET 1000, Buehler, Lake Bluff, USA) on an acrylic plate with a stick (DFL, Rio de Janeiro, Brazil). It was standardized a distance between cuts of 1.3 mm, being realized in both directions, forming sticks of approximately 1.0 mm2 of area and 1,0 cm of length.

The measured values were tabulated and submitted to a statistical test to test the normality of the data using the Shapiro-Wilks test. Since all data presented had a normal distribution ($p > 0.05$), which will be described in terms of their mean ± standard error. Caries lesion groups and healthy groups were compared using one-way ANOVA. The level of significance was set at $\alpha = 0.05$.

Acknowledgment. We thank the Department of Biomaterials of USP for the use of their equipment.

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Photobiomodulation in the cell viability of ST88-14 Schwann cells

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Immature Schwann cells (SC) are the glial cells derive from Schwann cell precursors, that have an important achievement and itself is directly involved in the axonal regeneration after a peripheral nervous injury when the desintegration of nerve fiber structure has developed. Neverthless, during an activation of a nerve repair course, the distal nerve loses his repair-supportive scope and start to decreasing the regenerating potential. The interact of the SC with axons is demanding for the optimal target of regenerate and constitute a trigger to attenuate neuropathic pain, caused by prolonged inflammatory reaction and neuromuscular disorders. The treatment with photobiomodulation has shown, in previous studies, that the SC is able to respond differently to laser irradiation reliant on irradiation parameters. Where different levels of energy suggested an enhancing of the viability of various SC cells lines.

Objective: The aim of this study was to evaluate the effects of photobiomodulation in cell viability of SC ST88-14 cell line, in red and infrared laser parameters.

Methods: ST88-14 cells line were cultured in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% fetal bovine serum. The cells were counted and divided into three experimental groups: (1) control group (non-irradiated), (2) 660 nm photobiomodulation group (irradiated) and (3) 780 nm photobiomodulation group (irradiated). The cells were irradiated with GaAlAs diode laser (660 and 780 nm, 40 mW, 1.6 J, 80 J/cm^2, 40s). Cell viability were assessed 24, 48 and 72h after irradiation by 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazolium bromide (MTT) assay.

Results: The results revealed that a greater increase in cell viability was found at 48h for both experimental lasers groups (p<0.0001) in comparison to the control group. Moreover, at 72 h, only 780 nm laser group had a significant increase (p=0.009) in comparison to all other experimental groups.

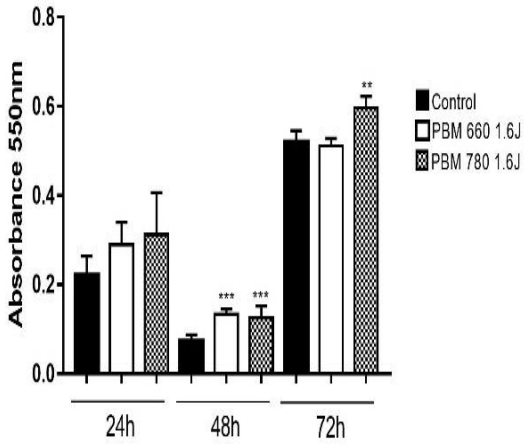

Fig. 1. Cell viability in SC in different sets of laser parameters; data expressed as mean ±SEM (***p<0.0001, **p=0.009 vs. control group)

Conclusion: The present findings demonstrated that the photobiomodulation, employed under the parameters described, was able to enhance cell viability of the SC. Combining different parameters could be useful, but further studies should be conducted to allow a better understanding about SC modulation.

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Healing evaluation after photobiomodulation in pressure injuries

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Pressure injuries are a major challenge for health professionals. Its high incidence is considered a serious public health problem and a negative indicator in the quality of nursing care[1-2].The main objective of this study was to evaluate the healing after photobiomodulation with red LED in pressure injuries stages 3 and 4 of patients hospitalized in a public hospital in the State of São Paulo.It is a randomized controlled clinical study divided into two distinct groups.

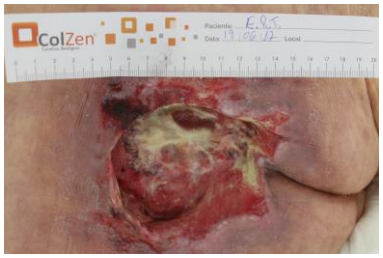
CG (conventional group) and GLED (LED treatment group), totaling 12 patients between 18 and 70 years old and skin phototype I to IV. The GC procedures were performed once daily with physiological solution, hydrogel with sodium alginate and cellulose membrane as the secondary coating. . While in GLED the dressings were done twice a day, cleaning with physiological solution and red LED (λ= 664 ± 20 [nm], P= 15.0 mW, I= 4.8 mW/cm^2, H= 2.9 J/cm^2, E= 9.0 J, t= 10 min).The evaluation of healing was performed by measuring the area, pH and temperature of the lesion, and applying the PUSH, FUGULIN and TISS-28 scale

Based on the initial results it was found that there were no significant differences in the temperature and pH of the wounds in both groups. Regarding the area, there was decrease of the wound in both groups, but without statistical significance between them. The data showed that the evolution of the PUSH scale was favorable to the healing process in the LED group. compared to the group C.

Group LED patient before photobiomodulation

Group LED patient after photobiomodulation

There were differences between the groups (p = 0.004), with the scores 34.00 ± 4.00 in the GC and 31.00 ± 6.00 in the GLED, indicating that the healing process was more evidenced in GLED. There was no suitable patient on the TISS-28 scale



Group C patient before conventional dressing

Group C patient after conventional dressing

There was no suitable patient on the TISS-28 scale.Photobiomodulation was as effective as the conventional treatment proposed, emphasizing the practicality and quality of its healing process.However, further studies with larger samples are required for this therapy to be included as standard procedure in the hospital environment.

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DENTINAL REMINERALIZATION ASSESSED WITH OCT AND SPECKLE

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The lesion of dental caries is characterized by the demineralization of the dental enamel and the degradation of the dentin. Dental caries lesions formation is an intermittent process that can result on progressive destruction of the tooth. The development of dental caries is a result of the accumulation of bacteria on the teeth and the frequent ingestion of sugar. The diagnosis of caries disease should be based on the interaction of factors and not only in the presence of lesions, since the lesions are manifested before the appearance of their visible manifestation. The process of diagnosis of caries is complex, it involves an interpretation of a set of data, clinical, symptoms and complementary examinations. The professional must ally the acquirer knowledge and distinguish the disease in order to close a diagnosis. Currently the gold standard for diagnosis is the clinical examination, associated with anamnesis and radiographic findings. The optical coherence tomography (OCT) is a diagnostic technique used dentistry that uses hard and soft tissue images. The OCT provides information about the enamel´s defects as well as their extent in depth. Speckle images have been sensitive to even minimal loss of minerals through the enamel by analyzing the scattering patterns of coherent light. The objective of this study is to investigate and compare the diagnosis of dentin caries using the OCT technique and the Speckle images as well as to develop a methodology that identifies and quantifies caries lesions in a non-invasive, non-destructive way, with low cost and running in real time. To perform this study, it will be used 32 vestibular dentin samples of bovine teeth. After being cleaned, cuted and polished, the samples will be divided into 4 groups and immersed in 30 ml of a cola-based soda for 10, 20, 30 and 40 minutes, twice a day, for 7 consecutive days and half the surface will be protected by two layers of nail base. Each sample will be analyzed by OCT and laser speckle.

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Biofotônica aplicada às Ciências da Saúde

PROTOCOL TO EVALUATE THE EFFECT OF CONDITIONED-MEDIUM FROM LASER-TREATED MACROPHAGES IN MESENCHYMAL STEM CELLS VIABILITY

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Macrophages have an important role in the various phases that occurs after muscle injury. In the beginning of the inflammatory process, macrophages acquire a pro-inflammatory phenotype (M1). At this point, macrophages will have a role in phagocytosis of tissue debris and activation of myogenic progenitor cells. In later phases, macrophages acquire an anti-inflammatory phenotype (M2) and will have an important role in the regeneration of muscle tissue. So, the modulation of the two macrophages phenotypes is important for the complete recovery of muscle tissue after injury.

Photobiomodulation (PBM) and cellular therapies with mesenchymal stem cells (MSC) have shown promising results in modulation of the inflammatory process. Both, PBM and MSC promote alteration of macrophages, from M1 to the M2 phenotype. Moreover, the combination of both therapies presented better results in previous reports compared to each therapy isolated, when applied to injured sciatic nerve. The combined effect of the application of these therapies in muscle injuries is little known.

Objective. Compare the effect of conditioned-medium, from M1 macrophages (phenotype 1) submitted to PBM using 660nm and 780 nm lasers diodes, in the viability of MSC also submitted to PBM using the same dosimetric parameters.

Methods. Peripheral blood mononuclear cells (PBMC) will be isolated from human peripheral blood using Ficoll-Paque method. PBMC will be cultured for 2h in RPMI medium allowing monocytes to attach to the culture plate. The medium will be changed and monocytes will be kept for 7 day in RPMI with 10% FBS and 50ng/ml of M-CSF to differentiate to macrophages.

Macrophages will be treated with 1 µg/mL LPS (*E. coli*) and 0.2 µg/mL (IFN-γ) for 24h to induce M1 phenotype. Macrophages will then be submitted to PBM using 660 nm (70mW, 25.025J/cm^2, 1 J) and 780 nm (70mW, 25.025J/cm^2, 1 J) diode lasers and cultured at 10^4cell/cm^2. The conditioned-medium (CM) will be collected 24 h after irradiations from non-activated human macrophages; M1 macrophages, M1 macrophages submitted to PBM at 660nm and M1 macrophages submitted to PBM at 780nm. MSC cells cultures will be submitted to the same PBM conditions, cultured at 10^4cell/cm^2 in the presence of the CM, for 24 and 48 h. Alterations in cells viability will be analyzed with MTT assay.

This study was submitted to Nove de Julho University Ethics Committee.

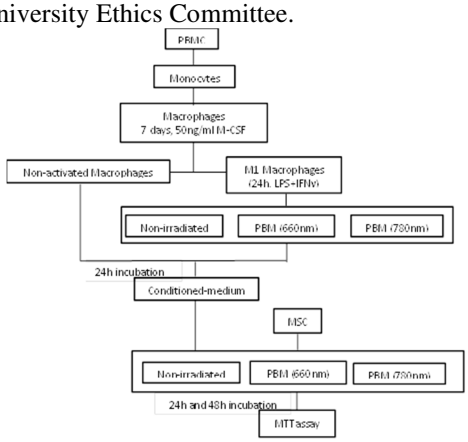

Figure 1. Flow chart of the experimental procedure.

Expected results. Conditioned-medium is expected to promote alterations in viability proliferation after the application of PBM in MSC.

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Computer vision applications for laser speckle images of tooth lesions

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This study presents computer vision techniques applied to laser speckle images of tooth aiming lesions diagnostics

The most prevalent disease in human history is carious lesions, affecting virtually 100% of all the population at least once in a lifetime. Detecting incipient lesions is still a clinical challenge since in its early stages; the changes in the microstructure of the enamel are almost imperceptible even for a trained clinician.

When rough surface reflects a laser beam, a phenomenon is known as speckle occurs. A random cluster of bright and dark spots generated by constructive and destructive interference characterizes laser speckle but the patterns of such clusters contain information about the microstructure of the surface and sub-surface of the sample.

Objective: the goal of this work is to demonstrate recent advances in the field of computer vision applied to laser speckle images

Material and Methods. Teeth samples were illuminated with a laser source ($\lambda = 633$ nm). The laser was placed at about 76 cm from the sample A lens expanded the laser beam to illuminate a larger area of the sample. A Canon T3i camera fitted with a Canon macro 100 mm lens captured the image of the sample. The camera performed no binning.

We applied different computer vision techniques and statistical approaches to enhance the contrast between the sound and lesioned tissue.

Results: The chi-squared distance analysis of the pixels (Fig. 1) demonstrates a segmentation of a laser speckle image of a decayed tooth.

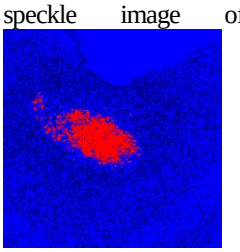

Fig. 1: segmentation of laser speckle image of a lesioned tooth

Image segmentation plays an important role as part of clinical diagnostic. This is the division of an image into specific regions. In the case of images obtained by coherent lighting, such as the laser light, there are no methods of segmentation developed. Nevertheless, our approach demonstrates it is possible to apply a known method of image segmentation to LSIs.

Conclusion: This technique shows itself to be quite promising for the construction of a diagnostic device.

Acknowledgment. We thank the FAPES for grant number 2015/25180-7

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III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

EFFECT OF LASER IRRADIATION ON FLUORETES COMPOUDS FOR WHITE SPOT LESION TREATMENT

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The use of diode laser can promote the catalysis of remineralization of enamel prisms and leave the substrate more responsive to fluoride. Fluoride therapy is already well established in the literature, however the protocols of use are extensive requiring several sessions, which makes it difficult for patients to adhere to treatment. The association of laser use and fluoride may accelerate the therapeutic process of caries lesions on dental enamel.

The objective of this study will be to evaluate, in vitro, the microhardness values of different forms of topical application of fluoride on lesion of artificial caries in bovine dental enamel irradiated with diode laser. The specimens will be prepared for the formation of the artificial caries lesion according to Featherstone model and treatment with pH cycling. In the experimental groups will be used the diode laser emitting wavelength at 808 nm.

Afterwards, they will be divided randomly into 7 groups (n = 10): treated with laser (G1), neutral fluorine gel 2% and laser (G2), fluorinated phosphate gel, 1.23% and laser (G3) (G4), fluoride and laser varnish (G5), laser and glycerin solution (G6), water, laser and water (G7). The microhardness (KNH) of the enamel will be evaluated with a Knoop penetrator and 0.098N load, by means of 5 readings in different areas of the same surface. The analysis will be performed after 7, 14, 21, 28 days and its stability after one week of the end.

For the surface characterization will be used electromicrography, confocal microscopy and energy dispersion. The data will be analyzed by the ANOVA test and the means will be presented in absolute values and ± standard deviation.

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PHOTOBIOMODULATION IN THE CLOSURE OF ULCERS AFTER MINOR AMPUTATIONS IN PATIENTS WITH DABETES MELLITUS

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Introduction: Diabetes Mellitus (DM) is currently one of the most prevalent public health problems, with an increasing incidence of 300 million individuals affected by 2030. DM is associated with life-threating complications and co-morbidities, among which, the foot ulcers are the most challenging. If untreated, the diabetic foot ulcer can lead to lower limb amputation. Even after the amputation, the ulcers take several months or years to heal, what increases the risk of infection and additional amputations. Recently the use of laser as an alternative method to accelerate the healing of diabetic ulcers has shown promising results.

Objective: To evaluate the effect of photobiomodulation laser in the closure of ulcers after minor amputations in patients with DM.

Method: The sample consisted of 19 patients with DM, who fit in the inclusion criteria, undergoing medical follow-up at the outpatient clinic of the Mandaqui Hospital. The patients were randomly divided into two groups: group A - which received conventional treatment plus photobiomodulation (λ= 660nm, P= 108mW, A= 0,04cm^2, I= 2,7W/cm^2, H= 108J/cm^2, t= 40s, E= 4,32J) and group B - which received the conventional treatment). Weekly, before the treatment, the lesions were evaluated, also the capillary glycaemia, and pH in the center of the ulcer. Then, the ulcers were photographed with a conventional camera and with a thermographic camera. An evaluator performed the lesion surface measurement using ImageJ software and data was sent for statistical analysis. After the total closure of the lesions the patients will be followed for a period of 6 months with follow-up at 1 month, 3 months and 6 months.

Results: Data on gender, age, smoking, temperature of the lesion before and after laser application were not significantly different between the groups. The closure rate was different (p = 0.015) between groups. In group A the final area was greater than the initial area (45.70% ± 87.3%) while in group B there was a reduction of area between the last and first day of treatment (-44.20% ± 39.0%) (Fig. 1).

Group A showed glycaemia results inversely proportional to the area and perimeter with a moderate correlation (ratio coefficient = -0.581), also, the glycaemia results were inversely proportional to pH (coefficient of relation = -0,456).

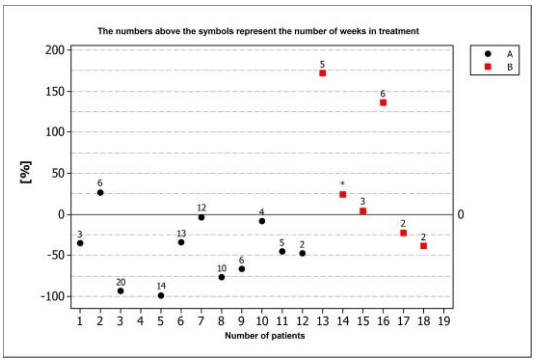

Fig. 1: Area closure rate (%) of lesion.

Conclusion: Photobiomodulation laser was effective in reducing the total lesion area by accelerating the healing process, thus reducing the risk of new amputations.

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EVALUATION OF ANTIMICROBIAL PHOTODYNAMIC THERAPY ON PERIODONTAL POCKETS – CLINICAL TRIAL

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Introduction. Periodontitis is an inflammatory disease of the supporting tissues of teeth induced by microorganisms organized in biofilm and influenced by the immune response of the host. Mechanical periodontal treatment does not completely remove periodontal pathogens, and in specific cases, the use of systemic antimicrobials may be necessary. Aiming to reduce the use of antibiotics, antimicrobial photodynamic therapy (aPDT) appears as an alternative and can be used as adjuvant of periodontal treatment. PDT is based on the use of photoactive substances, known as photosensitizers (PS) that bind to the target cell and produces reactive oxygen species followed by light irradiation in resonant wavelength. The purpose of this study was to investigate the antimicrobial potential of PDT in periodontal pockets.

Methods. This clinical trial, approved by the Human Research Ethics Committee of the Uninove University (1.517.902) included 30 patients with chronic periodontitis who were under follow-up with periodontal treatment in the Dental Clinic of the University. Each patient had at least 3 incisors with probing depth greater than 4 mm selected for the experimental procedures. Microbiological evaluations were performed to quantify microorganisms before and after irradiation. The methylene blue (MB) PS (100 μM) was used in aqueous solution and associated with a surfactant (MBS). The PS was deposited in the periodontal pockets with a syringe and a pre-irradiation time of 1 min was adopted. Then, the laser with radiation emission at wavelength of λ = 660 nm and output radiant power of 100 mW was used in 3 irradiation times - 1, 3 and 5 min. Each irradiation point was approximately 0.4 cm^2, which resulted in radiant exposure of 15, 45 and 75 J/cm^2, respectively. The irradiation had a constant power density of 250 mW/cm^2.

Results. The results demonstrated the absence of antimicrobial effect with aqueous methylene blue mediated PDT. On the other hand, MB associated to the surfactant produced microbial reduction in the group irradiated for 5 min ($p<0.05$). In conclusion, the clinical use of PDT may be limited by factors that reduce the antimicrobial effect.

Conclusion. Forms of irradiation and stability of the PS play important role on clinical aPDT.

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ROLE OF PHOTOBIOMODULATION ON THE PRESENCE OF M2 MACROPHAGES IN SKELETAL MUSCLE OF ELDERLY RATS AFTER LESION

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According to the IBGE, between 2005 and 2015, the proportion of people over 60 years of age in Brazil grew faster rate than the world average. This continuous increase in the elderly populacional presents a major challenge to health cérvices. Areinha leads to a decrease in muscle mass, defined as sarcopenia, and consequently an increased risk of falls and injuries. Skeletal muscle repairs in elderly individuais is slow and not always successful. This fact has been related to a prolonged stay of inflammatory cells in the local of the lesion. Neutrophils and macrophages are considered crucial cells for the repair process and targets for rehabilitation therapies. Photobiomodulation (PBM) has been extensively used in the treatment of muscular lesion in the younger population, however few studies have evaluated its effect on muscle lesion in the elderly.

Objective. To evaluate the effect of PBM on the presence of repair profile macrophages after acute lesion in muscle of elderly rats.

Methodology. Thirty-five Wistar rats with 24 months of age were divided in three groups: control (n = 5); cryolesion (n = 15); cryolysis + PBM treatment (n = 15). The cryolesion is a well-established model in the literature that mimics an muscular lesion. PBM treatment (780 nm, 40 mW, 10 seconds and 0.4 J / point, total energy of 3.2J) was daily performed at 8 points around the lesion. After 1, 3 and 7 days, the animals were euthanized and the TA muscles removed for analysis of the infiltrated M2a macrophage by the immunoblotting (CD206+). The images were analyzed with the aid of Image J. This study was approved by the ethics committee from UNINOVE (AN 0002/2014).

Results. An increase in the number of M2a macrophages (CD206+) was observed in the muscle of animals receiving PBM treatment within 3 days compared to untreated animals. In the periods of 1 and 7 days there was no significant difference between the treated and untreated groups.

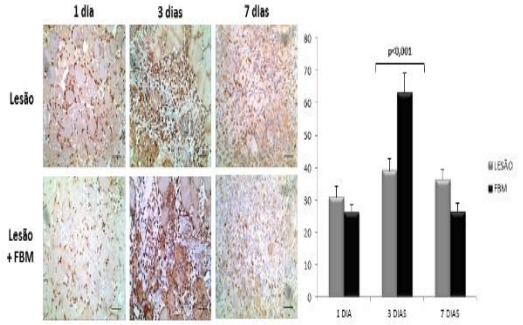

Fig. 1. Mean number of positive cells (CD206+). Results presented in mean and standard deviation.

Conclusion: PBM was able to modulate the presence of M2a macrophages within 3 days, which is considered crucial in the transition phase of M1 to M2 profiles. This modulation can be considered positive, since under normal conditions the macrophages of this profile reach the site of the delay process in the elderly.

Acknowledgment. Grant Fapesp number: 2013 075021; Grant Fapesp number: 2014 123811

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Effect of biomodulation on asymptomatic apical periodontitis on functional activity of lung structural cells stimulated *in vitro* with House Dust Mite (HDM).

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Background: Approximately 350.000 hospitalizations occur in Brazil annually due to asthma, being the fourth cause of hospitalizations, and the third among children and young adults. Asthma is a chronic inflammatory disease characterized by lower airway hyper responsibility and variable airflow limitation. Asymptomatic apical periodontitis is caused by pulpal necrosis, with consequent inflammation and destruction of the periapical tissues. Bacteria are able to survive and maintain the periapical infectious process despite the therapeutic measures applied in cases of well-managed endodontic treatment, mainly *Enterococcus faecalis*. To date, there are no papers correlating the reduction of asthma symptoms in patients with asymptomatic apical periodontitis**. Objective**: The objective of this study will be to evaluate the effect of biomodulation on the functional activity of fibroblasts and pneumocytes II incubated with *Enterococcus faecalis* lipopolysaccharide (LPS) and stimulated *in vitro* with House Dust Mite (HDM). **Methods**: Three male Balb / C mice will be used to obtain the primary culture, which will be euthanized and the lung removed. Cell cultures will be separated into 7 experimental groups arranged in 24-well plates occupying 6 wells each group. The Dust Mite and laser (HDM + LPS), House Dust Mite (HDM), Enterococcus faecalis (LPS), House Dust Mite and Enterococcus faecalis (HDM + LPS) , House Dust Mite, Enterococcus faecalis and laser (HDM + LPS + L). After 1 hour of stimulation with HDM (30µg / ml), the cells will be incubated with LPS (10µg / ml) and after 4 hours irradiated or not with laser. A diode laser with output area of 0.785 cm2, output power of 30 mW, wavelength of 808 nm will be used. The irradiation time will be 60 seconds, resulting in a total energy dose of 3 J. After 24 hours the supernatant will be collected for the levels of IL-4, IL-5, IL-10, IL-13, TNF -α and KC, using ELISA kit. If the data are normal, ANOVA - one way and will be used $\alpha = 0,05$.

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III FORUM INTERNACIONAL DE LASERTERAPIA e
III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

Photobiomodulation and Transcutaneous electrical nerve stimulation associated to therapeutic exercises in quality of life and pain in patients with knee osteoarthritis. A Systematic Review and Meta-analysis.

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Background: Osteoarthritis can be considered as a chronic disease with several disturbs characterized by joint cartilage degeneration with slow progression associated to painful symptoms. Several electrophysical agents (EPAs) have been studied associated to therapeutic exercises to improve pain and quality of life. The most EPAs studied are Photobiomodulation (PBM) and Transcutaneous Electrical Nerve Stimulation (TENS).

Objective: To compare the effectiveness of PBM and TENS associated to therapeutic exercises in quality of life and pain in patients with knee osteoarthritis.

Method: A Systematic Review of the randomized clinical trial (RCT) was performed following the "Preferred Reporting Itens for Systematic Reviews" (PRISMA) criteria aged from January 1st, 1980 to July 31st, 2017. Al the eligibility criteria were performed by two reviewers with the purpose to evaluate the risks and bias.

Results: At the first search, 6.613 studies related to PBM and 8.739 related to TENS were found. After the second screening, 106 studies related to PBM and 53 related to TENS were selected. After the third screening, remained only 4 studies to PBM and 8 to TENS to do the Meta-analysis. Al steps of screening followed rigorous criteria from PRISMA with Rob Table and Jadad Score. The results related to pain showed significant improve pain for 4 weeks of treatment when compared TENS versus PBM ($p=0.001$). For 6 weeks of treatment showed better results for PBM when compared to TENS $p=0.001$. For 8 weeks of treatment the results showed improve of pain to TENS when compared to PBM ($p<0.05$). The results related to Quality of Life for 4 weeks of treatment, the PBM showed better results $p<0.001$. For 6 weeks of treatment the PBM showed significant results when compared to TENS ($p<0.001$). **Conclusion:** The PBM showed better results when used up to 4 and 6 weeks of treatment. TENS showed significant results when compared to PBM up to 8 weeks of treatment. Both, PBM and TENS associated to therapeutic exercises showed to be a valuable source to improve the quality of life and pain in patients with knee osteoarthritis.

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Gene Expression of Inflammatory Markers in the Treatment of Chronic Periodontitis in Animal Model

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Introduction

Periodontal disease is an inflammatory response on gingival tissues directed to microorganisms that colonize the teeth. This disease leads to reabsorption of the alveolar bone process resulting in dental loss, and can generate systemic inflammatory changes. Mechanical periodontal treatment does not completely remove periodontal pathogens, and sometimes the use of antimicrobials is necessary. In order to reduce the use of antibiotics, antimicrobial photodynamic therapy (PDT) can be used as an adjuvant to periodontal treatment. Photodynamic therapy combines the use of a photosensitizer (FS) with a light source to generate reactive oxygen species and destroy cells. Protoporphyrin IX NetNI (PpNetNI) is a derivative of protoporphyrin IX, which has affinity sites for binding to the microbial cells. The objective of this study was to investigate the effects of PDT as an adjuvant to the treatment of periodontal disease.

Methods

Thirty healthy Wistar rats were used in this study (CEPA UNINOVE AN0029 / 2015). Periodontitis was induced by the placement of a cotton ligature around the first mandibular molar. After 7 days, the ligature was removed and all animals received the followed treatments: 1) SRP and irrigation with PpNetNI, 10μM; and 2) SRP, 10 μM PpNetNI followed by irradiation. The irradiation was performed with an LED (630 nm) with power 400 mW (irradiance 200 mW/cm^2, radiant exposure 18 J/cm^2). Rats were euthanized 24 h, 48 h and 7 days after treatment. The gingival tissue was surgically removed and frozen in nitrogen. Specimens were analyzed for IL-1β, IL-6 and IL-10 gene expression, quantified by real-time polymerase chain reaction (RT-PCR).

Results

Twenty-four hours after treatment, there is an increase in IL-1β levels, and this difference increases dramatically after 48h. On the other hand, there is no difference in IL-1β level 7 days after treatment. IL-10 exhibits a different behavior from the other markers, 24 h after treatment, an expressive increase of IL-10 can be seen in the FS group, but 7 days later the PDT group is at high levels that collaborate with the standard of resolution of the inflammatory process of the periodontal tissue that led to increased tissue repair.

Conclusion

PDT accelerated inflammatory response time by accelerating the acute phase of inflammation, which advanced the repair and remodeling phase.

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Quality of life evaluation in patients with spinal cord injury submitted to photobiomodulation treatment

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A spinal cord injury is a tragic event that can profoundly affect a patient's life, with long-term physical, psychological, social, environmental, and financial implications. Spinal cord injury may result in neurological impairment of all body systems and functions below the level of the neurological injury, causing loss of motor function, decreased mobility, increased morbidity, decreased expectation and quality of life.

The study involved 25 patients. The volunteers were randomly allocated to either the control group or treatment group. Evaluations were conducted before and after the intervention using electromyography to assess the motor response. The treatment group received phototherapy and irradiation was administered to the injury site transcutaneously at a wavelength of 808 nm using a Quantum diode laser (Ecco Fibras e Dispositivos, Brazil).

The Quality of Life Questionnaire (WHOQOL- bref) is a tool that clearly assesses the perspective of people's lives through reported results and has been validated in several studies to measure the quality of life in individuals with spinal cord injury.

Results

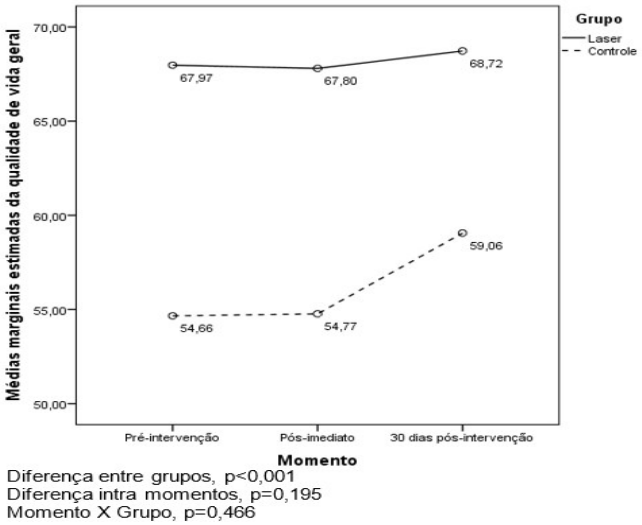

Fig. 1. Association between a general quality of life between the moments of patients with spinal cord injury after photobiomodulation.

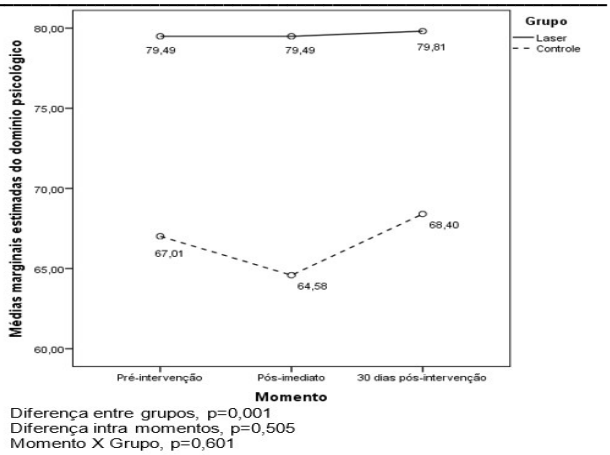

Fig 2. Evaluation of the domain psychological between the moments of patients with spinal cord injury after low intensity laser intervention

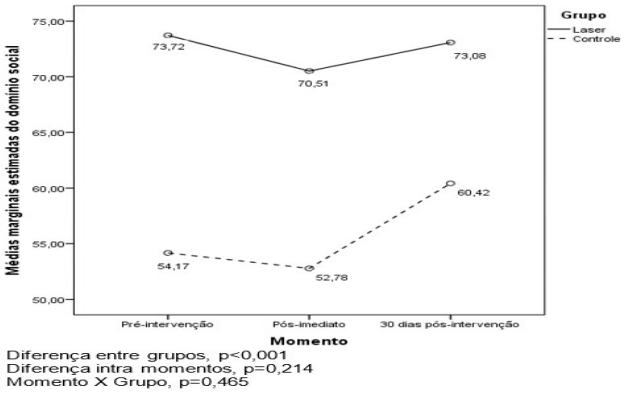

Fig 3. Evaluation of the domain social between the moments of patients with spinal cord injury after low intensity laser intervention.

Conclusion. This study showed that photobiomodulation improved the quality of life of individuals with spinal cord injury.

Acknowledgment. We thank University Nove de Julho (São Paulo, Brazil) for the use of their equipment

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III FORUM INTERNACIONAL DE LASERTERAPIA e
III Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde

Effect of photodynamic therapy (aPDT) on infected dentin in deciduous teeth: a pilot study

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Background. Caries is the most common oral disease in the world, and its treatment has been widely studied. Photodynamic therapy (PDT) associates a photosensitizer dye with laser therapy providing antimicrobial action, and Papacarie® gel is an alternative for removal of carious tissue preserving the affected dentin. The association of methylene blue dye with Papacarie® originated PapaMBlue, which can be combined with a laser irradiation and may be effective for the treatment of caries in children.

Objective. The objective of this study was to evaluate the clinical and microbiological effect of photodynamic therapy on dentin infected by caries lesion in deciduous teeth.

Material And Methods. Twenty-two children with deciduous molar caries were randomly divided into two groups, which received different treatments: group 1 low-speed drill, group 2 Papacarie® and PDT. For microbiological analysis, dentin samples were removed before and after the interventions. The samples were cultured in anaerobiosis and the CFU/ml count was performed. The Student's T-test was used to compare the means.

Results. A significance level of 95% ($p < 0.05\%$) was considered for all analyzes. There was a decrease in the CFU/mL counting when PDT was used to treat carious lesions in deciduous molars, when compared to conventional treatment with a low-speed drill.

Conclusion: Association of PDT with PapaMBlue has potentiated the antimicrobial action in a minimally invasive treatment and this study brings a new treatment option for caries in deciduous teeth with shorter working time than conventional treatment.

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Acknowledgment. We gratefully thank Universidade Nove de Julho for its technological support.

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Protocol for randomized, double-blind, placebo-controlled clinical trial to determine the effectiveness of light-emitting diode (LED) photobiomodulation in pain control, facial edema, trismus and quality of life after extraction of retained lower third molars

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Background: Removal of third molars included, although very common, is a surgery that generates great morbidity to patients for causing pain, edema and trismus. Photobiomodulation (FBM) has shown excellent results in the control of postoperative sequels; however, there are no studies on the effectiveness of the use of light emitted by diode (LED) in this clinical situation.

Objective: To evaluate the effectiveness of FBM with LED in the control of pain, facial edema, trismus and quality of life resulting from the extraction of third molars retained.

Methods: A randomized, double-blind, placebo-controlled clinical trial involving 34 adult patients seeking FO-UFRGS for the removal of lower third molars included and meeting the eligibility criteria and agreeing to participate in the study will be conducted. A single surgeon will perform all surgical procedures, one examiner will perform initial evaluation and reevaluations (blind as to the experimental group) and another the application of LED. Previously the surgeries will be performed the facial and mouth opening measurements of all patients and after, they will be submitted to the surgical protocol. Immediately after the surgeries, the patients will be randomized by means of envelopes in two groups. In the LED group the patients will receive daily LED applications (intra oral with 660nm and extra oral with 850nm) from the immediate postoperative to 7 days after the surgical procedure. In the control group the patients will be treated in the same way as in the LED group, however, the person in charge of the application will simulate intraoral and extraoral irradiation with the LED kept off.

Results: Pain (EVA and NRS-101), postoperative edema, spasm, temperature, dysphagia and hematomas, as well as the impact of the surgical procedure on patients' quality of life will be evaluated after 1, 2, 5 and 7 days. For the analysis of the oral health impact profile (OHIP-14 questionnaire) and anxiety analysis (Beck anxiety inventory -BAI) the questionnaires will be applied preoperatively and 7 days after treatment. Initial descriptive analyzes will be performed considering all variables measured in the study, both quantitative (mean and standard deviation) and qualitative (frequencies and percentages). Later, the appropriate statistical tests will be applied for each specific analysis. In all tests, the significance level of 5% probability or the corresponding p-value will be adopted. All analyzes will be performed using the statistical software SAS for Windows, version 9.1.3.

Conclusions: In spite of the several beneficial effects of LEDs in the pain repair and control process described in the literature, no studies were found to evaluate the effect of the use of LEDs in the postoperative period of included third molar surgeries. This is a relatively new technology and they are still under investigation especially in clinical trials so this study will make an important contribution to the topic.

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Sensitization of glucose sensors as a pathway for increased uptake of methylene blue in *Candida albicans* with multidrug efflux systems

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Introduction

The field of antimicrobial therapies is constantly challenged by the phenomenon of microbial resistance and the emergence of emerging pathogens. Photodynamic therapy (PDT) is a new promising strategy to microbial inactivation based on the use of photosensitizer (PS) in the presence of oxygen and activation by light to form reactive oxygen species. Photoprocesses due to PDT are highly lethal to microbial cells, these are called Type I reactions - with formation of free radicals - and Type II reaction - with the formation of singlet oxygen. The accumulation of intracellular photosensitizer (PS) can be affected by the biofilm of *C. albicans* but the glucose sensor - SRR (sugar receptor-repressor), via glucose repressor and via adenylate cyclase – may be a pathway for better PDT performance.

Methods

Strains of *C. albicans* ATCC 10231, YEM 12, YEM 13, YEM 14 and YEM 15 were grown aerobically on Sabouraud agar and incubated at 30 ° C for 24 h. Microbial inoculants were divided into 4 groups with and without glucose: Control; Irradiation only; PS toxicity and PDT groups with 3-time irradiation. After going through the proposed treatments, the colony forming units were counted, converted and the data were submitted to statistical analysis (ANOVA) and Tukey's test. To verify the presence of MB in and out of the fungal cell, fluorescence spectroscopy and flow cytometry assays were performed.

Results

As super expressive strains of the Major Superfamily Facilitator had a greater ability to accumulate MB in their cytoplasm, however, as strains that overexpress ABC showed greater resistance to photooxidative stress.

Conclusion

PDT is an effective alternative in the inactivation of *C. albicans* and the glucose can affect the photodynamic effect. The MB present inside the cell increases photodynamic inactivation, however, its effect depends on the characteristics of the strain.

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CAPACITY OF THE M2A MACROPHAGE SUPERNATANT TO ALTER THE FUSION OF MYOGENIC PRECURSOR CELLS WHEN SUBMITED TO PHOTOBIOMODULATION

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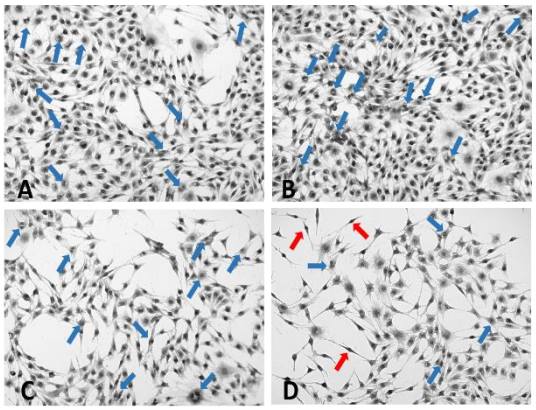
The change in macrophages polarization from M1 (proinflammatory) to M2 (anti-inflammatory/repair) is a very important event in the muscle repair process and coincides with the change from the proliferative stage to the differentiation of myogenic precursor cells (MPC). Photobiomodulation has been shown to generate good results in the muscle repair process, but little is known about its effects on macrophage products, and especially about how these products act in the differentiation of MPC.

Objective. To evaluate the capacity of the M2a macrophages supernatant submited to photobiomodulation to alter the fusion of myogenic precursor cells.

Methodology. J774 macrophages activated with 0,1 µg/mL of IL-4 (M2a phenotype) and non-activated were plated in concentration of $2,8x10^5$ and incubated for 24h. After this period, the macrophages were washed, centrifuged and irradiated with PBM with 660 nm and 780 nm (1 J, 70 mW, 17.5 J/cm^2, 15s). This procedure gave rise to cultures representing the groups: control (non-activatedand and non-irradiated); M2a (activated and non-irradiated); M2a + 660 nm (activated and irradiated) and M2a + 780 nm (activated and irradiated), which were incubated 24 hours more. The supernatant from these cultures was added to the culture of MPC (mioblast of cell line C2C12 - $0,03x10^4$) which was then incubated for a period of 72h. The MPC was colored with a crystal violet solution and images were taken of the plates for evaluation.

Results. MPC cultivated with supernatant of macrophages M2a and macrophages control showed the higher cell density, with some cells in fusion process (2 or more nucleus) characterizing the proliferative phase of myogenesis. On the other hand, MPC cultivated with supernatant of M2a macrophages + PBM with 660 nm and 780 nm, demonstrated lower cell density, some cells in fusion and development of first-degree muscle fibers (especially in the group treated with supernatant of M2a macrophages irradiated with PBM of 780 nm). In these groups, it was possible to observe fusiform morphology, cytoplasmic extensions and alignment in nucleus of cell. These results characterize the cellular differentiation process.



Experimental groups: control (A); M2a (B); M2a + 660 nm (C) and M2a + 780 nm (D). Red arrows indicate well differentiated cells with fusiform morphology. Blue arrows indicate cells in process fusion.

Conclusion. PBM, especially irradiation with 780 nm, stimulated macrophages to produce products that diferentiate myogenic precursor cells.

Acknowledgment. Grant Fapesp number: 2013/07502-1; Grant Fapesp number: 2015/21219-6. Grant Fapesp number 2017/07535-8.

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Evaluation of the effectiveness of natural compounds as photosensitizing agents in antimicrobial photodynamic therapy

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This study aimed to evaluate the feasibility of using herbal compounds, such as: lemon oil, riboflavin, hydrolyzable tannin, curcumin, hypericum and cranberry, as photosensitizing agents in Antimicrobial Photodynamic Therapy (aPDT).

Solutions of each FS were analyzed in a spectrophotometer to determine the absorption band, between 350 and 700nm and to determine the light source ideal for irradiate of each compound. Serial dilution of the substances were tested in *E. faecalis* suspension to evaluate any antimicrobial effect in the absence of light.

After initial spectroscopy, the light sources were chosen. Three LEDs emitting in 405nm, 470nm and 660nm were used and a bacterial suspension was teste for each wavelength with 10J of energy, 26 J/cm^2 of energy density and 0.3, 1 and 3 W/cm^2 of power density.

In 96 wells plate a *E. faecalis* suspension of 10^9 cells/ml, received 100µl of each compound and were irradiated with each light source using the followed parameters 10J, and 26 J/cm^2.

A TTC (triphenyl hydrochloride tetrazolium) viability test were used to evaluate bacterial death.

The compounds were also test, in bovine dental tissue (dentine) to evaluate possible stain after use. Dentine samples (5x5mm) were covered with each compound for 15 minutes, washed in distillated water and measured using a spectrophotometer.

Results

The spectroscopy analysis showed the following absorption bands for each compound: Hypericum UV-550and 640-700nm; riboflavin UV-540 nm; lemon UV-530 and 650-700; hydrolysable tannin UV-530 and 640-700 nm; curcumin UV-500 and 630-700nm. (FIG 1)

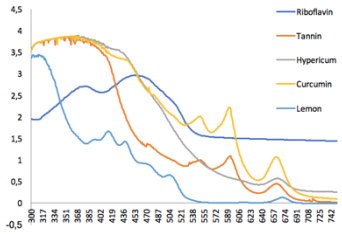

Fig1: Absorption band of each compound

The results showed none of the herbal compounds tested, at any concentrations (1:1 to 1:10), was toxic for *E.faecalis* suspension. Also, none of the light sources used, in the absence of the compound, promote any bacterial death at the tested parameters.

However, when the compounds were irradiated a significant bacterial reduction were found.

None of the compounds presented dentine stain, when used with an appropriate concentration.

In conclusion, the herbal compounds: hypericum, lemon oil, riboflavin, tannin and curcumin have antibacterial effects when irradiated with visible light at 405, 470 and 660nm. Herbal compound seems to be and alternative approach for aPDT.

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Evaluation of endodontic treatment with photodynamic therapy (PDT) in pulmonary inflammation modulation in an experimental model of asthma.

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Background: In the last decades there has been an increase in the prevalence of allergic diseases in developed countries. The 'hygiene hypothesis' suggests that childhood infections may have a protective effect on the development of allergies. Thus, Th1-profile cytokines released during infection may inhibit the release of Th2-profile cytokines as in asthma. Bacteria are able to survive and maintain the periapical infectious process despite the therapeutic measures applied in well-conducted endodontic treatment cases. Photodynamic therapy (PDT) can be used to eliminate microorganisms present in the root canal system. **Objectives:** This study aims to evaluate the endodontic treatment associated with PDT in the modulation of pulmonary inflammation in an experimental model of asthma. **Methods:** Sixty - four, male Balb / C mice (± 25 grams) were divided into 8 groups (n = 8): 1. Basal, 2. Asthma (A), 3. Apical Periodontitis (PA), 4. PA + endodontic treatment TE + PDT, 6. A + PA, 7. A + PA + TE, 8. A + PA + TE + PDT. Apical periodontitis will be induced performing the pulpectomy of the left first molar (21 days). Asthama will be induced by ovalbumin (OVA) and aluminum hydroxide, subcutaneously (days 0 and 7) and nebulization with OVA (3 x / week, for 4 weeks). Endodontic treatment will be performed with calcium hydroxide and PDT with methylene blue (0.005%) associated with red diode laser (λ=660 nm, energy density 6, 369 J / cm, with 9 J at the point, delivered in 90s. In the 7th days, euthanasia will be performed for morphological analysis of the lung and the mandible. The total and differential counts of inflammatory cells will be evaluated in bronchoalveolar lavage (BAL) and serum. Cytokines IL-4, IL-5, IL-10, IFN-γ, TNF-γ, IL-1 and IL-6 in BAL supernatant and mucus production as well as collagen deposition in the airways will also be evaluated.

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Interaction of Methylene Blue and Fluconazole on Photodynamic Therapy

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Background and objective. Combining PDT to conventional antimicrobial drugs may be a promising strategy to improve the antimicrobial efficiency promoted by each of the therapeutic modalities. Thereby the objective of this study was to investigate the interaction between fluconazole and methylene blue for application in photodynamic therapy.

Material and Methods. A methylene blue in water solution (25 μM or 100 μM) combined to 100 μg/mL fluconazole were exposed to LED radiation (λ=660 nm, 85 mW/cm^2, 1 to 8 min). The solutions were analyzed using a UV-VIS absorption spectrophotometer and a fluorescence spectrophotometer. The ROS production was indirectly estimated using 13.3 μM N,Ndimethyl-4-nitrosaniline (RNO) and 15 mM L-histidine. Following irradiation, ROS formation was determined by absorbance at 440 nm.

Results. The irradiation of MB solution promoted a reduction of absorbance of MB leuco band (292 nm), MB dimer band (610 nm) and MB monomer band (664 nm). Similar behavior was observed in MB+FLU solution, whereas no alterations were observed in FLU solution. Loss of absorbance at 292 nm and 664 nm, after 8 min of irradiation was 18% for both MB and MB+FLU solution.

MB fluorescence also reduced in function of irradiation time in both MB and MB+FLU solution. The signal intensity at 690 nm reduced 20% after 8 min of irradiation.

RNO degradation showed similar ROS production in MB solution with and without FLU.

Table 1. ROS production in MB and MB+FLU solutions determined by absorbance at 440 nm

Irradiation time	MB (SD)	MB+FLU (SD)
0 min	0.45 (0.01)	0.45 (0.01)
1 min	0.33 (0.01)	0.34 (0.01)
2 min	0.23 (0.02)	0.22 (0.02)
4 min	0.12 (0.01)	0.13 (0.01)
8 min	0.11 (0.01)	0.11 (0.01)

Conclusion. Irradiation of MB solution promoted loss of absorbance and fluconazole association did not alter this characteristic. Fluconazole did not affect ROS generated following methylene blue irradiation.

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Efeito da terapia fotodinâmica na periodontite apical em modelo experimental de doença pulmonar obstrutiva crônica (DPOC)

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Background: There is a strong association between chronic lung diseases and plaque index of patients and correlation between loss of periodontal insertion and reduction of lung function. In this way, several experimental models have been proposed to know the interrelationship of the immunological/inflammatory processes of pulmonary diseases and periodontitis, as well as the discovery of new therapeutic options. However, there are still few studies that relate endodontics and pulmonary diseases.

Aim: This study aims to evaluate the effect of low intensity laser therapy (LBI) associated with endodontic treatment on asymptomatic apical periodontitis in an experimental model of COPD.

Methods: C57BL/6 mice will be induced for COPD to orotracheal aspirates of cigarette smoke extract for 60 days (2 times/week). The extract of cigarette smoke will be obtained by diluting three cigarettes in 3 ml of PBS. The PAA will be induced by opening the pulp chamber with a LN # ½ drill under general anaesthesia and removing all material from the interior with the aid of an endodontic file of # 8 and 10 and irrigation with 1 ml of NaOCl at 2, 5% and leave it open to the mouth. After 21 days of PAA induction, endodontic treatment with root canal preparation with endodontic files of # 10 and 20 and irrigation with 1 ml of 2.5% NaOCl, intracanal medication with Ca (OH) 2, PDT and sealing the teeth with photopolymerizable resin. The PDT will be performed with the application of methylene blue in 0.01% purified water, with a non-bevelled needle with silicone stop and starting from the apical portion to the coronary within the root canals, waiting for 3 minutes for FS action. The irradiations will be performed with the red laser diode (= 660 nm) with output power of 40 mW (MM Optics Twin Laser,). The laser will be conducted to the interior of the root canal through a probe (optical fiber) for 60 seconds. The application will be performed with shuttle movements inside the channel. The animals will be divided into groups: G1 (n=5) Basal; G2 (n=5) Induction of asymptomatic apical periodontitis (PAA) without endodontic treatment (ET); G3 (n=5) NAIVE; PAA + TE; G4 (n=5) PAA + TE + Photodynamic therapy (PDT); G5 (n=8) COPD G6 (n=8) COPD + PAA; G7 (n=8) COPD + PAA + TE; G8 (n=8) - Experimental COPD + PAA + TE + PDT. After 7 days of endodontic treatment the animals will be euthanized for morphological analysis of the lung and the mandible. The cytokines IL-4, IL-5, IL-6, IL-10, IL-13, IL-1β, IFN-γ, TNF-α and mucus production will be evaluated. Total and differential counts of inflammatory cells in BAL and serum will be performed. The frequency of inflammatory cells will be assessed by flow cytometry.

Funding. Universidade Nove de Julho.

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EFFECTS OF RED AND INFRARED LASER TREATMENT ON MUSCLE REPAIR PROCESS AFTER ACUTE LESION

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Biological alterations that take place in the musculature after a lesion follow a series of phases comprised of the destruction of tissue integrity, cell proliferation and remodeling. Throughout these phases, some immune system cells exhibit specific functions, such as macrophages. Interleukin-6 (IL-6), a pro-inflammatory cytokine mainly produced by M1 macrophages, is responsible for leukocyte recruitment to the lesion area (monocytes and lymphocytes), killer T cells activation and stimulation of cell proliferation. However, a detrimental increase of this cytokine can damage the tissue. Photobiomodulation (PBM) has been extensively studied in tissue repair framework, but there is still the need to research the best dosimetric parameters for this therapy in the muscle lesion process and to understand its role in relation to the main biological mediators of this process, such as IL-6.

Objective. Compare the effect of PBM using red and infrared laser on the IL-6 gene expression in acute muscle lesions.

Methodology. Wistar rats were divided in 4 groups: control (without lesion, without treatment), muscle lesion without treatment, muscle lesion treated with PBM (660nm - 70mW; 1 J/point, 8 points), muscle lesion treated with PBM (780nm - 70mW; 1 J/point, 8 points). Evaluation was performed at 2, 4 and 7 days after treatment. Following animal euthanasia, the tibialis anterior muscle was removed and processed for analysis by qPCR method.

Results. Two days after treatment, there was an increase in IL-6 gene expression in lesion groups compared to the groups without lesion. In contrast, the groups submitted to treatment with PBM of 660 nm presented a decrease in IL-6 gene expression in relation to lesion groups without treatment in this same period. Four days after treatment, there was an increase in this gene expression in the groups submitted to treatment with photobiomodulation of 780nm compared to the lesion groups and lesion + PBM with 660nm groups.

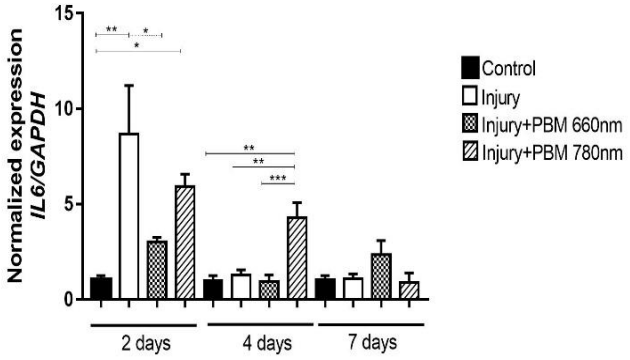

Fig. 1. IL-6 gene expression in muscle lesion model after 2, 4 and 7 days period of photobiomodulation treatment. p<0.05*, 0.01**, 0.001***.

Conclusion. The treatment with PBM of 660nm was caused a decrease in IL-6 gene expression 2 days after muscle lesion. However, there was observed an increase in the expression of this gene after infrared treatment of 780nm in the period of 4 days after the treatment. These results may help the understanding of the mechanisms of this therapy in different lesions and repair types.

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Effect of infrared laser photobiomodulation (780nm) on the quantity of myonuclei and blood vessels during compensatory hypertrophy

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Introduction: The satellite cells (CS) assist in the regeneration of muscle fiber by its capacity for proliferation and differentiation. However, in compensatory hypertrophy there are controversies regarding the participation of these cells in this process. The evaluation of the quantity of myonuclei and blood vessels may contribute to a better understanding of remodeling of this tissue in hypertrophy.

Objective: To analyze the quantity of myonuclei and blood vessels during the process of compensatory hypertrophy

Method: Wistar rats weighing 242.5g ± 13.59 were submitted to bilateral ablation surgery of the synergistic muscles of the hind paws preserving the m. Plant that suffered the overload. The animals were divided into three groups: control, hypertrophy group without irradiation (H) and hypertrophy group and irradiation in the left plantar muscle (H + LLLT), both analyzed after 7 and 14 days. The irradiation was applied to the left hind paw immediately after surgery and only one daily application. The irradiation parameters were: λ = 780nm; 40mW, 10J / cm2, 10s per point -8 points with total energy of 3.2 J. For the analysis of myonuclei and blood vessels the sections were stained with HE (hematoxylin-eosin). The ImageJ program was used for the quantification of myonuclei and blood vessels.

Results and discussion: it was observed (Fig. 1A) an increase in the number of myonuclei in the H and H + LLLT groups at 7 and 14 days when compared to the control group. There was an increase in the number of myonuclei in the H + LLLT group when compared to the H group in 7 and 14 days. Nakano et al. (2009) also found an increase in the number of myonuclei after laser irradiation (830nm) after muscular atrophy. Bruusgaard et al. (2010) also demonstrated an increase in the number of myonuclei in their studies with muscle hypertrophy but without laser irradiation. In relation to the blood vessels (Fig. 1B) there was an increase in the number of vessels in the H and H + LLLT groups at 7 and 14 days when compared to the control group. When compared to each other there was an increase in the number of vessels in the H + LLLT group at 7 and 14 days in relation to the H group, being in agreement with the findings of Nakano et al. (2009).

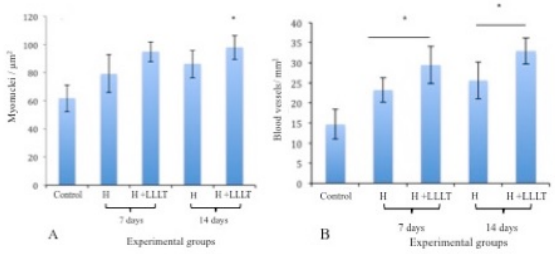

Fig.1 (A) Quantification of the number of myonuclei. Values expressed as mean and standard deviation * p <0.05. (B) Quantification of the number of mature vessels (objective 10x, counted in 5 fields). Values expressed as mean and standard deviation for (*) p <0.05.

Conclusion: Infrared laser irradiation (780nm) induced an increase in the number of myonuclei and blood vessels after 7 and 14 days.

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Aknowlegements: Capes and UNIN OVE

EFFECT OF INTRAVASCULAR LASER IRRADIATION OF BLOOD (ILIB) ON HEMODYNAMIC VARIABLES IN HYPERTENSIVES PATIENTS: STUDY PROTOCOL FOR A DOUBLE-BLIND RANDOMIZED TRIAL

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Background: High Blood Pressure is a multifactorial condition with high morbidity and low control rates. Currently, its treatment is based on a combination of non-pharmacological and pharmacological treatment. As nondherence to tratament is identified as a main cause of uncontrolled arterial hypertension, which is a significant risk factor for cardiovascular events, it´s necessary to look for methods that facilitate this process in order to promote improvement in the quality of life of hypertensive patients. Studies have shown that the photobiomodulation (PBM) is capable of inducing a photobiological response within the cells that modifies the micro and macrovascular response, in addition to this, evidences showing systemic effects on intravascular laser irradiation of blood (ILIB). In the hypothesis that PBM may influence blood pressure levels, and perhaps facilitate adherence to treatment, the present study proposes a clinical research protocol to evaluate the effects of PBM on hemodynamic parameters of normotensive and hypertensive patients attending a university outpatient clinic, who will undergo ILIB sessions.

Methods/design: Patients attending the Integrated Health Outpatient Clinic of the Nove de Julho University, located in the city of São Paulo, Brazil, will be invited to participate in the study. A specific clinical record will be filled with data characterizing the sample, cardiovascular risk, blood pressure measurements and heart rate. The sample will be composed of 30 patients, 10 normotensive and 20 hypertensive patients, who will be randomized and divided into 2 groups: "Hypertensive 15" and "Hypertensive 30" and in all groups we will have randomization of patients that will be part of the intervention group, or of the placebo group. Patients in the intervention group of both groups will be submitted to PBM in the form of ILIB with the use of a specific bracelet for application in the radial artery region at the frequency of 1 application every 2 days completing a cycle in 10 days which should be repeated after 20 days. Blood pressure and Heart rate will be assessed before and after each session. The evaluators to carry out each phase of the study will be different and without previous contact, as well as the analysis of the results will be done by a researcher who will not participate in the procedure of data collection. After this, they will be grouped into categories using Microsoft Office Excel 2007. Data will be analyzed through descriptive and inferential statistics and compiled into tables and / or charts, with the SPSS software version 24.0. The ANOVA test with repeated measurements will be performed to test differences between groups and a paired t-test will be performed to test differences within the group. The level of significance for the statistical analysis will be set at 5%.

Acknowledgment: We thank the Nove de Julho University.

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PHOTOBIOMODULATION ASSOCIATED WITH CARBON BIOMATERIAL MAY IMPROVE THE BONE HEALING PROCESS

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Key-words: Photobiomodulation; LLLT, Activated carbon, Mechanical properties, Bone repair.

Introduction: Bone diseases such as fractures and bone defects may result from several reasons, where the repair process is normally long and painful. The most used therapies are based either on the implantation of a biocompatible prosthesis or through the insertion of a biomaterial in the local injury. However, those treatments involve extended and costly surgical intervention. Thus, the association of two low cost techniques such as the use of activated carbon fiber (ACF) as bone biosubstitute and the application of the low-level laser therapy in order to assist the bone repair can be an alternative to overcome those problems.

Material and Methods: The study was performed by induction of a bone defect in rat tibias and their subsequent treatment with ACF and laser therapy. Five different groups of rats were studied: control (CTL), untreated Injury (NT), Injured treated with activated carbon fiber felt (ACF), Injured treated with lasertherapy (L6J) and Injured treated with association of ACFF and laser therapy (ACF+L). All groups were evaluated by histological and biomechanical properties of bone after the healing process and by phosphatase alkaline level (ALP).

Results: The NT group presented lowest values of stress at break, besides histological changes related to disorganization of the tissue. Gradually, the groups L6J, ACF and ACF+L showed to improve their mechanical properties in comparison to CTL group. The group ACF+L presented the highest value of stress at break, organized histological aspects and increasing the levels of ALP.

Conclusion: Thus, the association of low level laser and activate carbon fiber seemed to assist the process of bone healing in experimental model in rats tibia.

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SLEEP BRUXISM: MORPHOGIAL AND PSYCHOSOCIAL EVALUATION AFTER THE PHOTOBIOMODULATION THERAPY IN CHILDREN AND ADOLESCENTS.

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INTRODUCTION

Sleep bruxism(SB) is characterized by mandibular movements, such as involuntary clenching or grinding of teeth, and it is been commonly found in children. Respiratory and stomach problems have been associated with SB, although psychological issues, as anxiety has been associated too (1). An alternative to treatment can be photobiomodulation therapy. It becomes interesting because it is not invasive and has been showing good results in problems related to muscular tissues.

AIM

This study aim to evaluate morphological and psychosocial aspects present in children and adolescents diagnosed with sleep bruxism and their response to photobiomodulation therapy.

MATERIAL AND METHODS

This project will be evaluated by the Research Ethics Committee of Universidade Nove de Julho.

Sample

The sample will be composed by children and adolescents from 6-15 years old, divided in: Group 1-without SB; Group 2 - With SB, treated with red LED; Group 3 - With SB, treated with infrared LED; Group 4 - With SB, treated with occlusal splint. They must be enrolled in the dentistry clinic of the University of Nove de Julho. Volunteers will be excluded if they use drugs, such as anti-inflammatory, myo-relaxants, corticosteroids, anticonvulsants, antidepressants, chronic diseases of muscular character or that compromise the patient's motor coordination. The calculation of the sample will be based on a previous pilot study.

Sleep bruxism diagnoses

The diagnosis will be based on the parents' report of grinding and clenching teeth and presence of wear facets in permanent dentition.

Morphological aspects

- Bite Force

The maximum bite force will be measured by a digital gnatodynamometer for unilateral measurement.

-Eletromyography

The masseter and temporal muscles will be evaluated with a portable electromyography. The signal capture will be 10 seconds for each movement, and in chewing.

Psychosocial aspects

-Anxiety will be evaluated with Staic questionary, property to age.

-Salivar cortisol e dopamine levels will be measuared with an enimaimunoassay kit by ELISA.

-Salary will be ask to parents by an anamnesis.

Photobiomodulation therapy

Group 2 will be submitted to initial/final analysis of the morpho-psychosocial variables in red LED therapy, with dimensions 3cm X 6cm, using a plate containing 6 LEDs with a wavelength of 650 nm +/- 20 nm, 7-minute operation, optical spot diameter of 5mm +/- 2mm and optical output of 2 ~ 5mW, with a dose of 2.675 J / cm2. Group 3 will use an infrared LED (wavelength 850nm +/- 20 nm, dose = 2.675 J / cm2.

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Xanthene dyes combination for photodynamic optimization on *Streptococcus mutans* suspensions

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Background: Antimicrobial photodynamic therapy (ADT) is an adjunct alternative treatment for dental caries. In order to enhance the action of this method, the association of dyes could be a feasible option in this field. **Aim:** The objective of this study was to evaluate the potential of the combination of rose bengal (RB) and erythrosine (ER) dyes on the planktonic suspension of *Streptococcus mutans*. **Material and methods:** A light dose (96J / cm^2) (L) was tested in a short period (40 s) by a high power photopolymerizer with the dyes combination (RB = 1.5 μM and ER = 3.5 μM) to an adjusted inoculum of bacteria. Isolated photodynamic effect of ER (ADT ER = ER+L-) RB (ADT RB = RB+L-) and combinated dyes (ADT RB+ER+L+); light isolated effect (RB-ER-L+); dyes isolated effect (RB+L-; ER+L-; RB+ER+L-) and control group (RB-ER-L-) served as all studied groups. Aliquots of each group was submitted to bacterial count after 48 hours and data submitted to ANOVA test followed by Turkey-test with significance level at 5%. **Results:** It was verified that the photodynamic groups presented bacterial reduction; however, the only group associated with two dyes at low concentrations presented bacterial eradication and a statistically significant difference in relation to the other groups ($p < 0.05$), confirming a higher bactericidal potential when in combination. Other groups demonstrated no significant bacterial reduction in comparison to control group. **Conclusion:** The association of xanthene dyes is a viable possibility for the *in vitro* control of cariogenic bacteria.

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Effects of photobiomodulation in pain after tendinitis induction by collagenase in rats

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Keywords: Photobiomodulation, LLLT, Tendinitis, Tendinophaty, Pain, Neurokinin, COX-2.

Introduction: Tendinopathies are alterations in tendon health, where the most commonly used treatment is the pharmacological treatment for pain relief with unsatisfactory results due to its side effects in prolonged use. The search for non-pharmacological therapies in the treatment of these diseases assumes a prominent role in the medical area. Low level laser therapy (LLLT) have being a promising therapy in the modulation of acute and chronic inflammation with no adverse effects.

Objective: The objective of this study was to study the effect of LLLT on the gene expression of COX-2, neurokinin 1 and on the improvement of functional parameters.

Material & Methods: Male Wistar rats weighing between 150 and 200g were used, from the UNINOVE Animal Ethics Committee AN0037. The animals were separated into 4 groups: Control, with healthy tendon (CTL), untreated tendinitis (NT) and Tendinitis treated with Sodiun Diclofenac (DIC) or LLLT (L3J) in the following irradiation parameters : (Laser CW, 830nm, 107.14J / cm2, 3J, 100mW and beam diameter = 0.028cm). In order to induce tendinitis, the animals of the NT, DIC and L3J groups were anesthetized and received transcutaneous injection of collagenase (100µg / animal) in the posterior region of the paw in the calcaneus tendon. The treatments started immediately after induction of tendinitis and continued daily until the 7th day. The animal paw compression test (Randall & Selitto) was performed until the 7th day and then the animals were euthanized and the tendon was removed for analysis.

Results and Discussion: Animals of the NT group showed increased expression of COX-2 and NK1 (223.260100 ± 10.692710 UA) compared to the CTL group (100 ± 4.0251 UA). Both DIC and L3J groups presented a reduction in the expression of COX-2 and NK1 (88.656130 ± 11.444890UA and 56.871650 ± 32.277470UA), respectively, compared to the NT group. The L3J group presented improvement in the functional parameters of the paw compression (1.56667 ± 6.908208g), with similar values to the control group (-0.8 ± 3.051119g) when compared to the NT group (-24.04 ± 12 , 89178g).

Conclusion: LLLT modulated the expression of the COX-2 inflammatory enzyme by controlling the painful process by reducing neurokinin expression and improving certain functional parameters.

Acknowledgment. We thank the University of Nove de Julho for support.

Funding: CNPq process: 426903 / 2016-1

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Effects of low-level laser therapy on the bone healing process in rats under the influence of chronic alcohol intake

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Abstract: Chronic alcohol consumption is associated with prolonged hospital stays, high number of referrals to intensive care units and harm in the tissue repair process. Excessive chronic alcohol consumption decreases bone formation through a direct toxic effect on the function of osteoblasts, besides changing the signaling pathways related to the inflammatory response. Thus, it is necessary to investigate the treatments wich present an osteogenic potential and are able to accelerate the process of bone repair in alcoholics. There are evidence that Low-level laser therapy (LLLT) can stimulate bone cells and accelerate the repair process, but there are no studies that support the implementation of this promising therapeutic intervention in the bone tissue of experimental models of alcoholics. The present study aimed to evaluate the effects of LLLT in the bone repair process in rats undergoing chronic excessive alcohol intake. For this, thirty *Wistar* rats were submitted to a surgical procedure to perform bone defect and distributed into three groups: control (n = 10), alcohol (n = 10) and alcohol + LLLT (n = 10). Each group was divided into two groups of 5 animals each, for analysis 7 days post-lesion and another 14 days post-lesion. Alcohol administration was held by a bottle alcohol (20% solution), 3 days a week for 45 days. The two groups (n = 20) undergoing the oral administration protocol alcohol had a blood sample collected at the end of the experiment to check the serum levels of ethanol. Histological, morphometric and immunohistochemistry were performed. In the histological and morphometric evaluation the laser-treated group showed a better histological pattern and a higher amount of newly formed bone compared to alcohol group. An intense RUNX2 immunoexpression was observed in the laser-treated group, 7 days after the surgery. There was no statistical difference in the morphometric analysis among the groups. In conclusion, LLLT, showed an osteogenic potential in bone healing of alcoholic rats also.

Keywords: Alcohol, bone tissue, low-level laser therapy, photobiomodulation. Acknowledgments: We thank the Brazilian funding agency FAPESP for the financial support of this research.

Randomized controlled clinical trial oh halitosis treatment with photodynamic therapy in bronchiectasis patients

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Introduction: Bronchiectasis (BQT) is a chronic lung disease in which irreversible and permanent abnormal dilatation of the bronchi and bronchioles occurs. The etiology of BQT is often idiopathic and the most frequent extrinsic etiologic factor is the aspiration of infected mucus. The pulmonary lobe BQT is full of secretion, producing fetid sputum and consequently generated halitosis, which until now has not been studied. Halitosis is characterized as an unpleasant odor being a sign or symptom of imbalance. The objective of this study is to reduce bacterial load by treating halitosis to reduce pulmonary exacerbations.

Method: This is a case-control clinical study where 24 patients were evaluated: 12 BQT patients in treatment at the Universidade de São Paulo General Hospital – São Paulo, Brazil, and 12 healthy patients, matched in age and gender, for the control group. For all patients, halitosis was assessed with Oralchroma™. Individuals with halitosis underwent treatment with tongue scraping and aPDT. The photosensitizer was methylene blue (0.005%) with the laser THERAPY XT-EC® (660nm, 9J, 100mW for 90 seconds per point, 320J/cm^2, 3537mW/cm^2). Six points in the tongue dorsum were irradiated with a distance of 1cm between them. No parameter was correlated with halitosis. Data were not normal and the Mann Whitney test was used. Baseline halothosis data were compared after PDT and after periodontal treatment. The p value <0.05 was considered statistically significant. The results of this study demonstrated that the application of photodynamic therapy to the back of the tongue was significant in healthy patients when compared to the bronchiectasis patients, reducing the presence of volatile sulfur compounds. In the bronchiectasis population, PDT was not able to cause immediate reduction of all CVS in bronchiectasis patients.
Clinical Trials Number: NCT02514226.

Figures.

Fig.1.a Fig.1.b

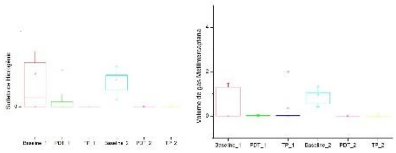

Fig. 1. (a) Comparison of the initial SH2 levels between groups, (b) Methylmercaptan levels before and after treatment in both groups.

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Effect of photobiomodulation and its association with pharmacological therapy in an experimental model of chronic asthma

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Asthma is characterized by chronic inflammation of the airways, reversible airflow obstruction, and airway hyperresponsiveness. Currently the most efficient treatment is the use of corticosteroids as Fluticasone (FT)[1]. Photobiomodulation (PBM) is relatively new, with no side effects and demonstrates effectiveness in reducing inflammatory parameters[2]. The aim of this studt was evaluate the effects of PBM and its combination with FT in an experimental model of chronic pulmonary allergic inflammation.

Seventy Balb/C mice were divided into 7 groups: control, FT, PBM, OVA, OVA+PBM, OVA+FT and OVA+PBM+FT. We induced inflammation by sensitization with ovalbumin - OVA (day 0 and day 14) and orotracheal challenge on the 21th day (3 days/week for 5 weeks). We treated with FT (100 µg/kg - intranasal) one hour before challenge with OVA, and one hour after the challenge we applied diode laser (660nm, 30mW and 3J/cm^2) at three points: below the trachea and one point in each lung lobe. Twenty-four hours after the last treatment, the animals were anesthetized for collection of bronchoalveolar lavage (BAL) and lungs. Functional and structural parameters such as total and differential cell count, cytokine levels in BAL, mucus production, collagen deposition and pulmonary mechanics were analyzed. The data were submitted to the One-way ANOVA test followed by the Newman-Keuls test. Significance levels adjusted to 5% ($p<0.05$).

We observed a reduction in the total number of cells in BAL ($p<0.001$). There was a significant decrease in macrophages ($p<0.05$) with best result in OVA+PBM ($p<0.001$), lymphocytes ($p<0.05$), with greater reduction in the groups associated to the laser ($p<0.001$), neutrophils and eosinophils ($p<0.001$). Reduction in the production of proinflammatory cytokines IL4, IL5, IL-1β, TNF-α ($p<0.001$) and IL13 ($p<0.01$) was observed in the groups treated with laser ($p<0.001$). Also an increased level of IL-10 ($p<0.01$) in the groups treated with FT ($p<0.001$). There was reduction in collagen fiber deposition and mucus production in the airways in all the treated groups ($p<0.001$). In the evaluation of pulmonary mechanics, the OVA+PBM group presented a better result, with a significant decrease in the respiratory and pulmonary systems ($p <0.001$).

Due to the high rates of systemic side effects caused by corticosteroid uses, it is importante to develop new anti-inflammatory therapies with immunomodulatory activity as an alternative to treat asthma [1]. Studies have shown that PBM provides an improvement in bronchial asthma [2,3].

Our results demonstrated that PBM was effective in modulate inflamatory parameters and improves pulmonary mechanics compared with conventional treatment.

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Acknowledgment. We thank the University Nove de Julho for the scholarship.

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PHOTOBIOMODULATION IN THE CLOSURE OF ULCERS AFTER MINOR AMPUTATIONS IN PATIENTS WITH DABETES MELLITUS

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Introduction: The International Diabetes Federation estimates that there are 415 million people with diabetes mellitus (DM) worldwide. One of the most common complications of DM is non-traumatic lower limb amputation (NTLLA). In Brazil, data from the Ministry of Health (2016) show that 80% of NTLLA are due to the complications of DM. Since the 90's positive results have been reported in the use of photobiomodulation in wound healing, which can minimize such amputations if the wound do not became a complication.

Objective: To verify the effects of photobiomodulation on the healing of diabetic foot ulcers.

Material and Methods: This is a case report, patient SSS, 54 years old, male, with a diagnosis of type 2 DM - non-insulin dependent and systemic arterial hypertension, with diabetic foot history 7 years ago, late postoperative open transmetatarsal amputation on the right foot (2010), with full healing in more than one year. In February 2017 he was hospitalized for hallux amputation with left foot fasciotomy (necrosis and infection); on March 21 was made transmetatarsal amputation on his left foot due to infection progression. Started laser treatment at the immediate postoperative. The treatment consisted of wound cleaning, followed by laser irradiation (Therapy XT, DMC, Brazil, λ=660nm, P=108mW, I=2.7W/cm2, H=108J/cm2, E=4.32J, t=40s, A=0.04cm2) once a week, with application mode in contact on the edges of the entire ulcer, followed by occlusive dressing (Membracel®, Brazil), surgical sponges and crepe bandage.

Keywords: Wound; diabetic foot; low-level laser therapy; area; temperature; glycemia

Results: There was a total closure of the lesion after 8 applications whose initial area it was 44.281cm² and an initial perimeter of 42,417cm. The temperature of the lesion was reduced from 31.9°C to 30.3°C and absence of the pain was reported. The Visual Numerical Pain Scale was used and the use of oral analgesics was reduced after the second application. During treatment, capillary glycemia **was monitored,** with results of around 200mg/dl and initial CRP of 15.9mg/dl to 0.8mg/dl.

Conclusion: It was possible to verify that the photobiomodulation promoted a better local analgesia and allowed complete closure of the ulcer in 8 weeks, unlike the previous amputation of the patient, which fully healed in more than 1 year when treated conventionally. More patients are being monitored with a randomized and controlled clinical trial about healing process after amputations in diabetic patients.

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3. Ventura MR; Gomes MT; Perez ST; Veloza MCG; Silva DFT; França CM; EFEITOS DA FOTOBIOMODULAÇÃO EM CICATRIZAÇÃO DE ÚLCERA PÓS AMPUTAÇÃO PARCIAL DE DEDOS DO PÉ ESQUERDO EM PACIENTE PORTADOR DE DIABETES MELLITUS TIPO 2: RELATO DE CASO

Effects of photobiomodulation in the osteoclastogenic properties of cells derived from oral squamous cell carcinoma (SCC)

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Photobiomodulation (PBM) has been used in mucositis treatment, a common disease associated with the treatment of oral squamous cell carcinoma (OSCC). However, there is still controversy regarding the effects of PBM in tumor cells that may have remained after surgery and/or radio and chemotherapy. Additionally, oral squamous cell carcinoma is an invasive tumour that can infiltrate bone by the modulation of genes related to the osteoclastogenic potential.

Objective. To **e**valuate the effect of PBM with recommended parameters to treat mucositis in the mRNA expression levels of *IL-11* and *PTHrP* genes in the oral squamous cell carcinoma cell line (OSCC) SCC9. both related with the osteoclastogenic potential of cells,

Materials and Methods. Cells were cultured in DMEM/F12 supplemented with fetal bovine serum (10%) and hydrocortisone (400 ng/mL). For gene expression analyses, irradiated cells (InGaAlP diode laser 660nm-40mW-4J/cm^2 and GaAlAs diode 780nm-40mW-4J/cm^2 and 70mW-4J/cm^2, Twin-laser® MM Optics) and non-irradiated cells (control) were incubated for 9 days in 9cm culture dishes. After this period, total RNA was extracted and submitted to reverse transcription polymerase chain reaction (RTq-PCR).

Results. Cells treated with PBM in all parameters exhibited lower expression levels of *IL-11* gene when compared to non-irradiated cells. However, *PTHrP* gene expression was only decreased in irradiated cells with 780nm-70mW-4J/cm^2. PBM promoted an inhibitory effect in *IL-11* and *PTHrP* gene expression which can be associated with a decrease in the osteoclastogenic potential of the SCC9 cell line.

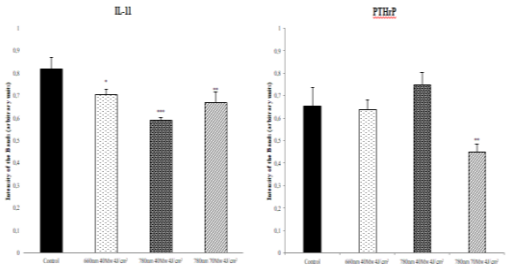

Fig. 1. Expression of *IL-11* and *PTHrP* genes in control (non-irradiated) and SCC9 cells treated with different parameters of PBM. Results are expressed in mean ± SD of two independent experiments in duplicate usingANOVA/Tukey ($p < 0.05$).

Conclusion. In the experimental conditions performed in this study, PBM was associated with a decrease in *IL-11* and *PTHrP* genes, which can be associated with a inhibitory effect of PBM in the invasion ability of OSCCand may be and proved to be a reliabletool for mucositis treatment.

Funding. Grant Fapesp number: 2013/07502-1

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Effects of photobiomodulation in the proliferation and migration of cells derived from oral squamous cell carcinoma (SCC)

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Photobiomodulation(PBM) has been used to prevent and treat mucositis, a common inflammation disease of the gastrointestinal tract associated with the treatment of oral squamous cell carcinoma (OSCC). However, the effects of PBM in the tumor cells that could be remained in the tissue after surgery and/or treatment with radio and chemotherapeutic remain controversial.

Objective. To analyze the effect of PBM (parameters recommended to treat mucositis) in the proliferation and migration capacity of the oral squamous cell carcinoma cell line SCC9.

Materials and Methods. Irradiated cells (Lasers InGaAlP diode 660nm-40mW-4J/cm² andGaAlAs diode 780nm-40mW-4J/cm² and 70mW-4J/cm²,Twin-laser® MM Optics) and non-irradiated cells were plated in culture slides with 8 wells and incubated for 1 and 3 days at the concentration of 2,12 x10^4cells/well. After this period, cells were submitted to the BrdU assay. Cellular migration was evaluated by the wound assay and irradiated and non-irradiated cells were plated in 6 wells plates at the concentration of 2 x 10^5cells/well (duplicate). After 3 days, the wound was made (cross shaped) with a 10µL tip. The plates were photographed until the wound area was completely filled by SCC9 cells. Measurement of the area was done with the software NIS - elements D 3.1 (Nikon, Tokyo, Japan) under inverted phase contrast microscopy.

Results. There is no significant difference in the number of positive cell to BrdU in the irradiated and non-irradiated SCC9 cells after the experimental periods of 1 and 3 days. Cell migration of non-irradiated cells began after 48 hours and concluded the complete closure of the region after 192 hours of culture. Cells irradiated with the 660nm laser showed higher migration ability when compared to control and laser 780nm. Cells irradiated with 780nm (70mW-4J/cm²) showed lower migration capability when compared to control and the wound was closed after 242 hours.

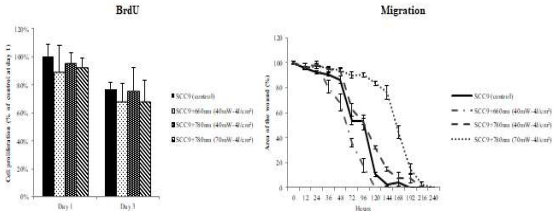

Fig. 1. **(a)** Percentage of BrdU positive cells. Results represent mean ± SD of two independent experiments. **(b)** Migration of SCC9 cell lines after 240 hours of culture. The results are the mean of the percentage remaining of the original wound area of each group after the evaluation periods. The results refer two independent experiments in duplicate (ANOVA/Tukey).

Conclusion. The application of the laser in the parameters recommended for mucositis under this conditions and periods did not increased the proliferation of the SCC9 cells and the PBM with 780nm-70mW-4J/cm² was able to decrease cell migration. The PBM in these experimental conditions proved to be a safe resource for the treatmentof mucosits.

Funding. Grant Fapesp number: 2013/07502-1

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3. J.A. Zecha et al., Support Care Cancer. 2781, 24 (2016).

Polarized laser versus uniform electric field in cell culture: wound assay

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Introduction: Electromagnetic fields activate multiple signaling pathways that are fundamental in cell polarization, migration, and wound healing[1]. Currently, photobiomodulation (PBM) is one of the non-invasive therapies and its benefits in wound healing are well reported in the literature. Many *in vivo* and *in vitro* studies about the PBM have as goal accelerates biochemicals reactions and collagen production[2].

Objective: To compare the effect of laser photobiomodulation with the electric field effect of the same intensity and direction in the culture of human dermal fibroblasts.

Method: Primary human fibroblasts isolated from a child's skin were cultured and maintained in exponential growth until they reached monolayer confluence on 18 x 18 mm sheets (Sigma-Aldrich, St. Louis, MO). A cell-free area (wound assay) with a thin point (p20) was created, and immediately after, the cells were divided into 5 groups: two were subjected to PBM (λ = 660nm, P = 50mW, A = 2.54 cm², I = 19.7 mW/cm², H = 2.0J/cm², t = 100s, E = 5J) polarized parallel or perpendicular to the wound; two were submitted to the parallel or perpendicular electric field; and the last one was a control group (without laser or electric field). The electric field was created with a device of metal plates parallel to each other, which was introduced in a Petri dish of diameter of 100mm. The blade with the cells was inserted for 100s between the metal plates, which were subjected to a potential difference of 10.1V. Milli-Q water was used as the dielectric medium, which allowed to create a uniform electric field of 2V/cm, which is very close to the value of the electric field of the polarized laser used in this experiment. After 18 hours, all groups were stained with Schiff Periodic Acid (PAS), for analysis of cell migration and ordering.

Results: The qualitative analysis of the images showed a greater ordering in the groups treated with laser in comparison to the groups treated with electric field. Migration was also higher in the laser groups, especially parallel to the wound. However, when compared to the control group, all groups presented greater alignment in relation to wound and greater migration, too.

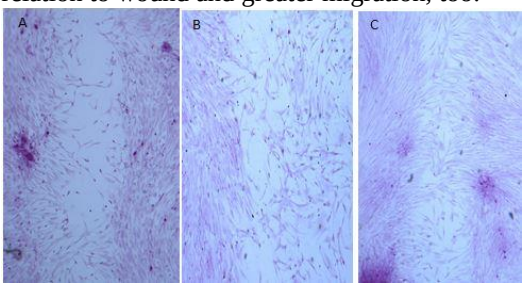

Fig. 1: Control (A); electric field parallel (B) and laser parallel (C).

Conclusion: Both the electric field and the polarized laser have effects on the ordering and migration of human dermal fibroblasts, but the parallel direction seems to be optimize such effects.

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Evaluation of endodontic treatment with photodynamic therapy (PDT) in pulmonary inflammation modulation in an experimental model of asthma.

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Background: In the last decades there has been an increase in the prevalence of allergic diseases in developed countries. The 'hygiene hypothesis' suggests that childhood infections may have a protective effect on the development of allergies. Thus, Th1-profile cytokines released during infection may inhibit the release of Th2-profile cytokines as in asthma. Bacteria are able to survive and maintain the periapical infectious process despite the therapeutic measures applied in well-conducted endodontic treatment cases. Photodynamic therapy (PDT) can be used to eliminate microorganisms present in the root canal system. **Objectives:** This study aims to evaluate the endodontic treatment associated with PDT in the modulation of pulmonary inflammation in an experimental model of asthma. **Methods:** Sixty - four, male Balb / C mice (± 25 grams) were divided into 8 groups (n = 8): 1. Basal, 2. Asthma (A), 3. Apical Periodontitis (PA), 4. PA + endodontic treatment TE + PDT, 6. A + PA, 7. A + PA + TE, 8. A + PA + TE + PDT. Apical periodontitis will be induced performing the pulpectomy of the left first molar (21 days). Asthama will be induced by ovalbumin (OVA) and aluminum hydroxide, subcutaneously (days 0 and 7) and nebulization with OVA (3 x / week, for 4 weeks). Endodontic treatment will be performed with calcium hydroxide and PDT with methylene blue (0.005%) associated with red diode laser (λ=660 nm, energy density 6, 369 J / cm, with 9 J at the point, delivered in 90s. In the 7th days, euthanasia will be performed for morphological analysis of the lung and the mandible. The total and differential counts of inflammatory cells will be evaluated in bronchoalveolar lavage (BAL) and serum. Cytokines IL-4, IL-5, IL-10, IFN-γ, TNF-γ, IL-1 and IL-6 in BAL supernatant and mucus production as well as collagen deposition in the airways will also be evaluated.

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Effects of photobiomodulation in the cancer stem cell properties of oral squamous cell carcinoma cells

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The oral squamous cell carcinoma (OSCC) represents 95% of the tumors of oral cavity. The treatment options for this malignancy are surgery, chemotherapy and/or radiotherapy. However, these treatments have several side effects, including mucositis, an inflammation on the mucosa of the digestive tract, which causes a lot of pain to the patient, compromising the continuity of the treatment. To minimize these effects and to prevent mucositis, photobiomodulation (PBM) has been used with promising results. However still controversy exists regarding the effects of PBM in the tumor cells that could be remained in the tissue after treatment. Recently, it was reported that oral squamous cell carcinoma (OSCC) presents a subpopulation of tumor initiating cells, called as cancer stem cells (CSC), responsible for tumor growth, metastasis and resistance to therapy.

Objective. The aim of this study was to investigate the effects of PBM in the CSC properties of OSCC cell lines (SCC9, CA1 and Luc4).

Materials and Methods. The cells were cultured in medium DMEM/F12 supplemented with fetal bovine serum (10%), hydrocortisone (400 ng/mL) and RM supplement. The cultures were irradiated with laser InGaAlP diode (660nm) and GaAlAs diode (780nm) (Twin-laser® MM Optics) and plated to clonogenic and sphere formation assay as well as to perform RT-qPCR to stem cell related genes. Non-irradiated cultures were used as control. Three independent experiments were performed in triplicate.

Results. There was no significant difference between the number of colonies and spheres formed in irradiated groups in relation to control. Additionally, *CD44*, *Bmi1* and *Oct4* mRNA expression levels were similar in both, irradiated and control groups, although a slightly decrease in gene expression was observed in PBM 780nm group.

Conclusions. PBM, in the experimental condition performed here was not associated with the modulation of the CSC properties and genes related to the maintenance of this cell subpopulation in OSCC.

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EFFECTS OF PERIODONTAL TREATMENT ON EXACERBATION FREQUENCY AND LUNG FUNCTION IN PATIENTS WITH CHRONIC PERIODONTITIS: STUDY PROTOCOL OF A 1-YEAR RANDOMIZED CONTROLLED TRIAL

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Background: Chronic obstructive pulmonary disease (COPD) has been associated with periodontal disease (PD), and periodontal treatment (PT) has been connected to reduction of lung disease exacerbations. Bronchiectasis has many clinical similarities with COPD but, although it is also a chronic lung disease, to date it has not been studied with relation to PD. The aim of this study is to evaluate whether PT associated with photodynamic therapy (PDT) reduces the number of exacerbations, improves pulmonary function, periodontal clinical parameters and quality of life after 1 year of periodontal treatment follow-up. **Methods**: Bronchiectasis patients will undergo medical anamnesis and periodontal examination. Participants with periodontitis will be divided into two groups and PT will be performed as G1 control group ($n = 32$) – OHO (oral hygiene orientation) + supragingival treatment + simulation of using photodynamic therapy (PDT); G2 experimental ($n = 32$) – scaling and root planing + PDT + OHO. Lung function will be assessed both at baseline and after 1 year by spirometry, exacerbation history will be analyzed through clinical records monitoring. Three instruments for quality of life assessment will also be applied – *Saint George's Respiratory Questionnaire* and Impact Profile Analysis Oral health (OHIP-14). It is expected that periodontal treatment can improve the analyzed parameters after 1 year.

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EVALUATION OF MUSCLE ACTIVITY, BITE FORCE AND SALIVARY CORTISOL IN CHILDREN WITH BRUXISM BEFORE AND AFTER LOW LEVEL LASER APPLIED TO ACUPOINTS: STUDY PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL

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INTRODUCTION

Sleep bruxism(SB) is characterized by mandibular movements, such as involuntary clenching or grinding of teeth (1). New treatments have been investigated by literature as an alternative. Photobiomodulation therapy is one of them(2).

AIM

The aim of this study is to evaluate the effects of low intensity lasertherapy in children with sleep bruxism.

MATERIAL AND METHODS

This project will be evaluated by the Research Ethics Committee of Universidade Nove de Julho.

The children will be randomly allocated to four groups, each with 19 participants: Group 1 will receive low-level laser therapy at acupuncture points; Group 2 will use an occlusal splint; Group 3 will receive placebo laser therapy; and Group 4 will be the control group without bruxism. Low-level laser (786.94 nm, 0.04 cm^2, 70 mW and 20 s per points) will be applied directly on the skin at six acupuncture points on each side. The BTS TMJOINT electromyograph will be used to determine muscle activity and a digital gnathodynamometer will be used to measure bite force. Two saliva collections will be performed in the participant's home at 9 am and prior to going to sleep, with at least one hour of fasting and followed by oral hygiene with water. Evaluations will be performed before as well as one and six months after treatment. The findings will be computed and submitted to statistical analysis. Descriptive statistics will be used first for the determination of point estimates, precision and validation using analysis of variance (ANOVA) for the evaluation of residuals and parametric tests will be used for the determination of pre-treatment conditions. Interval estimates will be used for the variables of interest to determine the prevision of the estimates and perform comparisons. When necessary, transformation methods or non-parametric tests will be used in the data analysis. The chi-square test, Student's t-test and ANOVA will be employed, with the level of significance set at 95% ($p < 0.05$).

Trial registration: The protocol for this study was registered with Clinical Trials number NCT02757261 on 8 april 2016.

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Biofotônica aplicada às Ciências da Saúde

USE OF LOW-LEVEL LASER THERAPY ON CHILDREN AGED ONE TO FIVE YEARS WITH ENERGY-PROTEIN MALNUTRITION

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The investigation of mechanisms that can reduce the impact of malnutrition on the defenses of the organism is of the utmost important and interest to public health. The objective of this research is to evaluate the effect of low-level laser on the saliva of children aged one to five years with energy-protein malnutrition.

Introduction. Episodes of malnutrition in early childhood, with consequent calcium, phosphate and vitamin A, C and D deficiencies, can increase one's susceptibility to dental caries through three probable mechanisms: defects in tooth formation, delayed tooth eruption and alterations in the salivary glands[1]. As saliva is the main defense factor of the oral cavity, a reduction/change in its physical properties can cause immunological disorders that affect an individual's defense capacity[2]. Studies have demonstrated that salivary immunoglobulin A (IgA) also plays an important role in the immunity of the oral mucosa[3]. Indeed, patients with IgA deficiency can experience recurring upper airway, lower airway and gastrointestinal infections[2,3]. With regard to an increase in salivary IgA, low-level laser intensifies the activation of B-lymphocytes, which differentiate into plasma cells, thereby contributing to the increase in immunoglobulin levels[3].

Objective. Evaluate the effect of low-level laser on the saliva of children aged one to five years with energy-protein malnutrition.

Methods. An experimental cross-sectional study is proposed, which will be conducted at the Center for Educational and Nutrition Recovery in the city of Maceió, state of Alagoas, Brazil, and University Nove de Julho in the city of Sao Paulo, Brazil. This project has been approved by CESMAC ethics committee (CAAE 71961317.1.0000.0039). The sample will consist of 50 male and female malnourished children aged 12 to 71 months. The nutritional reference standard for assessing children's nutritional status will be from the World Health Organization[4]. Saliva will be collected between 9 and 11 am using the drainage method. Salivary flow values will be analyzed as: Normal, Low and Hyposalivation[5]. Concentrations of salivary IgA in all samples will be measured using a commercial enzyme-linked immunosorbent assay (ELISA) and will be analyzed[6]. Low-level laser will be administered by contact in a continuous way using the Photon Lase III device (DMC Equipamentos LTDA®, São Paulo, Brazil). Laser (central wavelength=808nm; irradiated area=0.40 cm2; radiant energy=4J, beam spot on target=0.04cm2; multimodal beam profile; irradiation at aperture= 2500 mW/cm2; aperture diameter = 0.2cm) will be administered for 10 seconds on four intraoral points and four extraoral points in the region of the parotid glands bilaterally as well as one intraoral point and one extraoral point in the regions of the submandibular and sublingual glands. The first session will occur after the collection of saliva. The second and third sessions will be performed seven and 14 days after the first session, respectively. The final saliva collection will be performed after the third laser session.

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EFFECT OF LOW INTENSITY LASER ASSOCIATED WITH PHYSICAL TRAINING IN THE TREATMENT OF RATS WITH INDUCED MONOARTHRITIS

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Studies suggest that local and systemic inflammation causes autonomic changes in the development of monoarthritis. The release of pro inflammatory cytokines imply damage to the cardiovascular system. Non-invasive therapies such as low level Laser terapy (LLLT) and aerobic training (AT) seem promising treatment in these conditions.

AIM: Evaluate the association of non-pharmacological therapies of aerobic training and laser therapy on the inflammatory process and its influence on cardiovascular autonomic regulation on experimental model of monoarthritis. **METHOD:** Male Wistar rats (n=38) were divided into sedentary control (C=6); monoarthritis (MO=8); MO + LLLT (MOL=8), MO + AT (MOT=8) and MOT + LLLT (MOTL=8). MO was induced by intra-articular injection of zymosan (1mg/50 µL saline) into the right knee joint. AT treatment were done with with moderate AT treadmill for 8 weeks (5 days/week) and LLLT was applied at 660 nm (5mW power, power density of 0.1 W/cm2 power density of 2.5 J/cm2, 0.04 cm2 spot area and irradiation time 20 sec, twice a week). Functional capacity, arterial pressure (AP), heart rate (HR), variability pulse interval (VAR IP), low frequency band (LF) and morfomonucleares cells were measured. **RESULTS:** Trained rats showed increased functional capacity in the maximum stress test (MO, INICIAL 9.17±1.9 min and FINAL 8.19±1.3 min; MOTL, INICIAL 7.8±0.9 min and final 16.7±1.9 min) ($p<0.0001$). HR values (C, 343±25 bpm; MO, 386±29 bpm; MOL 346±30 bpm; MOT 324±15 bpm; MOTL 328±19 bpm) ($p<0.0382$) indicating an improvement of basal frequency. VAR IP (C, 90.9±31.7 ms; MO, 39.1±7.6 ms; MOL 33.1±12.7 ms; MOT 96.2±6.4 ms; MOTL 108.9±29.0 ms) ($p=0.0009$) indicating improvement in autonomic modulation. LF% (C, 13.60±1.3 %; MO, 20.8±3.5 %; MOL 12.5±1.1 %; MOT 16.1±1.6 %; MOTL 13.6±1.0 %) ($p=0.0674$) shows increased sympathetic activity. MN cells (C, 162.5±60 cellx10^4/ml; MO, 471.7±138 cellx10^4/ml; MOL, 109±22 cellx10^4/ml; MOT, 165.5±61 cellx10^4/ml; MOTL, 81.2±30 cellx10^4/ml) ($p<0.0068$) decrease in LLLT group. **CONCLUSION:** Acute monoarthritis caused sympathetic activity in MO, suggesting an early autonomic dysfunction. AT associated with LLLT had beneficial effects on functional capacity, autonomic modulation, cardiovascular conditioning and reducing inflammation, conditions that contribute to systemic health.

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Postoperative spine surgery applying lasertherapy could prevent complications?

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Background: Worldwide over 1 million patients were submitted to spine surgery every year, with a rate failure over 40%. Post-laminectomy epidural adhesion is implicated as a main cause of ''failed back surgery syndrome'' and complications´ risk. Postoperative epidural fibrosis is a foremost causative factor of low-back pain. The postoperative epidural scar can lead to recurrent radicular pain and physical impairment. The literature showed studies signalizing that Low-Level-Laser-Therapy (LLLT) is a successful tool to assist the inflammatory process, wound healing and dehiscence. The aim was to evaluate the photobiomodulation effects in spinal surgery.

Methodology: Clinical trial with 48 patients, who underwent to laminectomy, were divided into 2 groups: LLLT-on in 25 randomized patients, Power = 40mW, Fluence= 7.44 J/cm^2 was applied on the laminectomy site and surrounding on the cutaneous lesion. In the second group, 23 patients with LLLT were off. In those groups, pre and post LLLT (on or off) use in the 2nd and 5th days, a digital temperature and visual analogue scale were carried out. The drainage output were collected in the first and second days, following surgery in both groups.

Results: The results indicated temperature decrease, pain relief and accelerated wound healing in laser group and diminished drainage output. Lasertherapy facilitates the wound cicatrization, due to a prompt resolution while acute inflammation.

Conclusion: The LLLT applications promoted a better wound healing, reduced drainage output, diminish lesion inflammation and analgesia post spine surgery.

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Photobiomodulation in the chronic neuropathic pain refractory to clinical treatment

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Introduction

Injury to peripheral nerve injury can persist with neuropathic pain and "phantom pain" that is difficult to treat, since medications produce intense side effects, compromising physical therapy and quality of life. Adjuvant therapy with Photobiomodulation (PBM) may reduce the need for drug administration. The analgesic effects of PBM irradiation are well documented in several clinical and laboratory experiments. The aim was to evaluate the response of PBM in patients with chronic neuropathic pain refractory to conventional clinical treatment.

Methodology

A pilot study with 5 patients (22-49 years), diagnosis of traumatic injury on the hand, reporting long-lasting "shock" and "needle" pain, with no response during the use of potent analgesics, with high score in the the Visual Analogue Scale (VAS), resulting in impairment in daily living activities (DLA) (Barthel and Lawton scales). The patients were evaluated before and after each session, when they were submitted to the Diode Laser in the infrared, once a week.

Results

Among the PBM wavelengths for pain treatment, 830 nm seems to be the best indicated. High energy showed better results. In all cases, there was analgesia post PBM with significant improvement by the evaluation of VAS (Table 1 a) and DLA (Table 1 b), and were discharged without medications, with follow up of 12 months, denoting no return of symptoms.

(a)

Patient	Period of injury	Session	Medication	VAS Pre PBM	VAS Post PBM
P1	6 months	15	amitriptilina, carbamazepina clorpromazina	5	1
P2	1 year	3	amitriptilina, carbamazepina clorpromazina	6	3
P3	2 years	4	gabapentina, carbamazepinaa mitriptilina	7	0
P4	4 years	3	gabapentina, carbamazepina amitriptilina	8	0
P5	12 years	9	gabapentina, carbamazepina amitriptilina	8	0

(b)

Patient	BARTHEL PRE PBM	BARTHEL POST PBM	LAWTON PRE PBM	LAWTON POST PBM
P1	45	100	7	17
P2	45	90	7	17
P3	55	100	9	24
P4	55	100	9	24
P5	55	100	9	24

Table 1. Characteristics of injury (a) and Barthel and Lawton Scale – DLA (b).

Conclusion

PBM is able to reduce pain level in a non-invasive way, without side effects, besides improving the quality of life, proving to be a method of great applicability in this type of pathology.

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