



# *Proceedings of BIF*

Volume VI



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**Prefácio:**

Em 2021, diante do cenário desafiador imposto pela pandemia do COVID-19, o 7º Fórum Internacional de Biofotônica será realizado 100% online. Serão 10 h de atividades científicas divididas entre 2 dias de evento, com palestras curtas e devidamente ajustadas ao formato digital. Teremos 4 salas simultâneas: a principal, com palestras ministradas por renomados especialistas em biofotônica, a sala dos trabalhos aprovados para apresentação oral, a sala dos resumos, no qual os participantes poderão clicar e acessar todos os resumos submetidos e aprovados; e a sala dos expositores, em que nossos parceiros poderão apresentar os novos produtos e comercializá-los. Todas as salas terão moderadores de tão vasta experiência quanto os palestrantes. Nessa edição, também será realizado o 3º Encontro Latino-Americano de Biofotônica e o 2º Encontro BRICS de Biofotônica, com o objetivo de compartilhar experiências e discutir sobre novos meios de colaboração em toda a América Latina, Rússia, Índia, China e África do Sul. Também não podíamos esquecer de nossa missão humanitária em tempos tão difíceis: houve a opção de fazer uma doação ao "Amigos do Bem" a partir de nosso formulário de inscrição. Preparamos esse evento com muito carinho e dedicação.

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## 7º Fórum Internacional de Biofotônica (FIB)/ - 7<sup>th</sup> Biophotonics International Forum (BIF) 2021.

1

EFFECTS OF ANTIMICROBIAL PHOTODYNAMIC THERAPY ON ANTIBIOTIC RESISTANCE IN AN INVERTEBRATE ANIMAL MODEL.

Necchio BR (1), Garcez AS (1)

(1) São Leopoldo Mandic.

### **Abstract**

The increase in the number of bacterial strains resistant to antibiotics has been becoming a global public health problem and a search for alternative methods of microbial control, with photodynamic therapy (PDT) and a need in various areas of health. The objective of this work was to evaluate, on a resistant bacterial strain of *Escherichia coli*, the effect of a PDT pretreatment associated with antibiotics in a freshwater vertebrate animal model. Initially, *in vitro* antibiotic resistance of *E. coli* was determined through the halo of inhibition test and minimal inhibitory concentration to the following antibiotics: (penicillin) amoxicillin, amoxicillin + clavulanic acid, clindamycin and cephalexin. Antibiotic resistance will also be evaluated when the bacterial strain is previously treated with PDT using a LED emitting at 660 nm and with 100mW of power associated with a methylene blue photosensitizer. For an *in vivo* analysis, *Danio rerio* (zebra fish), better known as fish paulistinha, will be contaminated with a resistant strain of *E. coli*, and treated with antibiotics, PDT, a combination of PDT and antibiotics or with saline solution as a control. They will be evaluated as the fish wax curve over a period of 7 days to determine the effect of the combination therapy

**Key words:** Photodynamic Therapy, Antibiotics, Fish

**Study type:** Experimental study in animals, Experimental study *in vitro*



## 7º Fórum Internacional de Biofotônica (FIB)/ - 7<sup>th</sup> Biophotonics International Forum (BIF) 2021.

2

LOCAL PHOTOBIO-MODULATION, BUT NOT VASCULAR, REDUCES IL-4 AND INCREASES IL-10 IN ALLERGIC RHINITIS.

Schapochnik A (1), Klein S (1), Brochetti R (1), Alonso PT (1), Damazo AS (2), Destro MFS (1), Franco ALS (1).

- (1) Universidade Nove de Julho, Programa de Pós-graduação de Biofotônica aplicada às Ciências da Saúde;
- (2) Universidade Federal do Mato Grosso.

### Abstract

Allergic rhinitis (AR) is defined as inflammation and/or dysfunction of the nasal mucosa and is a worldwide health problem with an impact on patients' quality of life. Available treatments have considerable side effects and, in more severe cases, are not effective. Thus, Photobiomodulation (PMB) appears as an adjuvant therapy, with no adverse effects and has good results for several inflammatory diseases, including those of the respiratory tract. Considering that RA is not only a disorder located in the nose and nasal cavities and has a systemic component, this study aimed to compare the effects of vascular FBM (VPMB) in the caudal artery and local (LPMB) in the nostril on the development of RA. We use the LED device (Light Emitting Diode) from the Bio Lambda LED star brand, model Black Box Mini in the wavelength 660 nm, radiant power 160 mW, power density 38.5 mW / cm<sup>2</sup>, energy density 5.8 J/ cm<sup>2</sup> with Continuous Emission. For this purpose, adult male Wistar rats were submitted or not to RA by intradermal injection of Ovalbumin (OVA) plus aluminum hydroxide as an adjuvant dissolved in saline solution (from day 1 to 13). After immunization, nasal challenge was performed from day 14 to 21 through daily intranasal instillation of OVA. Rats treated with VPMB and LPMB were irradiated with a LED device in the tail artery and nostril for 3 consecutive days immediately after the OVA challenge. Our results showed that treatment with FBML, and not FBMV, reduced the level and gene expression of IL-4, increased IL-10, which contributed to improve RA symptoms. In this context, the proposed study provided subsidies to propose an effective therapeutic alternative for the treatment of AR with LPMB and not VPMB that does not present any additive effect that justifies its use.

Financial support: CNPq 305099/2017-5.

**Key words:** Allergic rhinitis, photobiomodulation, Light Emitting Diode, inflammation

**Study type:** Experimental study in animals



**7º Fórum Internacional de Biofotônica (FIB)/ - 7<sup>th</sup> Biophotonics International Forum (BIF) 2021.**

3

THE USE OF PHOTOBIO-MODULATION THERAPY BY BLOOD TRANSVASCULAR IRRADIATION IN AN INDIVIDUAL WITH COVID-19. CASE REPORT.

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**Abstract**

Due to the high impact of SARS-CoV-2 infection on the health of those affected and the absence so far of specific therapies, effective, non-invasive, prophylactic or even adjuvant methods should be among the priorities of the treatment of COVID-19, highlighting photobiomodulation therapy (TFBM). This scientific work aims to present a case report of a 58-year-old male patient JVF, smoker, with a diagnosis of Covid19 confirmed by RT-PCR, submitted to photobiomodulation therapy by LED light irradiation at a length of 660nm, about the sublingual vessels, during the hospital stay. Chest tomography showed more than 50% involvement of the lung parenchyma. The clinical evolution of respiratory impairment and routine laboratory tests at the of Itupeva's Municipal Hospital were evaluated. It was observed that the patient undergoing TFBM showed improvement in oxygen saturation in serial arterial blood gases, progressive reduction in the need for oxygen supplementation, and preservation of renal function. Thus, TFBM can present itself as an adjuvant therapy in the treatment of patients with covid-19, and further clinical studies are needed to prove its effectiveness in the treatment of Covid-19.

**Key words:** photobiomodulation therapy, Covid-19, low level laser therapy

**Study type:** Case report/Case series





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4

INFLUENCE OF THE CULTURE ON ANTIMICROBIAL THERAPY WITH BLUE LIGHT (ABLT) ON THE PERIODONTOPATHIC BACTERIA *AGGREGATIBACTER ACTINOMYCETENCOMITANS*.

Salviatto-Costa LT (1), Silva SRS (1), Prates RA (1), Deana AM (1)

(1) Universidade Nove de Julho, Programa de Pós-graduação de Biofotônica aplicada às Ciências da Saúde.

### Abstract

Periodontal disease (PD) is a chronic inflammatory disease caused by bacterial biofilm which is highly prevalent around the world. Antimicrobial therapy with blue light (aBLT) is based on the interaction of light with endogenous photosensitizers produced by microorganisms, such as metal-free porphyrins and flavins, in the generation of reactive oxygen species (ROS) and cell elimination. The goal of this work is to evaluate the potential of bacterial death by aBLT and the influence of BHI (brain heart infusion) culture media and blood agar on the death curve of the periodontopathic bacteria *Aggregatibacter actinomycetencomitans*. For this work, we use a violet LED emitting at  $403\text{nm} \pm 15$  with 1W of radiant power, the irradiance of  $588,2 \text{ mW} / \text{cm}^2$ , and irradiation duration of: 0, 1, 5, 10, 30 and 60 minutes in 2 different groups: *A. actinomycetencomitans* cultivated in BHI and *A. actinomycetencomitans* in blood agar. The plates were incubated in microaerophilia, in a bacteriological greenhouse, with a temperature regulated at  $37^\circ \text{C}$  during a period of 48h to count the colony-forming units (CFU / mL) and performed in triplicate. Spectroscopy and fluorescence microscopy were also carried out to investigate the presence of photosensitizers inside the microorganisms. Results: There was no statistical difference in the survival fraction of the colonies when *A. actinomycetencomitans* was cultivated in different culture media ( $p > 0,05$ ), however when the irradiation time reached 30 minutes ( $1.058 \text{ J/cm}^2$ ), a biological and statistical reduction of microorganisms in both media was observed ( $p < 0,05$ ). The absorption spectrum and the analysis of fluorescence microscopy give indications of the presence of endogenous porphyrins inside these bacteria, regardless of the culture media.

**Key words:** Antimicrobial Blue Light Therapy, endogenous photosensitizer, *Aggregatibacter actinomycetencomitans*

**Study type:** Experimental study in vitro



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5

PHOTOBIMODULATION THERAPY LED COMBINED WITH BIOMATERIAL AS A SCAFFOLD PROMOTES BETTER BONE QUALITY IN THE DENTAL ALVEOLUS IN AN EXPERIMENTAL EXTRACTION MODEL.

Dalapria V (1), Marcos RL (1), Silva ACA (1), Marinheiro N.R.S (1), Pinto R.S (1), Sales R.S (1), De França L.S (1), Bussadori, SK (1), Deana A.M (1)

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### Abstract

**Introduction:** The loss of the dental element causes deformity and bone atrophy, bone grafting immediately after tooth extraction will enable rehabilitation with implants to restore mastication and aesthetics. Biomaterials have ideal characteristics for use in bone regeneration strategies, as such materials serve as a scaffold for the growth of bone tissue, enabling the proliferation of blood vessels and the delivery of nutrients to cells in the interior of the graft. Photobiomodulation accelerates bone healing, activating osteoblasts, decreasing osteoclastic activity and improving the integration of the biomaterial with bone tissue. The aim of the present study was to evaluate the effect of photobiomodulation with LED  $\lambda = 850$  nm on the bone quality of Wistar rats submitted to molar extraction with and without bone graft with hydroxyapatite biomaterial (Straumann® Cerabone®). **Methods:** Forty-eight rats were divided into five groups ( $n = 12$ ): Baseline (no interventions); control (extraction) (basal and control were the same animal, on different sides); LED (extraction + LED  $\lambda = 850$  nm); biomaterial (extraction + biomaterial) and biomaterial + LED (extraction + biomaterial + LED  $\lambda = 850$  nm). Euthanasia occurred 15 and 30 days after extraction induction. **Results:** The ALP analysis showed improvement in bone formation in the control and biomaterial + LED groups in 15 days ( $p = 0.0086$  and  $p = 0.0379$ , Bonferroni). In addition, the LED group had better bone formation compared to the other groups at 30 days ( $p = 0.0007$ , Bonferroni). In the analysis of AcP, all groups had lower resorption compared to the baseline group. Bone volume increased in the biomaterial, biomaterial + LED and basal groups compared to the control group at 15 days ( $p < 0.05$ , t test). At 30 days, the basal group had greater volume compared to the control and LED groups ( $p < 0.05$ , t test). The LED combined with the biomaterial improved bone formation in the histological analysis and decreased bone degeneration, promoting an increase in bone density and volume. **Conclusion:** LED may be an important therapy to be combined with biomaterials to promote bone formation, along with other known benefits of this therapy, such as pain control and the inflammatory process. supporting and dissipating chewing forces for predicting the success of rehabilitation with implant and the primary stability of the implants.

**Key words:** LED; scaffold, Straumann; Bone density; Micro CT; Extraction; Photobiomodulation

**Study type:** Experimental study in animals



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6

RED LASER PHOTOBIO-MODULATION PROMOTES EARLY HOSPITAL DISCHARGE IN PATIENT WITH 85% OF THE BODY SURFACE BURNED: A CASE REPORT.

Silva VCC (1), Barros FC (1)

(1) Faculdade Inspirar.

### Abstract

**BACKGROUND:** Skin burns represent a public health problem, with high morbidity and mortality. Burns also generate psychological repercussions, both for the accident cause and unsightly scars left. The skin is the organ that provides the first protective barrier of the entire body, in addition to maintaining hydration and temperature control. Thus, both for protection and for aesthetic reasons, skin rehabilitation is essential in burn patients' treatment. The faster this barrier is re-established, the faster the patient will be close to full recovery, and the less sequelae s/he will have. **OBJECTIVE:** To report the case of a burn patient. **METHODOLOGY:** a 45 years old man suffered an accident caused by a cooking gas explosion on 04/28/2021. As a consequence, he had 85% of his body surface burned, with areas of second- and third-degree burns. He was admitted at the Adult Burn Treatment Center (CTQ-A) of the Souza Aguiar Municipal Hospital, in Rio de Janeiro. The patient received laser therapy on the entire burned area. The treatment was performed with the equipment Fluence (HTM), wavelength of 658 nm, power of 180 mW, scanning mode, two consecutive applications of 35J / cm<sup>2</sup> each (70J / cm<sup>2</sup>, total), twice a week by a physiotherapist, since his admission until hospital discharge. The patient also received all necessary medical care during the entire hospital stay. He was followed up through photographs and evaluated through the Lund Browder table. **RESULTS:** After 8 weeks, the patient had only 7% of the body area burned, without complete healing. He was discharged on 07/07/2021, only 70 days after his accident (10 weeks). On that date, skin recovery was complete (100%), with minimal sequelae. The patient had a complete range of motion of the entire upper limb, including the hands. Also, cutaneous attachments were reestablished, as the rapid growth of new hairs. During hospitalization, the patient was infected by COVID-19, but he also recovered very well. **CONCLUSION:** Considering the severity and extension of the patient burn, discharge was expected approximately 220 days after the accident (average hospital stay in CTQ-A), but the patient recovered in just 70 days. Therefore, we attribute this early discharge to the laser therapy used in the rehabilitation of this patient, which promoted not only fast, but also quality healing.

**Key words:** Phototherapy, Laser Therapy, LLLT, Physiotherapy, Dermatology.

**Study type:** Case report/Case series



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7

THE EFFECT OF INFRARED LED PREEMPTIVE FOTOBIMODULATION IN IMPACTED LOWER THIRD MOLAR TEETH SURGERY: CONTROLLED CLINICAL TRIALS, DOUBLE-BLIND, RANDOMIZED.

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**Abstract**

Introduction: The lower third molar teeth surgery is the last teeth to be born in the oral cavity, and its removal is indicated to prevent cysts and pericoronitis, especially in impacted cases. The pain, edema, and trismus are associated with surgery. Usually analgesics, anti-inflammatory, and physiotherapy are indicated. The photobiomodulation post-surgery is effective to reduce edema, trismus, and pain. Objective: The aim of this study is to evaluate the preemptive use of infrared LED on orofacial tissues to prevent pain, trismus, and edema. Methodology: This randomized, double-blind clinical trial, randomized, double-blind evaluated the impact of preconditioning the tissues involved on impacted lower third molar teeth surgery to prevent these unwanted effects. The participants were divided into two groups, and 1h before the surgery, the treated group received photobiomodulation with infrared LED 850nm, 8J, 80s, and the control group used a similar device without irradiation. On the second and seventh day after the surgery, the participants were evaluated and received the corresponding treatment. Results: After the second day, the treatment group demonstrated a significant pain reduction in relation to the placebo group ( $p = 0.006$ , Mann-Whitney), visual analogic scale value 0 and 2 responsive, there was no significant change in trismus. The treatment group showed on the seventh-day post-surgery facial measurements statistically equal to pre-surgical values (initial= 15,76cm e final= 15,84cm). Conclusion: This study demonstrated that the conditioning of the orofacial tissues involved in third molar surgeries using infrared LED with 850nm wavelength 8J, 80s, performed one hour before the surgical procedure, showed positive results in reducing postoperative pain.

**Key words:** third molar, preemptive photobiomodulation, impacted third molar, Infrared LED.

**Study type:** Clinical Trial



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8

TRANSCUTANEOUS LOW-POWER LASER IRRADIATION IN DOMESTIC ANIMALS – A REVIEW.

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- (2) Laboratory of Cell Biology and Tissue, São José dos Campos, SP, Brazil.

### **Abstract**

The ILIB (Intravascular Laser Irradiation of Blood) is a practice developed in humans extrapolated to animals. The aim of this review is to verify what is described in the literature about the clinical application of ILIB therapy in domestic animals. Methods Qualitative literature review in PubMed and Google Scholar databases, over a 10-year period, with the following descriptors: LLLT, laser therapy, veterinary, animals, ILIB, intravascular, percutaneous laser and blood. Only studies that addressed the main theme were considered. Results Among the different applications of light in the animal organism, ILIB therapy has been applied in the treatment of animals, aiming to obtain the same benefits that it promotes in the human organism. The results found in the literature review did not demonstrate the use of ILIB in animals for the treatment of pathologies in veterinary medicine. ILIB was initially studied in human cardiovascular diseases in the reduction of ischemic areas of myocardial infarction, improving the rheological properties of blood and microcirculation. The therapy has been shown to significantly increase the activation of the enzyme Superoxide Dismutase (SOD), promoting systemic homeostasis and, consequently, preventing disease. The animal body is expected to respond similarly to the human body to ILIB therapy. The researchers argue that the veterinarian must understand the fundamentals and physical properties of the laser in order to correctly define the dosage, the appropriate light release parameters and, thus, ensure therapeutic efficacy in different animal pathologies. The unavailability of data on the different species treated in veterinary medicine is highlighted here, such as: the most adequate extravascular bed for the application of this therapy; time required for exposure to blood flow; body score; circulating volume, thickness of the extract corneous, varied coats, in order to ensure the efficient delivery and absorption of light energy in the animal organism to obtain benefits similar to what occurs in the human organism. However, the lack of knowledge and training in the use of this therapy can lead to therapeutic errors and even biosafety accidents. Conclusions: the lack of scientific studies and recognized clinical trials on the use of ILIB therapy in animals shows the need for more studies to validate this technique in different animal species.

**Key words:** laser irradiation, intravascular, blood, percutaneous, modified ILIB, animals

**Study type:** Review



## 7º Fórum Internacional de Biofotônica (FIB)/ - 7<sup>th</sup> Biophotonics International Forum (BIF) 2021.

9

IS PHOTOBIMODULATION AN EFFECTIVE TREATMENT AGAINST RHYTIDS? - A SYSTEMATIC REVIEW.

Sena MM (1), Roque MB (1), Fabretti YSV (3), dos-Santos CC (1), Pereira L (1), Raimundo JS (1), Alves TVO (1), Pavani C (1)

(1) Universidade Nove de Julho, Programa de Pós-Graduação em Biofotônica Aplicada às Ciências da Saúde

### **Abstract**

**INTRODUCTION:** The formation of rhytids is part of the skin aging process. This process includes the reduced production and increased degradation of collagen and elastic fibers in the dermis. Although unavoidable, wrinkles can be improved in appearance through cosmetic procedures such as ablative and non-ablative lasers. In general, these treatments aim to stimulate fibroblastic activity by thermal tissue injury being accompanied by several adverse events and a long recovery period. Photobiomodulation (PBM), in turn, consists of a nonthermal noninvasive treatment that triggers intracellular photo biochemical reactions which increase cell metabolism. As a consequence, PBM generates increased collagen synthesis and reduced levels of matrix metalloproteinases. Thus, PBM may be considered a relevant therapeutic alternative to promote the improvement of the photoaged skin. **OBJECTIVE:** Evaluate and compare the protocols effectiveness through the results obtained by quantitative evaluation of periorbital region wrinkles. Describe the main parameters used in PBM for facial rejuvenation. **METHODS:** A systematic review was conducted from May to September 2021 using PubMed, Embase, MEDLINE/Bireme, SciELO, Cochrane Library and Web of Science databases. The search strategy involved the use of the most used descriptors for this technique. The following inclusion criteria for studies were selected: a randomized clinical trial design, no restriction on language and year of publication, and the quantitative analysis of the skin surface at the periorbital region as outcome. **RESULTS:** Five studies presenting the evaluated outcome were found. The following wavelengths/wavebands were used: 590 nm, 633 nm, 660 nm, 830 nm, 411 – 777 nm, 611 – 650 nm and 570 – 850 nm. The application protocol was also quite variable, with a study having daily applications for 12 weeks, totaling 84 treatments; three studies made 2 applications per week, two of them for 4 weeks and the other for 15 weeks, totaling 8, 8 and 30 treatments, respectively; and one applied 3 times a week for 4 weeks, totaling 12 sessions. In all studies significant changes in the depth of wrinkles and/or in the texture of the skin surface were observed, demonstrated by percentage improvements ranging between 10% and 36%. **CONCLUSION:** PBM proved to be well-tolerated, safe and effective as a nonablative therapeutic strategy for facial rejuvenation, especially in improving skin texture and depth of wrinkles in the periorbital region.

**Key words:** LLLT, Skin aging, Photoaging, Phototherapy, Rejuvenation, Wrinkles.

**Study type:** Review



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GENE EXPRESSION OF NATURAL KILLER CELL LIGANDS IN ORAL SQUAMOUS CELL CARCINOMA CELL LINES SUBMITTED TO PHOTODYNAMIC THERAPY.

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### Abstract

**Background:** Oral Squamous Cell Carcinoma (OSCC) is the most common tumor in the oral cavity. Despite the advances in treatment, the 5-years overall survival is 50%patients, mainly due to the late diagnosis. Photodynamic therapy (PDT) is a minimally invasive therapy that has been indicated to treat some types of cancers, preserving functional and anatomical integrity, with few side effects and low toxicity. In addition, studies have shown that PDT can modulate the immune system although the effect on the modulation of NK cell ligands, which could increase its cytotoxicity is unknown. **Aim:** The aim of this study was to evaluate the effects of PDT on cellular viability and gene expression of NK cell ligands in two OSCC cell lines. **Methods:** OSCC cell lines were divided in the following groups: Control (no treatment), 5-ALA (incubation with 1mM 5-ALA for 4h), LED (irradiation with BioLambda equipment LedBOX model (Brazil),  $660 \pm 9$  nm,  $0.0255\text{W}/\text{cm}^2$ ,  $6\text{J}/\text{cm}^2$  and 240s) and PDT (1mM 5-ALA+irradiation with LED). After 24h of treatment, cellular viability was evaluated by Alamar Blue and Crystal Violet assays. In addition, after 4, 12 and 24h of treatment, RNA was extracted and the expression of ULBP1, ULBP2, ULBP3, ULBP4 and MICA/B was investigated by RT-qPCR. **Results:** Cellular viability decreased significantly in the PDT group in both cell lines. The gene expression data showed an increase in the expression levels of ULBP1 gene in Ca1 after 4h and a decrease in the expression of the ULBP1-4 in PDT group after 12 and 24 h. In the Luc4 cell line, increased expression of ULBP1, ULBP3, ULBP4 genes was noticed after 3 and 12 h of treatment in the PDT group. **Conclusion:** PDT is able to decrease OSCC cellular viability and modulates the expression of NK cell ligands, mainly after 3 and 12h of treatment, suggesting that PDT could improve NK cell cytotoxicity. However, further studies are needed to address this hypothesis.

**Key words:** Keywords: Oral Squamous Cell Carcinoma, photodynamic therapy, 5-ALA, natural killer cell ligands.

**Study type:** Experimental study in vitro



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NATURAL KILLER CELL CYTOTOXICITY IN ORAL SQUAMOUS CELL CARCINOMA CELL LINES SUBMITTED TO PHOTODYNAMIC THERAPY.

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### Abstract

**Background:** Oral squamous cell carcinoma (OSCC) is the most frequent type of oral cancer, with an aggressive behavior and associated with a high mortality rate. OSCC treatment is surgery associated with radiotherapy. However, many patients develop recurrence and resistance to the available therapies. There is a growing body of evidence demonstrating the effectiveness of photodynamic therapy (PDT) as a treatment modality for OSCC early-stage tumors. PDT promotes not only the death of malignant cells but also, activates the immune system promoting immunological surveillance, via the induction of stress-induced ligands in malignant cells. Natural killer cells are involved in anti-tumoral immunity and few studies have shown that PDT improves their killing capability. However, the association of PDT and NK cytotoxicity is unknown in OSCC. **Aim:** The aim of this study was to investigate the effects of PDT in the cellular viability of OSCC cell lines as well as their susceptibility to NK cytotoxicity after PDT. **Methods:** OSCC cell line CA1 was divided in the following groups: Control (no treatment), 5-ALA (1mM 5-ALA), LED (irradiation with LED, 660nm, 100mW, 35.5mW/cm<sup>2</sup>, and 1.5 to 18 J/cm<sup>2</sup>) and PDT (1mL 5-ALA+ irradiation with LED). After 24h of treatment, cellular viability was evaluated by MTS and Alamar Blue. NK cytotoxicity was evaluated using the NK92-M1 cell line at different effector: target ratios by the Calcein-AM release assay. **Results:** Cellular viability in CA1 cells submitted to PDT decreased significantly with the increase of the radiant exposure. To investigate the effects of PDT in NK cytotoxicity, we further selected the parameters 1.5 and 3J/cm<sup>2</sup> as they promoted a lethal dose of 25% and 30%, respectively. NK cytotoxicity was not increased in PDT groups when compared to Control, 5-ALA and LED. **Conclusion:** PDT mediated by 5-ALA decreases OSCC cellular viability. However, this therapy was not able to increase NK cytotoxicity. **Funding:** Universidade Nove de Julho UNINOVE, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Cód. nº001/Proc: 88882.365373/2019-01), FAPESP (08540-8)

**Key words:** photodynamic therapy, natural killer cell, oral squamous cell carcinoma

**Study type:** Experimental study in vitro





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PHOTOBIMODULATION AS AN ADJUVANT TOOL IN THE HEALING OF DIABETIC ULCERS: AN IN VIVO AND IN VITRO STUDY.

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### Abstract

Introduction: Diabetic ulcers represent 60% of non-traumatic lower limb amputations, with a high morbidity and mortality with significant losses in quality of life and high socioeconomic impact. The conventional treatment used is usually painful and long, requiring additional treatments that provide short-term benefits. Photobiomodulation therapy (PBMt) is a low-cost and easy-to-handle tool that has analgesic, anti-inflammatory and biomodulatory effects, favoring the healing of diabetic ulcers. Objective: Evaluation of PBMt on ulcer healing and in vitro modulation of skin fibroblasts from diabetic patients at the HU-USP wound clinic. Methods: Cross-sectional and interventional study of 14 patients (CAAE: 85121318.20000.5467) from the HU-USP wound clinic. After signing the consent form, patients underwent clinical evaluation and PBMt was initiated (660 nm, 1.4 J, 14 applications – twice a week). To measure the ulcer retraction rate, digital photographs were taken considering the 1st and last day PBMt (Image J). In addition, a skin biopsy from the triceps sural region of diabetic and non-diabetic patients (control) was collected for fibroblast culture. The cells were cultivated in DMEM high glucose medium (25 mM) and incubated at 37°C and 5% CO<sub>2</sub>. After reaching confluence, cells were submitted to PBMt under plate. After 24h, viability was evaluated by the MTT assay, morphology by immunofluorescence and cell migration by the scratch assay. The results were analyzed by the Wilcoxon test (non-parametric), one-way ANOVA (IBM SPSS 20). FAPESP (2018/18483-1). Results: After PBMt, patients showed improvement in secretion and odor, in addition to significant wound retraction. Furthermore, data showed a decrease in pain intensity in patients. In vitro diabetic and non-diabetic fibroblasts showed fusiform and elongated morphology. However, when its viability was evaluated, there was a significant decrease in the number of diabetic fibroblasts compared to non-diabetics, however when submitted to PBMt, a significant increase in number and cell division of diabetic fibroblasts were observed. Furthermore, cell migration was significant in the treated group compared to the untreated after 24h of PBMt. Conclusion: PBMt promoted healing and significant improvement in pain in diabetic patients. The treatment proved to be able to act on the proliferative and migratory capacity of diabetic fibroblasts when compared to untreated ones, proving to be an efficient and promising adjuvant tool.

**Key words:** healing, diabetes, pain, photobiomodulation, ulcers.

**Study type:** Experimental study in vitro.



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EVALUATION OF DENTAL ENAMEL MICROPROPERTIES AFTER BLEACHING WITH 35% HYDROGEN PEROXIDE AND DIFFERENT LIGHT SOURCES: AN IN VITRO STUDY.

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**Abstract**

Introduction: tooth whitening assumes a prominent position in aesthetic treatments in dentistry, due to the current concept of the smile based on white teeth. Thus, over the years, new techniques for tooth whitening using a light source have emerged, capable of breaking down the pigments responsible for tooth darkening. Background: To evaluate the tooth enamel surface morphology after the action of 35% hydrogen peroxide with and without LED activation. Material and Methods: 70 bovine incisors with an enamel surface of 4x4x3 mm were used, prepared for reading superficial microhardness and roughness. Specimens were randomly distributed and divided into 6 experimental groups (n = 10); G1 = artificial saliva; G2 = 35% HP - 2 sessions (3x15'); G3 = 35% HP - 2 sessions (3x15') + blue LED; G4 = 35% HP - 2 sessions (3x15') + green LED; G5 = 35% HP - 2 sessions (3x20') + violet LED; G6 = Violet LED - 2 sessions (3x20'). The results were analyzed by the Anova, Wilcoxon, Dunnett and Tukey tests ( $\alpha = 0.05$ ). Results: The G3 group showed a greater change in microhardness. Regarding roughness, the biggest mean difference between groups occurred in G2, G4 and G6. Optical microscopy showed a smooth enamel surface in groups G2, G4 and G6. Conclusions: changes in the enamel surface were observed in relation to microhardness, but without significant changes in roughness, where the LED (green and violet) resulted in a smooth surface.

**Key words:** tooth whitening, superficial morphology, light, photoradiatio

**Study type:** Experimental study in vitro



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PHOTOBIMODULATION PROMOTES STRUCTURAL PROTECTION AND MODULATES THE INFLAMMATORY RESPONSE IN AN EXPERIMENTAL MODEL OF COLITIS.

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### Abstract

Introduction: Inflammatory bowel diseases (IBD) are chronic and multifactorial diseases characterized by dysfunction of the intestinal mucosa and impaired immune response. The ulcerative colitis (UC) is one of the chronic inflammatory diseases of the intestine and rectum with a difficult therapeutic approach, being currently available, costly and ineffective therapies. It is observed that in situations of intestinal tissue injury, translocation of microorganisms to the intestine occurs when recruiting inflammatory cells to the injured site. The treatment of UC is difficult and new therapies are needed. Objective: Thus, in this study we evaluated the effects of photobiomodulation to treat UC. For this, we quantified the levels of pro- and anti-inflammatory mediators in the intestinal mucosa. Methods: For this purpose, adult male Wistar rats were submitted to UC induced by sodium dextran sulfate (SDS) added to the drinking water on days 0, 2, 4 and removal at day 6. The treatment with LED was performed daily for 90 s from day 6 to 9 on the right and left sides of the ventral surface and in the external anal region. Results: Our results showed that LED treatment attenuated the inflammatory process by preventing the increase of IL-1 $\beta$  and IL-6 levels caused by SDS and accelerated its resolution by markedly increasing IFN- $\gamma$  and TGF- $\beta$  levels, reducing the inflammatory infiltrate and ulcerations of the intestinal mucosa, demonstrating protective actions on the epithelial barrier. Conclusions: Thus, LED treatment promotes structural protection and modulates the inflammatory response of the colitis, constituting a potential non-invasive and low-cost combined therapy to help patients achieve disease remission. Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper. Financial support: CNPq 305099/2017-5.

**Key words:** Key words: Ulcerative colitis; Photobiomodulation; Light emitting diode; Interleukins; Inflammatory bowel diseases;

**Study type:** Experimental study in animals



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LED REDUCES MYELOPEROXIDASE ACTIVITY AND EICOSANOIDS RELEASE IN AN EXPERIMENTAL MODEL OF CORTICOSTEROID-RESISTANT ASTHMA.

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**Abstract**

Introduction: Asthma is a chronic inflammatory disease characterized by reversible airway obstruction, smooth muscle hyperreactivity and increased mucus production. Glucocorticoids are potent anti-inflammatory drugs used in the treatment of asthma; however, some patients have a profile of resistance to the anti-inflammatory actions of corticosteroids, developing a more severe type of asthma denominate corticosteroid-resistant asthma (CRA). Thus, new therapies are needed and photobiomodulation (PBM) emerges as an alternative therapy based on previous studies of our group. Objective: This study aimed to evaluate the effect of PBM using infrared light emitting diodes (LED) in the development of CRA. Methods: Male Wistar rats were randomly divided into 4 groups: basal group, non-manipulated control rats (n=6); CRA group: asthmatic rats (n = 6); CRA+LED group: asthmatic rats treated with LED (n = 6) and CRA+DEXA group: asthmatic rats treated with dexamethasone (n = 6). For this, groups of rats were sensitized and challenged with ovalbumin plus Freud's adjuvant for the induction of CRA; and treated with LED directly in the respiratory tract on the skin (wavelength 810 nm; power 100 mW; density energy 5 J/cm; total energy 15 J; time 150 s) or with dexamethasone (1 mg/kg, ip). Results: Our experimental model was able to induce neutrophilic asthma. Conventional corticosteroid treatment did not reverse cell migration into the bronchoalveolar lavage as well as did not reduce leukotriene B4 levels. On the other hand, the treatment with LED reduced cell migration to the alveolar space, myeloperoxidase activity and levels of leukotriene B4, thromboxane B2, and prostaglandin E2. Conclusion: In conclusion, we showed promisor effects of LED when irradiated directly in the respiratory tract as adjuvant treatment of corticosteroid-resistant asthma. Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper. Financial support: CNPq 305099/2017-5.

**Key words:** Key words: Corticosteroid-resistant asthma, Photobiomodulation, Infrared light emitting diode, Interleukins, Mast cells, Eicosanoids.

**Study type:** Experimental study in animals.



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### EFFECT OF RED LIGHT EMITTING DIODE ON THE MODULATION OF INFLAMMATION IN SKIN BURNS

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#### **Abstract**

**Introduction:** Burns are a global public health problem, occurring around 1 million accidents with burns in Brazil, which corresponds to 38% of the main diseases treated in the country's health system. Burns can be defined as traumatic injuries resulting from thermal, electrical, chemical or radioactive trauma, which partially or totally destroy the skin and its appendages. Among the therapies that promote the repair of burned tissue quickly, effectively and reduce treatment costs, the use of Light Emitting Diode (LED) stands out. **Objective:** The objective of this study was to evaluate the effects of a photobiomodulation protocol using red LED on inflammatory cells during the healing process of skin burns. **Methodology:** Twenty Wistar rats were randomly divided into control group (CTRL) (n=10) and red group (RED) (n=10), with subgroups (n=5) for each time of euthanasia (7 and 14 days). Treatment animals were daily irradiated (630nm ±10nm, 300mW, 9 J/cm<sup>2</sup> per point, 30 seconds, continuous emission mode) at 4 wound angles (total: 36 J/cm<sup>2</sup>). After specimen removal, histological sections were stained with hematoxylin and eosin for quantitative analysis of the inflammatory infiltrate (neutrophils and lymphocytes) under light microscopy. **Results:** There was a greater number of inflammatory cells in the irradiated groups when compared to CTRL in the two evaluation times (7 and 14 days), with a statistically significant difference only at 14 days (p = 0.02). **Conclusion:** In conclusion, the red LED was able to modulate the number of inflammatory cells, however, this therapy seems not to be efficient in reducing the number of neutrophils and lymphocytes during the process of cutaneous burn repair. Specific studies using other protocols are needed to assess the effects of red light on the inflammatory response of skin burns.

**Key words:** Burns, LED, Photobiomodulation, Inflammation.

**Study type:** Experimental study in animals



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TOTAL MOUTH PHOTODYNAMIC THERAPY MEDIATED BY RED LED AND PORPHYRIN IN INDIVIDUALS WITH AIDS

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**Abstract**

**INTRODUCTION:** Due to the immune changes resulting from HIV/AIDS infection, systemic and local infections throughout the body are common. The use of High Activity Antiretroviral Therapy has been widely used during treatment, which, added to the use of antibiotics, antifungals and the patients' own immunocompromised state, cause important changes in the oral microbiota. The emergence of pathological microorganisms and with high resistance to drug therapies are frequent and cause serious damage to the oral health of these patients. In this sense, Antimicrobial Photodynamic Therapy (aPDT) appears as a promising alternative in the control of these oral infections. **PURPOSE:** The aim of the study was to test the effectiveness of an therapeutic protocol for total oral aPDT mediated by a 660 nm red LED (Light Emitting Diode) associated with porphyrin in individuals with AIDS. **METHODS:** Patients were selected by exclusion criteria and randomly distributed into groups to test the effectiveness of antimicrobial aPDT with 50 µg/ml porphyrin associated with the red LED. Before and after the treatments, saliva samples were collected and processed in duplicate in selective culture media. **RESULTS:** Colonies were counted and the results obtained in log<sub>10</sub> CFU/ml and tested statistically. **CONCLUSION:** It was concluded that aPDT was effective in reducing oral enterobacteria, in addition to reducing *Streptococcus* spp. and general count of microorganisms, when considering the numbers of TCD4 and TCD8 lymphocytes.

**Key words:** Keywords: Photodynamic Therapy; Porphyrin; AIDS; HIV.

**Study type:** Clinical or experimental protocol



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HYDROXYPROPYL METHYLCELLULOSE POLYMER AFFECTS METHYLENE BLUE AGGREGATION IN FORMULATION FOR ORAL USE.

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### Abstract

**BACKGROUND:** Methylene blue (MB) is the most studied phenothiazinium photosensitizer (PS) for microbial inactivation by Photodynamic Therapy (PDT). The cell death induced by PDT is due to oxidative species generated by light activation at a specific wavelength and intensity in the presence of oxygen. It is known that MB concentration influences the aggregation behavior, as well as the medium in which it was conveyed. Aggregation interferes with the photochemical action and affects the effectiveness of PDT. Some strategies have been used to overpass this, such as the development of formulations. Currently, polymeric systems are considered indispensable for PS delivery. Thus, analyzing the physicochemical characteristics related to the aggregation state of MB in formulation containing different polymer concentrations, has relevance for targeting optimized formulations to develop clinical protocols for PDT. **AIM:** Evaluate the aggregation of MB, through the dimer to monomer ratio (D/M), conveyed in formulation for oral use, containing different concentrations of the hydroxypropyl methylcellulose (HPMC) polymer. **METHODS:** The MB 0.005% was conveyed in oral formulation containing xylitol, methylparaben, propylene glycol, sodium dodecyl sulfate and water, in addition to the polymer HPMC in concentrations of 1%, 3% and 5%. In duplicate, absorption spectra between 250 nm and 800 nm were recorded in a UV-Visible UV-1800 spectrophotometer (Shimadzu, Japan) using a 2 mm cuvette. The D/M were determined by absorption wavelength values at 614 nm (dimer) and 662 nm (monomer). **RESULTS:** In formulations containing 0.005% MB, the D/M ratio value found in the presence of 1% HPMC was  $0.580 \pm 0.001$ , changing to  $0.611 \pm 0.001$  by increasing the concentration of HPMC to 3% and reaching  $0.617 \pm 0.002$  with 5% HPMC. Thus, when increasing the concentration of HPMC polymer, an increase in the D/M ratio is observed due to a greater aggregation of MB even without changing its concentration in the oral formulation. **CONCLUSION:** The D/M ratio of MB conveyed in oral formulation presents greater aggregation as the concentration of HPMC polymer is increased, demonstrating that the medium in which the PS is conveyed influences the MB aggregation behavior, to emphasize the importance of improving protocols for PDT to investigate the photochemical response. Further adjustments to this formulation will be necessary to control MB aggregation.

**Key words:** Photodynamic Therapy, Photosensitizer, Methylene blue, Aggregation, Polymer

**Study type:** Experimental study in vitro



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### AGGREGATION OF PHENOTIAZINIUM DYES IN AQUEOUS MEDIA.

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#### **Abstract**

##### INTRODUCTION

Photodynamic therapy is based on the use of a photosensitizing agent (PS). This agent is activated by visible light with specific parameters, generating reactive oxygen species that culminate in its cytotoxic effect. Depending upon the medium PS may aggregate, which affects PDT effectiveness. In particular, the aggregation status (dimer to monomer ratio) of the methylene blue affects the photochemical behavior (type I or II reactions). Thus, understanding the aggregation status of phenothiazinium compounds are relevant for the development of more effective clinical protocols. OBJECTIVE: Evaluate and compare the aggregation of the phenothiazinium compounds Methylene Blue (MB), Azure A (AA), Azure B (AB) and Dimethyl Methylene Blue (DMMB) in different aqueous media. METHOD: In a UV-1800 UV-Visible spectrophotometer (Shimadzu, Japan) absorption spectra between 500 and 800 nm were registered. The MB, AA, AB and DMMB 20µg/mL solutions were prepared in water, Phosphate Saline Buffer - PBS, physiological solution (NaCl 0.9%), sodium dodecyl sulfate (0.25%) and urea (1mol/L). The dimer-monomer ratio (D/M) was determined by the absorption values of each of the PS, being A590/A664 for MB, A588/A630 for AA, A593/A645 for AB and A573/A652 for DMMB. RESULTS AND DISCUSSION: Comparing the PS D / M was lower in MB, higher in DMMB and intermediate for AB and AA. As they are more hydrophilic, the variation of D / M values of MB, AA and AB among mediums is not very high. On the other hand, for DMMB it was possible to observe a significant change in D / M in the studied media, being smaller in SDS (0.32) and slightly larger in urea (1.18), intermediate in water (1.55), greater than water in PBS(1.99) and maximum in saline solution(2.30). Comparing the different media, D / M was lower in SDS (between 1.55 to 0.3), higher in water, PBS and saline solution, with the highest values being in saline solution (2.30 to 0.48) and PBS (1.99 to 0.47). Small differences in saline and PBS may be related to pH, but this variable was not analyzed in this study. CONCLUSION:SDS reduced D/M in phenothiazinium dyes, while saline solution and PBS increased it. MB presented the lowest aggregation while DMMB the highest. These data suggest that the correct choice of a medium for a PS may help improve PDT efficacy, which needs to be proved by further biological studies.

**Key words:** Photobiomodulation, aggregation, phenothiazinium

**Study type:** Experimental study in vitro





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THE ROLE OF PHOTOBIMODULATION ON THE RESOLUTION INFLAMMATORY PROCESS DURING ACUTE LUNG INJURY INDUCED BY SEPSIS.

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**Abstract**

Introduction: Sepsis is one of the most common causes of acute lung injury (ALI) leading to high mortality. Pro-resolving mediators play an important role in the restoration of lung homeostasis. ALI treatment is still a clinical health problem, so new treatments are needed. Objective: Here, we evaluated the role of photobiomodulation treatment on the resolution process of ALI. Methods: For this, male Balb/c mice were submitted to lipopolysaccharide (LPS) (ip) or vehicle and irradiated or not with light emitting diode (LED) 2 and 6 h after LPS or vehicle injection, and the parameters were investigated 7 days after the injections. The dosimetry parameters of LED: Radiant Power 100 mW; Continuous operation mode (CW); Wavelength  $660 \pm 10\text{nm}$ ; Total Radiant Emission 15 J; Area  $2.8 \text{ cm}^2$ ; Energy density  $5.35 \text{ J/cm}^2$ ; Irradiance  $33.3 \text{ W/cm}^2$ ; Exposure time 150 seconds. Results: Our results showed that after 7 days of the LED treatment the levels of IL-6 and IL-17 were decreased, while the levels of lipoxin A4 were increased without alter Resolvin E2. Thus, our results showed that LED treatment modulates the lung resolution process, which is important to re-established the lung homeostasis.

**Key words:** Photobiomodulation; Light emitting diode, lung injury

**Study type:** Experimental study in animals)



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EFFECT OF INTERACTION BETWEEN RED AND INFRARED LASER LIGHT ON COLLAGEN DEPOSITION IN LESIONS CAUSED BY BOTHROPS LEUCURUS SNAKE VENOM.

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### Abstract

**INTRODUCTION:** The treatment of local lesions caused by venom snakes of the genus *Bothrops* is considered a challenge, since serum therapy has more expressive effects on systemic alterations. In this context, photobiomodulation is used and shows satisfactory results that are associated, among other characteristics, with increased deposition and organization of collagen fibers and fibroblasts. **OBJECTIVE:** Evaluate the effects of red and infrared wavelengths, separately and in association, on collagen deposition in mice muscle submitted to inoculation of *B. leucurus* snake venom. **METHODOLOGY:** The work was approved by CEUA/UESC (026/20). 112 mice (25 to 30 grams) were used and the venom was inoculated in the gastrocnemius muscle in all them, at a dose of 0.6mg/kg, diluted in 50 µl of saline solution. The animals were divided into a control group and three groups treated with the following lasers: 1) red ( $\lambda=660$  nm) (GV), 2) infrared ( $\lambda=808$  nm) (GI) and 3) red ( $\lambda=660$  nm) + infrared ( $\lambda=808$  nm) (GVI). Each group (with 28 animals) was divided into four subgroups, according to duration of treatment application (one, two, three and six days). Diode laser (0.1 W, CW, 1J/point, DE: 10 J/cm<sup>2</sup>) was used, applied at 24hour intervals. Euthanasia was performed 24 hours after the last treatment session in each subgroup, for collection of the gastrocnemius muscles and subsequent histological processing. The blades were stained with Picrosirius red and photographed. ImageJ software was used to measure the collagen area. To assess the difference between all groups, the normality test was performed, followed by the Kruskal-Wallis and Mann-Witney test with Bonferroni correction. **RESULTS:** There was a significant difference in collagen levels (measured in pixel) between the control group (median 6.4 and mean 6.87) and groups GV (median 9.75 and mean 11) and GVI (median 14.08 and mean 15.17). Among those treated, there was a significant difference between GVI and GI (median 9.17, mean 9.73). **CONCLUSION:** Photobiomodulation stimulates collagen deposition in muscle tissue subjected to the bothropic venom action and the association between red and infrared laser culminates in more expressive effects when compared to the use of invisible length individually.

**Key words:** Photobiomodulation, Bothrops, Tissue repair

**Study type:** Experimental study in animals



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### EVALUATION OF THE IMPACT OF CATALASE INHIBITOR IN ANTIMICROBIAL PHOTODYNAMIC THERAPY

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#### Abstract

Background: Photodynamic Therapy (PDT) is a therapeutic modality with the mechanism of action based on the activation of a photosensitizer by a light source, elevating it to the excited state that can interact with molecular oxygen indirectly, via load transfer by reaction with biomolecules and generation of radicals forming reactive oxygen species, or directly, with singlet oxygen formation. The antioxidant enzymatic system is one option to protect against reactive species, with catalase being a representative. Aim: Evaluate the impact of catalase inhibition on methylene blue-mediated photodynamic therapy of *S. aureus* suspension. Method: *S. aureus* (ATCC 25923) was sown in TSA and incubated at 37°C for 24 hours. After this period, bacteria were inoculated in 5 mL of TSB and subsequent incubation at 37°C for 18 hours. From this inoculum, a 1:10 dilution was made in TSB, incubated at 37°C for 4 hours. The optical density was measured at 630nm, adjusted between 0.08 and 0.1, and diluted to final concentration in the assay at 1:200. The catalase inhibitor (3-amino-1,2,4-triazole) was solubilized in saline until a final concentration of 10µg/mL and kept in contact with the inoculum for 10- and 20-minutes inhibition period (IP). Methylene blue (MB) was solubilized in saline until final concentration of 50µg/mL. The pre-incubation time of the inoculum with MB was 10 minutes, at room temperature and in the dark. Irradiation was performed with 660nm LED (81.9 J/cm<sup>2</sup>, 91mW/cm<sup>2</sup>). After treatment, the inoculum was serially diluted and sowed by the droplet method in TSA and incubated at 37°C for 24 hours. Data Analysis: The comparison of values of continuous variables between the groups was made by the Variance Analysis test (one-way ANOVA), with Tukey's post-test. The significance level adopted for the statistical tests was 5% (p<0.05) and the maximum acceptable variation coefficient of the assays was set at 25%. Results: In the 10-minute IP, the PDT group reduced 0.47 log and the Combined Therapy group (CT: MB and inhibitor) 1.03 log; in the 20-minute IP, the reductions were 1.18 log and 0.74 logs, respectively. According to the statistical tests applied, the reductions were significant compared to the growth control group, but did not represent any difference between them. Conclusion: In the inhibition times evaluated, combined therapy did not obtain significant bacterial reduction compared to PDT, and it was necessary to study higher inhibition times.

**Key words:** Photodynamic Therapy, Catalase Inhibitor, Methylene Blue

**Study type:** Experimental study in vitro



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### EFFECT OF FOTOENTICINE AND METHYLENE BLUE ON ANTIMICROBIAL PHOTODYNAMIC THERAPY FOR ACINETOBACTER BAUMANNII CONTROL

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#### Abstract

Introduction: Antimicrobial photodynamic therapy (aPDT) has been considered an alternative for the treatment of skin infections caused by *Acinetobacter baumannii*. However, it is necessary to search for photosensitizers to enhance their effects. Fotoenticine (FTC) is a new photosensitizer, derived from Chlorine e-6, effective in aPDT against Gram-positive bacteria. However, there is a lack of studies on its action on Gram-negative bacteria, which tend to be more resistant to the action of photosensitizers. Objectives: The aim of this study was to test the FTC in the aPDT on *Acinetobacter baumannii* and compare its effects to Methylene Blue (MB), a photosensitizer already approved for clinical use. Methods: For this, the following tests were performed: 1) aPDT in planktonic cultures; 2) Bacterial cell membrane permeability test and analysis by confocal microscopy to assess the internalization of photosensitizers; 3) aPDT in biofilms, determining cell viability by counting Colony Forming Units (CFU); 4) In vivo assays to assess the effects of aPDT on burn injuries infected by *A. baumannii* in *Galleria mellonella*. Data analysis: Data were analyzed by ANOVA and Tukey test. Results: As a result, it was observed that aPDT with FTC reduced 2 log (CFU/mL) of *A. baumannii* in planktonic cultures, while MB led to complete inhibition. Both photosensitizers were able to penetrate bacterial cells. The aPDT with MB reduced 4 log of *A. baumannii* in biofilms, whereas with FTC it had no effect on the number of cells in the biofilms. In vivo, only aPDT with MB had an antimicrobial effect on burn injuries infected by *A. baumannii* in *G. mellonella*, increasing larvae survival by 35%. Conclusion: It was concluded that aPDT with FTC had antimicrobial action only in planktonic cultures of *A. baumannii*. Within the parameters tested in this study, the antimicrobial activity of aPDT with FTC was lower than MB in in vitro and in vivo assays.

**Key words:** *Acinetobacter baumannii*, photodynamic therapy, photosensitizers.

**Study type:** Experimental study in animals, Experimental study in vitro



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### RESEARCH PROTOCOL APPLICATION OF LOW-LEVEL LASER IN WOMEN WITH GENITOURINARY MENOPAUSE SYNDROME

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#### **Abstract**

**INTRODUCTION:** Postmenopausal Genitourinary Syndrome (PGS) defines a set of and signs associated with an estrogen deficit involving alterations in organs genitourinary and that results in several urinary, genital, and sexual alterations. Brazilian women live about a third of their life after menopause, where hormonal changes occur along with clinical manifestations, characterized by vaginal and vulvar dryness, burning, discomfort, vulvovaginal irritation, lack of lubrication, dyspareunia, dysuria, pollakiuria, and recurrent urinary infections. Fractionated photothermolysis and radiofrequency systems, alone or in combination were tested to improve PGS. **OBJECTIVE:** The goal of this project is to evaluate the clinical response of patients with symptoms of genitourinary menopause syndrome after the application of photobiomodulation in the vagina and its introit. **METHOD:** In this randomized, double-blind, placebo-controlled study protocol. Women over 50 years of age who are in the postmenopausal period (amenorrhea for at least 12 months, with no pathology involved) with one or more symptoms of PGS. Participants included in the study will be randomly divided into two groups: group A, which will receive photobiomodulation with a vaginal diode laser and its introit and group B (placebo) with the laser device turned off. Both treatments will be maintained for 4 consecutive weeks, as shown in Figure 1. The treatment group (n=30) will receive four consecutive applications, using laser diode DMC (808 nm), 4J per point, 100mW of power, 510mW/cm<sup>2</sup>, beam area of 0.2cm<sup>2</sup>, 8 sites in the external vagina, for the 40s in each site, once per week for 4 weeks. The Placebo Group (n=30) will be handled as treated, but with the laser turned off. The life quality will be analyzed by using a visual analog scale (VAS), female sexual functioning index (FSFI-6), urinary incontinence questionnaire (ICIQ-SF), Vaginal Health Index Score (VHI) and compared between groups. Also, the vaginal temperature will be measured using a thermal camera, the pressure of the pelvic floor force (vaginal dynamometer) and a 1-hour Pad Test performed to quantify the urinary loss. All data will be analyzed regarding its distribution and an appropriate inferential test will be applied. With this procedure, we intend to obtain an overall better life quality and diminished symptoms in women with PGS.

**Key words:** Menopause, photobiomodulation, genitourinary syndrome

**Study type:** Clinical or experimental protocol



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### THE USE OF PHOTONIC THERAPIES AS AN ADJUNCT IN THE TREATMENT OF OSTEONECROSIS OF THE JAWS ASSOCIATED WITH THE USE OF ANTIRESORPTIVE MEDICATIONS IN CANCER PATIENTS: A SERIAL CASE REPORT

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#### Abstract

Introduction: Medication-related osteonecrosis of the jaw (MRONJ) is a complex oral complication in patients on current or previous use of antiresorptive or antiangiogenic drugs, presenting exposed bone in the maxillofacial region and that has persisted for more than 8 weeks. Thus, MRONJ can be an adverse reaction of cancer therapies being the oral cavity an important target organ. Many treatments for MRONJ have been proposed, but a gold standard treatment has not yet been available. The low level laser therapies (PBM and aPDT) can be applied, isolated or in association, at different time points during the MRONJ lesions management. Objective: to present three clinical cases of diagnosed stage 3 MRONJ lesions in cancer patients in use of bisphosphonates and treated with photonic therapies (PT) – with an association of PBM and aPDT applied at pre, trans and post-surgery times. Methods: The pre-surgery treatment was conducted applying the PT to control the infection (aPDT) and to stimulate the tissue (PBM) surrounding the lesion leading to bone sequestration formation. When bone sequestration became visible on imaging exams (after an average of 8 weeks of pre-surgical PT), a minimally invasive surgery was conducted to remove it. Then a trans-surgical aPDT was conducted. After that, the post-surgery PT, similar to the pre-surgical, was applied due to preventing reinfection (aPDT) and also improving the healing by biostimulation (average of 4 to 8 weeks, until complete lesion closure). All the PT were done using the Therapy EC (DMC) (P=100mW, spot size=0.028cm<sup>2</sup>, punctually and in contact). The PBM was applied with red (660nm) or infrared laser (808nm) 2J per point, each point at 1cm apart, around the lesion (2 to 5 times a week). For the aPDT, a 0.01% methylene blue solution was used during 5 min, then the irradiations were done with 9J per point, 3 points (on lingual, vestibular and in the center) with red laser (660nm) 3 times a week. Results and Conclusion: The protocol lead to complete regression of the lesion in all cases, with no recurrences. In addition, the aPDT and PBM favored the surgical procedure by controlling infection and stimulating the rapid formation of granulation tissue, which promoted the detaching of the bone sequestrations allowing the identification of healthy tissue in the surgical bed, and also a more conservative surgical procedure. The combination of PBM and aPDT showed to be efficient in the treatment of MRONJ lesions

**Key words:** MRONJ, photobiomodulation, aPDT

**Study type:** Case report/Case series



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### CLINICAL EVALUATION OF LASER THERAPY IN TEMPOROMANDIBULAR DISORDERS

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#### **Abstract**

**INTRODUCTION:** Laser therapy can be used as a non-invasive treatment for Temporomandibular Disorders (TMD), which presents pain as the main symptom. **OBJECTIVE:** to evaluate the effectiveness of laser therapy in patients with TMD assisted in the clinic of the Orofacial Pain Control Service of UEPB. **METHODOLOGY:** The patient was submitted to a questionnaire on TMD assessment, using the Visual Analog Scale (VAS) to assess pain, before and after laser therapy sessions. To assess muscle tension, palpation was used as a diagnostic method for changes in muscle sensitivity before and after laser therapy, through the Jansen scale, which recommends 0- no pain, 1- mild discomfort, 2- moderate pain and 3 -severe pain. Mouth opening was assessed using a digital pachymeter, where measurements were taken before and after laser therapy. The sample consisted of 20 patients, 10 for Group 1 (laser with infrared emission – 830 nm) and 10 for Group 2 (laser with red emission – 660 nm). **RESULTS:** After 12 treatment sessions, the evolution of the muscle sensitivity threshold showed a statistically significant difference ( $p < 0.05$ ) for group 1 and group 2. Laser therapy in Group 1 improved mouth opening by an average of 4.643 mm, while in Group 2, the mean was 3.71 mm per patient, obtained through the t test, with a significance level of 5% ( $p < 0.05$ ). Regarding pain, assessed using the SVF, there was a statistically significant improvement ( $p < 0.05$ ), with the initial average pain in Group 1 assessed at 8.4 per patient and after laser therapy, 1.4; while in Group 2, the mean initial pain was 8.1 and after the laser therapy sessions, 1.9. It was found that in group 1, 90% had severe TMD while in group 2 it was only observed in 70%, in relation to wear of the dental element, the majority (60%) was bruxism; on auscultation, the click on the left side and the main disocclusion on the right side was by canine guidance (69.2%). **CONCLUSION:** Infrared emission laser and Red emission laser were both effective and statistically significant ( $p < 0.05$ ), at a 5% significance level, in the treatment of pain and mouth opening, making further studies necessary, especially randomized clinical controls, evaluating the efficacy of different dosages and clinical protocols for the application of low-intensity laser in TMD's.

**Key words:** Pain assessment, Low power laser irradiation, Temporomandibular Joint Dysfunction Syndrome.

**Study type:** Clinical Trial





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**EFFECTS OF THE ASSOCIATION OF PHOTOBIO-MODULATION AND STRENGTH TRAINING ON GLUCOSE TOLERANCE IN MICE WITH DIET-INDUCED OBESITY**

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**Abstract**

**INTRODUCTION.** Obesity is a chronic disease, defined as an abnormal or excessive accumulation of fat in the body. More than one billion adults worldwide are overweight, of which 500 million are considered obese. The vast majority of individuals with type 2 diabetes mellitus (T2DM) are also obese and these patients have abnormalities in insulin sensitivity and glucose metabolism. Recently, our group showed that strength training (ST) promotes better control in blood glucose levels and increased insulin sensitivity. In addition, we also showed that the use of photobiomodulation therapy (PBMt) in both red and infrared wavelengths was also able to improve insulin resistance and glucose tolerance. **AIM.** Thus, the aim of this work is to associate PBMt and ST and investigate the effect of this association on glucose tolerance in Swiss mice with diet-induced obesity. **METHODOLOGY.** For this, 42 animals randomly divided into 2 groups were used: 1) Chow (n=10), fed with commercial chow, received neither PBMt nor training, and 2) CAF (n=32), fed a cafeteria diet for a period of 14 weeks. After 8 weeks of intervention with diet, the CAF group was divided into Sedentary Sham (n=10) who did not receive PBMt or training, Trained Sham (n=11) who performed only the stair climbing physical training, Trained PBMt (n=11) who were trained and received the PBMt. The ST was performed on a ladder and consisted of 6 weeks, 3 times a week. Before each exercise session, the animals that received PBMt underwent 60 seconds of whole-body therapy inside a blanket made of 20 diodes with a red wavelength (660 nm; 11.64 mW/diode) and 20 with an infrared wavelength (850 nm, 22.89 mW/diode). The total energy delivered per PBMt session was 41.43 J/animal. At the end of 14 weeks, the animals were submitted to an intraperitoneal glucose tolerance test. **RESULTS.** The CAF group (34.694±8739) had worse glucose tolerance (mg/dLx120min) than the Chow group (18092±2238, p<0.05). The glucose tolerance of the ST (31181±4636) and ST+PBMt (32071±5569) groups was not different from the CAF group (p>0.05). **CONCLUSION.** We conclude that the association between PBMt and stair climbing ST did not affect glucose tolerance in Swiss mice fed a high-fat diet.

**Key words:** type 2 diabetes mellitus, photobiomodulation, strength training

**Study type:** Experimental study in animals



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EFFECT OF PHOTOBIO-MODULATION THERAPY ON CONVENTIONAL OR EXPERIMENTAL (CELL SHEET TISSUE ENGINEERING) GUIDED BONE REGENERATION IN DIABETIC RATS

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### Abstract

Diabetes mellitus (DM) impairs bone repair and requires auxiliary treatments, such as guided bone regeneration (GBR) techniques. However, the ideal material or surgical technique to control the stability and predictability of bone formation has not been found yet. Thus, new regenerative therapies, such as tissue engineering, have been evaluated in order to replace and/or improve the existing ones. Stem cells organized in Cell Sheets (CS) allow the cells to be transported, keeping their physiology as close to natural as possible, preserving their extracellular matrix fully preserved, exempting the use of additional scaffolds. In addition, the Photobiomodulation Therapy (PBMT) has been shown to be beneficial as an adjunctive therapy in the repair of different types of damaged tissues. Therefore, the aim of this study was to evaluate the effect of PBMT in the repair of critical bone lesions in rats with streptozotocin-induced DM and treated by conventional GBR [commercial porcine collagen membrane (CoLM)] or with CS of human dental pulp stem cells (hDPSCs). "Mature" CS (mCS) were obtained cultivating hDPSCs with 20µg/ml of Vitamin C for 10-15 days and "immature" CS (iCS) cultivating for 7 days. The mCS were analyzed by Live/Dead®, flow cytometry and Alizarin red in vitro. DM was induced in vivo in rats and critical injuries were performed on both parietal bones and treated according to eight randomized groups: 1) Negative Control: untreated injury; 2) PBMT: received only PBMT; 3) CoLM: received only CoLM; 4) CoLM+PBMT; 5) mCS: received only mCS; 6) mCS+PBMT; 7) iCS: received only iCS and 8) iCS+PBMT. PBMT was applied 0, 48 and 96h post-surgery (808nm, 40mW, 3s, 4J/cm<sup>2</sup>, 0.12J/point, 4pt). The animals were euthanized at 30 and 60 days after surgery and were analyzed by MicroCT, HE, histochemistry and immunohistochemistry. Data were analyzed statistically ( $p \leq 0.05$ ). The viability, maintenance of undifferentiation status and cell function of CS's cells were confirmed in vitro. In vivo, PBMT had significant effects on repair: on relative bone volume (BV/TV;  $p = 0.0064$ ) and number of trabeculae (Tb.N;  $p = 0.0068$ ) in the CoLM+PBMT group and on relative bone volume ( $p < 0.0001$ ) and trabecular thickness (Tb.Th;  $p < 0.0001$ ) in the PBMT group. In iCS+PBMT groups, there was abundant bone formation at 60 days, similar to that of the CoLM+PBMT group. In conclusion, the association of PBMT was beneficial both in conventional GBR and in experimental tissue engineering (iCS).

**Key words:** Diabetes mellitus, Guided Bone Regeneration, Tissue engineering, Stem Cell, Cell Sheet, Dental Pulp Stem Cell, Photobiomodulation Therapy, PBMT.

**Study type:** Experimental study in animals



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**CLASSIFICATION OF MELANIN IN THE SKIN THROUGH A DEVICE**

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**Abstract**

Abstract: There are several ways to classify the amount of melanin present in individuals, which can be applied in clinical methodologies, visual comparisons, common sense or regional clothing. The Fitzpatrick Scale currently used classifies the concentration of the melanin pigment present, in graduations that start with the smallest amount equivalent to 1 for the highest concentration, with a value of 6. The present study aims to develop a device capable of classifying and quantifying the amount of melanin present in volunteers, in order to obtain a quantitative numerical scale to be represented through the parameters and dimensions of the Fitzpatrick Scale.

**Key words:** Melanin; Fitzpatrick scale; Skin; Phototype.

**Study type:** Protocol study



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### EFFECT OF PHOTOBIMODULATION THERAPY ON GUIDED BONE REGENERATION IN DIABETIC RATS

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#### Abstract

**Introduction:** Diabetes mellitus (DM) impairs bone repair and requires auxiliary treatments, such as guided bone regeneration (GBR) techniques. However, the ideal material or surgical technique to control the stability and predictability of bone formation has not been found yet. Thus, new adjunct therapies, such as Photobiomodulation Therapy (PBMT), have been proposed in order to improve the existing GBR techniques/materiais. **Objects:** the aim of this study was to evaluate the effect of PBMT in the repair of critical bone lesions in rats with DM and treated by conventional GBR [commercial porcine collagen membrane (BioGide®, CoIM)]. **Methods:** DM was induced by streptozotocin injection in vivo in rats and critical injuries were performed on both parietal bones and treated according to four randomized groups: 1) Control: untreated injury; 2) PBMT: received only PBMT; 3) CoIM: received only BioGide®; 4) CoIM+PBMT. PBMT was applied 0, 48 and 96h post-surgery (808nm, 40mW, 3s, 4J/cm<sup>2</sup>, 0.12J/point, 4pt). The animals were euthanized at 30 and 60 days after surgery and were analyzed by HE and histochemistry (Masson Trichrome) with the aid of a quantification software. Data were analyzed statistically ( $p \leq 0.05$ ). **Results and Conclusion:** a statistical difference in the "total bone area" was found, where CoIM+PBMT in 30 days was greater than the Control group in 60 days ( $p=0.0148$ ). This could clinically indicate a reduction to half of the bone repair time, when associating PBMT with the conventional GBR technique using a commercial membrane. The PBMT showed to be beneficial as an adjunctive therapy in the guided bone regeneration in rats with Diabetes mellitus.

**Key words:** Diabetes mellitus, Guided Bone Regeneration, GBR, Photobiomodulation Therapy, PBMT.

**Study type:** Experimental study in animals



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EVALUATION OF THE EFFECT OF PHOTODYNAMIC THERAPY MEDIATED BY VEHICULIZED METHYLENE BLUE IN SURFACTANT MEDIUM AS A COADJUVANT IN THE TREATMENT OF PIECES WITH APICAL PERIODONTITIS AND PRESENCE OF FISTULAS CLINICAL TRIAL, RANDOMIZED, BLIND CONTROLLED TO DOU

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### Abstract

When we speak of Endodontics, we refer to the science and art that includes the etiology, prevention, diagnosis, and treatment of pathological alterations of the dental pulp and its repercussions in the periapical region and consequently in the body. As one of the pillars we have the control of the infection, in case there is no prevent it and treat it when it exists. When the dental disease is already installed, affecting the periapical tissues, causing inflammation of the same, it is when we refer to apical periodontitis. When we refer to the presence of a fistula, it is generally the product of an injury. Infections or inflammations can also cause a fistula to form, also called an intraoral sinus tract. A dental fistula is a small canal that forms from the infected area of the tooth, which usually coincides with the apex of the root, to the outer surface of the gum. The passage acts as a reservoir for microorganisms and their products and as it fills, a small bump forms on the gums. All procedures that take time and supplies and are often unsatisfactory. It is for this reason that photodynamic therapy (PDT) appears as an adjunct in endodontic treatment. Being a non-invasive treatment that has a photosensitizer and a light source for the formation of reactive oxygen species that cause bacterial death. The main limitation of this technique is the formation of dimers that decrease the effectiveness of the therapy. On the other hand, sodium dodecyl sulfate (SDS) showed the ability to reduce this effect of dimer formation. That is why the reason for this study is to validate the photodynamic effect of methylene blue conveyed in SDS at 0.25% for the treatment of patients with apical periodontitis and the presence of fistula, in order to eradicate persistent microorganisms in the root canals. and these treatments are of better evolution. The methodology will consist of the selection of 30 teeth with a diagnosis of apical periodontitis and the presence of a fistula. Patients will be randomly assigned to two groups as follows: Group I, patients undergoing conventional root canal treatment (n = 15) and Group II, patients undergoing conventional root canal treatment combined with antimicrobial photodynamic therapy (n = 15). Clinical findings will be counted due to the absence of symptoms, the presence of a fistula and radiographic parameters.

**Key words:** KEY WORDS: Periodontitis, Fistula, Endodontic treatment, Photodynamic therapy Methylene blue.

**Study type:** Clinical Trial



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**EFFECT OF PHOTOBIMODULATION ON THE SALIVARY GLANDS OF PATIENTS WITH XEROSTOMIA AND HYPOSALIVATION INDUCED BY THE USE OF ANXIOLYTIC DRUGS**

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**Abstract**

Depression is the most common mental illness with antidepressants in the first line of treatment of most depressed patients and this therapeutic class is inevitably associated with side effects and adverse reactions with xerostomia being a symptom that seems to be transverse to them all. Saliva performs multiple functions and plays a vital role in protecting the health of the soft and hard tissues of the oral cavity. Reductions in salivary flow are most often manifested as symptoms of dry mouth and this is the subjective complaint called xerostomia. Although xerostomia is the most frequent indication of reduced salivary production, it is not invariably associated with hyposalivation. The user of antidepressant drugs has a number of important systemic and oral complications. Among the most frequent oral complications we have xerostomia. Treatment for salivary changes remains unknown, but low-level laser therapy has been shown to be effective in improving salivary flow in patients with xerostomia due to diabetes, Sjogren's syndrome, and chemotherapy and radiotherapy for head and lung cancer. neck. This randomized controlled trial aims to evaluate oral symptoms related to salivary gland function and mucosal condition of depressed patients, as well as the effects of photobiomodulation on salivary flow. Sixty patients will be included in the protocol, after signing the Informed Consent Form (ICF), will undergo anamnesis, physical evaluation and oral health self-perception questionnaires and symptoms related to salivary gland function and then will be divided into two groups: Photobiomodulated (FBM) (n = 30); will have their larger salivary glands irradiated with laser Diode (808nm, 4J per point, 40s) and placebo (PCB) (n = 30), which will be subjected to a simulation, where the application protocol will be repeated, but with the laser off, we will perform previous and post treatment sialometries to compare saliva volume and biochemistry analysis, where we will measure total protein and calcium.

**Key words:** photobiomodulation, laser, xerostomy

**Study type:** Clinical Trial



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EFFECTS OF PHOTOBIOMODULATION ON THE PREVENTION OF THE SKIN PRESSURE INJURY IN PATIENTS WITH A DIAGNOSIS OF COVID-19: A RANDOMIZED, CONTROLLED, AND DOUBLE-BLIND CLINICAL STUDY PROTOCOL.

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**Abstract**

Introduction: The high incidence of pressure injuries (PI) is considered a serious public health problem and a negative indicator of the quality of nursing care. Objective: This study aims to verify the preventive effects of the use of photobiomodulation (FBM) in areas more susceptible to the development of PI in patients hospitalized with COVID-19. Methods: This is a controlled, randomized, and blind clinical study including hospitalized participants with risk of developing PI according to the Braden scale. Participants will be randomized into 2 groups: Group 1 - Control (n=70) in which the hospital's standard operating procedures for the prevention of LPP will be performed; and Group 2 - FBM (n=70) the same procedures as the group control and also FBM will be performed once a day, for 10 minutes in each of the 3 regions most commonly affected by LPP, that is, sacral and calcaneal (bilaterally). The FBM will be performed using a plate with 132 LEDs of 660nm and 132 LEDs of 850nm (each LED has P=8 mW; E=4.89J, radiant exposure= 9.6 J/cm<sup>2</sup>; irradiance 16 mW /cm<sup>2</sup>, 10 min). The incidence of PI will be evaluated every 48 hours after hospital admission for a period of 1 month or until hospital discharge if it occurs before this period. The time of onset of LPP will also be evaluated; the possible correlations of anthropometric data measurements and incidence of LPP. The data will be statistically evaluated.

**Key words:** pressure injury, photobiomodulation, prevention, LED, phototherapy.

**Study type:** Clinical Trial



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ANALYSIS OF THE SALIVARY BIOCHEMISTRY OF PATIENTS SUBMITTED TO PHOTOBIMODULATION FOR THE TREATMENT OF HYPOSALIVATION INDUCED BY ANTIHYPERTENSIVE DRUGS

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**Abstract**

The continuous use of medications to control chronic diseases, among them Systemic Arterial Hypertension (SAH), can determine hyposalivation. We carried out research using photobiomodulation (FBM) to stimulate the production of saliva and consequently increase its flow, using two experimental groups: Placebo Group (n=15) and FBM Group (n=25). The study consisted of applying the low power laser to the three pairs of major salivary glands - parotid, submandibular (both external) and sublingual (internal). Initial and final collections were performed - the first during the initial consultation and the last after the fourth application, both consisting of two stages - stimulated and non-stimulated. The application was done once a week for 4 weeks and sialometry after the last application, as well as pH evaluation. The parameters used in this research were: Diode Laser, 808nm, 4J per spot. The irradiation was distributed in 3 points on the parotids, 2 points on the sublingual's and 2 points on the submandibular is, totaling 20 points. The treated group showed a significant increase of 35% (non-stimulated) and 35% (stimulated) in salivary volume. Both groups showed no change in pH. We concluded that MBSF was effective in increasing salivary flow, suggesting that this may be a way to treat these patients, minimizing oral health problems and promoting quality of life for them. The project for the Post Doctorate is to evaluate the biochemistry of the saliva that was collected in the doctoral project

**Key words:**

photobiomodulation, salivary glands, protein, calcium, biochemistry,

**Study type:** Clinical Trial



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### THE USE OF LOW INTENSITY LASER IN PAIN CONTROL AND IN ACCELERATION OF ORTHODONTIC MOVEMENT

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#### **Abstract**

Introduction: Pain and prolonged duration of orthodontic treatment are factors that prevent patients, whether young or adult, from performing them. Low-intensity laser came as one of the alternatives to minimize these effects, controlling pain and accelerating orthodontic movement. Objective: To describe the main ways to use low-intensity laser to control pain and accelerate orthodontic movement. Methodology: This work was carried out through a literature review of several scientific articles found in the available databases. Laser is an acronym for light amplification by stimulated emission of radiation and is an electromagnetic radiation source with some special characteristics that differ from other light sources: monochromaticity, collimation and coherence. From the absorption of low power laser radiation by cellular chromophores, photobiomodulation occurs through the induction of photochemical reactions. Although the mechanisms involved are not completely elucidated, there is evidence that the main mechanism is the increase in oxidative metabolism through the photo-oxidation of cytochrome C, a mammalian mitochondrial membrane protein that participates in electron transport during ATP synthesis. Results: The AsGaAl laser at a wavelength greater than 800 nm was observed with the best analgesic effects. The most frequent dosimetry was in the range between 4-8 J/cm<sup>2</sup>. It was also found that there was a reduction in pain in six of the eight studies included in their literature review, with the best results being found with the use of the Gallium-Aluminum Arsenide laser. Low-power lasers have been shown to reduce post-treatment pain in the first three days after orthodontic separation, according to some studies. The dose, which is the ratio between the energy emitted by a laser beam and the irradiated surface area, for analgesic purposes, has been close to 4 J/cm<sup>2</sup>, but the application protocol depends on the patient's response. Regarding the time of exposure to tissues, there is a predominance of times ranging between 10 and 30 seconds. Conclusion: Low-intensity laser therapy in orthodontics proved to be a good adjuvant in post-orthodontic care pain control and orthodontic movement accelerator, leading to greater adherence to treatment by patients.

**Key words:** Lasers, Low-Level Light Therapy, Orthodontics.

**Study type:** Review





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CLINICAL EVALUATION OF SURFACTANT VEHICLE EFFECT ON ANTIMICROBIAL PHOTODYNAMIC THERAPY ON ENDODONTIC RETREATMENT: CLINICAL PROTOCOL FOR RANDOMIZED CONTROLLED DOUBLE BLIND CLINICAL TRIAL

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**Abstract**

Endodontic infection is defined as the invasion/colonization of microorganisms in the dental pulp, being responsible for pulp and periapical infection that occurs when there is microbial persistence in the root canal system. Among the currently known endodontic retreatment methods, manual and mechanized retreatment, associated or not, and the use of solvents and intracanal medications can be mentioned. As these techniques are similar, the level of failure in retreatment ends up being high. In this sense, antimicrobial photodynamic therapy (aPDT) serves as an adjuvant in endodontic retreatment. It is a non-invasive technique that uses a photosensitizer and a light source to form reactive oxygen species that cause bacterial death. However, the main limitation of the technique is the formation of dimers that reduce the effectiveness of the therapy. On the other hand, sodium dodecyl sulfate (SDS) showed the ability to reduce this dimerization effect. Therefore, the aim of this study will be to evaluate the photodynamic effect of methylene blue conveyed in 0.25% SDS for the treatment of patients with chronic periapical periodontitis in order to eradicate persistent microorganisms in previously filled root canals. The methodology will cover a sample consisting of 30 patients with unsatisfactory endodontic treatment with chronic periapical periodontitis. These patients will initially undergo mechanized endodontic retreatment. After that, they go through aPDT. Patients will be randomized and divided into 03 groups, as follows: 1) mechanized endodontic retreatment (REM) and aPDT with methylene blue (n=10); 2) REM and aPDT with methylene blue in 0.25% SDS (n=10) and 3) REM with placebo irradiation (n=10). Microbiological results will be evaluated by microbial counts before and after treatment and clinical findings for absence of symptoms and radiographic parameters. Data will be treated statistically for comparison between groups. As a primary outcome, it is expected that there will be a reduction in the intracanal microbial load.

**Key words:** Photodynamic antimicrobial chemotherapy (PACT), red laser, methylene blue, oral bacteria, antimicrobial

**Study type:** Clinical or experimental protocol



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**EFFICACY OF LOW LEVEL LASER THERAPY IN THE TREATMENT OF INFERIOR ALVEOLAR NERVE INJURIES:  
AN INTEGRATIVE LITERATURE REVIEW**

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**Abstract**

Introduction: The inferior alveolar nerve (IAN) is responsible for the innervation and sensitivity of the lower teeth, dental papillae, periodontium and supporting bone tissue. Damage to the IAN can result from oral surgical procedures and the complication commonly reported by patients is sensorineural disorder (paresthesia or hypoesthesia). Objective: The objective of this article is to conduct an integrative review of the literature on the efficacy of low-level laser therapy in the treatment of injuries to the inferior alveolar nerve. Methodology: MEDLINE, LILACS, BBO, IBECs and SciELO databases were used to search the articles with the following descriptors: mandibular nerve, inferior alveolar nerve, low-level laser therapy, photobiomodulation therapy, laser phototherapy, low-level laser, low-level light therapy, low-power laser therapy, low-power laser irradiation, laser biostimulation, low-intensity laser and photobiomodulation. The search was limited to articles in English, Portuguese or Spanish, published in the last 10 years, such as clinical trials, case series and case reports. Results: Ten articles met the inclusion criteria (eight randomized clinical trials, one non-randomized clinical trial and one case series). The cause of the injury was variable - in eight studies it was bilateral sagittal osteotomy, in one was the extraction of the lower third molar, and one included different etiology. In the nine studies that had a control group, six followed the split-mouth methodology and another three received laser or placebo irradiation throughout the entire length of the mandible. Data analysis: The best results were observed in general sensitivity tests using the Visual Analog Scale. When the influence of the postoperative period on nerve recovery was evaluated, studies attested to an improvement in the condition regardless of time. However, it is important to emphasize that the early start of treatment, after surgery, brought greater benefits. Although most articles have pointed out treatment efficacy, there was great variability in laser therapy protocols, which represents a considerable limitation. Furthermore, some studies performed little or no monitoring period. Conclusion: The use of low-level laser is effective in the treatment of iatrogenic injuries in the IAN, especially when therapy is started immediately after the injury. However, more studies are needed in order to establish a protocol for the use of laser.

**Key words:** Mandibular Nerve, Mandibular Nerve Injuries, Low-Level Light Therapy

**Study type:** Review



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PHOTOSENSITIZER CONVEYED IN SURFACTANT IMPROVES THE ANTIMICROBIAL EFFECT OF PHOTODYNAMIC THERAPY

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**Abstract**

**INTRODUCTION:** Periodontitis is an inflammatory disease that affects the supportive tissues of the teeth in response to the presence of microorganisms. The gold standard treatment is scaling and root planning. To reduce the use of antibiotics, antimicrobial photodynamic therapy has been studied as an adjunct in periodontal treatment. The main limitation of the technique is the formation of dimers that decrease the effectiveness of the photosensitizer, and sodium dodecyl sulfate has been shown to decrease dimerization. **OBJECTIVE** The aim of this study was to evaluate the photodynamic effect mediated by methylene blue in sodium dodecyl sulfate as an adjuvant treatment of periodontitis. **METHODS:** This clinical trial was performed with forty participants and all of them received scaling and root planning. After 40 days, photodynamic therapy was applied with real irradiation or placebo irradiation. The random allocation was in the following groups: 1) group treated with photodynamic therapy with methylene blue in sodium dodecyl sulfate; or 2) with photodynamic therapy with methylene blue aqueous solution; 3) and group treated with photosensitizer without light irradiation and 4) treated with photosensitizer in sodium dodecyl sulfate without light irradiation. The photosensitizer was in contact for 1min and the irradiation time or not 2min. The laser's wavelength was 660nm and 100mW of output power. Quantitative microbiological evaluation was performed by subgingival biofilm cultivation before and immediately after irradiation procedures. Primary outcome was microbial count and secondary outcomes were clinical probing depth, clinical attachment level and bleeding on probing. **RESULTS:** Preliminary results show that the antimicrobial action of methylene blue conveyed in sodium dodecyl sulfate improved antimicrobial effect of antimicrobial photodynamic therapy. **DATA ANALYSIS:** Analysis of variance followed by Tukey test for confirmation post hoc were performed to statistically analyze data. **CONCLUSION:** In conclusion, sodium dodecyl sulfate as a vehicle to methylene blue mediated antimicrobial photodynamic therapy showed high antimicrobial effect against periodontal bacteria in the clinical environment.

**Key words:** Antimicrobial photodynamic therapy, methylene blue, sodium dodecyl sulfate

**Study type:** Clinical Trial



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### EXPERIMENTAL PROTOCOL FOR EVALUATION OF THE EFFECTS OF PHOTOBIMODULATION ON LOWER LIMB ISCHEMIA

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#### Abstract

Introduction: Peripheral arterial occlusive disease (PAOD) is a late manifestation of atherosclerosis in the lower limbs, due to the progressive decrease in the blood supply that reaches the tissues. Current treatments for PAOD are not as effective in the long term and many patients progress to severe ischemia, with a risk of amputation. Photobiomodulation (FBM) has a positive effect on angiogenesis, but its effects on neoangiogenesis (angiogenesis and arteriogenesis) of ischemic tissues have not yet been properly evaluated. Objective: To evaluate the effect of FBM on neoangiogenesis, by analyzing angiogenesis and arteriogenesis, also considering the motor response and the effects on morphological aspects and muscle repair, in a model of hind paw ischemia induced by femoral artery ligation in rats. Methods: 55 male Wistar rats will be randomly distributed into 3 groups: Control (5); Ischemia (25) and Ischemia + FBM (25). The animals will be adapted for 3 days in relation to functional tests. The animals in the Ischemia Groups will undergo surgical interruption of the femoral artery in the right hind paw. The animals in the Ischemia + FBM group will be irradiated with laser after surgery, 5 sessions per week, for 2 weeks from the immediate postoperative period. Irradiation will be applied along the path of the femoral artery from the proximal point to the distal point of the surgical procedure topography, every 1 cm, with a 660 nm laser in the distal segment of the thigh, and 808 nm, in the root of the thigh, (4J,100mW, 133J/cm<sup>2</sup> per stitch). Oximetry, thermography, hyperalgesia, and macroscopic evaluation of ischemia will be performed on days 1, 3, 5, 7, and 14 postoperatively. Functional analyzes (of mobility) will be performed on days 7 and 14 after surgery. Serum dosages of IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$  and removal of the adductor thigh and gastrocnemius muscles for histological evaluation (diameter of muscle arteries, and remodeling, inflammatory cell count, myonecrosis and immature fibers) and immunohistochemistry (CD31 for angiogenesis and alpha-SMA for arteriogenesis), as well as the comparison of the weight of the tibialis anterior muscles, will be performed after euthanasia on days 1, 3, 5, 7 and 14. The results will be statistically treated.

**Key words:** Keywords: Atherosclerosis, neoangiogenesis, angiogenesis, arteriogenesis, limb ischemia, photobiomodulation, laser.

**Study type:** Experimental study in animals



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### EFFECTS OF GREEN LIGHT ON MELANOMA OF C57BL6 MICE

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#### **Abstract**

Visible light is an electromagnetic radiation in the 400 to 700 nm range that photosensitizers melanin, generating reactive oxygen species (ROS) and damage in membranes and DNA both by type I and type II mechanisms. Therefore, it may be possible to use visible light to disturb the survival and growth of pigmented melanoma lesions, even though there is no information regarding the wavelength's effectiveness. Nevertheless, this hypothesis needs to be tested, since visible light can also stimulate tumor growth by photobiomodulation mechanisms. The focus here was to verify the effects of photosensitization of melanin in B16F10 cells and melanoma induced in C57BL/6 mice. The cell viability was demonstrated by the MTT assay after irradiation with visible light (466, 532 and 630nm). After approved by Ethics Committee in Animal (250991), mice were divided into groups with carcinogenesis induction (C+) or control (C-), exposed (I+) or no (I-) to green light photobiomodulation sessions. Tumor measurements and light doses in a homemade radiator were performed at 7-day intervals. After seven weeks, skin samples were collected from all groups for histopathological analysis. Pigmentation was quantified by the ImageJ program and the size of irradiated C + and C + tumors were quantified with a pachymeter. Statistical analysis was performed in the Origin 7.0 program at  $p < 0.05$ . Green light (532 nm) caused death in 30% of the B16F10 cells. In mice, C-/I+ group showed initial pigmentation but no histological changes after irradiation. C+/I- showed tumor progression up to 3 times the initial volume while C+/I+ decreased it by 20%. The results suggest the possibility of phototherapy for melanoma despite the need for a long-term study for further clinical testing in humans.

**Key words:** melanoma, green light, phototherapy

**Study type:** Experimental study in animals



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### AMOXICILLIN INTERACTS TO METHYLENE BLUE AND MAY REDUCE THE EFFICACY OF ANTIMICROBIAL PHOTODYNAMIC THERAPY

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#### Abstract

Antibiotics (ATBs) are medicines used to treat bacterial infections. As a result of over and misuse, bacteria resistant to ATBs have increased more and more. In this context, antimicrobial photodynamic therapy (aPDT) has become an adjuvant treatment option. Therefore, it is necessary to increase the knowledge about the possible interactions between the ATBs and aPDT, the sensitizer agent. Since the generation of reactive oxygen species is essential to aPDT efficacy, quantifying the production of these species in the presence of the ATB is the first step to evaluate possible interactions. Quantify photoinduced production of hydroxyl radicals using methylene blue (MB) as photosensitizer in the presence of amoxicillin (AMX). The production of hydroxyl radical was determined by registering absorption spectra to evaluate the bleaching the RNO probe, when MB (Sigma-Aldrich) is irradiated alone or in the presence of AMX (Aurobindo laboratory, capsule, 500mg) in aqueous solution. The irradiation was performed with a laser (Quantum, Ecofibras®, Brasil) at  $\lambda$  660nm,  $I = 307\text{mW/cm}^2$  and  $H = 18,6\text{J/cm}^2$  for 720s. The data were collected both in the presence and absence of oxygen. After data analysis the RNO bleaching constant (CB) was determined in all cases. For statistical analysis, Shapiro-Wilk was used for normality tests. The descriptive data were presented as mean and standard deviation. For comparison between groups Two-way ANOVA test was used followed by Tukey post hoc. The OriginPro 2021 software was used to perform data analysis and graph preparation. The CB is proportional to the amount of hydroxyl radical produced. The CB was significantly higher in MB alone ( $1.11 \times 10^{-3} \pm 1.80 \times 10^{-4}$  mol/L.s) than in the presence of AMX ( $4.93 \times 10^{-4} \pm 3.75 \times 10^{-5}$  mol/L.s). When the measurements were performed in absence of oxygen, the CB for MB alone significantly reduced ( $4.34 \times 10^{-4} \pm 1.73 \times 10^{-4}$  mol/L.s) while the MB-AMX constant showed no change ( $3.95 \times 10^{-4} \pm 1.32 \times 10^{-4}$  mol/L.s). RNO bleaching by MB is oxygen dependent, which implies that the effect is related to the reactive oxygen species (hydroxyl radical) production. In the presence of AMX, MB presented reduced bleaching constant due to MB-AMX interactions. In order to deeply understand these interactions further investigations are needed. These days suggest that AMX may reduce the aPDT effect when antibiotic therapy is associated with aPDT, however the extension and clinical impact of this reduction also still needs further investigation.

**Key words:** antibiotics, amoxicillin, hydroxyl radical, antimicrobial photodynamic therapy, aPDT, methylene blue

**Study type:** Experimental study in vitro



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### INFLUENCE OF PHOTOBIOMODULATION ON SIGNAL TRANSDUCTION PATHWAYS IN VITRO

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#### **Abstract**

**Introduction:** Wound healing is a dynamic process aimed at replacing damaged tissue and is prone to interferences from underlying pathological conditions such as Diabetes Mellitus. Chronic diabetic foot ulcers (DFUs) are among the most severe and costly complications of diabetes and is a regular cause for hospitalization and lower limb amputation. At a cellular level, DFUs are characterized by the reduced production and function of cytokines and growth factors and their receptors, and alterations in signal transduction pathways, leading to decreased cellular migration, proliferation and viability and increased oxidative stress and cell death. Photobiomodulation (PBM) has shown to improve and hasten chronic wound healing. It has also been shown to reduce inflammation and oxidative stress. **Objectives:** This study investigated the effect of PBM at 660 nm on various cell signaling pathways in wounded fibroblast cell models. **Methods:** WS1 human fibroblasts (ATCC®, CRL-1502™) were modeled into wounded (W), diabetic wounded (DW) and hypoxic diabetic wounded (HDW). Cells were irradiated at a wavelength of 660 nm with a fluence of 5 J/cm<sup>2</sup> (irradiation time 454 s; power output 100 mW; spot size 9.1 cm<sup>2</sup>; power output density 11 mW/ cm<sup>2</sup>; energy 45.4 J). Unirradiated cells served as controls (0 J/cm<sup>2</sup>). Post-irradiation, cells were incubated for 24 and 48 h and subjected to different analytical methods to determine the effect on the TGF- $\beta$ /Smad, JAK/STAT, PI3K/AKT/mTOR, and AKT/FoxO1 signaling pathways. **Results:** PBM increased migration rate, proliferation, fibroblast differentiation, and cell survival, as well as increased vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF), and enzymic antioxidants superoxide dismutase (SOD), catalase (CAT), and heme oxygenase (HMOX1). Post-PBM at 660 nm there was activation of the JAK/STAT, PI3K/AKT/mTOR, and AKT/FoxO1 signaling pathways, with no effect detected on the TGF- $\beta$ /Smad pathway. **Conclusion:** This study provides a novel insight into the molecular framework underlying tissue regeneration under diabetic conditions in vitro in response to PBM at 660 nm. Activation of these signaling pathways lead to increased cell activities resulting in hastened wound closure. Further research into specific signal transduction pathways and activation of defined molecules in these pathways is an important step in understanding how PBM affects cellular processes and further promotes and advances the field of PBM.

**Key words:** photobiomodulation, diabetes, wound healing

**Study type:** Experimental study in vitro





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### EFFECTS OF TRANSCUTANEOUS VASCULAR PHOTOBIO-MODULATION ON A MUSCLE MASS DURING THE COMPENSATORY HYPERTROPHY PROCESS IN SKELETAL MUSCLE

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#### **Abstract**

Skeletal muscle is a dynamic and adaptive tissue capable of altering its characteristics to meet its diverse functional demands. Compensatory hypertrophy (CH) occurs due to excessive mechanical load on a muscle, promoting an increase in the size of muscle fibers. Photobiomodulation (PBM) has demonstrated beneficial effects on muscle tissue during CH however there is little information about the transcutaneous vascular application. The aim of this study was to evaluate the effect of transcutaneous vascular photobiomodulation (VPBM) on the muscle mass ratio during the CH process. Wistar rats were divided into three groups: control group (n=5), hypertrophy (H) group (n = 10) and Hypertrophy + VPBM group (n = 10). CH was induced through the ablation of synergist muscles of the plantaris muscle. The preserved plantaris muscle below the removed muscles was submitted to excessive functional load. VPBM was performed with a low-level laser (AsGaAl,  $\lambda = 780$  nm; 40 mW; energy density 80 J/cm<sup>2</sup>; 80 seconds; 1 point, energy 3.2 J). Animals were euthanized after seven and 14 days. The plantaris muscles were removed, weighed on a semi-analytical scale and submitted analysis to determine the weight ratio of the left and right muscles (L/R). The results showed a reduction in muscle mass in groups H and H + VPBM when compared to the control group after 7 days. At 14 days a reduction in muscle mass was observed in group H compared to the control group. The H + VPBM group kept its muscle mass similar to the control group values. In conclusion, the VPBM applied for 14 days maintained the muscle mass ratio at levels close to those found in the control group.

**Key words:** Skeletal muscle, Low-Level Light Therapy, Hypertrophy.

**Study type:** Experimental study in animals





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### VASCULAR PHOTOBIO-MODULATION ON CREATINE KINASE, ASPARTATE AMINOTRANSFERASE AND GRANULOCYTES AFTER MUSCLE INJURY IN AN ANIMAL MODEL

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#### Abstract

**Introduction:** The punctual/local method of photobiomodulation therapy (PBM) administration has demonstrated benefits on the modulation of important cytokines and growth factors during the skeletal muscle repair following an acute injury. Evidences suggest that the use of non-invasive vascular photobiomodulation (VPBM), well known as modified ILIB (Intravascular or Intravenous Laser Irradiation of Blood), has effects on systemic diseases, immune system and inflammatory process which could improve the muscle repair. **Objective:** The aim of the present study was to evaluate the effects of preventive and therapeutic VPBM on biomarkers and granulocytes after an acute injury in tibialis anterior muscle of rats. **Methods:** Wistar rats (n=35) were randomly divided into four experimental groups: control, Injury, Previous VPBM+Injury and Injury+VPBM after. The tail vein of the animals was transcutaneous irradiated using a low-level AlGaAs diode laser (780 nm, 40 mW, 0.04 cm<sup>2</sup>, 3.2 J, 80 s) and the procedure was performed in different period, prior and after the injury induction. Blood samples were collected at 2 and 7 days following the cryoinjury procedure and submitted to a hematology analyzer to obtain the relative granulocytes count. Furthermore, the serum concentrations of creatine kinase (CK) and aspartate aminotransferase (AST) were measured with an automatic analyzer. **Results:** The Injury group showed higher relative granulocytes count at day 2 in comparison with the treatment groups (Previous VPBM+Injury and Injury+VPBM/after). CK concentration in the Injury+VPBM/after group was lower at 2 and 7 days and AST levels were lower only at day 2 in comparison to the Injury group. Previous VPBM+Injury group showed lower CK and AST levels when compared to Injury and Injury+SPBM after groups at all evaluated periods. The Previous VPBM+Injury group also showed lower CK and AST concentrations at day 7 in comparison to the Control group. **Conclusion:** VPBM was able to reduce the CK and AST levels and the number of granulocytes specially after 2 days and the effect was more pronounced in the previous VPBM group.

**Key words:** NILIB, systemic photobiomodulation, photobiomodulation, low-level light therapy, muscle injury, muscle repair.

**Study type:** Experimental study in animals



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### GAIT ANALYSIS OF AN EXPERIMENTAL MODEL OF SPINAL CORD INJURY AFTER VASCULAR PHOTOBIO-MODULATION

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#### **Abstract**

**Introduction:** Spinal cord injury (SCI) results from a trauma that promotes discontinuity of the spinal canal, leading to functional and sensory losses in the areas below the injury. Secondary processes resulting from the SCI are therapeutic targets. Photobiomodulation resources (FBM) used in the treatment of SCI demonstrated improvement in functional recovery, reduction of the inflammatory process and in oxidative stress. The vascular FBM (VFBM) is a systemic modality of the use of resources of light which has already shown positive effects in many clinical conditions including improved immune response, reduced inflammatory process, normalization of cell membrane potential and increase in ATP production. **Objective:** To analyze the effects of VPBM on gait functionality in the immediate phase of SCI in an experimental model. **Methodology:** for this study 15 Wistar rats were divided into 3 groups as follows: control (n=5), SCI (n=5) and SCI+FBMV (n=5). The gait functionality analysis was performed at 1, 3, 7, 14, 21, 28 and 35 days after the SCI induction. The SCI VFBM group was irradiated with Low Intensity Laser (AsGaAl, 780 nm, 80 J/cm<sup>2</sup>, 40 mW, for 80 seconds, totaling 3.2J energy in a single point) for 14 consecutive days, being applied in the tail artery region. The Basso-Beattie-Bresnahan Index (BBB) was used for the gait functionality evaluation of the right lower limb (MID) of the animals. Data normality was verified using the Kolmogorov-Smirnov test, with parametric data expressed as mean and standard error. The comparison of functional tests between groups was performed using the ANOVA with a significance level of  $p \leq 0.05$ . **Results:** Both the SCI and the SCI+VFBM group showed a decreased motor function compared to the control group ( $P < 0.0001$ ). At 14 days after SCI, there was an acceleration of the functional recovery of the MID in the SCI VFBM group when compared to the SCI group ( $p = 0.0042$ ), and this recovery was maintained in the following assessments: 21 ( $p = 0.0001$ ), 28 ( $p = 0.0002$ ) and 35 days ( $p < 0.0001$ ) after SCI. In conclusion, the results showed a therapeutic potential of VFBM performed immediately after the SCI in the gait functionality recovery.

**Key words:** Spinal cord injury, Photobiomodulation, Low Power Laser, Gait analysis.

**Study type:** Experimental study in animals



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### COMPARISON AMONG OZONETHERAPY, LASERTHERAPY AND COMBINED THERAPY IN THE TREATMENT OF KNEE RHEUMATOID ARTHRITIS. AN EXPERIMENTAL STUDY

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#### **Abstract**

**Introduction.** Rheumatoid arthritis (RA) is a chronic inflammatory, autoimmune, systemic and progressive disease that causes irreversible destruction of cartilage and bone. Cytokines are fundamental in inflammation, being responsible for the synthesis and excessive release of metalloproteinases (MMPs). Experimental in vivo studies using photobiomodulation therapy (LLLT) and ozone therapy have demonstrated positive effects on the modulation of the inflammatory process. **Objectives.** To analyze the in vivo and in vitro effects of photobiomodulation and ozone therapy on the levels of the following inflammatory markers: Cox-2, IL-6, IL-10, TNF- $\alpha$ , MMP-3, MMP-9 and MMP-13, collagen II, disintegrin, ADAMTS 1, 4 and 5, TIMP and PCR; on tissue histology and on the mechanical properties of tissue. **Materials and methods.** Forty-eight male Wistar rats will be divided into 6 groups, G1 (healthy control), G2 RA (rheumatoid arthritis), G3 RA LLLT (rheumatoid arthritis + photobiomodulation therapy), G4 RA O3 (rheumatoid arthritis + ozone therapy), G5 RA LLLT + O3 (rheumatoid arthritis + photobiomodulation therapy + ozone therapy) and G6 (RA + corticosteroid). The animals in groups G2, G3, G4, G5 and G6 will be submitted to an RA model in which they will receive 3 injections of the lesion-inducing solution on days 0, 7 and 28 which will be applied to the knee joint. After induction, the animals will be subdivided into 5 groups with 8 animals in each group. The treatment of groups G3 (RA LLLT), G4 (RA O3), G5 (RA LLLT + O3) and G6 (RA + corticosteroid) will begin immediately after the last induction and a laser with a wavelength of 808 nm and energy of 10J and/or 0,05ml of the O2-O3 mixture at a concentration of 20 $\mu$ g O3/ml O2 will be used. The rats will be euthanized using thiopental (100 mg/kg) and lidocaine (100mg/ml). Protein and gene expression analysis of Cox-2, IL-6, IL-10, TNF- $\alpha$ , MMP-3, MMP-9 and MMP-13, collagen II, disintegrin, ADAMTS 1, 4 and 5, TIMP and PCR will be performed by western blotting and PCR techniques. An evaluation of tissue histology and tissue mechanical properties will also be carried out. The data found will be subjected to the Shapiro-Wilk test, analysis of variance (ANOVA) and comparison between groups using the Tukey post hoc test.

**Key words:** Photobiomodulation; Ozone therapy; Rheumatoid arthritis; Cytokines; Metalloproteinases; Chondrocytes.

**Study type:** Experimental study in animals.



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**LOW POWER LASER AND AMBER LED IN THE TREATMENT OF FACIAL TELANGIECTASIAS: A COMPARATIVE STUDY**

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**Abstract**

Telangiectasias are visible dilations of arterial or venous capillaries with reddish and bluish tones, which differ from other vascular manifestations due to their size. Phototherapy has been widely used in the treatment of this pathology, LED therapy and low power laser therapy are examples of non-invasive treatments that use light as a stimulatory therapy for the affected cells. The purpose of this study was to compare the results between the amber colored LED (590nm) and the red low power laser (658nm) in the treatment of facial telangiectasias. Therefore, based on the scientific literature, a treatment protocol was elaborated, carried out in ten sessions, two a week, for five weeks, at the Esthetics clinic of the Centro Universitário do Espírito Santo - UNESC. Three female models were selected, aged between 22 and 54 years, phototypes II and III, with accentuated telangiectasias across the face. The protocol consisted of skin cleaning with cleansing gel, physical exfoliation, toning, laser therapy on the right hemiface for 16 seconds at each end of the telangiectasias and application of amber LED on the left hemiface for 4 minutes, ending the protocol with application of serum with vitamin C all over the face. The study showed greater effectiveness of the red laser compared to the amber LED, with no total remission of telangiectasias, but considerable reduction in the size of the lesions, as well as the underlying redness. There was also an improvement in the texture and freshness of the skin on the right side, in all patients. It is concluded that, even though it is a more accessible photodynamic therapy with aesthetic indication for the treatment of telangiectasias, the amber LED is less effective in reducing facial lesions than the red laser.

**Key words:** capillary dilation, led therapy, laser therapy, phototherapy

**Study type:** Clinical or experimental protocol



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**PHOTOBIMODULATION THERAPY PARTICIPATES IN THE TISSUE ORGANIZATION AND MAINTENANCE OF MECHANICAL PROPERTIES IN AN EXPERIMENTAL MODEL OF SKIN INJURY**

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**Abstract**

The skin is a lining tissue that is permanently acted on by the environment, often aggressively, leading to tissue damage. Usually, in its repair process, structural changes occur that evolve into the development of a scar. Photobiomodulation therapy (PBM) is a resource used to modulate the inflammatory process, aiding in the skin repair process. However, the effect of PBM on the mechanical properties of the skin and its impact on tissue quality have not yet been compared. The objective of this work was to evaluate the effect of photobiomodulation therapy on the maintenance of mechanical properties and tissue organization in an experimental model of skin lesion. Wistar rats, between 150g to 200g, with 3 months of age were used. After anesthetizing, 2 sharp injuries were made on the animal's back using a surgical scalpel. The animals were divided into 5 groups of 5 animals each, namely: Control without injury (CTL), Untreated scar (NT), Scar + anti-inflammatory (DIC), scar + 1J laser (PBM1J) and scar + 3J laser (PBM3J). Pharmacological treatment and laser therapy were performed immediately after lesion induction and daily irradiation was maintained until the seventh day. After 28 days, the animals were euthanized with an overdose of the same anesthetic and the tissue was immediately removed for histological analysis and tensile tests. Results: The NT group showed reduced mechanical properties and alterations in histological analyses. All treated groups showed improvement in tissue organization when compared to the NT group. The PBM1J group showed a significant improvement in mechanical properties and histological organization. We conclude that the use of sodium diclofenac did not alter the skin's mechanical properties. However, photobiomodulation therapy showed an expressive improvement in mechanical properties (Fmax and Dmax) in addition to an improvement in tissue organization.

**Key words:** Scar, Laser Therapy, Collagen, Mechanical Properties, Sodium Diclofenac

**Study type:** Experimental study in animals



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### USE OF PHOTOBIO-MODULATION (PBM) THERAPY ASSOCIATED WITH CARBON/GRAPHENE OXIDE COMPOSITE BIOMATERIAL IN BONE REPAIR IN OSTEOPENIA MODEL

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#### Abstract

Osteoporosis mainly affects postmenopausal women due to the marked decrease in bone mineral density (BMD), with a higher occurrence of fractures and difficulty in union. It can be considered a public health problem, generating significant costs with prevention, surgeries and long-term treatments. Photobiomodulation (PBM) can contribute positively and has been widely studied to determine effective protocols for osteogenesis, being a non-invasive and low-cost method. Biomaterials can replace bone tissue and favor the repair process. Previous studies (in vitro) carried out by the group using Carbon Biomaterials and Composite Carbon / Graphene Oxide (CB) proved to be interesting, but the in vivo effects are not yet known. Objective: To evaluate the effects of PBM associated with CB implanted in bone repair after induction of a non-critical lesion in the tibia of osteopenic rats and whether they can improve tissue response and accelerate the repair process. Materials and Methods: Wistar rats were randomly distributed into groups: SHAM (ovariectomy simulation); NL (ovariectomy (OVX)); NT (untreated bone lesion); PBM (808 nm, 200J/cm<sup>2</sup>, 100mW, 6J, 60s), C (carbon), PBM + C, G (graphene), PBM + G. The animals were sacrificed three times (30-60-90 days). Blood and tibiae were collected for the following analyses: Biochemistry: ALP (alkaline phosphatase), TRAP (tartrate acid resistant phosphatase) and Functional: Mechanical property (3-point bending test). Results: After 30 days of bone injury, all treated groups showed a significant increase in ALP levels and a considerable drop in TRAP in relation to TN, favoring the repair process. In groups treated for 60 days, ALP had values close to or lower than NT, while TRAP significantly decreased compared to NT. For 90 days, all groups were balanced between markers. For mechanical properties (Maximum Strength / Maximum Deformation) the groups C + PBM and G + PBM at 30 days had a decrease in strength when compared to groups PBM, C and G, but at 60 days all treated groups had higher fracture resistance than NT, except for G + PBM. After 90 days, all groups had similar results to each other. Conclusion: the group treated only with PBM showed benefits in healing and repair, and in the groups with associated treatments PBM + CB, no damage to bone formation was observed. The clinical use of the association of CB with PBM seems promising, and perhaps a future study option for its use in large bone losses.

**Key words:** photobiomodulation, osteoporosis, graphene,

**Study type:** Experimental study in animals



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**EFFECT OF PHOTOBIMODULATION COMBINED WITH PHYSICAL THERAPY ON FUNCTIONAL PERFORMANCE IN CHILDREN WITH MYELOMENINGOCELE: A PROTOCOL RANDOMIZED CLINICAL BLIND STUDY**

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**Abstract**

Myelomeningocele is a severe type of spina bifida, resulting from improper closure of the neural tube. This condition drastically affects the structures of the spinal cord resulting in deficiencies. The combination of these deficiencies results in an overall decrease in mobility and functional participation amongst this population. Physiotherapy plays an essential role in rehabilitating people with MMC. The current literature shows that resources such as photobiomodulation (PBM) may support the rehabilitation of neurological conditions. The aim of the proposed study is to evaluate the effects of photobiomodulation (PBM) combined with physical therapy on functional performance in children with low lumbosacral myelomeningocele. Materials and methods: This is a protocol randomized clinical blind study that will include 30 individuals of both sexes, aged between 5 to 8 years, diagnosed with low and sacral lumbar myelomeningocele and capable of performing the sit-to-stand task. The participants will be randomly assigned into two treatment groups: PBM + physiotherapeutic exercises and sham PBM + physiotherapeutic exercises. Irradiation will be carried out with a light emitting diode (LED) at a wavelength of 850 nm, energy of 25 J per point, 50 seconds per point and a power of 200 mW. The same device will be used in the placebo group but will not emit light. Muscle activity will be assessed using a portable electromyography (BTS Engineering) and the sit-to-stand task will be performed as a measure of functioning. Electrodes will be positioned on the lateral gastrocnemius, tibialis anterior and rectus femoris muscles. The Pediatric Evaluation of Disability Inventory will be used to assess functional independence. Quality of life will be assessed using the Child Health Questionnaire—Parent Form 50. Changes in participation will be assessed using the Participation and Environment Measure for Children and Youth. The data will be analyzed with the aid of GraphPad PRISM. Discussion: The results of this study can contribute to a better understanding of the effectiveness of PBM on functioning and quality of life in children with myelomeningocele. ClinicalTrials.gov Identifier: NCT04425330.

**Key words:** Myelomeningocele, Physical Therapy, Physical Functional Performance, photobiomodulation

**Study type:** Clinical or experimental protocol





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### PHOTODYNAMIC THERAPY IN SIMPLE HERPES RESISTANT TO DRUG TREATMENT

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#### **Abstract**

Lip herpes is a very common condition caused by the herpes simplex virus (HSV). It is a chronic condition that causes blistering vesicle lesions in the lip and perioral region and its transmission occurs through direct contact with the virus. In the event of triggering factors such as excessive sun exposure, infections, stress, trauma, hormonal changes and fever, the virus that remains latent in regional ganglia migrates to skin and/or mucosa cells and triggers lesions. The viral cycle lasts from 7 to 15 days in 21 days, its self-resolution occurs. Treatment is done with the use of topical and oral antivirals. It is a common infection and its presence has a negative impact on the quality of life of affected individuals, as it causes pain and discomfort, which can lead to malnutrition and dehydration. Photodynamic therapy aims to promote microbial reduction, using methylene blue with a photosensitizing agent and low-power red laser, and thus contributes to speeding up repair. This report describes the case of a 35-year-old male patient who presented to the dental office complaining of herpes lesions in the upper and lower lip, in the crust phase, using topical and oral acyclovir for 7 days with painful symptoms. quite exacerbated. Bioxtra oral gel was used to remove the crusts and then methylene blue gel was applied at a concentration of 0.01%, leaving it for 5 minutes pre-irradiation, and then laser irradiation red with energy of 9J per point, these being 1 cm apart from each other, 2J of red laser was also applied around the lesions. After the intervention, the patient was able to open his mouth and was released with a prescription for Admuc. Within 24 hours, the lesions were in the final stage of healing and the patient reported a significant improvement in pain. It is concluded that the use of photodynamic therapy is a great adjuvant resource for accelerating tissue repair and has an important role in improving the quality of life of the patient.

**Key words:** photobiomodulation, simple herpes, drug resistance

**Study type:** Case report/Case series





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PHOTOBIO-MODULATION THERAPY ASSOCIATED WITH THE USE OF GRAPHENE AND CARBON COMPOSITE BIOMATERIALS IN BONE REPAIR IN AN EXPERIMENTAL MODEL OF INDUCED OSTEOPENIA – MORPHOLOGICAL AND BIOMECHANICAL ASPECTS

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### Abstract

Bone lesions are more frequent in women after menopause and in elderly women due to the lack of estrogen hormone, which causes a decrease in bone density. Some experimental work showed that Photobiomodulation (PBM) could be interesting for helping bone repair. Some works that used carbonaceous materials with controlled porosity are investigated with the purpose of a bone substitute. The aim of this study was to evaluate the effect of Photobiomodulation Therapy associated with the use of composite carbon and graphene biomaterials on biomechanical and morphological aspects, in a model of injury in tibiae of ovariectomized rats. Materials and Methods: For the analysis of mechanical (flexion test and determination of elastic modulus) and morphological (Scanning Electron Microscopy) properties, Wistar rats aged 80-90 days, with mass between 240- 250g and randomly distributed in the following groups: SHAM (ovariectomy simulation); NL (ovariectomy (OVX)); PBM (6J; 60s; 100mW; 808nm; 200 J/cm<sup>2</sup>); C (Carbon); G (Graphene Oxide); PBM+C and PBM+G. After 7 days of ovariectomy, bone damage was induced in both tibias. After the different treatments, the animals were euthanized at different times (30, 60 and 90 days) and the tibia was removed for analysis. All samples were obtained from another line of research. Preliminary Results: There was little significant difference in apparent bone density in the groups associated with PBM in the period of 30, 60 and 90 days in relation to NT, NL, C, G and a difference in the PBM+G group in relation to PBM+ C within 30 days. The modulus of elasticity data was confirmed using 2 techniques for testing. An increase in the modulus of elasticity was verified for the PBM, G and PBM+G groups in the three periods, using the flexion technique, compared to the NT group, indicating greater stiffness. Specifically, the PBM group stood out in the increase in the elastic modulus in relation to the NT group in the three periods. Preliminary conclusion: From these preliminary results, we observed that the material treated with PBM, with Graphene composite presented stiffness superior to the other treated groups in the period of 60 and 90 days. Isolated Photobiomodulation Therapy showed improvement in mechanical properties 90 days after the start of treatment.

**Key words:** Photobiomodulation (PBM), Low Intensity Laser, bone repair, ovariectomy, Activated charcoal, Graphene, Mechanical properties.

**Study type:** Clinical Trial



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**PHOTOBIMODULATION IN THE PROCESS OF SKIN ELEVATION: TISSUE REGENERATION**

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**Abstract**

Introduction: Skin flap is a surgical practice commonly used for tissue repair. The loss of skin flaps (CR) due to tissue necrosis is a serious complication and may occur with mortality and occurs in 2 to 20% of the procedures performed. The main cause is tissue hypoperfusion combined with ischemia-reperfusion injury. There is currently no adjuvant treatment that improves the survival of skin flaps, without side effects or significant risks. The effect of photobiomodulation stimulation on endothelial progenitor cells, responsible for neovascularization and maintenance of vascular endothelial function in the cutaneous flap may be an outcome to improve the healing process favoring tissue reconstruction. Objective: This study aimed to evaluate the effect of Low Intensity Laser on skin flaps in mice. Methodology: Forty-eight adult male mice (Balb/C, 20 – 25g) were used, divided into two groups: Control (n=24) and Experiment (n=24). We increased the skin flaps on the back of the animals of both groups and submitted to irradiation with a low intensity laser in the Experiment group. In the Control group there was only the simulation of the application. Photobiomodulation in the flap pedicle had a wavelength of 660 nm, radiant exposure of 2 J/cm<sup>2</sup> for a total time of 20 seconds in scan, in the internal vascular pedicle of the flap. After treatment, the animals were separated into 3 subgroups (according to the day of euthanasia) containing 8 animals each, within the respective Control and Experiment groups. The animals were euthanized on the 4th, 7th and 10th postoperative day. Results: Treatment with photobiomodulation caused a decrease in necrosis at all postoperative evaluation times with increased interleukin-6 (IL-6) in mice that were not submitted to photobiomodulation therapy and reduction in tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-10 (IL-10). Conclusions: There was a significant reduction in inflammatory cells in the groups that received photobiomodulation treatment, corroborating the thesis of the protective effect of low-intensity laser in the healing process of the flap.

**Key words:** photobiomodulation, low intensity laser, surgical flaps, necrosis.

**Study type:** Experimental study in animals



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EFFECT OF PHOTOBIMODULATION THERAPY IN BALANCE BETWEEN EFFECTOR AND REGULATORY T CELLS IN EXPERIMENTAL MODELS OF CHRONIC OBSTRUCTIVE LUNG DISEASES

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### Abstract

Photobiomodulation (PBM), can be used in lung diseases, due to the low cost and absence of effects. This therapy aims at a negative policy of cytokines, chemokines, and transcription factors. It is known that Treg lymphocytes suppress other effector cells (Th1, Th2 and Th17) and inflammatory cells for tissues, as well as the release of inflammatory mediators. In this sense, the objective was to evaluate Foxp3 Treg cells, as well as the production of IL10 in the lung after PBM in obstructive diseases such as asthma and COPD. Some parameters were studied in Balb/C with asthma and C57BL/6 with COPD submitted to the diode laser (660nm, 100mW, 180s) for 15 days. The protocol used for asthma induction consisted of sensitization with ovalbumin (day 0 and day 14) and orotracheal challenge on day 21 (3x per week/5 weeks) and that of COPD in the application of extract from the cigarette smoke orotracheal pathway (3x per week/7 weeks). Bronchoalveolar lavage (BAL) and lungs were collected for analysis. The data were submitted to the One-way ANOVA test followed by the Newman-Keuls test. Significance levels adjusted to 5% ( $p < 0.05$ ). PBM reduced the number of inflammatory cells, the levels of IL1- $\beta$ , TNF- $\alpha$ , IL-6, IFN- $\gamma$ , ( $p < 0,001$ ), LTB4 ( $p < 0,01$ ), IL-5 and IL-4 in asthma ( $p < 0,001$ ), and increased IL-10 ( $p < 0,001$ ). We also observed a decrease in collagen and mucus ( $p < 0,001$ ). there was a significant increase CD4+CD25+Foxp3+ and CD4+IL-10+. On the other hand, PBM reduced the number of inflammatory cells, the levels of IL1- $\beta$ , TNF- $\alpha$ , IL-6, IFN- $\gamma$ , MCP-1, GM-CSF ( $p < 0,001$ ), KC/CXCL1, LTB4 ( $p < 0,01$ ) and increased IL-10 ( $p < 0,001$ ). We also observed decrease of collagen, mucus, bronchoconstriction index, alveolar enlargement, CD4+, CD8+, CD4+STAT4+ and CD4+IFN- $\gamma$ + in COPD. Thus, PBM treatment can be used as an immunotherapeutic strategy for asthma and COPD, through the possible mechanism of expansion of regulatory T cells and reduction of T effectors population cells in lung.

**Key words:** Photobiomodulation, CD4+, CD8+, COPD.

**Study type:** Experimental study in animal



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### REGULATORY MECHANISM OF PHOTOBIOMODULATION THERAPY IN AN EXPERIMENTAL MODEL OF CHRONIC ASTHMA

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#### **Abstract**

**INTRODUCTION:** It is largely known that photobiomodulation (PBM) has beneficial effects on allergic pulmonary inflammation. Our previous study showed an anti-inflammatory effect of the PBM in an acute experimental model of asthma, and we see that this mechanism is partly dependent on IL-10. However, it remains unclear the activation of regulatory T cells mediated by PBM in a chronic experimental model of asthma. However, it is still unclear that this IL-10-dependent mechanism may come from the increase in regulatory T cells induced by PBM in an experimental model of chronic asthma. **OBJECTIVE:** In this sense, the objective of this study was to verify the anti-inflammatory role of the photobiomodulation in the pulmonary inflammatory response in a chronic experimental asthma model. **METHODS:** The protocol used for asthma induction was the administration of OVA subcutaneously (days 0 and 14) and intranasally (3 times / week, for 5 weeks). On day 50, the animals were sacrificed for the evaluation of the different parameters. The PBM used was the diode, with a wavelength of 660 nm, power of 30 mW and 3J for 60 s/point, in three different application points. **RESULTS:** Our results showed that PBM increases macrophages, neutrophils, and lymphocytes in the BAL. Moreover, PBM decreased the release of cytokines by the lung, mucus and collagen in the airways and pulmonary mechanics. When we analyzed the percentage of Treg cells in the group irradiated with laser, we verified an increase in these cells, as well as the release of IL-10 in the bronchoalveolar lavage. **CONCLUSION:** Therefore, we conclude that the use of PBM therapy in chronic airway inflammation attenuated the inflammatory process, as well as the pulmonary functional and structural parameters, probably due to an increase in Treg cells.

**Key words:** Photobiomodulation, Asthma, T - reg

**Study type:** Experimental study in animals



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### EFFECT OF PHOTOBIMODULATION ASSOCIATED WITH OPIOID ON THE CONTROL OF PAIN CAUSED BY TENDINITE INDUCED IN AN EXPERIMENTAL MODEL

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#### **Abstract**

Pain caused by tendinopathies is frequent and difficult to be treated, but there are a variety of therapies and treatments to alleviate this symptom, the most used today are anti-inflammatory drugs and opioids, as they present pain relief quickly, but momentarily, presenting risk due to adverse effects, which can cause severe signs and symptoms and even irreversible effects to the individual's health. Photobiomodulation therapy appears to be a promising therapy for pain modulation, as so far, no adverse effects have been observed when compared to anti-inflammatory drugs and opioids. The search for new therapies for the treatment of pain assumes a prominent role in the medical field. This study aims to evaluate and compare the action of opioids associated with Photobiomodulation in pain modulation. Methodology: Male Wistar rats were distributed in seven (7) experimental groups, namely: I - Healthy Control Group (CTL); II - Untreated Tendinitis Group (NT); III - Tendinitis group treated with Photobiomodulation (PBM) in the parameters of 808 nm, 3 J, 100 mW; IV - opioids-treated Tendinitis Group (OP); V - Tendinitis group treated with PBM associated to OP (PBM + OP); VI - Tendinitis group treated with Naloxona associated to PBM (NX + PBM) and VII - Tendinitis Group treated with naloxona (NX). Tendinitis was induced from the transcutaneous injection of collagenase type I in the region of the calcaneus tendon and the treatments were administered according to the description of each experimental group. After 8 hours of tendinitis induction, local allodynia was measured. At the end of the experiment, the animals were euthanized and the tendons removed for analysis. Gene expression of B1, NK1, MOR receptors and myeloperoxidase (MPO) levels were evaluated.

**Key words:** Pain, tendinopathies, photobiomodulation, mechanisms, opioids, NK1 receptor, B1 receptor, MOR receptor, inflammatory mediators.

**Study type:** Experimental study in animals



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MONITORING OF CHEMOTHERAPY TREATMENT FOR BREAST CANCER PATIENTS USING RAMAN SPECTROSCOPY AND MULTIVARIATE ANALYSIS

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### Abstract

Introduction: It has been proven that Raman spectroscopy and Multivariate Analysis (specially PCA) can be used to discriminate between healthy and cancer cells, thus, these tools seem to be promising for medical research. In this research, we use Raman Spectroscopy and PCA to distinguish between healthy and breast cancer serum blood samples, also, we propose with the same tools, the monitoring of breast cancer patients in chemotherapy treatment. Aim of the study: Monitoring breast cancer patients undergoing chemotherapy treatment using Raman spectroscopy and multivariate analysis. Methodology: All the blood serum samples required for this study were obtained at the Instituto Mexicano del Seguro Social, a public health institution. We collected 5 samples of healthy people, 5 from diagnosed breast cancer patients (with no treatment) and 2 from a breast cancer patient in chemotherapy treatment. For the spectra measure, we use the Raman system Horiba Jovin Von Ivon with a laser of 830 nm and an Olympus microscope. To ensure statistically representative sampling, we collected data on 3 different points of each sample. We saved the spectra in a database and were processed applying normalization, smooth and baseline correction. After that, the PCA implementation was made in Matlab, it shows the data in terms of their principal components, making it easier to discriminate between health and sick cancer patients. Preliminary results: Calculating and plotting the mean spectrum of healthy people vs the mean spectrum of breast cancer patients, we can find biochemical differences. The main information obtained by PCA allows us to discriminate between cancer and healthy spectra. Plotting the principal component loadings each point shown by the PCA is a different spectrum and are identified by their color, a color for healthy spectra and other for cancer ones, the resulting algorithm obtains that points that are from the same class tend to cluster. Finally, for the monitoring of the chemotherapy treatment, we pretend to apply PCA to all the spectra collected, points of the patient under treatment should get closer to the cluster of healthy spectra as the chemotherapy is received. Conclusion: In this study, Raman spectroscopy and PCA allow us to discriminate between blood serum samples of healthy people and breast cancer patients, we pretend that the monitoring could show if the patient responds to treatment

**Key words:** Raman spectroscopy, Multivariate Analysis.

**Study type:** Clinical Study