

IV FÓRUM INTERNACIONAL DE LASERTERAPIA

PROCEEDINGS OF FIL vol. IV



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Os conceitos emitidos neste proceedings são de inteira responsabilidade dos autores
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Programa de Pós-Graduação em Biofotônica Aplicada às Ciências da Saúde
Universidade Nove de Julho

Prefácio

O IV FÓRUM INTERNACIONAL DE LASERTERAPIA se constituiu num fórum de discussão de temas relacionados à Biofotônica e suas aplicações em Ciências da Saúde, voltado a divulgar as amplas possibilidades de pesquisa e aplicação resultantes de uma visão interdisciplinar aplicada à essa temática. O evento teve como público os pesquisadores, docentes e discentes de graduação e pós-graduação, bem como os profissionais de saúde dos serviços privados e do Sistema Único de Saúde (SUS). Contou com a inscrição de 1179 participantes de 20 diferentes instituições. Ao aproximar diferentes perfis, buscou-se maior integração entre a teoria desenvolvida na academia e a prática verificada no contexto da saúde visando a ampla inserção da Biofotônica. A massiva participação do corpo discente da graduação e pós-graduação da UNINOVE promoveu a disseminação das tecnologias dentre os futuros profissionais de saúde. As discussões de caráter científico e tecnológico foram abordadas, proporcionando assim melhoria da formação dos pesquisadores e profissionais envolvidos nessa área de conhecimento. A conferência contou com 83 submissões de trabalhos científicos, dos quais 68 foram aceitos para apresentação em forma de poster ou oral. O evento foi gratuito, alcançando, portanto, um grande número de pessoas.

O IV Fórum Internacional de Laserterapia teve uma carga horária total de 18 horas, composta de apresentações plenárias, apresentação oral de trabalhos científicos e apresentação de pôsteres científicos que contribuíram com o intercâmbio de informações científicas e tecnológicas e estreitamento dos laços entre grupos de pesquisa no Brasil e no exterior. O apoio do setor produtivo industrial se deu pela participação das principais empresas de equipamentos biomédicos para fototerapia. Com a apresentação de 68 (sessenta e oito) trabalhos científicos, os docentes e discentes divulgaram os resultados mais atuais dos projetos de pesquisa realizados em suas instituições e por consequência, melhoraram a qualidade da produção devido à discussão entre importantes nomes da pesquisa nacional e internacional. A conferência

este ano abordou um tema de alta relevância para a Biofotônica, que foi um aprofundamento no conhecimento dos mecanismos de ação pelos quais a luz atua no tecido biológico. Este é um dos focos para elevar cientificamente o Programa de Pós-graduação da UNINOVE bem como outros programas brasileiros que participarão do nosso evento. A programação foi voltada para as áreas da saúde que podem se beneficiar das tecnologias de aplicação da luz em saúde, a saber: Medicina, Odontologia, Enfermagem, Fisioterapia, Educação Física, Biomedicina, Biologia, Física, Química, dentre outras. Visto que esta é uma área multidisciplinar, podemos entender que a participação das diversas vertentes do conhecimento científico promoveu um diálogo amplo e construtivo. A comissão organizadora do evento foi composta por Professores Doutores atuantes na área de Biofotônica em âmbito internacional. Os membros organizadores do evento estão listados a seguir. O corpo de palestrantes contará com um grupo de altíssimo impacto científico, como o Prof. Dr. Leonardo Longo (Florença, Itália) e Prof. Dr. Diego Longo (Florença, Itália), o Prof. Dr. Tianhong Dai (Harvard University), Prof. Dr. Adenilson de Souza da Fonseca (Universidade Estadual do Rio de Janeiro), Profa. Dra. Rosane Lizarelli (FUSCAR) e a Profa. Dra. Sandra Kalil Bussadori (UNINOVE), dentre outros.

Programação

	08/nov			09/nov	
08:00	Recepção		08:00	Recepção	
			08:30	Apresentação de trabalhos Fisioterapia e medicina	
09:00	Abertura	Profa. Dra. Kristianne Porta - Biofotônica UNINOVE			
09:30	Laser na Odontologia	Profa. Dra. Sandra Kalil Bussadori - Biofotônica UNINOVE	09:30	Perspectivas futuras da FBM	Prof. Dr. Leonardo Longo - Instituto di Laser Medicina di Firenze (I.L.M.)
10:30	Café				
11:00	Avaliações de Pôsteres		11:00	Neurofotobiomodulação	Dr. Diego Longo - Diretor do A - Studio Longo de Fisioterapia
12:00	Tecnologia e PDT (Research Day)	Profa. Dra. Silvia Nuñez - Universidade Brasil	12:00	Almoço	
13:00	Almoço		13:30	PBM na estabilidade genômica	Prof. Dr. Adenilson Souza Fonseca UERJ
14:30	Apresentações Oraís Odontologia		14:30	Laser na fisioterapia	Prof. Dr. Paulo Sérgio Bossini - NUPEN e UFSCAR
15:30	Café				
16:00	Antimicrobial applications of Blue Light against bacterial resistance	Prof. Dr. Tianhong Dai - Harvard Medical School / Wellman Center for Photomedicine	16:00	FBM na harmonização orofacial	Profa. Dra. Rosane Lizarelli - IF-USP São Carlos
17:00	Encerramento do dia		17:00	Encerramento do congresso	

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Profa. Dra. Silvia Cristina Núñez (Universidade - SP)

Prof. Dr. Adenilson Fonseca (UERJ)

Prof. Dr. Tianhong Dai (Harvard University, EUA)

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5	Guilherme Henrique Cardoso Fernandes, Solange Almeida dos Santos, Jheniphe Rocha Caires, Stefanny Gonçalves da Silva, Gabriel Nobre Chicuta and Paulo De Tarso Camillo de Carvalho	THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON SPERM MOTILITY, AND INTEGRITY OF THE PLASMA MEMBRANE AND ACROSOME IN CRYOPRESERVED BOVINE SPERM

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Solange	Almeida dos Santos	3. EFFECT OF PHOTOBIMODULATION THERAPY IN DISINTEGRINS AND METALLOPROTEINASES, IL3, AND EXPRESSION OF TYPE II COLLAGEN, IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS OF THE KNEE INFILTRATED WITH

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		5. THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON SPERM MOTILITY, AND INTEGRITY OF THE PLASMA MEMBRANE AND ACROSOME IN CRYOPRESERVED BOVINE SPERM
		6. EFFECT OF THE PHOTOBIMODULATION ASSOCIATED WITH IMPLANTATION OF MESENCHYMAL ADIPOSE-DERIVED STEM CELLS IN EXPRESSION OF MMPs AND DECREASE DEGRADATION OF TYPE II COLLAGEN IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS
Patricia	Almeida-Mattos	51. Photobiomodulation and Carbon Biomaterials: effects on biomechanical properties and bone healing
		52. Reduction of inflammatory process and allodynia associated with phototherapy in experimental model of tendinitis
Carol	Alves	69. PROPOSED LABORATORY METHODOLOGY FOR OBTAINING DENTINA AFFECTED BY CARIES AND VALIDATION THROUGH LASER SPECKLE AND OCT
Eduardo	Alves Brigidio	19. SYSTEMIC INFLAMMATORY RESPONSE DURING DIABETIC FOOT TREATMENT: PHOTOBIMODULATION VERSUS CONVENTIONAL
Carolina	Antunes	4. Photobiomodulation in Oral Mucositis in Patients with Head and Neck Cancer: A Systematic Review

Andréia	Araujo	67. Effect of photobiomodulation (780nm) on the expression of IL-6 And TNF- alpha during the compensatory hypertrophy
Renato	Araujo Prates	2. Evaluation of daily photobiomodulation in stem cell properties of oral squamous cell carcinoma cell lines
		25. Evaluation of the effect of photobiomodulation on the flow and salivary pH of patients with Diabetes Mellitus and xerostomia
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		77. EVALUATION OF REMINERALIZATION OF SPOT LESION WITH 808 nm DIODE LASER
		78. Photodynamic therapy has an overt antimicrobial effect on P. gingivalis: a systematic review of experimental models
		81. PHOTODYNAMIC THERAPY FOR THE ENDODONTIC TREATMENT OF PRIMARY TEETH: A CLINICAL TRIAL
Viviane Aparecida	Arenas Rodrigues	53. PHOTSENSIBILIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC THERAPY - COMPARATIVE STUDY.

Paulo	Assirati	51. Photobiomodulation and Carbon Biomaterials: effects on biomechanical properties and bone healing
Isabella Sena	Avelar	18. EVALUATION OF PERIODONTAL TREATMENT ASSOCIATED WITH PHOTODYNAMIC THERAPY IN SYSTEMIC PARAMETERS OF INFLAMMATION IN EXPERIMENTAL MODEL OF ASTHMA
Luciane	Azevedo	40. Antimicrobial photodynamic therapy in perimuni-implantitis and non-neoplastic proliferative lesion: a case report
		42. Effect of photobiomodulation therapy on hypertrophic scar of the lip: a case report
Claudia	Bagnarolli	33. Effects of photobiomodulation in hypertensive patients - A Systematic Review
Vinicius	Barbosa	23. ENHANCEMENT OF THE ACTION OF METHYLENE BLUE aPDT BY SELECTING SUITABLE EXPERIMENTAL PARAMETERS
Camila	Basilio Okamoto	81. PHOTODYNAMIC THERAPY FOR THE ENDODONTIC TREATMENT OF PRIMARY TEETH: A CLINICAL TRIAL
Darcio	Bauleo	33. Effects of photobiomodulation in hypertensive patients - A Systematic Review
Gabriela	Benedito Machado	75. Evaluation of chamomile and fig extracts combined to photobiomodulation therapy to minimize the effects of UV-A radiation in keratinocytes.
Caroline	Bento Correa	23. ENHANCEMENT OF THE ACTION OF METHYLENE BLUE

		aPDT BY SELECTING SUITABLE EXPERIMENTAL PARAMETERS
Alexandre	Bergamo	78. Photodynamic therapy has an overt antimicrobial effect on P. gingivalis: a systematic review of experimental models
Maria	Biffi	16. EVALUATION OF THE EFFICACY OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN TREATMENT OF PERI-IMPLANTITIS: A CONTROLLED, RANDOMIZED, BLINDED CLINICAL TRIAL.
Nathalia	Bim	16. EVALUATION OF THE EFFICACY OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN TREATMENT OF PERI-IMPLANTITIS: A CONTROLLED, RANDOMIZED, BLINDED CLINICAL TRIAL.
Greice	Bitencourt	86. ADMINISTRATION OF LOW-LEVEL LASER ON MUSCLES OF MASTICATION FOLLOWING THE INDUCTION OF INITIAL FATIGUE: PROTOCOL FOR A RANDOMIZED, CONTROLLED.
Carol	Brandit	77. EVALUATION OF REMINERALIZATION OF SPOT LESION WITH 808 nm DIODE LASER
Auriléia	Brito	44. Photobiomodulation and its interaction with leukotriene receptor antagonist in an experimental model of chronic asthma
		45. Effectiveness of photobiomodulation in the increase of T reg and IL-10 cytokine in an experimental model of chronic asthma
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Sandra	Bussadori	12. Assessment of halitosis level after photodynamic therapy and tongue scraper in bronchiectasis patients
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38. THE EFFECT OF PHOTOBIMODULATION ON THE FATIGUE OF INDIVIDUALS WITH MULTIPLE SCLEROSIS

39. Pilot study on the effect of photobiomodulation therapy in multiple sclerosis

55. EFFECT OF SURFACE TREATMENT WITH CO₂ LASER ULTRAPULSED IN COMPOSITE RESIN REINFORCEMENT RESISTANCE

58. Evaluation of the effect of photobiomodulation in the control of pain in patients with oral lichen planus: a clinical, controlled, randomized, double blind study.

59. Evaluation of photobiomodulation in salivary production of patients with antihypertensive drug-induced xerostomy: study protocol for a randomized, controlled blind clinical trial

60. Analysis of the effects of photobiomodulation in patients with TMD: case report

62. ACTION OF PHOTODYNAMIC THERAPY WITH RED LED ON HALITOSE CONTROL: CLINICAL CONTROLLED AND RANDOMIZED TEST.

63. Remineralization of early enamel caries lesions induced by bioactive particles: an in vitro Speckle analysis

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		54. Evaluation of Photobiomodulation in the treatment of oral lichen planus: a randomized, controlled, double blind study.
Monique	C. G. Veloza	19. SYSTEMIC INFLAMMATORY RESPONSE DURING DIABETIC FOOT TREATMENT: PHOTOBIMODULATION VERSUS CONVENTIONAL
Lyvia	C. R. Perez	19. SYSTEMIC INFLAMMATORY RESPONSE DURING DIABETIC FOOT TREATMENT:

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Marcia	Cabral	69. PROPOSED LABORATORY METHODOLOGY FOR OBTAINING DENTINA AFFECTED BY CARIES AND VALIDATION THROUGH LASER SPECKLE AND OCT
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Thalita	Campos	4. Photobiomodulation in Oral Mucositis in Patients with Head and Neck Cancer: A Systematic Review
Cristina	Capelo	52. Reduction of inflammatory process and allodynia associated with phototherapy in experimental model of tendinitis
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Dhuane	Caroline Monteiro da Silva	10. PHOTOBIO-MODULATION AMELIORATES LUNG INFLAMMATION IN SEPSIS-INDUCED ACUTE RESPIRATORY DISTRESS SYNDROME
		21. COMPARATIVE STUDY BETWEEN PHOTOBIO-MODULATION AND VITAMIN C TO TREAT ACUTE LUNG INJURY EXPERIMENTALLY
Nicole	Carvalho	25. Evaluation of the effect of photobiomodulation on the flow and salivary pH of patients with Diabetes Mellitus and xerostomia
Ana Eliza	Castanho Garrini dos Santos	64. Evaluation of dental whitening supervised in the office, in upper canines with LED Violet (405nm) with and without the use of carbamide peroxide gel 35%
Lisyanne	Cavalcante	30. Photodynamic therapy with Bixa orellana extract and LED for the reduction of halitosis: a randomized and controlled clinical trial.
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		CONTROLLED AND RANDOMIZED TEST.
Paulo Francisco	Cesar	55. EFFECT OF SURFACE TREATMENT WITH CO2 LASER ULTRAPULSED IN COMPOSITE RESIN REINFORCEMENT RESISTANCE
Gabriel Nobre	Chicuta	5. THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON SPERM MOTILITY, AND INTEGRITY OF THE PLASMA MEMBRANE AND ACROSOME IN CRYOPRESERVED BOVINE SPERM
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Eduardo Saba	Chuffi	53. PHOTSENSIBILIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC THERAPY - COMPARATIVE STUDY.
Caroline	Coelho	86. ADMINISTRATION OF LOW-LEVEL LASER ON MUSCLES OF MASTICATION FOLLOWING THE INDUCTION OF INITIAL FATIGUE: PROTOCOL FOR A RANDOMIZED,CONTROLLED.
Jose Cabrera	Colasso	53. PHOTSENSIBILIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC THERAPY - COMPARATIVE STUDY.
Gabriela	Collina	23. ENHANCEMENT OF THE ACTION OF METHYLENE BLUE

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Fernanda	Cordeiro da Silva	38. THE EFFECT OF PHOTOBIMODULATION ON THE FATIGUE OF INDIVIDUALS WITH MULTIPLE SCLEROSIS
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Luciana	Corrêa	42. Effect of photobiomodulation therapy on hypertrophic scar of the lip: a case report
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Maria	Cristina Chavantes	38. THE EFFECT OF PHOTOBIMODULATION ON THE FATIGUE OF INDIVIDUALS WITH MULTIPLE SCLEROSIS
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Karen	Cunha	54. Evaluation of Photobiomodulation in the treatment of oral lichen planus: a randomized, controlled, double blind study.
Lucas	Cunha	50. Evaluation of survival in Escherichia coli cultures exposed to low power blue LED
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Andrezza Maria	Côrtes Thomé	34. Photobiomodulation prevents DNA fragmentation of alveolar epithelial cells and alters the mRNA levels of caspase 3 and Bcl-2 genes in acute lung injury
		41. Dichromatic laser radiation effects on infected pressure injury
Stefanny Gonçalves	da Silva	5. THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON SPERM MOTILITY, AND INTEGRITY OF THE PLASMA MEMBRANE AND ACROSOME IN CRYOPRESERVED BOVINE SPERM
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Keila	da Silva Canuto	37. Low-level lasers and the effects on migration and viability on MDA-MB 231 cells
Luiz Philippe	da Silva Sergio	34. Photobiomodulation prevents DNA fragmentation of alveolar epithelial cells and alters the mRNA levels of caspase 3 and Bcl-2 genes in acute lung injury
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Nasser Ali	Daghastanli	56. Fluconazol susceptibility of a resistant strain of Candida albicans is altered by PDT
Vanessa	Dalapria	66. Effect of LED on the bone repair of dental alveoli of rats after exodontia grafted with inorganic bovine bone and collagen membrane.
Caroline	Dantas	40. Antimicrobial photodynamic therapy in perimuni-implantitis and non-neoplastic proliferative lesion: a case report
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Katia	De Angelis	39. Pilot study on the effect of photobiomodulation therapy in multiple sclerosis
Kaline	de Brito Sousa	27. EVALUATION OF LED THERAPY ON THE VIABILITY OF J774 MACROPHAGES POLARIZED TO M1 PHENOTYPE
		28. DIFFERENTIATION OF MUSCLE CELLS TREATED WITH SUPERNATANTS OF IRRADIATED M2A MACROPHAGE
		76. Macrophage Light Absorption Spectrum
Daniela	de Fátima Teixeira da Silva	2. Evaluation of daily photobiomodulation in stem cell properties of oral squamous cell carcinoma cell lines
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Ana Paula Ligeiro	de Oliveira	18. EVALUATION OF PERIODONTAL TREATMENT ASSOCIATED WITH PHOTODYNAMIC THERAPY IN SYSTEMIC PARAMETERS OF INFLAMMATION IN EXPERIMENTAL MODEL OF ASTHMA
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Paulo	De Tarso Camillo de Carvalho	3. EFFECT OF PHOTOBIO-MODULATION THERAPY IN DISINTEGRINS AND METALLOPROTEINASES, IL3, AND EXPRESSION OF TYPE II COLLAGEN, IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS OF THE KNEE INFILTRATED WITH

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Elisabete	Dias	25. Evaluation of the effect of photobiomodulation on the flow and salivary pH of patients with Diabetes Mellitus and xerostomia
Isadora	Dias	86. ADMINISTRATION OF LOW-LEVEL LASER ON MUSCLES OF MASTICATION FOLLOWING THE INDUCTION OF INITIAL FATIGUE: PROTOCOL FOR A RANDOMIZED,CONTROLLED.
Tatiana	Dias Schalch	76. Macrophage Light Absorption Spectrum
Thais Helena	dos Santos	64. Evaluation of dental whitening supervised in the office, in upper canines with LED Violet (405nm) with and without the use of carbamide peroxide gel 35%
Danilo	dos Santos Teixeira	27. EVALUATION OF LED THERAPY ON THE VIABILITY OF J774 MACROPHAGES POLARIZED TO M1 PHENOTYPE
Ivone	Duarte	57. The impact on quality of life and psychosocial relationships in patients

		undergoing treatment for facial aging using photobiomodulation.
Janete	Esteves	20. THE ROLE OF PHOTOBIMODULATION ON THE PARAQUAT-INDUCED PULMONARY FIBROSIS
Mateus	Evaristo	52. Reduction of inflammatory process and allodynia associated with phototherapy in experimental model of tendinitis
Leonardo Trindade	Fabretti	56. Fluconazol susceptibility of a resistant strain of Candida albicans is altered by PDT
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Daniele	Fernanda Peron	78. Photodynamic therapy has an overt antimicrobial effect on P. gingivalis: a systematic review of experimental models
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Patrícia	Freitas	42. Effect of photobiomodulation therapy on hypertrophic scar of the lip: a case report
Regina Teresa	Fruet	16. EVALUATION OF THE EFFICACY OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN TREATMENT OF PERI-IMPLANTITIS: A CONTROLLED, RANDOMIZED, BLINDED CLINICAL TRIAL.
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Andrea	Gomes Oliver	38. THE EFFECT OF PHOTOBIMODULATION ON THE FATIGUE OF INDIVIDUALS WITH MULTIPLE SCLEROSIS
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José	Gonçalves	72. The Photodynamic Efficacy of Phenothiazinium Photosensitizers is directly proportional to the oxidative stress photoinduced.
Marcela	Gonçalves	30. Photodynamic therapy with Bixa orellana extract and LED for the reduction of halitosis: a randomized and controlled clinical trial.

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Adriana	Isabella	33. Effects of photobiomodulation in hypertensive patients - A Systematic Review
Lara	Jansiski Motta	64. Evaluation of dental whitening supervised in the office, in upper canines with LED Violet (405nm) with and without the use of carbamide peroxide gel 35%
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Maria	Varellis	25. Evaluation of the effect of photobiomodulation on the flow and salivary pH of patients with Diabetes Mellitus and xerostomia
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IV FORUM INTERNACIONAL DE LASERTERAPIA e
IV Encontro de alunos e ex-alunos do PPG
Biofotônica aplicada às Ciências da Saúde



Evaluation of daily photobiomodulation in stem cell properties of oral squamous cell carcinoma cell lines

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STOPIGLIA, RENATO ARAUJO PRATES, DANIELA DE FÁTIMA
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Recent studies have demonstrated that the Oral Squamous Cell Carcinoma have subpopulation of cells, called as cancer stem cells (CSC), responsible for tumor growth, metastasis therapeutic resistance¹. The conventional treatment for OSCC is surgery with radio/chemotherapy, both associated with oral mucositis (OM)². Some studies have demonstrated that photobiomodulation (PBM) act preventing mucositis, reducing pain and promoting wound healing, mainly when applied daily in oral mucosa³. However, some studies demonstrated that PBM can promote tumor progression by acting in neoplastic cells³.

Objective: The aim of this study was to evaluate *in vitro* the effects of daily PBM with dosimetric parameters used to treat OM in stem properties of OSCC cell lines.

Material and methods: Cell Culture: OSCC cell lines (SCC9, Luc4 and CA1) were cultivated in DMEM/F12 with 10% FBS, 1% antibiotic, 400 ng/ml hydrocortisone and RM+ supplement. PBM: Cells were irradiated using the LEDbox (BioLambda, São Paulo, Brazil) wavelength 660nm, power 80mW, power density 25.5mW/cm², energy density of 3 and 6J/cm² with 120 and 240 sec and total energy of 9.6 and 19.2J, respectively, for 3 consecutive days. Colony formation assay: After irradiation 200 cells/well were cultivated for 8 days in 6 wells plates. Colonies were fixed, stained with cristal violet and counted using the ImageJ software. Sphere formation assay: 1x10³ cells/well were plated in polyHEMA coated 24-well plates and cultivated for 15 days after

PBM. The sphere formations were counted and the spheres were captured by the ZOE Fluorescent Cell Imager (Bio-Raid, Singapore). Statistical analysis: Data distribution was verified by D'Agostino & Pearson and Shapiro-Wilk normality tests and significance difference between groups was calculated using T-Test and Mann-Whitney test.

Results: SCC9 cell line showed a significant decrease in the number of colonies after PBM with 3J/cm² and 6J/cm² in relation to control ($p=0,0023$, $p=0,0010$) and in the number of spheres with the same dosimetric parameters ($p=0,0004$, $p<0,0001$). LUC4 cell line have also demonstrated decrease in the number of spheres when treated with PBM with 6J/cm² when compared to control ($p=0,0017$) No difference in CA1 cell line was observed between control and PBM treated cells.

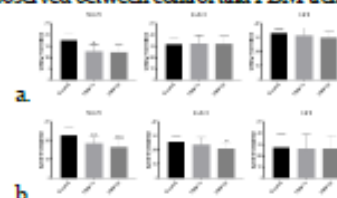


Figure: (a) Colony formation (b) Sphere formation.

Conclusion: PBM with 3J/cm² and 6J/cm² (doses used to treat mucositis) decreased the number of spheres in SCC9 and Luc4 cell lines, demonstrating that PBM do not promote the stem cell properties. Additional studies investigating the effects of PBM in CSC are necessary to understand its role in this subpopulation of cells in OSCC.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

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EFFECT OF PHOTOBIO-MODULATION THERAPY IN DISINTEGRINS AND METALLOPROTEINASES, IL3, AND EXPRESSION OF TYPE II COLLAGEN, IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS OF THE KNEE INFILTRATED WITH STEM CELLS DERIVED FROM ADIPOCYTES

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Introduction: Osteoarthritis is the most common arthritic condition. The matrix metalloproteinases (MMPs), including collagenases (MMP1 and MMP13), gelatinases (MMP2 and MMP9), and stromelysin (MMP3), mediate cartilage collagen breakdown, whereas aggrecanases, which are members of the A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family including ADAMTS1, ADAMTS4, ADAMTS5, ADAMTS8, ADAMTS9, and ADAMTS15, mediate loss of cartilage aggrecan. Thus, the importance of MMPs and ADAMTS in the pathogenesis and development of OA is widely demonstrated [1,2].

Objective: This study aimed to verify the action of PBMT in disintegrins and metalloproteinases, as well as IL3, with the expression of type II collagen, in an experimental model of osteoarthritis of the knee infiltrated with stem cells derived from adipocytes.

Method: Adipose-derived stem / stromal cells (ADSCs) and were collected from three rats Fisher 344 male. The biologic characteristics of ADSCs were analyzed by flow cytometric analysis. 50 Rats Fisher 344 female were distributed into five distinct groups, containing 10 animals in each group, being that: (Control) comprised healthy animals; (OA) Osteoarthritis Group; (OA PBMT) Osteoarthritis Group with treatment by PBMT; (OA ADSCs) Osteoarthritis Group with treatment by injection of ADSCs; (OA ADSCs PBMT) Group Osteoarthritis with treatment by injection of ADSCs associated with the PBMT. The animals were subjected to OA (papain solution, 4%) and the OA ADSCs and OA ADSCs PBMT group received intra-articular infiltration of 10×10^6 cells diluted in 100 μ l of DMEM modified medium with SFB and Penicillin-streptomycin were irradiated with PBMT (wavelength of 808 nm, power 50 mW, energy 42 J, for both groups. In the present invention relates to the use of PBMT in the joint space of the rats' right and left knees. Euthanasia was performed 7 days after the first application and the articular cartilages were extracted for protein expression by ELISA pro-inflammatory cytokines IL-1 β , IL-6, TNF- α and anti-inflammatory IL-10 and RT / PCR for mRNA in SYR-sex-determining region Y, MMPs 13, SOX-9, ADMANTS-4, COL2-1 and in the anti-inflammatory cytokines IL-3 and IL-3 RA.

Results and discussion: The Associations between the expression of COL2-1 mRNA and MMP-13, MMP-1, ADAMTS-4 and SOX-9 mRNA expression were analyzed using Spearman correlation tests. We found that COL2-1

expression had a positive correlation with MMP-13 mRNA expression ($r = -0.9867$, $P = 0.0001$, Fig. 3B), MMP-13 mRNA expression ($r = -0.9981$, $P = 0.0001$; ADAMTS-4 mRNA expression ($r = -0.9920$ $P = 0.0001$; Fig. 3C) and SOX-9 mRNA expression ($r = -0.9860$, $P = 0.0001$). (1). In our study, the experimental OA model used provided a confirmation of the above-mentioned changes. The OA group showed upregulated expression protein for proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) and downregulated expression protein for the anti-inflammatory cytokine IL-10, IL-3 and IL-3 RA. Our findings also verify the upregulation of the mRNA expression of MMP-13 and ADAMTS-4, and the downregulation mRNA levels of SOX-9 and. In our model, alterations in COL2-1 mRNA expression could also be observed because the OA group showed COL2-1 mRNA levels below those observed in the control group. Such findings have provided us with a good comparison between the OA group and the groups treated with ADSCs, with or without PBMT. Finally, the anti-catabolism effect of SOX-9 in cartilage submitted to ADSCs associated with PBMT on the articular cartilage of rats submitted to an experimental osteoarthritis protocol was provided by the real-time PCR.

Conclusions: The PBMT associated with the intra-articular injection of ADSCs prevented degenerative modifications in the type II collagen joints and modulated the inflammatory process by means of the negative regulation of cytokines and MMPs 13 and ADAMTS -4, as well as promoted positive regulation in the anti-inflammatory cytokines IL - 3 and IL-3 RA. in rats with osteoarthritis.

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Acknowledgment: Universidade Nove de Julho

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PHOTOBIMODULATION IN ORAL MUCOSITIS IN PATIENTS WITH HEAD AND NECK CANCER: A SYSTEMATIC REVIEW

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Introduction: One of the side effects of treatment with radiotherapy and chemotherapy is Oral Mucositis (OM), an alteration in the oral mucosa which presents in the initial stage as erythema, and may progress to ulceration.¹ Besides experiencing pain, patients with OM may also have difficulty eating, thereby generating an impaired nutritional status. The condition is considered a potential risk for secondary infections, which can lead to treatment discontinuation, compromising the cancer treatment efficacy and quality of life of patients.² Exposure of connective tissue due to ulcer formation increases fungal and bacterial adhesion, exacerbating pain as well as the risk of sepsis.³ Within this context, low-level laser therapy has an anti-inflammatory and analgesic effect besides healing properties,⁴ reducing OM symptoms.

Objective: The purpose of this study is to carry out a Systematic Review about the scientific production related to photobiomodulation in Oral Mucositis, facilitating decision making and expanding the scope of its application in health services

Methods: The data collection was carried out between October 2017 and January 2018. The scientific productions were selected according to figure 1.



Fig. 1. Fig. 1. Flowchart for article search. São Paulo/ SP, 2018

Results. After reading title and abstract, we selected 15 articles that met the criteria for inclusion. Only randomized clinical studies were selected due to their higher level of evidence. As observed in the studies included in this work, the radiant exposure used most often was 2.0J/cm² (17.64%), followed by 3.0J/cm² (11.76%) and 4.0J/cm² (11.76%). Of all the studies analyzed, 8 applied the laser prophylactically, 1 therapeutically, and 6 studies applied the laser both for OM treatment and prevention.

Conclusion. Photobiomodulation is a proposal for the effective treatment of OM, but it is important to make low-level laser therapy parameters available in publications for reproducibility and clinical application.

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THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON SPERM MOTILITY, AND INTEGRITY OF THE PLASMA MEMBRANE AND ACROSOME IN CRYOPRESERVED BOVINE SPERM

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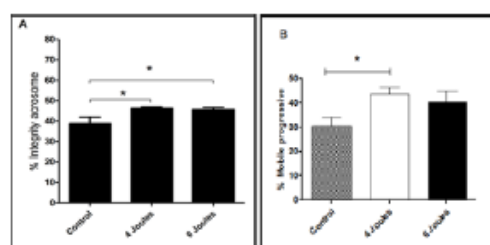
Introduction: Freezing changes sperm integrity remarkably. Cryopreservation involves cooling, freezing, and thawing and all these contribute to structural damage in sperm, resulting in reduced fertility potential. Low-level laser irradiation (LLLI) could increase energy supply to the cell and cause reactive oxygen species reduction (ROS), contributing to the restoration of oxygen consumption and adenosine triphosphate synthesis (ATP) in the mitochondria.

Objective: Our goal was to analyze the effects of low-level laser irradiation on sperm motility and integrity of the plasma membrane and acrosome in cryopreserved bovine sperm.

Method: We analyzed 09 samples of bull semen (*Bos taurus indicus*), divided into three groups: a control group without laser irradiation, a 4J group subjected to a laser irradiation dose of 4 joules, and a 6J group subjected to dose of 6 joules. Samples were divided for the analysis of cell viability and acrosomal membrane integrity using flow cytometry; another portion was used for motion analysis. Irradiation was performed in petri dishes of 30 mm containing 3 ml of semen by an aluminum gallium indium phosphide laser diode with a wavelength of 660 nm, 30 mW power, and energy of 4 and 6 joules for 80 and 120 seconds respectively. Subsequently, the irradiated and control semen samples were subjected to cryopreservation and analyzed by flow cytometry (7AAD and FITC-PSA) using the ISAS - Integrated Semen Analysis System.

Results and discussion: Results and discussion: We noted that LLLI with the wavelength of 660 nm, power 30 mW and doses of 4 and 6 joules was able to improve the percentage of live sperm cells evaluated by flow cytometry and maintain acrosomal membrane integrity. The analysis of straightness show that percentage of cell movement and motility a dose of 4 joules was more effective ($p < 0.05$). Flow cytometry showed an increase in the percentage of live sperm cells and acrosome integrity in relation to control cells when subjected to irradiation of low-power laser in two different doses of 4 and 6 joules ($p < 0.05$). The improvement in semen quality after LLLI has been illustrated previously described in several species: dog, bovine, rabbit, and turkey [1,2]. Using the

same energy and wavelength as in previous similar studies, we show additional evidence that LLLI may result in a significant increase in the percentage of live sperm cells, integrity of acrosome membrane and higher sperm motility. Sperm motility after LLLI has been investigated in several studies [1, 2]. However, some studies [2] showed negative outcomes regarding motility, these results may be related to the wavelength, laser power, energy density, irradiation time, as well as the experimental analysis conditions.



Fig(A): Shows the percentage of sperm with acrosome integrity under the total of living cells. (Newman-Keuls Test * $p < 0.05$). (B) Mobile percentage of progressive sperm analysis presenting the group not irradiated groups and subjected to low-level laser irradiation dose 4 and 6 joules group. Data shown are the mean \pm standard deviation. One-way ANOVA and Newman-Keuls post hoc analysis were applied. * $p < 0.05$ control group vs. 4 Joules group.

Conclusion: We conclude that LLLI may exert beneficial effects in the preservation of live sperm. A dose of 4 joules prior to cryopreservation was more effective than a dose of 6 joules in preserving sperm motility.

Aknowlegements: Capes and Uninove

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EFFECT THE PHOTOBIO-MODULATION ASSOCIATED WITH IMPLANTATION OF MESENCHYMAL ADIPOSE-DERIVED STEM CELLS IN EXPRESSION OF MMPs AND DECREASE DEGRADATION OF TYPE II COLLAGEN IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is an age-related chronic degenerative joint disorder associated with cartilage damage resulting in intense pain and disability [1].

Objective: This study aims to determine if photobiomodulation therapy (PBMT) can improve the bioavailability and chondroprotective benefits of mesenchymal stem cells injected into the knees of rats used as an experimental model of osteoarthritis (OA), as well as reduce the expression of matrix metalloproteinases (MMPs) and the degradation of type II collagen (COL2-1) in the cartilage.

Method: Adipose-derived stem/stromal cells (ADSCs) were collected from three male Fisher 344 rats and then characterized by flow cytometry. Fifty female Fisher 344 rats were distributed into five groups of 10 animals each. These groups were: control group, OA group, OA PBMT, OA ADSC group, and OA ADSC PBMT group. OA was induced in the animals using a 4% papain solution. Animals from the OA ADSC and OA ADSC PBMT groups received an intra-articular injection of 10×10^6 ADSCs and were treated with PBMT by irradiation (wavelength: 808 nm, power: 50 mW, energy: 42 J, energy density: $71.2 \text{ J} / \text{cm}^2$, spot size: 0.028). Euthanasia was performed 7 days after the first treatment, and articular cartilages.

Results and discussion: The use of PBMT alone and the injection of ADSCs resulted in the downregulation of pro-inflammatory cytokines and metalloproteinases in articular cartilage tissue, when compared to the OA group. They also caused the upregulation of tissue inhibitors of metalloproteinases 1 and 2 (TIMP-1 and TIMP-2), mRNA and protein expression of COL2-1 in articular cartilage tissue, when compared to the OA group.

The experimental OA model used here provided a confirmation of the abovementioned changes. The OA group showed upregulated expression of the mRNAs coding IL1 β , IL-6, and TNF- α and downregulated expression of the mRNA coding for the anti-inflammatory cytokine IL-10. We verified the upregulation of the mRNA expression of MMP-1 and MMP-2, and the downregulation of the mRNA levels of TIMP-1 and TIMP-2. Alterations in COL2-1 expression were observed, because the OA group showed lower COL2-1 mRNA levels compared to the control group. In addition, the role of ADSCs in

suppressing immunoreactions in MSCs has been studied, indicating their possible use in decreasing local inflammation in several musculoskeletal diseases [2].

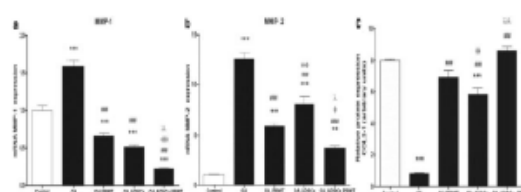


Fig. 1 Comparison of matrix metalloproteinase 1 (MMP-1) (a), MMP-2 (b), tissue inhibitors of mRNA levels in articular cartilage from OA ADSC PBMT group as measured by realtime polymerase chain reaction. Data are expressed as mean \pm standard deviation. Comparison of type II collagen (COL2-1) protein expression level (c) as measured by western blot in articular cartilage from the OA ADSC PBMT group. Data are expressed as mean \pm standard deviation.

The intra-articular injection of ADSCs combined with PBMT prevented joint degeneration resulting from COL2-1 degradation and modulated inflammation by downregulating cytokines and MMPs in the OA group.

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Acknowledgment. Universidade Nove de Julho - Uninove

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The influence of periodontal treatment associated with photodynamic therapy in experimental model of chronic obstructive pulmonary disease

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Background: The correlation between periodontitis and chronic obstructive pulmonary disease (COPD) has been studied. Conventional methods of periodontal treatment (PT) have been associated with adjuvants, mainly the photodynamic therapy (PDT) that emerges as a promising treatment with no side effects. The main objective was to evaluate if PT associated with PDT may influence in the modulation of pulmonary inflammation.

Methods: 64 C57Bl/6J mice were randomly divided into 8 groups: Basal; Periodontitis (P); P+PT; P+PT+PDT; COPD; COPD+P; COPD+P+TP; COPD+P+TP+PDT. COPD was induced by orotracheal instillation of 30µl of cigarette extract, 3 times/week for 7 weeks. Periodontitis was induced by the ligation technique in the lower left first molar, performed at the 3rd week after induction of COPD, and remained for 15 days. Periodontal therapy was performed with curette throughout the gingival sulcus of the molar. PDT was performed by inserting methylene blue Chimilux® (0.005%, 0.05mg/ml) into the gingival sulcus with 3 minutes of pre-irradiation diode laser (Therapy XT® DMC, São Carlos, Brazil, ANVISA 80030810157) Device specifications: single probe, λ: 660nm duty cycle, radiant power: 100mW, irradiance: 3,5W/cm², spot size : 0,02827cm², radiant exposure: 318J/cm², radiant energy per point: 9J, total energy: 18J. Treatment specifications: 90s per point, with 2 application points with direct in-contact probe, inside the mouth and

the treatment was realized once. Euthanasia was performed 51 days after the first induction of COPD. It was realized histomorphometrical analysis of lung, total and differential bronchoalveolar lavage (BAL) count, femoral lavage for medullar count, total and differential serum cell count. The frequency of inflammatory cells was assessed by flow cytometry. ANOVA one-way followed by the Student-Newman-Keuls test was used as statistical test.

Results: The P +PT+PDT group showed an increase in the total number of leukocytes in the BAL when compared to the P group ($p<0.05$). This increase was represented by lymphocytes CD3 ($p<0.05$) and neutrophils ($p<0.05$) followed by macrophages, however in a small amount ($p>0.05$). The regulatory cytokine IL-10 are also increased in these groups, however there was no difference compared with P group ($p>0.05$). The total count of femoral lavage was increased in P +PT and P +PT+PDT groups. Also, in COPD+ P +PT and COPD+ P +PT+PDT groups total cells count decrease in serum, ($p>0.05$). There were no difference among groups differential cell count in serum ($p>0.05$).

Conclusion: Conventional PT associated or not with PDT, was able to influence systemic response. Further studies are needed to understand the link with a regulatory process.

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PHOTOBIOMODULATION AMELIORATES LUNG INFLAMMATION IN SEPSIS-INDUCED ACUTE RESPIRATORY DISTRESS SYNDROME

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Abstract: Acute respiratory distress syndrome (ARDS) is characterized by rupture of the endothelium and alveolar injury as result by an uncontrolled lung inflammatory response causing gas exchange impairment. Treatment of ARDS is still a clinical health problem, so new therapies are needed. In this context, photobiomodulation has been showing good results for several inflammatory diseases, including lung diseases, and here we studied the effects of LED on ARDS induced by sepsis.

Introduction: Acute respiratory distress syndrome (ARDS) is characterized by rupture of the endothelium and alveolar injury. Among the main causes of ARDS, sepsis is highlighted. The restoration of normal lung function is very complicated and the treatment of ARDS is still a clinical health problem, so new therapies are needed. It is known that light-emitting diode (LED) treatment displays anti-inflammatory effects, and here we studied the effects of LED treatment on ARDS induced by sepsis.

Methods: Balb-c mice were injected with lipopolysaccharide (LPS) or saline (i.p.) and irradiated or not with LED on trachea and lungs, for 152 s, 2 and 6 h after the injections. Twenty-four hours after LPS or saline injections, local and systemic effects of LED treatment were investigated.

Results: We showed that LED treatment reduced LPS-induced neutrophils influx, decreased the

levels of IL-1 β , TNF- α , IL-17A and enhanced the levels of IFN-gamma in the bronchoalveolar fluid. We also observed reduced levels of resolvin D1 after LED treatment, and no differences were found in resolvin E2 and lipoxin A4 levels. Moreover, the tracheal hyperresponsiveness was reduced after LED treatment.

Conclusions: Our data showed the beneficial effect of short treatment with LED on ARDS caused by sepsis, and suggest that LED is a promisor tool to treat ARDS.

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Assessment of halitosis level after photodynamic therapy and tongue scraper in bronchiectasis patients

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Background: Halitosis is an unpleasant odor that emanating from the mouth. Bronchiectasis is a lung disease that are among the extra-oral causes of halitosis. To date, no studies have evaluated its treatment in adult population with bronchiectasis

Aim: The aim of this randomized, controlled clinical trial was to treat oral halitosis in bronchiectasis adults with photodynamic therapy (PDT) and tongue scraper.

Methods: Twenty-four bronchiectasis patients were randomized into 2 groups: G1- treatment with photodynamic therapy (PDT); G2- treatment with tongue scraping). Methylene blue was used as photosensitizer (0.005%) irradiated with THERAPY XT® laser (DMC, São Paulo, Brazil) at 660 nm, continuous wave, 9 J, for 90 s per point in the tongue, 6 points in contact, 100 mW, 3537 mW/cm² and 320 J cm². Halitosis were evaluated measuring volatile sulfur compounds using gas chromatography (Oralchroma, Abilit, Japan). A syringe inserted into the patient's mouth with the plunger fully inserted. The patient closes his mouth, breathes through his nose and waits with his mouth closed for 1 minute. The plunger was pulled out, the syringe is withdrawn from the patient's mouth and a needle is placed in the syringe that will inject the air collected in the Oralchroma for measure the volatile sulfur compounds to diagnostic the halitosis. After the treatment, a second evaluation were performed if the halitosis persists, participants received periodontal treatment. After one week, these patients are reassessed. Comparisons were made using the t Student test, with the level of significance of 5 % (p < 0.05).

Results: Thirty-nine bronchiectasis patients were evaluated, and twenty four was halitosis positive. In the present study, 43% of the patients are male while 57% are female, mean age ranges from 33 to 55 years, 62% Caucasian, 28% African and 3% Asian. In the marital status, 46% are married, 37% are single, 10% are divorced and 7% are widowed. 72% receive less than

three minimum wages, 22% receive more than 3 minimum wages and 6% do not respond. After aPDT both groups significantly reduced the levels of halitosis (p<0.05) for sulphide. This population has 49% of predicted VEF1 and 65% of predicted CVF. Thus, the ratio VEF1/CVF is 61% if compared with a healthy population. Thus, the pulmonary disease impacts 50% on quality of life of this population

Conclusion: The PDT and scraping treatment was effective in the immediate reduction of halitosis in both groups. PDT does not involve lingual papillae mechanical aggression that occurs with tongue scraping.

Financial support: This study was sponsored by Foundation for Research Support of the State of São Paulo (FAPESP:2015/20535-1)

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PHOTODYNAMIC THERAPY REVERSES THE NEGATIVE EFFECTS OF PERIODONTITIS ON THE UTERINE MICROENVIRONMENT DURING THE GESTATION

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Abstract: Periodontal disease (PD) is considered the most common localized and inflammatory dental disease caused by bacterial infection associated with dental plaque. Clinical evidence shows an association between maternal DP and adverse outcomes during pregnancy, especially preterm births and low birth weight. Thus, the objective of this study was to investigate the impact of PD on the uterine microenvironment and on the offspring, as well as the role of photodynamic therapy (PDT).

Introduction: Periodontitis (P) is one of the most common infectious diseases that affects the periodontium and gradually destroys periodontal tissues. Among several systemic effects occasioned by P, alterations during the gestation have been studied. PDT is characterized by the association of a light source with a photosensitizing agent in order to cause cell necrosis and microbial death. Thus, our objective was to evaluate the effect of PDT on the negative repercussions of periodontitis during pregnancy, mainly in the uterine microenvironment.

Methods: Ten days before pregnancy, periodontitis was induced by ligature technique, and subsequently the rats were caged overnight with a male. Pregnancy was confirmed by vaginal smear. The treatment with PDT was performed 15 days after the induction the ligatures. Pregnant rats non-manipulated were used as control. Pregnant rats were euthanized at day 18 of gestation and the uterus was removed in order to investigate the parameters. The photosensitizer methylene blue (0.005%, CHIMOLUX, DMC, São Paulo, Brazil) was

administered at the two sites (vestibular and lingual). After three minutes, the periodontal pockets were irradiated with a red laser (MM OPTICS; Wavelength 660 ± 10 nm; Radiant power 100 mW; Exposure duration 90s; spot size 0.02827 cm^2 ; Radiant energy 9 J; Irradiance 3.5 W/cm^2 ; Radiant exposure 318 J/cm^2 ; Total radiant energy 18 J).

Results: We showed that PDT had an important impact on the uterine microenvironment reducing the gene expression of IL-6, COX-1, COX-2 and NOS in the uterine tissue of pregnant rats with periodontitis. We also observed that PDT reversed the decreased level of IL-10 in the placenta.

Conclusions: Thus, our data showed the important role of oral health during gestation as well as PDT is an effective therapy. These studies might be useful in providing an important indicator of risk for future obstetric complications considering the impact in the offspring.

Funding: This study was sponsored by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2017/006444-9).

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ANALYSIS OF NON-INVASIVE THERAPY WITH LOW INTENSITY LASER IN AN EXPERIMENTAL MONOARTRITE MODEL IN THE WISTAR RAT KNEE ARTICULATION

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Monoarthritis is an autoimmune and degenerative disease caused by inflammation of the joint capsule. It is associated with musculoskeletal injuries, genetic factors, obesity and mechanical stress.

AIM: To evaluate the inflammatory profile in the right knee joint of wistar rats with induced experimental monoarthritis, using low intensity laser treatment (LILT). **METHOD:** Male Wistar rats (285g) were randomly divided into: control (C); monoarthritis (MO); MO + LILT (ML). Monoarthritis was induced by intra-articular injection of zymosan (1 mg / 50 µL saline) into the right knee joint. Treatment with LILT (660 nm, 5 mW, 2.5 J / cm², 20 sec). For the application of the laser, the rats were manually immobilized in the ventral decubitus position and the laser beam was applied 90° in a punctual and anteromedial way in the knee joint, in contact with the skin. The procedure was performed twice a week. After the 8-week laser treatment, euthanasia was performed and surgery was performed to remove the sample of synovium that was conditioned for fixation in 10% paraformaldehyde. Using standard operating protocol, the samples were dehydrated with systematic baths of 70% ethanol at increasing concentration to absolute, followed by systematic baths in xylol, substance miscible with the inclusion medium. The macroscopic samples were performed in paraplast at 58 °, using microtome for histological sections of 5 µm, made in quadruplicates. The laminae were stained with hematoxylin and eosin 60. Others, duplicate laminae passed through new baths of alcohols until staining with pricossirius red,

followed by xylol and subsequent assembly with coverslips. Approved by the Bioethics committee of Uninove protocol n° AN009/2014. **RESULTS:** Rats treated with LILT demonstrated better tissue organization and with fewer areas without the presence of tissues and decreased edema. (ML) They also presented a better organization of this tissue architecture, a marked decrease in birefringence and percentage of collagens.

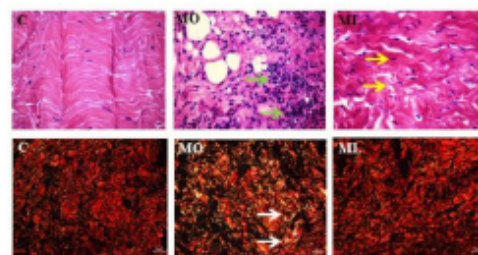


Figure 1. Images of synovial membrane sections stained with H & E and pricossirius red. Green arrow indicates areas of tissue absence and high concentration of inflammatory cells. Yellow arrow indicates better tissue organization. Collagen arranged in thick fibers; birefringence in yellow (white arrow) which is indicative of fibrosis.

CONCLUSION: These results suggest that LILT radiation could be a non-invasive treatment for monoarthritis.

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Evaluation of the efficacy of antimicrobial photodynamic therapy in treatment of peri-implantitis: a controlled, randomized, blinded clinical trial

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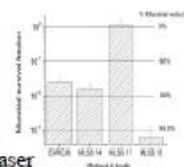
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The elimination of pathogenic microorganisms from the peri-implant system is one of the success factors of peri-implantitis (PI) treatment. Photodynamic antimicrobial therapy (aPDT) consists of the use of red light to inactivate photosensitizing bacteria (FS). PapaMBlue® was used in this assay as an FS mediator of this therapy.

We performed a randomized, blinded clinical trial to evaluate the efficacy of aPDT with this photosensitizer in the treatment of IP. The aim of this study was to evaluate the efficacy of antimicrobial photodynamic therapy in the treatment of peri-implantitis mediated by the photosensitizer PapaMBlue®. Implants with PI bag with depth ≥ 5 mm were randomly divided into two groups: group I received conventional treatment (scraping and removal of peri-implant calculus); group II received conventional treatment and photodynamic therapy (aPDT) with PapaMBlue®. Both groups initially received oral hygiene guidance and conventional treatment which was performed by peri-implant pocket curettage and lavage with sterile saline solution.



Before the application of laser and PapaMblue



After Laser + PapaMBlue® application

1. APDT + PapaMblue® decreased bacteria concentration in PI by 99.9%. Control group did not have reduction on the amount of bacteria after conventional peri-implant curettage treatment



Therapy EC, DMC, São Carlos, Brazil). $\lambda = 660$ nm, $P = 100$ mW/2 mm at each site, 30 J/cm^2 radiant exposure and power density $I = 250$ mW/cm²

CONCLUSION - APDT with photosensitizer PapaMblue® significantly reduces the amount of bacteria

Acknowledgment. This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil - CAPES – Finance Code 001. The authors acknowledge the financial support from The São Paulo Research Foundation (grant FAPESP - 2016/10269-5)

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Effect of led photobiomodulation on analgesia during labor : study protocol for a randomized clinical trial

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Introduction. Labor pain is one of the most intense pains experienced by women, which leads to an increase in the number of women opting to undergo a cesarean delivery. Pharmacological and nonpharmacological analgesia methods are used to control labor pain. Epidural analgesia is the most commonly used pharmacological analgesia method. However, it may have side effects on the fetus and the mother. Light-emitting diode (LED) photobiomodulation is an effective and noninvasive alternative to pharmacological methods. The aim of this research is To evaluate the effects of LED photobiomodulation on analgesia during labor.

Methods and analysis. This is a prospective study of clinical and activities will be conducted at the the Mandaqui Hospital (São Paulo, Brazil). In total, 60 women in labor admitted to a public maternity hospital will be selected for a randomized controlled trial. The participants will be randomized into 2 groups: intervention group [analgesia with LED therapy (n = 30)] and control group [analgesia with bath therapy (n = 30)]. The perception of pain will be assessed using the visual analogue scale (VAS), with a score from 0 to 10 at baseline, that is, before the intervention. In both the groups, the procedures will last 10 minutes and will be performed at 3 time points during labor: during cervical dilation of 4 to 5 cm, 6 to 7 cm, and 8 to 9 cm. At all 3 time points, pain perception will be evaluated using VAS shortly after the intervention. In addition, the evaluation of membrane characteristics (intact or damaged), heart rate, uterine dynamics, and cardiotocography will be performed at all time points.

Expected outcomes: The use of LED photobiomodulation will have an analgesic effect superior to that of the bath therapy.

Discussion: This study describes the protocol for a randomized controlled clinical trial aimed to evaluate

the effect of LED therapy on analgesia during labor. The advantages of using LED therapy for analgesia during labor include the ease of application, the feasibility of application by the same team that assists the patient during labor, the possibility of the patient choosing the position that feels the most comfortable, even under analgesia, and improved mobility; the patient can remain in the upright position to help the fetal descent. The main contribution of this clinical trial is the development of an analgesic intervention during labor that is effective and accessible for use in various public and private health services. The findings of this study may help develop protocols for analgesia during labor, thus allowing patients to not be afraid of pain and promote vaginal delivery.

This study will be the first to use photobiomodulation for analgesia during labor, and the results may help elucidate the correlation between phototherapy in pregnant women and fetal characteristics during therapy. In case of favorable outcomes, this approach can be used as a noninvasive and nonpharmacological alternative for analgesia during labor.

Trial registration: NCT03496857.

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EVALUATION OF PERIODONTAL TREATMENT ASSOCIATED WITH PHOTODYNAMIC THERAPY IN SYSTEMIC PARAMETERS OF INFLAMMATION IN EXPERIMENTAL MODEL OF ASTHMA

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Background: Periodontitis (P) and asthma (A) have high prevalence in the world population which implies high economic and social cost. Evidence suggests that P may be able to modulate the systemic immune response [1,2]. As the standard periodontal treatment (PT) may not have full efficacy over periodontal pathogens in deep pockets, photodynamic therapy (PDT) has been used with promising results, but the cellular mechanisms involved are still unclear. **Objectives:** To evaluate if PT associated with PDT is capable of interfere in systemic parameters of inflammation in an experimental model of asthma and periodontitis in Balb/c mice. **Methods:** After CEUAUninove approval (#020.2017), sixty-four Balb/c male mice, 2 months years-old and 25 g were divided into 8 groups (n = 8): B-BASAL; P; P + PT; P + PT + PDT; A; A+P; A+P+PT; A+P+PT+PDT. Periodontitis was induced with ligation technique. After 15 days, PT was performed. For the PDT, it was used methylene blue ChimioLux® (0.005%, 0.05mg/ml) for 3 minutes, pre-irradiation. The irradiation was performed only once by direct in-contact probe with red diode laser (Therapy XT® DMC, single probe, $\lambda = 660\text{nm}$, radiant power of 100mW, 35,38mW/cm² of power density, energy density of 6,369 J/cm², two irradiation points, inside the mouth, by buccal and lingual surface of the lower left first molar, with 0,0028cm², 9J per point, for 90s). After 43 days, all mice were euthanized. Total and differential blood-cells counts were performed, as well as the platelet count, femoral bone marrow cells count and the level of inflammatory cytokines (IL-4 and IL-10) in lung

homogenate was analyzed. Histological analysis of the mandible was made to characterize the P. For the statistical analysis one-way ANOVA followed by the Student-Newman-Keuls test was used. **Results:** There was an increase in the number of blood circulating eosinophils in group A when compared to group B (p <0.01) that characterizes experimental model of asthma. Periodontitis (p <0, 05) presented a lower amount of cytokine TNF- α in the gingiva when compared to the Asthma group. There was no difference in total and differential blood circulating cells, total platelet count and femoral bone marrow cells, lung edema and inflammatory cytokines (IL4 and IL10) in lung homogenate for all analyzed groups. **Conclusion:** These data have contributed to elucidate that periodontitis and asthma associated or not with periodontal treatment with PDT is not able to interfere in systemic parameters of mice Balb/c. Further studies are needed to understand the systemic regulatory process of these pathologies.

Statement: Approved by the animal ethics committee of UNINOVE, CEUA 020.2017.

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SYSTEMIC INFLAMMATORY RESPONSE DURING DIABETIC FOOT TREATMENT: PHOTOBIOMODULATION VERSUS CONVENTIONAL

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ABSTRACT: Diabetic foot (DF) is defined by the World Health Organization (WHO) as a situation of infection, ulceration and/or destruction of the deep tissues of the feet, associated with neurological abnormalities and various degrees of peripheral vascular disease, in the lower limbs of patients with Diabetes Mellitus (DM). It is one of the most frequent complications of DM, which, due also to its systemic repercussions, can necessitate lower limb amputations in patients; thus systemic inflammatory response becomes very important in the evolution of DF. The greater the systemic repercussion and the lower the control via treatment of these lesions, the greater the risk of a larger amputation (at the leg or thigh level). Photobiomodulation (PBM) has well-known, beneficial effects on wound healing and control of local inflammatory processes, but its effect on systemic inflammatory responses is still controversial.

KEYWORDS: Diabetic foot, Photobiomodulation, Systemic inflammatory response, Diabetes Mellitus, Inflammation

OBJETIVE: To compare the systemic inflammatory response of patients during DF treatment with and without the use of photobiomodulation.

METHODS: Five patients with DF were treated at two distinct infectious events. At the first event conventional treatment (antibiotic therapy, surgery and appropriate dressings) was used and at the second event conventional treatment associated with PBM. Laser (Therapy EC, DMC, Brazil) parameters used: 660nm; P= 108mW; I=2.7W/cm²; irradiation area= 0.04cm², continuous wave, radiant exposure= 108J/cm², E= 4.32J per point, exposure time= 10 s per point, once a week, with application points located on lesion edges with a distance of 1 cm between irradiated points. Irradiation was also performed in the ulcer bed (in the center, in the N, S, E and W directions). Leukogram and PCR tests were performed to monitor the systemic inflammatory process. The data were organized in comparative graphs of the two events and analyzed statistically (OriginPro 2017, from OriginLab Corporation, MA, USA. The significance level was 5%).

Approval from the Mandaqui Hospital Group's (CAAE 53351716.5.0000.5511). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

RESULTS: PARTICIPANT 01:

• Using PBM (8 sessions): the PCR peak was lower, although still high. He presented an intense cellular response (leukogram with an important peak and more intense than in the first event) and gradual decline with normal index around the tenth day.

• Conventional: It showed the beginning of the normal and peak leukogram frame around the tenth day, followed by fall with subsequent normalization of cellularity levels.

PARTICIPANT 02:

• Using PBM (1 session): starts with peak of PCR, with small drop on the second day of treatment. Normal leukogram.

• Conventional: PCR starts normal with peak on the third day (highest relative to the second event). Leukogram curve with peak at the onset of the event and decline and normalization as treatment progressed.

PARTICIPANT 03:

• In PBM use (15 sessions): Initial PCR peak much higher than in the first event, with faster decrease, although we only have two days of analysis. Leukogram with higher peak when compared to the first event. Pattern of similar curve decline in the two events.

PARTICIPANT 04:

• In the use of PBM (4 sessions): Peak of high PCR, with small fall (day 3), new elevation (day 6) and drop of the PCR at the levels of the first event (day 10). Leukogram curve even shows the pattern of the PCR curve.

• Conventional: PCR without changes in the dosages performed. Leukogram patterns in the event with the highest peak initially, but faster fall and normalization (day 5).

PARTICIPANT 05:

• Using PBM (30 sessions): PCR E Leukogram with lower peak. Leukogram shows the same pattern of response in both events.

• Conventional: Rapid fall and normalization of dosages (patient was submitted to the amputation of the affected limb).

CONCLUSION: IN FOUR OF THE PARTICIPANTS IN THE INFECTIOUS EVENT WITH CONVENTIONAL TREATMENT ASSOCIATED TO THE PBM THERE WAS REDUCTION OF THE LEVELS OF PCR, BEING IN TWO OF THEM (PARTICIPANT 01 AND PARTICIPANT 05) WITH STATISTICAL MEANING. IN RELATION TO THE RESULTS OF THE LEUCOGRAM STUDIES WITH MORE PATIENTS ARE NEEDED TO FURTHER INVESTIGATE THE SYSTEMIC EFFECTS.

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THE ROLE OF PHOTOBIO-MODULATION ON THE PARAQUAT-INDUCED PULMONARY FIBROSIS

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Abstract: Pulmonary fibrosis (PF) is a chronic and progressive lung disease characterized by progressive lesion of the pulmonary parenchyma, inflammatory infiltrate and interstitial fibrosis. Treatment of PF is still a clinical health problem, so new therapies are needed. In this context, photobiomodulation has been showing good results for several inflammatory diseases, including lung diseases, and here we studied the effects of LED on PF.

Introduction: Pulmonary fibrosis (PF) is a chronic and progressive lung disease characterized by progressive lesion of the pulmonary parenchyma, inflammatory infiltrate and interstitial fibrosis. It is triggered by the excessive and disordered deposition of collagen and other extracellular matrix components, which results in severe changes in the architecture of the alveolus wall. Several factors can trigger PF among them exposure to chemical agents such as paraquat. Due to the absence of an effective treatment, the objective of the study was to investigate the effect of treatment with photobiomodulation on the course of PF.

Methods: Adult male C57BL6 mice were submitted to the induction of PF by the administration of Paraquat (10mg / kg, ip) and after 7 days of induction, the mice were treated during 7 days with Photobiomodulation (LED). Device specifications: BioLambda Apparatus LEDsabr, Wavelength: 660 nm; Radiant Power: 160 mW; Power Density: 38,5 mW/cm²; spot area: 4,15 cm²; Density of energy: 5,8 J/cm²;

Continuous (cw); Total Radiant Emission: 24 J. Treatment specifications: Exposure time: 152 s; Irradiated points: 1 point; Irradiation Method: Direct skin contact; Anatomical location: trachea and lungs; Irradiation rhythm: punctual; Number of treatments: 1 day, seven applications.

Results: We showed that photobiomodulation reduced Paraquat-induced cell influx into the bronchoalveolar lavage and elevates the level of resolvin D1 without alter the levels of IL-6, TNF- α , IL-10, and IL-17A in the lung homogenates. In addition, did not alter the tracheal responsiveness.

Conclusions: The reduced cell migration induced by photobiomodulation might be attributed, at least in part, to elevated level of resolvin D1. Thus, photobiomodulation although did not alter some parameters, showed beneficial effects on the inflammation and more studies are needed.

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COMPARATIVE STUDY BETWEEN PHOTOBIOMODULATION AND VITAMIN C TO TREAT ACUTE LUNG INJURY EXPERIMENTALLY

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Abstract: The imbalance of oxidative and antioxidant species in lung tissue is an important event for the development of acute lung injury. The treatment is not effective resulting in high mortality. Thus, we investigated the efficacy of photobiomodulation in comparison with vitamin C to treat acute lung injury experimentally.

Introduction: The imbalance of oxidative and antioxidant species in lung tissue is an important event for the development of acute lung injury, which is characterized by rupture of the endothelium and alveolar injury resulting from an uncontrolled lung inflammatory response. The treatment is not effective resulting in high mortality. Thus, we investigated the efficacy of photobiomodulation in comparison with vitamin C to treat acute lung injury experimentally.

Methods: Adult male Bal/c mice were submitted to LPS injection (5µg/kg, ip, *Salmonella abortus equi*) and irradiated with LED or treated with vitamin C (150mg/kg, ip) 2 and 6 h after LPS injection. The parameters were investigated 1 day after LPS injection.

Results: Our results showed that both treatments reduced the cell influx into the alveolar space as well as the blood cellularity. LED treatment, but not vitamin C restored the cell influx in the bone marrow. Moreover, LED treatment reduced IL-

17, an important inflammatory cytokine, in alveolar lavage fluid, while vitamin C increased IL-10, an anti-inflammatory cytokine. No differences were observed in catalase and superoxide dismutase activity between both treatments.

Conclusions: Our data showed that both treatments had beneficial effects in the acute lung injury, but by different mechanisms. Thus, we might assume that photobiomodulation seems to be more advantageous if we consider costs and side effects.

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Experimental Parameters of Photodynamic Therapy with Methylene Blue in *Aggregatibacter actinomycetemcomitans* *in vitro*: Literature review

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Photodynamic Therapy (PDT) is a clinical treatment modality with potential application in the elimination of microorganisms. PDT acts by combining light and a photosensitizing agent (PS) in the presence of oxygen and wavelength light. *Aggregatibacter actinomycetemcomitans* (*A.a.*) is oral bacterium that can also be isolated from several non-oral infections. Several studies investigate the antimicrobial potential of PDT (aPDT) with methylene blue (MB) in biofilm and planktonic *Aa* culture.

Keywords: Photodynamic Therapy, Methylene Blue, *Aggregatibacter actinomycetemcomitans*

Object and objective (s): The objective of the present study was to review the literature in terms of the *in vitro* antimicrobial efficacy of the photodynamic therapy with MB in *A.a.*, evaluating the experimental parameters used.

Development: The bibliographic survey was performed in the Pubmed database, using the following terms as key words: photodynamic therapy and methylene blue and *Aggregatibacter actinomycetemcomitans*, from August 1 to September 10, 2018. The search resulted in ten articles, but only 4 of them performed an *in vitro* study.

Search results: The 3 studies found used MB as PS but at different concentrations (Alvarenga 34 µg / mL, Street 100 µg / mL and Umeda 1 µg / mL). In terms of equipment and dosimetry, Alvarenga used laser (660 ± 2 nm, 250mW / cm², 75J / cm²); as well as Street (670nm, 9.4J / cm²) while Umeda used high power LEDs (650nm, 1100wW / cm², 4 and 8J / cm²). The bacterial reduction found by the authors was almost 3 log₁₀ in biofilm in Alvarenga's article, 1.9 log₁₀ and 4.9 log₁₀ in suspension and biofilm, respectively, in

the Street article and 3 log₁₀ suspended in Umeda's article. In suspension, the Umeda protocol achieved a greater reduction than that of Street, with a 100-fold lower concentration and 1.2-fold higher radiant exposure. In biofilm the Street protocol presented a greater reduction than Alvarenga, using a concentration 3 times higher, a radiant exposure 9 times lower.

Conclusion and final considerations: The results presented in the articles show that the experimental parameters have influence on the microbial reduction found and that the most effective protocol depends on an appropriate combination of radiant exposure and FS concentration. It is possible that the irradiance also plays an important role in the experimental protocol, but due to the lack of this information in one of the articles, it was not possible to evaluate the influence of this parameter.

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ENHANCEMENT OF THE ACTION OF METHYLENE BLUE aPDT BY SELECTING SUITABLE EXPERIMENTAL PARAMETERS

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Photodynamic antimicrobial therapy (aPDT) is based upon the combination of a photosensitizer, light and oxygen. In Brazil, methylene blue (MB) is a well known photosensitizer due to its low cost and effectiveness. However, MB has its photochemical mechanism of action modulated by its state of aggregation, that is, depending on the medium in which MB is conveyed, there is a formation of monomers or dimers, also known as metachromacy. It is already known that reducing aggregation increases the effectiveness of aPDT with phenothiazinium photosensitizers, but dosimetry for the inactivation of *Candida albicans* is still necessary.

The objective of this work was to evaluate the irradiance effect in the inactivation of *Candida albicans*, when organized in biofilm.

C. albicans (ATCC 10231) was seeded in Sabouraud Agar Dextrose 48 hours prior to the experiment. After this period the cells were collected, counted in a Neubauer chamber and 0.15mL of a suspension (3×10^8 cells/mL) was added in two 48 well plates (previously treated for 24 hours with fetal bovine serum) with 0.3 mL of Sabouraud Broth Medium. The plates were maintained for 48 hours in an incubator for biofilm growth. The biofilms were treated with physiological solution (PS), methylene blue 0.005% in physiological solution (MB 5 - PS), oral formulation (OF) and methylene blue 0.005% in oral formulation (MB 5 - OF). A pre-irradiation time of 5 minutes was used and each of the plates was irradiated. Plate 01: 30 minutes with an LED of 640 ± 12.5 nm, 2.6 mW/cm^2 (Radiant Exposure 4.7 J/cm^2), Plate 02: 27 minutes with Biolambda LED 660 ± 10 nm nm, 37.3 mW/cm^2 (Radiant Exposure 60.4 J/cm^2). At the end, the biofilm was disassembled in phosphate buffered saline and diluted (10^{-1} to 10^{-5}) which were applied to Sabouraud Dextrose agar. The plates were held for 24h at 37°C and the number of CFU/mL counted. The experiments were performed in triplicate and three independent experiments.

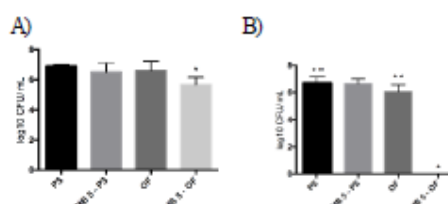


Figure 1: CFU/mL count after treatment with aPDT in different irradiances. A) 30 minutes with an LED of 640 ± 12.5 nm, 2.6 mW/cm^2 . B) with an LED of 660 ± 10 nm, 37.3 mW/cm^2 for 27 minutes.

It is observed in figure 1A that the treatment with methylene blue in the formulation of oral use in which there is less aggregation of the photosensitizer presented a reduction in the number of colonies of *C. albicans*, although it did not reach the inactivation of the microorganism. When another irradiation system with higher irradiance was used, for the same oral Thus, the results show that reducing aggregation with oral use formulation may increase the efficacy of methylene blue aPDT in *C. albicans* biofilm, as well as the use of adequate irradiation parameters. The use of this formulation in clinical practice may potentiate the treatment of patients with oral candidiasis.

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Evaluation of the effect of photobiomodulation of the major salivary glands on the flow and salivary pH of patients with Diabetes Mellitus and Xerostomia

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Introduction

Diabetics with decompensated blood glucose usually develop oral manifestations, such as: gingivitis, caries lesions, periodontitis, infections opportunistic and xerostomia. The success of any dental treatment of these patients is directly related to the quantity and quality of the saliva they produce. Salivary acidity has great diagnostic importance as it generates reflexes oral health, causing or even aggravating oral pathologies that the diabetic patient has already presents a predisposition. The ideal pH is essential to avoid, for example, the proliferation of pathogenic microorganisms like streptococcus mutans, the main bacterium that causes the disease caries, and the saliva buffer capacity. Regarding the treatment for salivary changes, there is a need for more studies, however, the laser has shown satisfactory results in the improvement in salivary flow and, consequently, in the quality of life of this patients.

Methods

18 diabetic patients with xerostomia were submitted to this study, signing Informed Consent Form, anamnesis, physical evaluation, and self-report perception of oral health and symptoms related to the function of the salivary glands, the patients were divided into two groups randomly: Photobiomodulates (FTB= 9); who had the irradiated salivary glands and Placebo (PCB=9); submitted to a simulation, with the laser off. Sialometries were performed, in a total of 4 collections. The pH analysis was performed on the first and last day of the protocol in the Non-Stimulated (NE) and Non-Stimulated-Laser (NEL) samples. The sialometries had a duration of 5' to compare the volume of saliva. The protocol established for this study were extra and intra-oral bilateral applications in the parotid glands, submandibular, and sublingual using Laser of Diode.

Wave-length	808 nm
Power (mW)	100mW
Radiant energy (J)	3.2
Exposure time (s)	32
Operation mode	Continuous

Table 1

Total radiated area per session (cm ²)	0,336 cm ²
Irradiance (cm ²)	3571mW/cm ²
Radiant exposure (cm ²)	114J/cm ²
Total energy per session (J)	38,4J

Table

2

Results

Preliminary results suggest that there was an increase in saliva NE and E volume of the patients. The placebo group showed no statistical differences compare the initial and final volumes of NE and E saliva. As for pH, no there were changes in the FTB and PCB groups.

Conclusion

This work shows that the photobiomodulation protocol of the salivary glands increased the flow with four laser applications and showed no changes in pH salivary, however, more studies are needed to better understand its effect.

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The effects of photobiomodulation on nitric oxide synthesis in satellite muscle cells cultivated in the presence of M1-macrophages conditioned medium

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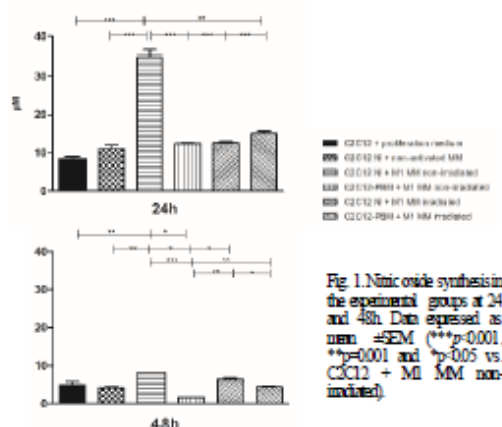
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After an acute skeletal muscle injury, the macrophages have a important role in the regeneration process due to the products secreted by them. Depending on their phenotype, macrophages may differentially influence the activation and differentiation of satellite muscle cells (SC). The treatment with photobiomodulation (PBM) has shown, in previous *in vitro* studies, the ability to modulate the inflammatory process through the reduction of expression and synthesis of important inflammatory mediators.

Objective: The aim of this study was to evaluate the effects of PBM on nitric oxide (NO) synthesis in C2C12 cells cultivated in the presence of M1 macrophages-conditioned medium.

Methods: For the activation of M1 profile, J774 macrophages were incubated with interferon- γ and lipopolysaccharide for 2h in medium containing 5% of Fetal Bovine Serum (FBS). After this period, the cells were removed and transferred to tubes, centrifuged (1200 rpm, 5 min., 10°C) and irradiated at the bottom with AlGaAs diode laser (780 nm, 70 mW, 1.75 W/cm², 0.04 cm² and 17.5 J/cm² for 15s, totaling 1 J). The conditioned-medium (MM) was collected 24h after the treatment with laser. Myoblasts C2C12 were cultivated in 10% SFB. The cells were counted and divided into six experimental groups: (1) control group, (2) C2C12+ MM non-activated, (3) C2C12 + M1 MM, (4) C2C12-PBM + M1 MM, (5) C2C12 + M1 MM irradiated and (6) C2C12-PBM+ M1 MM also irradiated. All the groups cells were centrifuged and the pellets (PBM groups) were irradiated with the same parameters. After the treatment, the cells were seeded in a 96-well plates and received 30% of MM (from non-activated, and irradiated or non-irradiated M1-macrophages). After, C2C12 cells were incubated in at 37°C, 5% CO₂ for 24 and 48h and at the end of this period the NO synthesis was assessed by Griess method.

Results: The results showed an increase in NO synthesis in the groups that received M1-MM non-irradiated at 24h and 48h. Furthermore, it was observed a decrease in C2C12 groups only laser irradiated in all periods evaluated. At 24h it was observed a decrease in NO synthesis in C2C12 cells groups irradiated and non-irradiated that received the M1-MM also treated. Moreover, when both cells were irradiated it was observed a decrease in NO synthesis at 48h after the treatment with PBM.



Conclusion: The present findings demonstrated that the photobiomodulation, used with the parameters described, was able to decrease the synthesis of NO in satellite muscle cells cultivated in the presence of M1 macrophages medium.

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EVALUATION OF LED THERAPY ON THE VIABILITY OF J774 MACROPHAGES POLARIZED TO M1 PHENOTYPE

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After the occurrence of an injury, the immune cells migrate from circulation to the affected site. The inflammatory process is complicated, but can be divided into phases that are characterized by the ordered migration of effector cells, vascular changes, and an increase in enzymes, cytokines and inflammatory mediators. 2 days after the occurrence of a tissue injury, the proinflammatory macrophage (M1) is the most numerous at the injury site, being responsible for the phagocytosis of cell debris, cytokine release, and growth factors that contribute to tissue repair. However, very intense or prolonged activation can have an inverse effect, which impairs repair. Several studies have pointed to Photobiomodulation (Laser and LED) as an important therapeutic source in the field of muscle and tissue injuries.

Objective: To evaluate the effect of LED therapy using wavelengths 850 nm (infrared) and 580 nm (amber) on cell viability of J774 macrophages polarized to the M1 phenotype.

Methodology: J774 macrophages were cultivated in DMEM supplemented with FBS 10% and polarized to M1 phenotype with LPS and IFN- γ . After this, LED therapy treatment (infrared 850 nm and amber 580 both with 70 mW, applied for 15 seconds for 1J or 30 seconds for 2J) was conducted and cells were stored in an oven at 37°C with CO₂ 5% for 48 hours. For viability analysis, the crystal violet method was used. Non-irradiated and non-polarized M1 macrophages served as control.

Results: Macrophages activated to the M1 profile presented lower cellular viability than the control group (not activated). Macrophages activated to M1 profile and irradiated with 2J infrared LED or 2J amber LED showed an increase in cell viability compared to only activated macrophages. The irradiation with 1J infrared LED reduced the viability of these cells.

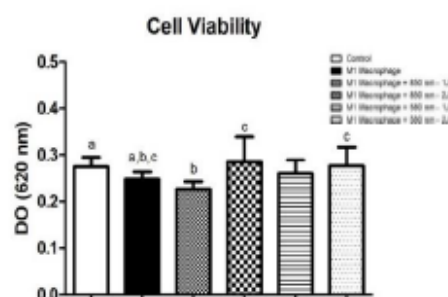


Fig. 1. Cellular viability (crystal violet) by J774 activated macrophages for 2h with LPS + IFN (M1) and irradiated with amber and infrared LEDs with energies of 1 and 2 J after 48 h of irradiation. A comparison was made between the control group and the activated group M1 and activated group M1 and activated M1 + LED treatment (infrared 1 and 2 J and amber 1 and 2 J). The t-test paired with Welch's correction was used. The same letters represent a statistically significant difference: a, b = $p < 0.01$; c = $p < 0.05$.

Conclusion: Amber and infrared LED irradiations with 2J were able to increase cell viability of J774 macrophages polarized to M1 phenotype.

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DIFFERENTIATION OF MUSCLE CELLS TREATED WITH SUPERNATANTS OF IRRADIATED M2A MACROPHAGE

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The occurrence of a muscle injury triggers a complex repair process. Macrophages play a crucial role in the initial and later steps of muscle repair which are characterized by a proinflammatory (M1) and anti-inflammatory (M2) profile, respectively. Concomitantly, myogenic precursor cells (MPC) need to be activated to differentiate into muscle cells that will replace the injured tissue. In this context, it is well known that mediators produced by M1 macrophages are related to the proliferation of MPC cells as well as to M2 macrophage products with their differentiation. On the other hand, while the role of Low-Level Laser Therapy (LLLT) on the muscle repair process has been extensively studied, its effects on macrophage products that may influence MPC differentiation are still unclear.

Objective: The objective of this study is to evaluate the effects of the supernatant of macrophage cultures, induced to M2a phenotype and irradiated with red laser (660 nm) and infrared (780 nm) using the same dosimetric parameters (70 mW, 17.5 J/cm², 1J), on MPC differentiation.

Methodology: J774 macrophages were cultured in DMEM with fetal bovine serum (10%). These cells were treated with IL-4 (0.1 µg/mL) for 24h to activate the M2a profile. After this period, the cells were irradiated with LLLT (660 nm and 780 nm) and cultured for another 24 hours. Non-irradiated and non-activated macrophages were used as controls. The supernatant of each group was added to the myogenic precursor cell line C2C12 and incubated for 72 hours. Cells were collected with TRIzol and stored at -70 °C to perform RNA extraction, complementary DNA synthesis and Real Time PCR to evaluate gene expression of *MyoD*.

Results: Myoblasts receiving macrophage supernatant from M2a + LLLT 660 nm showed decreased *MyoD*

gene expression relative to myoblasts cultured with supernatant from non-irradiated M2a macrophages. On the other hand, myoblasts treated with M2a + LLLT 780nm macrophage supernatant showed an increase in the expression of this gene relative to cells receiving LLLT 660nm treatment.

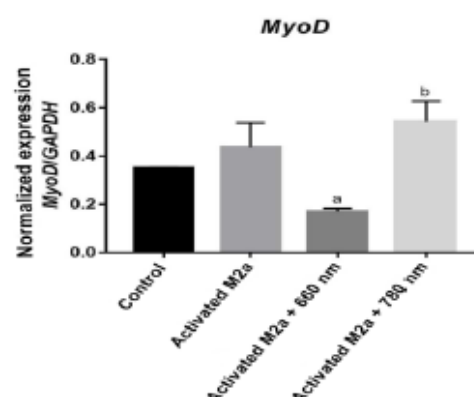


Fig. 1. Effect of laser irradiation on *MyoD* / *GAPDH* gene expression in C2C12 cell cultures (3×10^6) which received macrophage supernatant without activation (Control), with activation to M2a phenotype without irradiation and groups with M2a activation and irradiation with red and infrared LLLT. Evaluation after 72 hours after irradiation. Bars represent means and standard deviation of the 2 experiments. a= difference from the M2a group and b= difference from the M2a+660 nm group.

Conclusion: 780nm laser irradiation was able to generate superior effects when compared to 660nm laser use in MPC differentiation and this superiority may be linked to its ability to modulate production of M2 macrophage differentiation activating factors.

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Photodynamic therapy with Bixa orellana extract and LED for the reduction of halitosis: a randomized and controlled clinical trial.

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Halitosis, also known as bad breath, is a term used to define an unpleasant and foul odor that emanates from the mouth and may have local or systemic origin. This project aims to observe the presence of halitosis and to verify if the treatment with antimicrobial photodynamic therapy (aPDT) is effective against it. A total of 39 students or UNINOVE employees with a diagnosis of halitosis were selected, presenting sulfhydryde (SH₂) ≥ 112 ppb in gas chromatography. The patients were randomly divided into 3 groups of 13, which received different treatments: Group 1: aPDT applied in the region of the back and middle thirds of the tongue; Group 2: treatment with tongue scraper; Group 3: combined treatment of tongue scraper and aPDT. For aPDT urucum was used in a concentration of 20% (Fórmula e Ação®) applied in sufficient amount to cover the back and middle third of the tongue for 2 minutes for incubation, associated with a LED (Valo Cordless Ultradent®). Six points were irradiated on the back of the tongue with a distance of 1 cm between the points, considering the halo of light scattering and effectiveness of aPDT. The apparatus was previously calibrated at wavelength 395-480 nm for 20 seconds, energy of 9.6J and radiant energy of 6.37 J/cm² per point. The results of the halimetry were compared before, immediately after treatment and 7 days after. The Friedman test was used for the intragroup analysis and the Kruskal Wallis test for the intergroup analysis. In all groups, there was a statistically significant difference between the initial

sulfhydryde value and the value immediately after the treatment ($p < 0.05$). In Groups 1 and 3, there was difference between the value immediately after the treatment and the control of seven days. In Group 2, there was no statistical difference between these times (Fig. 1). There was no statistical difference between groups (Kruskal Wallis).

Clinical Trials number: NCT03346460.

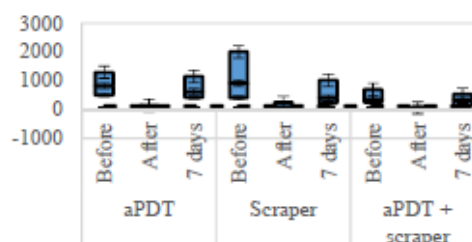


Fig. 1. Sulfhydryde levels (ppb) before, immediately after and seven days after treatment.

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Effects of photobiomodulation in hypertensive patients - A Systematic Review

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Abstract

High Blood Pressure (Hypertension) is a chronic disease of high prevalence caused by multiple factors and determined by elevated blood pressure levels, representing the main risk factor for the development of cardiovascular disease with possible target organ damage. Representing high costs to public coffers, this disease, difficult to control, represents a high morbidity and mortality rate. The treatment of such chronic noncommunicable disease is by the combination of non-pharmacological and pharmacological treatment. There are several studies that point out the difficulty of adherence to the treatment of hypertensive patients. In search of a new therapeutic alternative the use of low-intensity laser or photobiomodulation may be a possibility. With few studies and considered as one of the most important non-pharmacological therapeutic advances of the present day, the technique can allow an efficient antihypertensive treatment with reduced adverse effects when compared to the side effects that the pharmacological treatment promotes, being able to become an element important for a possible increase in the rate of adherence to treatment. The objective of this study was to perform a systematic review to analyze the effects of photobiomodulation in hypertensive patients.

Methods/design: A systematic review on the effects of photobiomodulation and arterial hypertension was carried out in three databases: PubMed, Lilacs, SCIELO and the Virtual Health Library, performing the PICO strategy, acronym for P (patient) - hypertensive patients, without restriction of gender, age and ethnicity; I (interventions) - use of devices

capable of performing use of devices capable of performing photobiomodulation by laser; C (comparison) - comparison between treatment groups and those receiving placebo and O - outcome - clinical improvement in hypertensive patients observed with reduction of blood pressure.

Results: Considering the inclusion criteria adopted, 5 studies were found, of which 80% deal with laser application in humans. Both studies evidence the use of photobiomassage in blood pressure control, but they do not clarify the pathophysiological processes that determine the reduction of blood pressure.

Conclusions: It was concluded that photobiomodulation is efficient in the treatment of hypertension and can be used as a hypotensive treatment, however, further research is needed to elucidate the subject, since the existing research does not fully clarify the mechanisms of the action of photobiomodulation.

Descriptors: Lasertherapy; Hypertension; Photobiomodulation.

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Acknowledgment. We thank the University Nove de Julho

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Photobiomodulation prevents DNA fragmentation of alveolar epithelial cells and alters the mRNA levels of caspase 3 and Bcl-2 genes in acute lung injury

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Acute lung injury (ALI) are defined as a pulmonary inflammation, could occur from sepsis and leads to pulmonary permeability and alveolar edema¹. The photobiomodulation (PBM) have been widely described in the literature for treatment of some inflammatory diseases², it could be a possible treatment for ALI. The aim of this study was to evaluate the mRNA levels from Caspase-3 and BCL-2 genes and DNA fragmentation in lung tissue from *Wistar* rats affected by ALI and exposed to low power infrared laser.

Methods. (CEUA/012/016) Photon laser III (D.M.C São Paulo; AsGaAl; 808nm; 100mW; 3.6W/cm²; 0.028cm² spot size; four points per lung, punctual by skyn contact). Adult male Wistar rats into 6 groups (n=5): control, PBM10 (10J/cm², 2J and 2 seconds), PBM20 (20J/cm², 5J and 5 seconds), ALI, ALI+PBM10 and ALI+PBM20. ALI was induced by intraperitoneal *E. coli* lipopolysaccharide (LPS) injection, after 4 hours, exposed to infrared laser. After 24 hours, the lung samples were collected and divided for mRNA expression of Caspase-3 and Bcl-2 and DNA fragmentation quantifications.

Results. Data show that Caspase-3 mRNA levels are reduced and Bcl-2 mRNA levels were increased in ALI after LPIL exposure when compared to non-exposed ALI group (Figures 2 and 3). DNA fragmentation was increased in inflammatory infiltrate cells and reduced in alveolar cell (Figure 4).

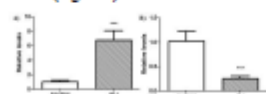


Fig. 1. (A) and (B) mRNA relative levels from Caspase 3 and Bcl-2, respectively from normal and acute lung injury (ALI)-induced.

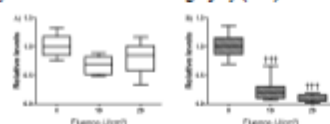


Fig. 2. Caspase 3 mRNA relative level in lung tissue after LPS-induction and PBM (808nm). A) 0 (control), 10 (10J/cm²), 20 (20J/cm²); B) 0 (ALI), 10 (ALI + 10J/cm²), 20 (ALI + 20J/cm²).

GAPDH and ALI were used for normalization ($\Delta\Delta Ct$). (†) when compared with ALI group.

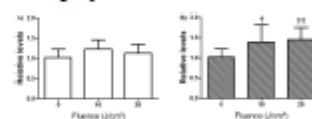


Fig. 3. Bcl-2 mRNA relative level in lung tissue after LPS-induction and PBM (808nm). A) 0 (control), 10 (10J/cm²), 20 (20J/cm²); B) 0 (ALI), 10 (ALI + 10J/cm²), 20 (ALI + 20J/cm²). GAPDH and ALI were used for normalization ($\Delta\Delta Ct$). (†) when compared with ALI group.

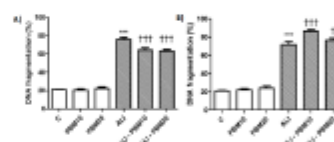


Fig. 4. Percentage of DNA fragmentation by TUNEL POD positive labeling quantification in A) alveolar cells and B) infiltrate area. C (control), PBM10 (10J/cm²), PBM20 (20J/cm²); Acute lung injury (ALI), ALI-PBM10 (acute lung injury and 10J/cm²), ALI-PBM20 (acute lung injury and 20J/cm²). (*) when compared with control group and (†) when compared with ALI group.

Conclusion. PBM can alter mRNA relative levels from genes involved in apoptotic process and DNA fragmentation in inflammatory and alveolar cells after LPS-induced ALI. Also, inflammatory cell apoptosis is part of a selective action, which could be part of anti-inflammatory effect induced by low-power lasers.

Funding. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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Tooth bleaching using violet led. Thermal variation and protocol effectiveness

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This work studied the variation in temperature in dental bovine pulp chamber during irradiation with a violet led source. The current clinical protocol for teeth whitening with violet led was tested in its effectiveness and safety.

The uses of bleaching agents in dentistry like carbamide or hydrogen peroxide are widely studied. Light sources like blue led and lasers (diode, argon) have already been used as speed agents, but having no effect on intrinsic stains. Recently a new bleaching protocol¹ was proposed using a violet light source ($\lambda = 410 \text{ nm}$) and was based in the absorbance of the energetic photons by stain molecules at this wavelength.

Materials and Methods. Bovine teeth were provided by disposal livestock. Teeth were cleaned from the soft tissues and stored in 0.4% timol, under temperature of 4°C. For the first experimental test, a thermopar was adapted inside the pulp chamber of three teeth which were continuously irradiated with a led source with output power of 400 mW and irradiance of 200mW/cm² for 180 s. The temperature inside the three pulp chambers as a function of time was taken and Pearson's correlation (R) was calculated. Four teeth were submitted to a bleaching protocol which consisted in 20 cycles of 60 s of violet light with 30 s interval between applications. The same led parameters described for temperature measurements were employed and the teeth color was measured before and after the protocol with the aid of a spectrophotometer (Easyshade, VITA Zahnfabrik). Color differences among specimens were verified by CIELAB system determining the ΔE as the following equation:

$$\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$$

where L represent the lightness of the object, a value represents the chromatic coordinate that varies from red (+) to green (-) and b value is determined in the

chromatic coordinate that varies from yellow (+) to blue (-).

Results. The temperatures inside the pulp chambers are plotted in figure 1. There was a strong positive correlation among the measurements ($R = 0.99$).

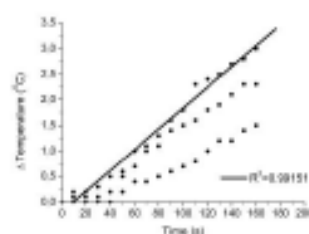


Figure 1 – Data of temperature variation as a function of time inside the pulp chambers of three bovine teeth.

There was no perceivable change in color ($\Delta E < 3.3$) for two specimens after the protocol application and no change was detected in shade for any specimen, as seen in table 1.

Table 1 – Color parameters for the studied specimens (S) IS – initial shade; FS – final shade.

S	L_1	L_2	a_1	a_2	b_1	b_2	ΔL	Δa	Δb
1	41.9	41.7	11.1	19.9	9.5	37.5	2.3	8.8	18.0
2	39.9	37.2	9.9	4.0	15.6	36.6	2.7	-13.9	-21.0
3	34.4	34.5	2.2	2.9	12.2	29.9	0.1	0.7	17.7
4	31.1	31.7	3.9	3.6	19.7	40.5	0.6	-0.3	20.8

Conclusions. Although some changes were observed in color parameters after treatment, violet led did not alter shade of bovine teeth using the current clinical protocol. Thermal variation inside the pulp chamber should not be a cause of concern during bleaching treatment with violet led.

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Low-level lasers and the effects on migration and viability on MDA-MB 231 cells

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Introduction: Low-level lasers are devices that emit monochromatic, coherent and highly collimated beams of light¹, and they are useful in several clinical procedures. Their wavelength, frequency, power, fluence and emission mode properties are determining to photophysical, photochemical and photobiological responses², but the molecular mechanisms involving biological laser-induced effects are not well understood yet. Also, there are some divergences on their use on oncological patients, or even on those who have an oncological historic. Recent studies show positive effects of low-level lasers on photodynamic therapy (PDT) and to treat radiotherapy side effects, but the knowledge about the molecular effects of these lasers on tumors is controversial.

Objective: this study aimed to evaluate the effects of low-level infrared (808nm) and red (660nm) lasers on viability and migration of breast cancer cells.

Material and Methods: MDA-MB 231 breast cancer cells were trypsinized and exposed to low-level red (660nm, spot size 0,028cm²) and infrared (808nm, spot size 0,028cm²) lasers at fluence of 25 J/cm² (7s) and 50 J/cm² (14s) in continuous wave, and emission of 100mW. To evaluate relative cell migration, after the irradiation those cells were plated in a plate of 6 wells and after 24h a crop in cross was made in each well. Percentage of migration was evaluated after 24h of incubation. To evaluate relative cell viability after exposure to lasers, those cells were plated on plates of 96 wells. After 24h of incubation, cell proliferation was evaluated by WST-1 method. The percentage of cell viability was evaluated after 2h. Kruskal-Wallis, followed by Dunn post-test was performed to statics analysis.

Results:

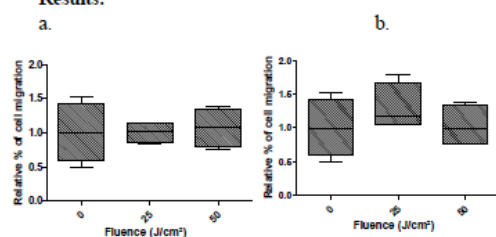


Figure 1: Relative percentage of migration of MDA-MB 231 cells after exposure to low-level (a) infrared (808nm) and (b) red (660nm) lasers at 25 and 50 J/cm².

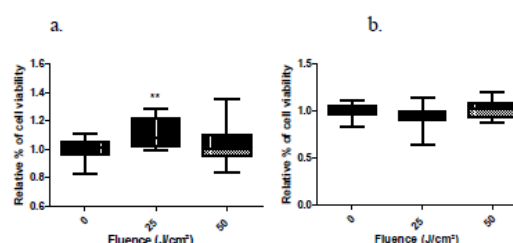


Figure 2: Relative percentage of viability in MDA-MB 231 cultures after exposure to low-level (a) infrared (808nm) and (b) red (660nm) lasers at 25 and 50 J/cm².

Conclusion: The results show that there was no significant modification of the relative migration of MDA-MB 231 cells after exposure to both low-level lasers. Low-level red laser exposure did not change the relative viability, but the relative viability was significantly increased at 25 J/cm² in MDA-MB 231 cultures exposed to infrared laser.

Funding: This study was supported by CAPES, Finance Code 001, CNPQ and FAPERJ.

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THE EFFECT OF PHOTOBIOMODULATION ON THE FATIGUE OF INDIVIDUALS WITH MULTIPLE SCLEROSIS

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Among the autoimmune diseases of the central nervous system stands out the multiple sclerosis, which is the second most common cause of physical incapability in young individuals. The inflammation and the oxidative stress contribute to destruction of the myelin sheath and consequently block or slow the synapses in the demyelination site, causing multiple sclerosis. Fatigue is one of the most common symptoms and can affect up to 90% of these individuals and is described as a feeling of physical and mental exhaustion that limits daily life activities, causing a negative impact on quality of life. Although the pharmacological treatment is able to promote balance between pro- and anti-inflammatory cytokines, a number of undesirable effects are still observed, so it can be state that the success of the treatment has not yet been achieved. Photobiomodulation (PBM) is a non-pharmacological therapy widely used for other chronic inflammatory conditions and may have a role in the treatment of multiple sclerosis.

Objective: To evaluate the fatigue of individuals with relapsing-remitting multiple sclerosis diagnosis undergone to a PBM treatment on the sublingual and radial artery region.

Methods:

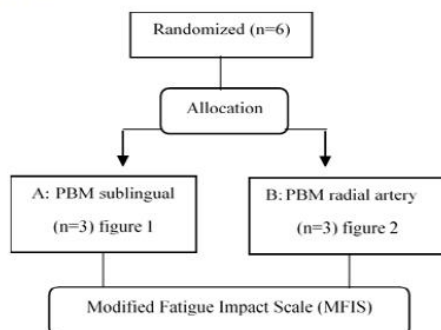


Figure 1. PBM sublingual



Figure 2. PBM radial artery.

Table1. Parameter photobiomodulation

Parameter	Sublingual	Radial artery
Wavelength	808 nm	808 nm
Energy	360,472J	360,472J
Time	1800 s	1800 s

Results:

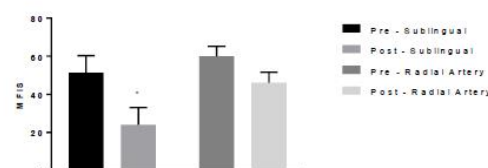


Figure 3. MFIS * significant differences pre and post PBM sublingual, $p = 0.0203$.

Conclusion: This study showed that sublingual PBM can have a positively influence on fatigue, and the initial results encouraged us to continue the investigation and increase the sample size.

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Pilot study on the effect of photobiomodulation therapy in multiple sclerosis

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Multiple sclerosis (MS) is a complex multifactorial, demyelinating and inflammatory disease of the central nervous system. While the unbalance of pro- and anti-inflammatory cytokines can be altered with pharmacological treatments, the tolerability to drug therapy and the accumulation of neurological disabilities are not yet properly controlled. Photobiomodulation (PBM) is a non-pharmacological therapy widely used for other chronic inflammatory conditions and may have a role in MS management.

Objective: of the present study was to investigate, in a prospective manner, the potential effect of photobiomodulation in MS.

Methods:

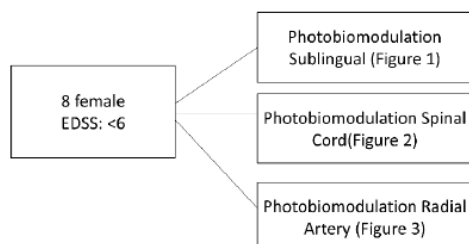


Table1. Parameter Photobiomodulation

Parameter	Sublingual	Radial Artery	Spinal Cord
wavelengths	808 nm	808nm	808nm
Irradiance	287W/cm	287W/cm	1433 W/cm
Time	1800 s	1800 s	1800 s
energy	360,472J	360,472J	1,799,848 J

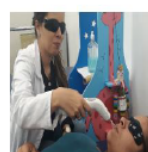


Figure 1



Figure 2



Figure 3

Results

The initial results showed no significant differences between the pre ($p = 0.7055$) and post ($p = 0.1502$) levels of nitrite in the 4 treatment groups (table1). Reports of patients: The patients referring less fatigue, muscle pain, tremors, decreased sensation of tingling in the limbs and headaches during therapy. However, these findings did not affect EDSS levels. There were no adverse events, discomfort or worsening in patients participating in this trial.

Table 1. levels of nitrite

Interventions	Individuals	Nitrite (Nit/mg prot)- Pre treatment	Nitrite (Nit/mg prot)- Post treatment
SUBLINGUAL PBM	3	0.0021	0.0013
	7	0.0030	0.0017
	10	0.00118	0.00078
SPINAL CORD PBM	1	0.00374	0.01487
	5	0.00372	0.00446
	6	0.00163	0.00168
RADIAL ARTERY PBM	9	0.00282	0.00065
	8	0.00066	0.00044

Conclusion:

This pilot study showed that photobiomodulation may positively influence symptoms of MS. The response seems to be individual and subjective, but initial results were encouraging to pursue the investigation. Larger number of subjects will be treated

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Antimicrobial photodynamic therapy in perimini-implantitis and non-neoplastic proliferative lesion: a case report

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Infections associated with orthodontic mini-implants are a great concern both for patients and professionals, sometimes leading to the loss of the anchorage device. This case report presents antimicrobial photodynamic therapy as a helpful tool in the control of the signs and symptoms of perimini-implantitis.

Background. Mini-implants extended the limits of the orthodontic treatment. With its extensive use, recently there has been an increase in reports of the failures of the technique. One commonly cited factor is tissue irritation around the anchorage devices. The accumulation of biofilm on the exposed surface can lead to mucositis and, as it progresses, to a perimini-implantitis. Such cases are usually associated with pain, edema in the perimini-implant mucosa and may compromise the stabilization of the device. Antimicrobial photodynamic therapy (aPDT) is a non-invasive antimicrobial therapy that may be beneficial in such infirmity.

Case report. A Caucasian 27-year-old female patient, good oral and general health, presented a mucosal irritation and hyperplasia around a buccal-shelf mini-implant on the right side of the mandible. She declared spontaneous pain (grade 7 in a visual analog scale), tissue growth and difficulty in local hygiene for the past ten months.

Treatment protocol. It was conducted three sessions of aPDT to recondition the tissue around the mini-implant, with 3-days intervals. For the aPDT, 0.02% methylene blue was applied in the affected area, with a pre-irradiation time of 5 minutes, followed by low power red laser irradiation (Therapy XT, DMC, São

Carlos, Brazil, 660 nm, 100 mW, spot size 0.043 cm², 9 J/point, 90 sec/point, at 4 points around the mini-implant). When inflammation ceased, the patient was referred for excisional biopsy of the non-neoplastic proliferative lesion related to the mini-implant.

Results. After nine days, there was a decrease in edema and erythema around the mini-implant, the patient reported no more spontaneous pain nor while handling the tissue, and regained the ability to conduct proper hygiene. No side effects have been reported.

Discussion. Few studies describe effective protocols for the treatment of mucositis and perimini-implant hyperplasia. Due to its high risk depending on patient's health and compliance, not to mention placement site and surgical technique, the investigation of therapeutic procedures to treat complications associated with mini-implants are essential in modern orthodontics.

Conclusion. Antimicrobial photodynamic therapy was effective in relieving the signs and symptoms of perimini-implantitis. Therefore, it may be a promising technique for recovering infections associated with temporary skeletal anchorage devices in orthodontics.

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Acknowledgment. We thank the Special Laboratory of Lasers in Dentistry (LELO-FOUSP) for the use of their equipment and academic support.

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Dichromatic laser radiation effects on infected pressure injury

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Infection is a delaying factor in the wound healing process, but the fact that it is infected or not is sometimes neglected at the time of low power laser irradiation. Thus, in this study was evaluated the effects of low power dichromatic laser on inoculated pressure injury.

Methods. (CEUA/007/2015) Experiment was conducted on male Swiss mice. Ischemia-reperfusion cycles were employed by external application of magnetic plates to cause pressure injury formation and wounds were inoculated (Figure 1).¹

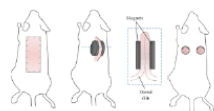


Fig. 1. IR-induced injury model

One day after inoculation wounds daily treated with therapeutic low power dichromatic laser (simultaneous emission in 660nm and 808 nm, spot size of 0.028 cm², at 140 J/cm², 20 seconds per point, at continuous emission). The animals were euthanized 14 days after wounding. It was evaluated wound contraction, epidermis thickness and bacterial survival.

Results. It was verified that 4 days after the injury the area of the wound in the inoculated group was significantly higher than the initial day, inoculated (Figure 2) and irradiated wounds showed neo-epidermis thinner than that of the inoculated and non-irradiated group (Figure 3), bacterial survival decreased in irradiated group (Figure 4).

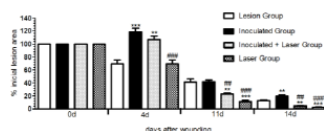


Fig. 2. Macroscopic analysis of wound healing in laser-exposed mice. Percentage of original wound area at 0, 4, 11, and 14 days after wounding (graphical representation per day). (**) $p < 0.01$, (***) $p < 0.001$ compared between the lesion group vs inoculated,

inoculated + laser and laser groups per day; (##) $p < 0.01$, (###) $p < 0.001$ compared between the lesion inoculated vs lesion, inoculated + laser and laser groups

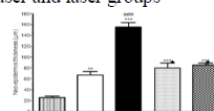


Fig.3. Microscopic analysis of wound healing. Measure of neo-epidermis thickness of the control, lesion, inoculated, inoculated + laser and laser groups at 14 days after wounding. (**) $p < 0.01$, (***) $p < 0.001$ compared between the control group vs lesion, inoculated, inoculated + laser and laser groups; (###) $p < 0.001$ compared between the lesion vs control, inoculated, inoculated + laser and laser groups; (lll) $p < 0.001$ compared between the inoculated vs control, lesion, inoculated + laser and laser groups

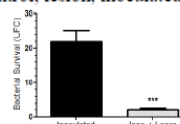


Fig. 4. Bacterial Survival of the inoculated and inoculated + laser groups at 14 days after wounding. (***) $p < 0.001$ compared between the control inoculated vs inoculated + laser

Conclusion. These results suggests that low power dichromatic red and infrared lasers improve healing by kill or inhibition of bacteria as well as accelerating wound healing, resulting in tissue repair.

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Effect of photobiomodulation therapy on hypertrophic scar of the lip: a case report

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Background: Wound healing is a complex process that aims to restore the functional and aesthetic condition of the damaged tissue. Multiple factors, local or systemic, can interfere on the path to a perfect healing and, as result, lead to tissue modifications such as keloids and hypertrophic scars. There are few reports about impaired wound healing of traumatic lesions on the lips, especially about the remodeling phase and the occurrence of hypertrophic scars on this area. Therefore, there is still no standard protocol for the management of this clinical condition. Photobiomodulation Therapy (PBMT) has been presented as a therapeutic option for impaired wound healing, since it modifies the biological response of the target tissue in a safe and non-invasive way.

Case report: A Caucasian patient, 66 years old, was sent to LELO-FOUSP complaining about lack of sensitivity and increase of volume on the lip due to a trauma. After a pathologist diagnosis of hypertrophic scar, it was suggested laser PBMT, in order to alleviate her symptoms.

Treatment protocol: The treatment was performed with simultaneous irradiation of a low power diode laser, 808 and 660 nm, 0.098 cm², emitting 100 mW each, 2 J/point (Therapy XT, DMC, São Carlos, Brazil), in 5 points (4 around and 1 in the center of the scar), for four months. The patient was asked to graduate (0-10) her discomfort concerning the sensitivity and volume in the affected area, being “0” no discomfort at all and “10” the biggest annoyance noticed since the trauma.

Results: From the 6th to the 16th week of treatment, she reported that her discomfort regarding low sensitivity on the lower lip regressed from 5 to 0.7 and volume from 6 to 1 (figure 1).

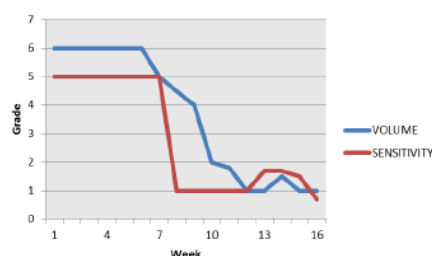


Figure 1: The graduation of discomfort regarding volume and lack of sensitivity on the lower lip during treatment.

Clinically, it was observed as benefit a more aesthetic aspect of the lips during the smile. Three months after finishing the treatment, the results were maintained. No side effects have been reported.

Conclusion: Laser PBMT, within the mentioned protocol, improved patient's perception regarding the sensitivity and volume of the lower lip with hypertrophic scar four months after trauma.

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Photobiomodulation and its interaction with leukotriene receptor antagonist in an experimental model of chronic asthma

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Asthma affects a significant portion of the population, with a high social and economic cost. According to data from the Brazilian Ministry of Health, asthma occupies the fourth place in hospitalizations in the Unified Health System¹. Studies of leukotriene receptor antagonists such as montelukast (MK) have contributed at different levels for the treatment of asthma. Photobiomodulation is a new therapy that demonstrates efficacy in lung diseases by reducing inflammatory parameters², with low cost and without side effects. The aim of this study was to evaluate the effects of PBM and its combination with MK in an experimental model of chronic pulmonary allergic inflammation.

Seventy Balb/C mice were divided into 7 groups: control, MK, PBM, OVA, OVA+PBM, OVA+MK and OVA+PBM+MK. We induced inflammation by sensitization with ovalbumin - OVA (day 0 and day 14) and orotracheal challenge on the 21st day (3 days/week for 5 weeks). We treated with MK - sodium montelukast (5mg/kg) by gavage one hour before challenge with OVA, and one hour after the challenge we applied diode laser (660nm, 30mW and 3J/cm²). Twenty-four hours after the last treatment, the animals were anesthetized for collection of bronchoalveolar lavage (BAL) and lungs. Functional and structural parameters such as total and differential cell count, cytokine levels, LTB4 and CysLT in BAL by ELISA, mucus production, collagen deposition and tracheal responsiveness to methacholine (MCh). The data were submitted to the One-way ANOVA test followed by the Newman-Keuls test. Significance levels adjusted to 5% (p<0.05).

We observed a reduction in the total number of cells in the BAL (p<0.001). There was a significant decrease in macrophages (p<0.05), with better results in OVA+PBM (p<0.001), lymphocytes (p<0.05), with

greater reduction in PMB (p<0.001), neutrophils and eosinophils (p<0.001). Reduction in the production of proinflammatory cytokines IL4, IL5, IL-1 β , TNF- α and IL13 (p<0.001). It also increased the level of IL-10 in the MK-treated groups (p<0.001). We observed a significant reduction in LTB4 levels in all treated groups (p<0.001) and CysLT in the PBM-treated groups (p<0.001), as well as the reduction in methacholine (MCh) tracheal responsiveness (p<0.001). There was a reduction in collagen fiber deposition and mucus production in the airways in all treated groups (p<0.001).

Due to the high rates of systemic side effects caused by the use of corticosteroids, it is important to develop new anti-inflammatory therapies as an alternative for the treatment of asthma¹. Studies have shown that PMB provides an improvement in bronchial asthma^{3,4}.

Our results show that PBM alone or associated with MK reduced lung inflammation, characterized by cellular quantification in BAL, cytokine levels (IL4, IL5, IL13, TNF α , IL-1 β and IL10), leukotriene levels, remodeling (mucus and collagen) as well as tracheal reactivity demonstrating a promising role for the treatment of asthma.

Funding. São Paulo Research Foundation (FAPESP)

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Effectiveness of photobiomodulation on the increase of Treg cells and IL-10 cytokine in an experimental model of chronic asthma

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Asthma is defined as a heterogeneous disease characterized by chronic inflammation of the airways. It has a high prevalence and a high economic and social cost¹. The photobiomodulation is relatively new and effective, of very low cost, without side effects and of possible use in the treatment of chronic pulmonary diseases². The aim of this study was to evaluate the effects of PBM in an experimental model of chronic pulmonary allergic inflammation.

Seventy Balb/C mice were divided into 3 groups: control, PBM, OVA and OVA+PBM. We induced inflammation by sensitization with ovalbumin - OVA (day 0 and day 14) and orotracheal challenge on the 21st day (3 days/week for 5 weeks). One hour after the challenge we applied diode laser (660nm, 100mW and 5J/cm²). Twenty-four hours after the last treatment, the animals were anesthetized for collection of bronchoalveolar lavage (BAL) and lungs. Functional and structural parameters, percentage of macrophages, neutrophils, T cells CD4+, eosinophils, CD4+CD25+Foxp3+ Treg cells, as well as IL-10 production by these cells in flow cytometry, cytokine release (IL-4, IL-5, IL-13 and IL-10) and LTB4 by ELISA, mucus production, collagen deposition and tracheal responsiveness to methacholine (MCh). The data were submitted to the One-way ANOVA test followed by the Newman-Keuls test. Significance levels adjusted to 5% (p<0.05).

We observed a significant reduction in macrophages-CD11b (p<0.01), lymphocytes T-CD4+ (p<0.05), neutrophils-LY6G (p<0.01) and eosinophils-Siglec-F (p<0.001). Increased transcription factor (Foxp3) as well as IL-10 cytokine, CD4+CD25+ lymphocytes T in BAL, reduced proinflammatory cytokine production IL4, IL5 and IL13 (p<0.001) and increased IL-10 (p<0.001). We observed a significant reduction in the LTB4 level (p<0.01), as well as the reduction in

the methacholine tracheal responsiveness (MCh) (p<0.001). There was a reduction in collagen fiber deposition and mucus production in the airways (p<0.001).

Our results demonstrate the effectiveness of 5J/cm² dose photobiomodulation in the modulation of chronic allergic pulmonary inflammation. PBM reduced lung inflammation, characterized by cellular quantification, CD4+CD25, CD4+CD25+Foxp3 and CD4+IL-10 in LBA, cytokine and leukotriene levels, remodeling (mucus and collagen), as well as tracheal reactivity demonstrating a role promising for the treatment of asthma.^{2,3,4}

Finally, we emphasize the importance of PBM use, since we could add new evidence, which may contribute to better elucidate the anti-inflammatory effect in asthma.

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Effect of tooth pulp stem cell associated with photobiomodulation in an experimental model of chronic obstructive pulmonary disease caused by smoking

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Chronic Obstructive Pulmonary Disease (COPD) affects more than 200 million people worldwide, is the fourth largest cause of death in the world and an increase in its prevalence and mortality is predicted in the coming decades¹. An innovative approach is the use of mesenchymal stem cells (MSCs), which offer the possibility of simultaneously targeting the inflammatory response and promoting regeneration of the structure². Photobiomodulation is effective, low cost, without side effects and possible use in the treatment of chronic pulmonary diseases³. The aim of the study was to analyze some inflammatory parameters in animals with COPD undergoing therapy photobiomodulation and stem cells from human dental pulp.

Eighty C57BL / 6 mice were divided into 8 groups: control, PBM, CT, CT+PBM, COPD, COPD+PBM, COPD+CT and COPD+PBM+CT. COPD was induced with orotracheal cigarette extract 3 times a week for 7 weeks, in the last two weeks the animals received daily irradiation with diode laser (660nm, 30mW and 3J/cm²). Twenty-four hours after the last treatment, the animals were anesthetized for collection of bronchoalveolar lavage (BAL) and lungs. Functional and structural parameters, percentage of macrophages, neutrophils, T cells CD4+, T cells regulatory CD4+CD25+Foxp3+, as well as IL-10 production by these cells in BAL flow cytometry, levels of GM-CSF, MCP1, IL-1 β , IL-6, IL-8, IL-10, TNF- α , IFN- γ and LTB4 by ELISA, collagen deposition and alveolar enlargement. Data were submitted to the One-way ANOVA test followed by the Newman-Keuls test. Significance levels adjusted to 5% (p < 0.05).

We observed a significant increase in macrophage-CD11b group COPD+PBM (p < 0.01) with higher increase in the groups associated with CT (p < 0.001), and significant reduction in lymphocytes T-CD4+ (p < 0.001) and neutrophils-LY6G only in the PBM-treated group (p < 0.05). We observed increased transcription factor (Foxp3), as well as IL-10 cytokine

in BAL (p < 0.01) with better results in the isolated treatment groups (p < 0.001). Reduced levels of GM-CSF, MCP1, IL-6, IFN- γ , TNF- α (p < 0.001), IL-1 β in the group DPOC+PBM (p < 0.01) and DPOC+PBM+CT (p < 0.001), IL-8, there was a reduction in the COPD+PBM group (p < 0.01) and an increase in the COPD+PBM+CT group (p < 0.001), we noticed a significant decrease in the level of LTB4 (p < 0.01) with better results in the COPD+CT group (p < 0.001). In addition, an increase in the anti-inflammatory cytokine IL-10 in the COPD+PBM (p < 0.001), COPD+CT (p < 0.01) and COPD+PBM+CT groups (p < 0.05). We observed a significant reduction in alveolar enlargement and deposition of collagen fibers in the airways (p < 0.001).

Our results show that PBM alone and in some cases associated with CT reduced pulmonary inflammation, characterized by cellular quantification, CD4+CD25+Foxp3 and CD4+IL-10 in BAL, levels of GM-CSF, MCP1, IL-1 β , IL-6, IL-8, IL-10, TNF- α , IFN- γ and LTB4, remodeling, as well as alveolar enlargement demonstrating a promising role for the treatment of COPD.^{1,2,3}

Finally, we emphasize the importance of the use of PBM, since we could add new evidence to modulate the inflammation and together with the use of CT to treat pulmonary emphysema in individuals with COPD.

Funding. São Paulo Research Foundation (FAPESP)

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***In vitro* study of chronic obstructive pulmonary disease caused by smoking: the effect of photobiomodulation associated with mesenchymal stem cells from deciduous teeth on the inflammatory mediators released by BEAS.**

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation, with destruction of the extracellular matrix, affecting the epithelium and contributing to alveolar destruction in COPD¹. Currently, therapies for COPD, such as corticosteroids or theophylline, improve the management of the disease, but do not prevent the progression of this disease due to the evolution of emphysema¹. As therapy, mesenchymal stem cells (MSCs) because they have modulatory immune function, can alter both innate and adaptive immune cell activity². Photobiomodulation (PBM) is relatively new and promising, with low cost, non-invasive and without side effects³. The aim of this study was to investigate the effects of PBM associated with treatment with MSCs derived from primary tooth, on inflammatory mediators in bronchial epithelial cells (BEAS) induced by cigarette smoke extract.

The human bronchial epithelial cell line (BEAS-2B) was cultured by 6 passages and plated (5x 10⁴ cells/well). After 24 hours of culture the cells were incubated with EFC (Cigarette Smoke Extract), 1 then irradiated with diode laser 808nm, 30mW, 60 seconds/well and/or CTMDD in the 3th passage which were extracted from the pulp of (MSCDD) and previously cultured. After 24 hours the culture supernatant was collected for the dosing of the mediators. The effects of the association of PBM with MSCDD on the release of cytokines (IL-6, IL-10, and IFN- γ) by ELISA were evaluated.

The EFC increased levels of IFN- γ (p <0.001), decreased IL-10 levels (p <0.001) and did not alter IL-6 cytokine levels, on the other hand the CTMDD-treated groups showed a significant increase compared to the other groups (p

<0.001). We also noted a significant decrease in IFN- γ (p <0.001) as well as the increase of the anti-inflammatory cytokine IL-10 (p <0.001) in the treated groups.

Our results demonstrated that PBM and CTMDD together modulate the production of cytokines in BEAS alone. The immunopathology of COPD is a complex and cross-talk between inflammatory and structural cells that trigger both cell migration and remodeling of the airways, with clinical consequences of irreversible airflow limitation and respiratory symptoms^{2, 3}. This process is probably triggered by various components of tobacco smoke. Increased understanding of non-pharmacological modulators will provide support for an effective therapeutic approach to COPD.

Thus, the study of stem cell and PBM treatment in chronic obstructive pulmonary disease in vitro, may become a reference for future experimental studies on the effects of 808nm laser, and in the future a promising treatment for patients with the disease.

Funding. São Paulo Research Foundation (FAPESP)

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Effect of a *Hovenia dulcis* Thunberg extract and low power lasers on *Escherichia coli* cultures

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Introduction. The use of antibiotic in a large range has brought concerns in worldwide due to the so-called selective pressure, what generates multiresistant bacteria, and high costs for health system¹. This justifies the searching for new antibacterial agents. Photodynamic therapy is a type of phototherapy based on action of three factors: photosensitizer, light and oxygen. Besides it is a low cost therapy, in general, the infectious agents do not develop resistance to photodynamic therapies. A number of photosensitizers have been proposed and some of them even are used successfully, including natural products, as vegetal extracts. **Objective.** The objective of this study was to evaluate the effects of a *Hovenia dulcis* Thunberg extract and low power lasers on *Escherichia coli* cultures proficient and deficient in DNA repair. **Material and Methods.** *Escherichia coli* cultures, proficient (AB1157) and deficient in endonuclease VI (JW1625), in exponential and stationary growth phases, were incubated with an extract of *Hovenia dulcis* Thunberg (30 minutes, 37 °C) and exposed to low power red (660nm) and infrared (808nm) lasers (25, 50 e 100 J/cm², 7, 14 and 21s of exposure, 100 mW, spot size of 0.028 cm²). After that, bacterial cells were spread onto Petri dishes, incubated, the units of forming colonies were counted and the survival fractions were calculated. Groups were compared by ANOVA and Bonferroni post-test considering p<0.05 as level of significance.

Results. Data suggest that the survival fractions in *E. coli* AB1157 cultures were not altered (data not shown), but incubation with the extract and exposure to infrared laser at the lower laser fluence (25 J/cm²) significantly decreased the survival fraction in JW1625 cultures in exponential growth phase.

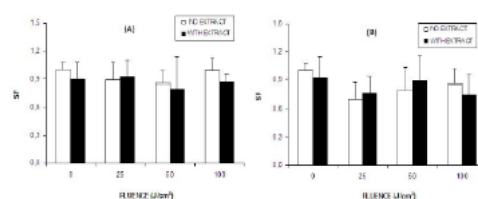


Figure 1. Survival fractions in *E. coli* JW1625 cultures incubated with a *Hovenia dulcis* Thunberg extract and exposed to low power red and infrared lasers in stationary growth phase. SF: survival fraction.

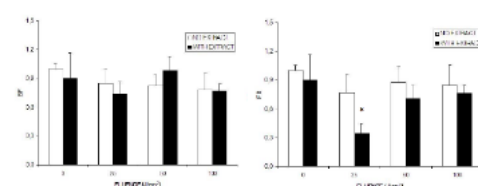


Figure 2. Survival fractions in *E. coli* JW1625 cultures incubated with a *Hovenia dulcis* Thunberg extract and exposed to low power red and infrared lasers in exponential growth phase. SF: survival fraction. (*) p<0.05 when compared with control group.

Conclusion. The results suggest that chemical substances in extract of *Hovenia dulcis* Thunberg could be activated by low power infrared laser and inactivate *E. coli* cells deficient in endonuclease VI.

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Evaluation of survival in *Escherichia coli* cultures exposed to low power blue LED

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Introduction: Low power lights are used in therapies based on their photobiomodulation effect. Low power LED (light emitting diodes) are used in clinical protocols for wound healing^{1,2}. However, there are few experimental data on blue LED on DNA. **Objective.** The objective of this study was to evaluate the survival in *Escherichia coli* cultures, proficient and deficient in DNA repair, exposed to low power blue LED.

Materials and

Methods: *E. coli* cultures proficient (AB1157) and deficient in endonuclease VI (JW1625) and endonuclease VIII (JW0704) were exposed to blue LED radiation at different fluences (160, 320 or 640 J/cm², 30, 60 and 120s, spot size of 0.28 cm², 1500 mW). Subsequently, aliquots were diluted sterile 0.9% NaCl solution, spread onto Petri dishes containing nutritive medium incubated (18h, 37 °C). After that, the forming colony units were counted and the survival fractions were calculated. Results are from 5 independent experiments. Groups were compared by ANOVA and Bonferroni post-test considering $p < 0.05$ as level of significance.

Results: Data in figure 1 suggest that the survival fractions in *E. coli* AB1157 cultures exposed to low power blue LED were not significantly altered ($p > 0.05$).

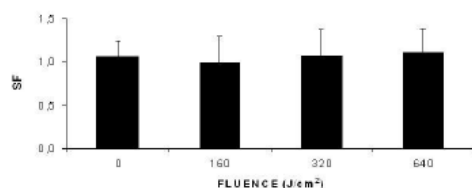


Figure 1. Survival fractions in *E. coli* AB1157 cultures exposed to low power blue LED. SF: survival fraction.

However, survival fractions in *E. coli* JW1625 and JW0704 cultures exposed to blue LED were

significantly decreased ($p < 0.05$) at the higher LED fluence (640 J/cm²) (figures 2 and 3).

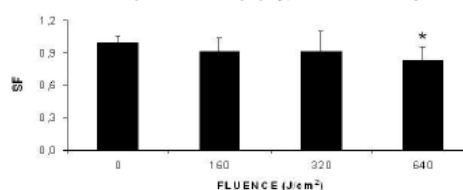


Figure 2. Survival fractions in *E. coli* JW1625 cultures exposed to low power blue LED. SF: survival fraction. (*) $p < 0.05$.

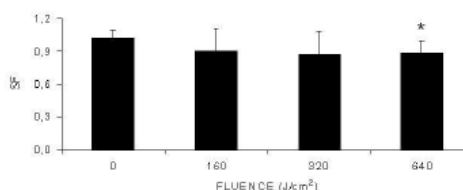


Figure 3. Survival fractions in *E. coli* JW0704 cultures exposed to low power blue LED. SF: survival fraction. (*) $p < 0.05$.

Conclusion: Our research suggests that exposure to low power blue LED could decrease survival in *E. coli* cultures deficient in repair of oxidative lesions in DNA.

Funding: Plano de Iniciação Científica e Pesquisa - UNIFESO and FAPERJ.

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Photobiomodulation and carbon biomaterials: effects on biomechanical properties and bone healing

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The association of two techniques such as the use of activated carbon as bone substitute and the application of the low-level laser therapy in order to assist the bone repair can be an alternative to overcome those problems.

The most used therapies on bone repair are based either on the implantation of a biocompatible prosthesis or through the insertion of a biomaterial in the local injury. However, those treatments involve extended and costly surgical intervention. The aim of this study was to verify the use of photobiomodulation therapy by low intensity laser associated with carbon biomaterial (AC) in the process of bone repair in rat tibias, assessing biochemical and biomechanical changes.

Methodology: The study was performed by induction of a bone defect in rat tibias and their subsequent treatment with AC and/or photobiomodulation (L). The animals were divided on groups: control (CTL), untreated Injury (NT), Injured treated with activated carbon (AC), Injured treated with laser therapy (L) - (810nm; 6J; 100mW) and Injured treated with association of AC and laser therapy (AC+L). All groups were evaluated by biomechanical properties of bone after the healing process and by phosphatase alkaline level (ALP).

Results: Groups L, AC and AC+L showed to improve their mechanical properties in comparison to CTL group (fig 1). The group AC+L presented highest value of stress at break and increasing the levels of ALP (fig 2).

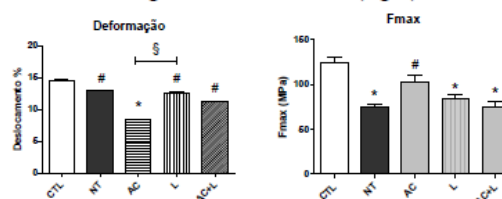


Fig. 1. *** $p < 0,05$ vs CTL, # $p < 0,05$ vs NT, § $p < 0,05$ vs CTL, * $p < 0,05$ vs NT, § $p < 0,05$ vs L

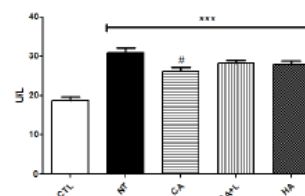


Fig. 2. ALP levels: *** $p < 0,05$ vs CTL

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

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Reduction of inflammatory process and allodynia associated with phototherapy in experimental model of tendinitis

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Tendinopathies are alterations in tendon health, where the most commonly used treatment is the pharmacological treatment for pain relief with unsatisfactory results due to its side effects in prolonged use.

The search for non-pharmacological therapies in the treatment of these diseases assumes a prominent role in the medical area. Low level laser therapy (LLLT) has been a promising therapy in the modulation of acute and chronic inflammation with no adverse effects. The objective of this study was to study the effect of LLLT on the gene expression of COX-2, neurokinin 1; MPO and on the improvement of functional parameters.

Material & Methods: Male Wistar rats (150-200g) were used. Animal Ethics Committee AN0037. The animals were separated into 4 groups: Control, with healthy tendon (CTL), untreated tendinitis (NT) and Tendinitis treated with Sodium Diclofenac (DIC) or LLLT (L3J) in the following irradiation parameters : (Laser CW, 830nm, 107.14J / cm²; 3J; 100mW and beam diameter = 0.028cm). To tendinitis induction, the animals received transcutaneous injection of collagenase (100µg) in the calcaneus tendon region. The treatments started immediately after induction and continued to 7th day. After, the animals were euthanized and the tendon was removed for analysis.

RESULTS

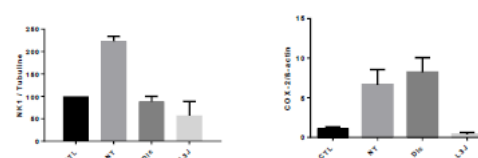


Fig1. reduction in the expression of COX-2 and NK1

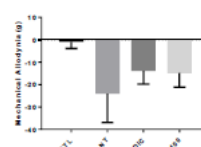


Fig2. improvement in the functional parameters when compared to the NT group.

Funding: CNPq Process: 426903 / 2016-1

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

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PHOTOSENSIBILIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC THERAPY - COMPARATIVE STUDY.

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Introduction

Oral diseases are commonly caused by the overgrowth of bacterial cells. Currently, a change in the prevention and treatment of oral diseases is sought. Conventional antimicrobial strategies generally act with the general elimination of microorganisms. Photodynamic Therapy appears as a promising antimicrobial alternative, since it is lethal only at the moment of photoactivation, involving only the microorganisms that absorb the dye. This work aims to investigate the photodynamic effect mediated by Proto-Porphyrin IX (Pp-IX), Methylene Blue (AM) and Fluorescein in *Streptococcus mutans*.

Methods

In this work, *S. mutans* ATCC 25175 was cultured in microaerophilic agar of brain and heart infusion (BHI) incubated at 37°C for 48 hours. The inoculums were prepared by collecting pure colonies that were suspended in buffered phosphate buffered saline (PBS) pH 7.2. The FS used were AM, Pp-XI and Fluorescein, which after being added to the inoculum provided the final concentration of 100 µM AM and Fluorescein and 10 µM Pp-IX.

The light source used was LED with wavelength of $\lambda = 630$ nm for Pp-IX, $\lambda = 660$ nm for AM and $\lambda = 470$ nm for Fluorescein. Six groups were used: (control group, irradiated with LED without FS group, FS without irradiation group, and PDT groups). The irradiation times of the PDT groups were 30, 60 and 120 s.

Results

The Control, LED and FS groups did not present significant differences ($P > 0.5$) with the FS used. The PDT groups with Porphyrin and with Fluorescein did not present significant microbial reduction after irradiation, while the PDT groups with AM presented a microbial reduction of 100% after 120 s of irradiation.

Conclusion

We can conclude that Pp-IX and Fluorescein had no effect on bacterial inactivation, therefore its use for PDT in *S. mutans* was not indicated in the parameters used in this assay. On the other hand, MA proved to be highly effective in the elimination of *S. mutans*.

Funding. FAPESP (2016/10269-5) and CAPES

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Evaluation of Photobiomodulation in the treatment of oral lichen planus: a randomized, controlled, double blind study.

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Oral lichen planus (OLP) is a chronic autoimmune mucocutaneous disease mediated by T lymphocytes with unknown etiology. Clinically, OLP, has different manifestations, including reticular, atrophic and erosive aspect, both associate with intense symptomatology. OLP treatment consist in topical and/or systemic use corticosteroids, which are associated with side effects. In addition, many patients become refractory and irresponsive to treatment. As an alternative therapy, Photobiomodulation (PBM) has shown promising results in OLP, controlling pain and clinical appearance. However, due to the absence of clinical studies with a satisfactory level of clinical evidence, the effect of PBM on OLP is still poorly understood.

Objectives: The objective of this study was to compare the efficacy of PBM (660nm) to corticosteroid therapy with clobetasol propionate 0.05% for the treatment of OLP.

Methods and Analysis: Fourteen patients with histopathological diagnosis of LPO were included in this study and randomized in two groups: Corticosteroid group (n = 6), in which research participants were treated with the steroid anti-inflammatory clobetasol propionate gel 0.05%, three times a day for 30 consecutive days and PBM with laser off to mask the treatment and PBM Group (n = 8), in which research participants were treated with low-intensity laser (660 ± 20nm, 100mW power, energy density 177J/cm², 5 seconds, 0.5J total energy per point) twice a week for 30 consecutive days and placebo gel 3 times a day during treatment to mask therapy. The clinical aspect of OLP was evaluated by scores according to Thongprasom et al.³ in the

following scores: 0 (absence of injury), 1 (hyperkeratotic lesions), 2 (atrophic area ≤ 1cm²), 3 (atrophic area > 1cm²), 4 (erosive area ≤ 1cm²) and 5 (erosive area > 1cm²). The functional evaluation was performed by scores according to Libelly et al.⁴ as: 0 (no difficulty), 1 (mild difficulty), 2 (moderate difficulty), 3 (severe difficulty) and 4 (impossible to perform specific function). The clinical aspect of OLP and the functional scores were performed at baseline (D0) and weekly during treatment (D7, D14, D21 and D30). The results were submitted to Shapiro-Wilk's normality test and ANOVA followed by Tukey's post-test.

Results: Most of the patients included in the study were female (13/14) and presented an average age of 63.6 years. A total of 51 lesions of LPO were included, with 15 Reticularis, 30 atrophic and 6 erosives. In the corticosteroid group, significant statistical difference was observed between the baseline and D21 (p = 0.01) and between Baseline and D30 (P = 0.0002). In the PBM group, there was significant statistical difference between D0 and D30 (P = 0.01). In the comparison between the corticosteroid and PBM group, in the different periods evaluated, no significant statistical difference was observed between the treatments.
Conclusion: Both the corticosteroids and the PBM are effective in the treatment of the LPO, being able to promote improvement in the clinical aspect of the lesions.

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EFFECT OF SURFACE TREATMENT WITH CO₂ LASER ULTRAPULSED IN COMPOSITE RESIN REINFORCEMENT RESISTANCE

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The objective of this study is to evaluate the bond strength of repairs made in composite resin after treatment of the failure surface with different bonding agents, taking into account the use of CO₂ laser as a form of surface treatment to repaired.

A silicon matrix with a standard resin block was made so that all specimens had the same size, after which 60 blocks of composite resin (Opallis) were made, measuring 5X5X5mm³. The resin blocks were diamond-tipped and were divided into 6 groups (n = 10) according to the surface treatment.

The appropriate groups were irradiated with ultrapulsed CO₂ laser with 14W of power, 0.01s pulse interval and 0.004 pulse time, and afterwards a repair with composite resin, Ambar Universal adhesive and Silane, was carried out in distilled water for 7 days.

The resin samples were fixed in a plate with a stick to be cut by the metallographic cutter, the distance between the cuts was standardized in 1.2 mm, being realized in both directions, forming sticks of approximately 1.0 mm² of area and 1, 0 cm in length.

Seven sticks of each specimen were selected for the microtensile test performed in a universal test machine at a speed of 1 mm / min until fracture. The maximum stress values were obtained by the ratio between the load recorded at the moment of fracture given in Newton (N)

and the area of the specimen (mm²). The crosssectional area of each specimen was measured after the test using the digital caliper. Data were tabulated and converted to MPa.

There were pre-test failures in all tested groups.

It was concluded that the use of the CO₂ laser as a surface treatment improved the bond strength in repairs made from composite resin in relation to the groups that only received treatments with the drill and associated with the silanization, and presented a significantly lower number of failure adhesive.

Conclusion: Within the limitations of the present study the CO₂ laser was shown to be effective in improving the bond strength of composite resin repairs.

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Fluconazol susceptibility of a resistant strain of *Candida albicans* is altered by PDT

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Background and objective. Combining PDT to conventional antimicrobial drugs may be a promising strategy to improve the efficiency promoted by each therapeutic modalities. The objective of this study was to investigate the whether PDT could affect fluconazole susceptibility of a resistant *C. albicans* strain.

Material and Methods. *C. albicans* strain used in this study was an azole-resistant strain YEM13 (overexpressing *MDR1*) and ATCC 90028, a reference strain susceptible to fluconazole. YEM13 cells previously incubated with 50 μ M MB for 10 min were irradiated with a irradiance of 75 mW/cm² for 6 min (sublethal parameter). The MIC to the antifungal fluconazole was determined by the broth microdilution method established by the European Committee on Antimicrobial Susceptibility Testing¹. *C. albicans* samples tested were YEM13, YEM13 after PDT, and ATCC 90028. Inocula with a concentration of $1-2 \times 10^5$ CFU/ml were incubated with 10 concentrations of fluconazole (from 0.125 to 64 μ g/ml). After 24 h the turbidity of the medium was measured in a spectrophotometer at 530 nm. MIC value was defined as the lowest drug concentration that promoted an inhibition of growth of $\geq 50\%$ compared to the drug-free control.

Results. The PDT conditions promoted no reduction of the viable cells. MIC values of tested samples are presented in figure 1. MIC values of *C. albicans* ATCC 90028 was 0.5 μ g/mL, and of YEM 13 cells that were not submitted to aPDT was 64 μ g/mL; both values agree with those described in the literature². Fluconazole was more active in *C. albicans* previously submitted to sublethal PDT. There was reduction of the MIC value from 64 μ g/mL to 32 μ g/mL. It is noteworthy that sublethal conditions were used

and even more promising results can be obtained with the optimization of the irradiation parameters.

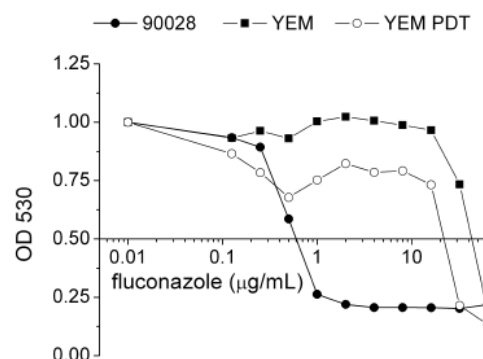


Fig. 1. Fluconazole susceptibility curves of *C. albicans* previously submitted to sublethal PDT. The intersection of the x-axis and the y-axis represents 50% inhibition of growth.

Conclusion. Our data showed that sublethal MB-mediated PDT could increase susceptibility to fluconazole in *C. albicans* strain presenting MFS family efflux system. The association between PDT and fluconazole may be an important alternative in the treatment of *C. albicans* strains that present resistance to this antifungal drug.

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The impact on quality of life and psychosocial relationships in patients undergoing treatment for facial aging using photobiomodulation.

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The aesthetics of the face may have effects on the quality of life of patients, as well as on their self-esteem. Despite being treated as futilities, aesthetic treatments may have strong and important influences on psychological and emotional levels, as well as on the well-being of people. The objective of the study was to evaluate the impact of a photobiomodulation aesthetic treatment for wrinkles on the quality of life and psychosocial relationships of the participants.

According to the World Health Organization (WHO), quality of life can be defined as the individual's perception of their position in life, goals, concerns and expectations in the cultural sphere and values. Many skin infections are not life threatening, but affect the quality of life of individuals. Today there are numerous treatments for skin aging, one of them is the use of Photobiomodulation Therapy. Due to that, this work aims to evaluate the impact of a photobiomodulation aesthetic treatment for wrinkles on the quality of life and psychosocial relationships of the participants.

Methods and analysis: Two questionnaires were applied to 37 women with periocular wrinkles. These questionnaires were applied before and after a treatment of 10 sessions using photobiomodulation with red (660 ± 10 nm) and amber (590 ± 10 nm) LEDs, with one colour only at each hemiface. The facial side to be treated with each different colour was randomized. The equipment used was the Cicatrillux Bionext LED boards Cosmedical (Maua, Brazil), in contact with skin. Each board features 36 LED units around $10\text{cm} \times 12\text{cm}$ area. Each LED has 5mW of power, $6.4\text{mW} / \text{cm}^2$ and the final radiant exposure is $3.8\text{J} / \text{cm}^2$ (10 minutes per session). The MelasQoL questionnaire used in this study is an adaptation to

wrinkles of the quality of life questionnaire for patients with Melasma (MelasQoL-BP), in which melasma was replaced by wrinkles. The Skindex-29 questionnaire is used to verify the quality of life in patients with cutaneous conditions. It was adapted for this research, 10 questions related to pathologies were removed, since there is no applicability in the study. Both questionnaires were subjected to reproducibility evaluation according to Kappade Cohen (1960). The evaluation of the reproducibility of the MelasQoL and Skindex-29 questionnaires revealed 50-60% of substantial agreement in questions. In the MelasQoL questionnaire the number of points assigned to each of the responses is higher the greater the patient's degree of dissatisfaction / discomfort. The same parameter was used in the adapted Skindex-29, but for this questionnaire the answers were divided into domains: Total, psychosocial and emotional.

Results and Conclusion: The data obtained through the MelasQoL questionnaire showed a statistically significant reduction of the total score, which indicates that the participants had lower dissatisfaction after the Photobiomodulation Therapy for wrinkles. The same occurred with data from the adapted Skindex-29, which showed a statistically significant reduction in the total score. Regarding the domains of the questionnaire, a statistically significant difference was observed only in the emotional domain.

Acknowledgment. We thank Cosmedical which kindly provided the LED device used in this research, and Tecnotests, the CK Electronic representative in Brazil, which kindly provided the devices for non-invasive analysis of the skin.

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Evaluation of the effect of photobiomodulation in the control of pain in patients with oral lichen planus: a clinical, controlled, randomized, double blind study.

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Oral lichen planus (OLP) is a chronic autoimmune mucocutaneous disease mediated by T lymphocytes with unknown etiology. Clinically, OLP, has different manifestations, including reticular, atrophic and erosive aspect, both associate with intense symptomatology. OLP treatment consist in topical and/or systemic use corticosteroids, which are associated with side effects. In addition, many patients become refractory and irresponsive to treatment. As an alternative therapy, Photobiomodulation (PBM) has shown promising results in OLP, controlling pain and clinical appearance. However, due to the absence of clinical studies with a satisfactory level of clinical evidence, the effect of PBM on OLP is still poorly understood.

Objectives: The objective of this study is to evaluate the effect of PBM in the control of pain in patients with OLP, through a clinical, controlled, randomized and double blind clinical trial.

Methods: Fourteen patients with histopathological diagnosis of OLP were included in this study and randomized into two groups. Corticoid group (n = 6), in which the participants of the study were treated with 0.05% clobetasol propionate three times a day for 30 consecutive days and PBM with laser tuned off to mask the treatment and PBM group (n = 8), in which the participants were treated with low intensity laser (660nm, 100mW, 177J/cm², 5s, 0.5J per point) twice a week for 30 consecutive days and placebo gel 3 times daily during treatment to mask the therapy. Pain was assessed by visual analogue scale (VAS) and by a

single examiner with no knowledge of patients allocation at baseline (D0) and weekly (D7, D14, D21 and D30) during treatment. Shapiro-Wilk normality test and ANOVA follow Tukey's post test were used. **Results:** The majority of patients included in the study were female (13/14) and a mean age of 63.6 years. A total of 51 OLP lesions were included, and classified as: reticular (15/51), atrophic (30/51), erosive (6/51). In the corticoid group, there was a significant decrease in pain at D30 in relation to the baseline (D0) (p = 0.003). In the PBM group, no difference in pain was observed between baseline (D0) and other periods. In addition, no difference was found between corticoid and PBM groups.

Conclusion: corticoid was more effective than PBM in the control of pain in patients with OLP, although it is still necessary to conclude this clinical study to evaluate the possible indication of PBM for treatment and control of pain in patients with OLP.

Ethics and dissemination: This protocol was approved (#2.375.410) by the Nove de Julho University (UNINOVE) Research Ethics Committee.

Registration details: NCT03320460.

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Evaluation of photobiomodulation in salivary production of patients with antihypertensive drug-induced xerostomy: study protocol for a randomized, controlled blind clinical trial

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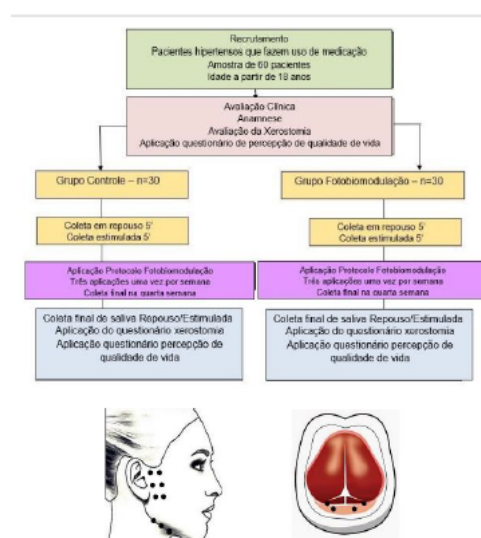
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Xerostomia is a quantitative and qualitative alteration of saliva, a symptom present in patients who make continuous use of medications to control chronic diseases, among them hypertension. Difficulties in chewing, swallowing, phonation and taste are present, besides the burning mouth syndrome, periodontal disease, root cavities and bad breath that will interfere in the quality of life, an difficult to socialize. Currently the methods presented for treatment of xerostomia are palliative, not consisting of effective treatment to use them. The objective of this work is to evaluate the efficacy of photobiomodulation in the production of saliva in patients with xerostomia induced by anti-antihypertensive drugs.

The method consists in the application of low-intensity laser in the parotid, submandibular and sublingual salivary glands. The parameters used are: Argon diode Laser, of the DMC brand, 808nm, 4j per point, continuously and in contact with the irradiated surface, resulting in the irradiance of 3571 MW/cm², distributed as follows: 6 points in each parotid, 2 points in each Sublingual (external) and other two in each submandibular (internal), totaling 16 extra oral and 4 intra oral, totaling 20 points. The exposure time will be 40s per point, corresponding to 800s per session and 3600s at the end of the four treatment sessions. The radiant exposure will be 142j/cm². There will be a control group. At the end of the sessions will be collected saliva sample and will be compared to the initial sample. The initial and final volumes will be compared, both from the unstimulated and stimulated collection. In addition to this measurement of the salivary flow, analyses of the mineral content – sodium, calcium and potassium, as well as the PH will be performed.



It is expected to obtain greater saliva secretion, as well as the increment of the biochemical elements of it. Reduction of halitosis and symptoms of burning mouth syndrome are also expected, since both tables, as described in the literature review, are present in patients with xerostomia.

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Analysis of the effects of photobiomodulation in patients with TMD: case report

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Introduction. Temporomandibular disorder (TMD) is a term used to define a number of clinical signs and symptoms that affect the masticatory muscles, the temporomandibular joint (TMJ), and associated structures [1]. Signs and symptoms of TMD are found in all ages; however, the prevalence of this disorder, considered low in children, increases with age in adolescents and young adults. The changes caused by the TMD, especially pain, can interfere in the quality of life of these patients [2]. Photobiomodulation therapy is a non-invasive, non-pharmacological treatment that, according to various studies, has shown beneficial results in the treatment of pain associated with TMD [3]. The application of laser therapy in patients with TMD has demonstrated the ability to relieve pain within minutes of its application, promoting significant well-being. Moreover, it is an adjuvant pain-relief treatment in which the analgesic action of the laser enables the patient to return to their duties, providing more comfort and a better quality of life [4]. The general purpose of this study is to evaluate of photobiomodulation therapy in the treatment of pain in patients between 15 and 25 years of age with TMD.

Methods and analysis. This is a prospective study of clinical and activities will be conducted at the premises of the clinic of the Escola de Odontologia of the Universidade Nove de Julho. The project will follow the regulatory standards for ethics research with humans and will be submitted to the Institutional Review Board of the university. For a diagnosis of the TMD, the Research Diagnostic Criteria for Temporomandibular Disorders questionnaire (RDC/TMD) [5] will be applied before any intervention.

Inclusion Criteria: Young people between 15 and 25 years of age with a diagnosis of TMD in group Ia and Ib (Chronic myofascial pain in accordance with the RDC-TMD) will be included in the study.

Exclusion Criteria: Group II (disk displacement of the Temporomandibular joint) and group III (arthralgia, arthritis, arthrosis). Individuals with dental-facial

anomalies who were in orthodontic or orthopedic treatment of the jaws or in psychological or physical therapy will be excluded. Individuals who were taking muscle relaxants or anti-inflammatory medications will also be excluded.

For the photobiomodulation Therapy, a laser, model THERAPY EC, (DMC®, São Carlos, SP, Brazil) , will be used. Twelve laser applications will be applied, with 2 sessions per week. A wave length of 808 nm ± 10 nm and power of 100 mW.

Results. The study will present a follow-up of the treatment outcome of 03 patients.

Conclusion. The photobiomodulation therapy promoted satisfactory results in the control of myofascial pain and could be presented as an effective and non-invasive treatment of TMD according to the therapeutic protocol used.

Trial registration: NCT01331031.

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ACTION OF PHOTODYNAMIC THERAPY WITH RED LED ON HALITOSE CONTROL: CLINICAL CONTROLLED AND RANDOMIZED TEST.

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Halitosis is the term used to describe any unpleasant odor relative to expired air regardless of its source. The prevalence of halitosis in the population is approximately 30%, of which 80-90% of the cases originate in the oral cavity resulting from proteolytic degradation by gram negative anaerobic bacteria. Antimicrobial photodynamic therapy (aPDT) has been widely used and with very satisfactory results in the health sciences, it involves the use of a non-toxic dye, called photosensitizer (FS), and a light source of a specific wavelength in the presence of the oxygen in the medium. This interaction is capable of creating toxic species that generate cell death. The objective of this controlled clinical study was to verify the effect of aPDT in the treatment of halitosis by evaluating the formation of volatile sulfur compounds with gas chromatography before and after treatment. Included in this study were young adults aged 18 to 25 years with a diagnosis of halitosis. The selected subjects were divided into 3 groups, G1 aPDT, G2 Scraper and G3 aPDT and scraper. All subjects were evaluated with Oral Chroma™ before and after treatment. For groups 1 and 3 0.05% methylene blue was used as a photosensitizer (165 µm), with 2 minutes pre-irradiation time. Four points were applied with the equipment calibrated with 660 nm, energy of 36 J, power of 400 mW.

Red LED was used for photodynamic therapy. The chi-square test and the Fisher's exact test were used to evaluate the association of categorical variables. Student's tStudent test and analysis of variance (ANOVA) were used to analyze the correlation

between the continuous variables and the correlation test by Pearson. In the analysis of the experimental differences in each group the Wilcoxon test was used. A significance level of 95% ($p < 0.05$) was considered for all analyzes. A statistically significant decrease in halitosis level was observed immediately after treatment for all groups ($p < 0.05$), but halitosis levels returned to baseline after 7 days ($p < 0.05$). It was possible to conclude an improvement in the halitosis levels immediately afterwards for all the studied groups, but the result did not remain in the control of 7 days.

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Acknowledgment. We thank the UNINOVE, FAPESP and CNPq.

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“REMINERALIZATION OF EARLY ENAMEL CARIES LESIONS INDUCED BY BIOACTIVE PARTICLES: AN *IN VITRO* SPECKLE ANALYSIS”

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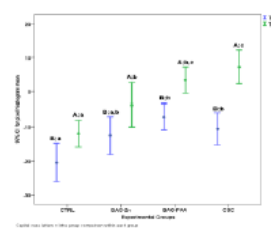
Tooth mineral is composed of calcium and phosphorus and the organic acids produced by the metabolism of cariogenic bacteria can diffuse into tooth's hard tissues and dissolve the carbonated hydroxyapatite mineral, causing the formation of caries lesions (1, 2). The increasing use of processed sugars and acidic foods and beverages can drastically increase the caries lesion (3). This current issue represents the main reason why it is of key importance to develop innovative remineralising strategies able to deliver calcium and phosphates into the enamel and dentine lesion in a more accurate and specific manner (4). One of the most promising materials with great remineralising potential is bioglass. Indeed, several types of bioactive glasses have been incorporated into toothpaste formulations (5) as well as many other dental products (6, 7).

Objective: This study aimed at evaluating the remineralization effect induced by different bioactive glass fillers on simulated early caries lesions in enamel (EECL).

Forty sound bovine teeth were selected after consent and donation by freezer. Blocks of 6 x 6 mm² were carried out from the crowns, vestibular surface of bovine incisors were polished and teeth were isolated with two layers of acid resistant nail varnish. Vestibular surface were divided in two areas (sound and treated). The sound area was isolated with duct tape (3 x 3 mm²), and the treated area was firstly immersed into demineralization/remineralization solution (1 week) to create the EECL. Then the areas were treated with a slurry of different bioactive particles with deionized water for 1 week. Samples were analyzed by laser speckle after formation of EECL and compared to the sound area, and then the treated area was analyzed again by laser speckle.

Groups	
Control group	Non treatment (CTRL)
F-BAG group	Bioglass doped with zinc (BAG-Zn)
BAG-F-PAA Group	Bioglass doped with fluoro and carboxylated with polyacrylic acid (BAG-F-PAA)
EN0 Pan no PMA group	Calcium silicate cement (CSC)

After analysis with laser speckle, EECL showed a lower average intensity of the backscattered light. After remineralization, a higher average intensity of the backscattered light was observed, which was similar to the sound areas. Thus, it could be concluded that the different bioactive fillers were able to remineralize the demineralized surface of the teeth.



Conclusion

After one week of treatment with different types of BAGs on early enamel caries lesions, analysis of laser speckle imaging showed changes on the microstructure of the enamel, which were similar to the sound enamel surfaces. Such treatments might be able to induce enamel remineralization.

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Evaluation of dental whitening supervised in the office, in upper canines with LED Violet (405nm) with and without the use of carbamide peroxide gel 35%

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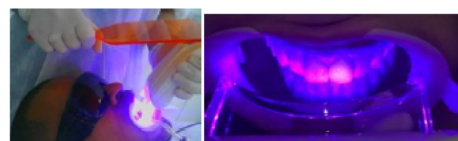
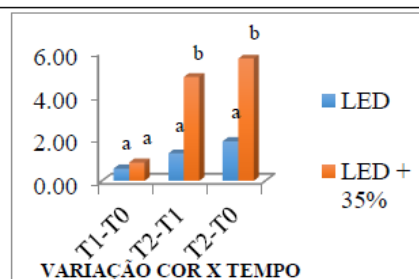
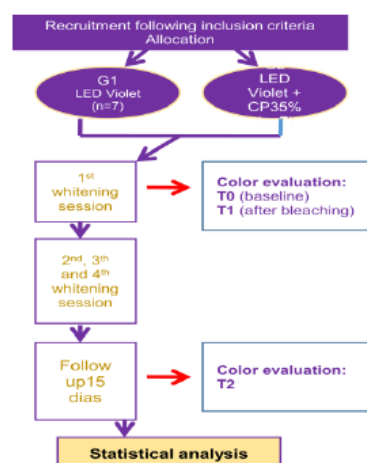
Introduction

The Violet LED is capable of producing tooth whitening without the use of chemical agent. However, according to the literature, the violet LED of 405 nm can also be used with the gel in different concentrations, which promotes a potentialization of results through colorimetric analysis and spectrometry. This wavelength coincides with the absorption peak of the dentin pigment molecules, interacting selectively, as in smaller molecules, making the teeth lighter.

The objective of this study was to evaluate colorimetric alterations after dental bleaching in canines (13), supervised in the office, using the Violet LED (405 nm) with and without the use of 35% Carbamide Peroxide (CP) gel.

Material and Method

After approval by the CEP-UNINOVE (#2.034.518).



Results

Statistical analysis was performed using the ANOVA test using the means of variation of the classical Vita scale. This study showed that for the right upper canines there is a statistically significant difference between G1 (LED) and G2 (LED + CP35%) considering whitening in T2 in relation to T0. However, there is no statistically significant difference when comparing bleaching between G1 and G2 in T1.

Conclusion

We conclude that G2 (LED + 35%) cleared more than G1 in T2.

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Evaluation of light absorption of oral squamous cell carcinoma cell line in nutritional stress

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Photobiomodulation (PBM) is based in the use of light to modulate different cellular process, including proliferation, migration and cellular viability. The cellular response to PBM is dependent on the absorption of light photons by chromophores as cytochrome c oxidase, responsible to increase the cellular metabolism. In vitro studies have demonstrated that PBM can promote or inhibit the malignant behavior of cancer cell lines, including oral squamous cell carcinoma cell lines. However, the effects of PBM in a specific cell type will be dependent of light absorption and thus, it is important to select the most suitable wavelength to be used during PBM. In addition, different culture conditions can also influence light absorption.

Objectives: To evaluate the effect of nutritional stress on the absorption spectrum in an oral squamous cell carcinoma cell line.

Methodology: The cell line CA1 was cultured in medium DMEM-F12 with 10% FBS, 1% antibiotic and RM supplement. To evaluate the effect of nutritional stress on the light absorption spectra, cells were cultured for 24 h under the following conditions: DMEM-F12 + 10% FBS, DMEM-F12 + 5% FBS, DMEM-F12 + 2.5% FBS and DMEM-F12 without serum. After this period, 1x10⁵ cells from each experimental condition were collected and suspended in 1 ml of phosphate buffer solution (PBS) to measure the absorbance in the Ocean Optics DH-2000-BAL spectrophotometer.

Results: CA1 cells showed a peak of absorption at 280nm and at 550nm. In addition, cells cultivated with DMEM-F12 without serum and with 2.5% of FBS showed higher absorbance intensity in relation to the cells cultivated with 5% or 10% (Figure 1).

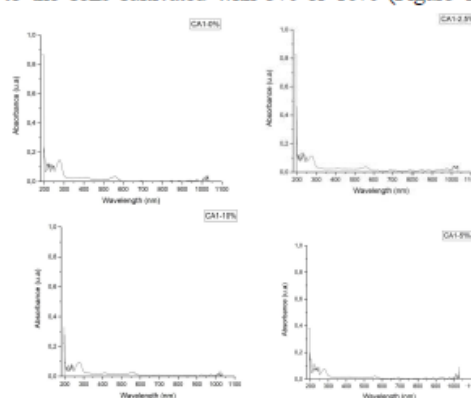


Figure 1: Evaluation of absorption spectrum of CA1 cell lines cultivated under different concentrations of FBS.

Conclusion: The nutritional stress influences the absorption of light in OSCC cell lines and thus, may interfere with the effects of PBM

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Effect of LED on the bone repair of dental alveoli of rats after exodontia grafted with inorganic bovine bone and collagen membrane.

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The loss of the dental element causes sharp bone changes with vertical and horizontal dimensional alterations, causing loss of volume and deformity of the bone architecture and atrophy of the alveolar process.

Studies show that 50% of this volume is lost in the first year after the exodontia, with greater intensity in the first 3 to 6 months, and may make it impossible to install a future implant without the need for invasive repair surgery with higher morbidity and high costs. immediate aesthetic changes to the patient.

The gold standard of regenerative surgeries is still the autogenous bone, however the use of biomaterials is consolidated with high predictability and bone quality formed; the bone substitute of choice has a low rate of resorption to preserve the bone architecture and allow osteoinduction, osteoconduction and osteogenesis in bone neof ormation.

Photobiostimulation has been used to create a series of positive effects that accelerate the healing of bone defects according to in vitro and in vivo studies, stimulate blood flow, recruit and activate osteoblasts, osteosynthesis, decrease of osteoclastic activity and improvement the integration of the scaffold material with the bone tissue in the remodeling process of the graft.

The aim of this study was to evaluate the efficacy and improvement of bone quality in male Wistar rats subjected to molar extraction with or without bone grafting with lyophilized inorganic bone and collagen membrane and to verify the therapeutic effect of photobiomodulation with 850nm LED on remodeling bone.

Materials and methods: Histological analysis, immunohistochemistry, infrared spectroscopy,

optical coherence tomography, micro CT and Speckle will be employed to evaluate bone repair.

We will use 48 animals divided into 5 groups, two G1 basal groups (without any intervention and treatment) and G2 control (performed only molar extraction) with 12 animals for both groups in order to minimize the use of animals. It will be done in a hemi-arch to the exodontia and in the hemi-arch opposite without interventions and treatments. In addition, 3 groups with 12 animals each, the G3 Exo / LED (carried out the extraction and treatment with LED $\lambda = 850 \text{ nm}$, power = 100 mW, exposure time = 60s, energy = 6J, radiant exposure = 30 J / cm^2 , 1 point irradiated 8 sessions 48/48 hours for 15 days), G4 Exo / Enx (carried out the extraction and grafting of the alveolus with the composite) and G5 Exo / Enx / LED (carried out the extraction, alveolus grafting with the composite and treatment with LEDs in the same parameters described above..

The animals will be euthanized in 15 and 30 days and the analyzes performed.

Results and Conclusions: The project in progress, without results and conclusions.

Keywords: bone regeneration, Laser Therapy, Photobiomodulation

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Effect of photobiomodulation (780nm) on the expression of IL-6 And TNF- α during the compensatory hypertrophy

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Introduction: Compensatory hypertrophy occurs due to the mechanical overload of the muscle, thus promoting the increase of the cross-sectional area of the fiber muscular. During this adaptation process, skeletal muscle is capable of release cytokines that will perform functions of control and coordination of pro-inflammatory responses interfering in muscle remodeling. The laser has been extensively studied, numerous studies have shown its effects on the modulation of cytokines.

Objective: To evaluate the effect of photobiomodulation (780nm) on the protein expression of IL-6 and TNF- α cytokines during compensatory hypertrophy.

Method: Wistar rats weighing $242.5g \pm 13.59$ were submitted to ablation of synergistic muscles of the Plantar muscle, which suffered the overload. The animals were divided into three groups: control, hypertrophy group without irradiation (H) and hypertrophy group with irradiation in the left plantar muscle (H + LLLT) and evaluated after 7 and 14 days of hypertrophy induction. The irradiation was performed immediately after the surgery and was followed daily until the end of the experimental period, for the irradiation used λ 780nm, energy density of $10 J / cm^2$, power of 40 mW, time of 10 seconds per point, being 8 application points on the skin covering the plantar muscle, totaling 3.2 J of energy. Levels of inflammatory markers were analyzed by the ELISA immunoenzymatic assay following the kit recommendations. The analysis was performed by the statistical test ANOVA followed by the Tukey test.

Results and Discussion: There was an increase in IL-6 expression in the H + LLL group when compared to the group only hypertrophy after 7 days, these findings are in agreement with the study by MITCHELL et al. (2013), who also found an increase in IL-6 in a model of muscle hypertrophy without irradiation. There was a decrease in TNF- α in the H and H + LLL groups when compared to the control group, in the Glass review study (2005), it is

reported that TNF- α may promote muscle mass decrease, which could justify the findings of this study in that the TNF- α decreased after 7 days of induction of hypertrophy. After 14 days there was no difference among the groups for both IL-6 and TNF- α .

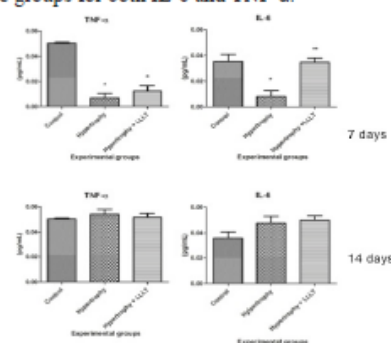


FIG.1 Expression of IL-6 levels after 7 and 14 days. Data presented in mean and standard error of the mean * $p < 0.05$. (ANOVA/ Tukey). Expression of TNF- α levels after 7 and 14 days. Data presented in mean and standard error of the mean * $p < 0.05$.vs control (ANOVA/ Tukey).

Conclusion: Photobiomodulation (780nm) induced the increased of IL-6 and decreased TNF- α expression after 7 days irradiation.

Aknowlegements: Capes and Uninove

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Evaluation of light absorption of primary human macrophages – a preliminary study

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Photobiomodulation (PBM) is a therapy based in the application of light to modulate inflammation and promote tissue repair. At the cellular level, the light photons will be absorbed by chromophores as cytochrome c oxidase, which will trigger an increase of ATP, reactive oxygen species, activation of transcription factors, cellular viability, proliferation and migration. The evaluation of the absorption spectrum by a specific cell type is important to select the most suitable wavelength to be used during PBM.

Macrophages have an important role in the inflammatory process. Different macrophage phenotypes act during the inflammatory process and are known as M1 and M2, with a pro-inflammatory and anti-inflammatory phenotype, respectively. Little is known about the effects of PBM and the light absorption spectra of primary human macrophages in both phenotypes.

Objective: Evaluate the absorption spectra of primary human macrophages.

Methods: Monocytes were isolated from human peripheral blood using Ficoll-Paque method. The cells were cultured for 2h in RPMI medium allowing monocytes to attach to the culture plate. Monocytes were kept for 7 days in RPMI with 10% FBS and 50ng/ml of M-CSF to induce their differentiation to macrophages. Next, macrophages were incubated with RPMI with 5% FBS for 48h for M0 phenotype; RPMI with 5% FBS with 1 µg/mL LPS (E. coli) and 0.2 µg/mL IFN-γ for 48h to induce M1 phenotype; or 0.1 µg/mL IL-4 for 48h to induce M2 phenotype. Cells were detached with 5mM EDTA, counted and placed in cuvettes with 2ml of RPMI with 5% FBS. The

absorbance was read in a spectrophotometer USB 2000+ (Ocean Optics®, USA).

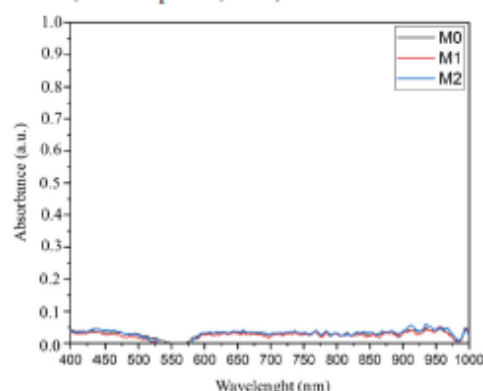


Fig. 1. Absorption spectra of primary human macrophages without activation (M0) and activated to M1 and M2 phenotypes.

Results: There were no differences between the absorption spectra of the three macrophage phenotypes evaluated. These primary cells have showed low light absorption, especially in the green/blue wavelengths.

Conclusion: Further studies should be performed to evaluate the best parameters of PBM in human macrophages with different phenotypes to achieve optimal PBM effects.

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PROPOSED LABORATORY METHODOLOGY FOR OBTAINING DENTINA AFFECTED BY CARDIAC AND VALIDATION THROUGH LASER SPECKLE AND OCT

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This is a validated protocol for disinfecting bovine dentin by means of biofilm of *Streptococcus mutans* (microbiological method). The validation of the protocol is performed through the different diagnostic tests: visual inspection, laser fluorescence and optical coherence tomography (OCT). To guard the study were 60 vestibular samples of bovine teeth ($n = 12$). The teeth are sanded on the vestibular surface to remove the enamel with exposure of the superficial dentin, obtaining a flat surface. The roots of the teeth are removed. Specimens are protected with inflammation of a colostrum on the vestibular face (control). The experimental groups will be composed of 2 factors of variation: cariogenic challenge time (7, 14 and 21 days) and type of dentin. Thus, the experimental groups were: G1 control group (caries), G2 (caries + fluoride), G3 (caries + Laser + fluoride), G4 (Caries + fluoride + Laser) and G5 (Caries + Laser). After the different times of cariogenic challenge, which are evaluated in their actions by healthy and affected by caries using different diagnostic methods and the results compared. A calibrated examiner will visualize all images bleeding through visual and current inspection by the OCT and Speckle laser and distinguish the healthy tissue from the caries

affected. After all the data entered will be applied the Pearson and Bland and Altman Correlation Statistical Test

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Evaluation of the metabolism of *Candida albicans* incubated for photodynamic inactivation with different glucose concentrations with the membrane efflux system ATP-Binding Cassette

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Candida albicans is an opportunistic commensal microorganism, often associated with severe infections in immunosuppressed individuals, responsible for the increase in the number of hospitalizations due to fungal infections increasing the number and size of major surgical procedures, and also due to the phenomenon of microbial resistance. Therefore, new techniques have been discovered, among them is photodynamic therapy, because the association of a photosensitizer (FS), oxygen and the correct light length cause cytotoxic damage to microorganisms. Studies have shown that depending on the concentration of glucose in the medium that *C. albicans* is inserted can modify its cellular respiration and the absorption of alternative sources of carbon, which can favor the entry of FS into the cell. This work aims to evaluate the inactivation of *Candida albicans* in different concentrations of glucose.

660nm for AM will be used. The samples will be divided in 7 groups, where 4 has no glucose (control group, irradiated group with LED without FS, group with FS without irradiation and the PDT group) and 3 groups with different concentrations of glucose with PDT. The LED group without FS will be irradiated for 18 min. The suspension of the PDT group without glucose will be stained for 30 min and irradiated for 6 min. In the glucose group the suspensions will be reserved with glucose for 80 min, then stained with FS for 10 min and irradiated for 6 min.

Funding. FAPESP (2016/10269-5) and CAPES

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Materials and Methods: In this work modified strains of *Candida albicans* YEM 14 and YEM 15 grown on Sabouraud Dextrose agar will be used and incubated at 36.5 ° C for 24 hours. The inoculum will be prepared by collecting from pure colonies and then suspended in buffered phosphate buffered saline (PBS) at pH 7.2. The FS is Methylene Blue (AM) that will be added to the inoculum with final concentration of 100µM. For irradiation LED that has a wavelength of $\lambda =$

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Oral health-related quality of life in patients with oral lichen planus treated with photobiomodulation

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Introduction. Oral lichen planus (OLP) is a chronic inflammatory disease that commonly affects the skin and oral mucosa [1,2]. The most typical involvement site for OLP is the buccal mucosa but any other oral cavity site can be affected [3]. The classical clinical presentation is reticular OLP, characterized by white lacy streaks, which are referred to as Wickham striae, normally surrounded by an erythematous border [4]. Atrophic and erosive/ulcerative OLP lesions are characterized by erythema associated with inflammation and/or epithelial thinning as well as mucosa ulceration, which is surrounded by keratotic striae on the periphery of the lesion [5]. Most importantly, these OLP presentations are associated with symptomatology ranging from a burning sensation to severe pain and rarely remit spontaneously [1,3,4]. The pain resulting of OLP is a fact that can influence daily life and directly interfere with patients' quality of life. The aim of this study is to analyze the perception of the quality of life the patients with OLP.

Methods and analysis. Fourteen patients with symptomatic and histopathological diagnosis of OLP were randomized into 2 experimental groups in a double-blind manner: Control group (G1) n=06 - clobetasol propionate 0.05% three times a day + placebo PBM twice a week (laser turned off to mask the treatment) for 30 consecutive days and Experimental group (G2) n=08 - PBM ($\lambda = 660\text{nm}$, power 100mW, radiant exposure: 177J/cm², and 0.5J per point) twice a week for 30 consecutive days and placebo gel 3 times daily during treatment to mask the therapy. The quality of life was measured by means of the Oral Health Impact Profile (OHIP 14) at baseline (D0) and at the end of treatment (D30). The OHIP 14 consist in 14 items that was used to determine the degree of impact of the different oral diseases on seven dimensions (two items per dimension). Dimensions (D): D1- Functional limitation; D2 - Physical pain;

D3- Psychological discomfort; D4 - Physical disability; D5 - Psychological disability; D6 - Social disability; D7 - Handicap [5,6]. Shapiro-Wilk normality test and Mann Whitney test was used to evaluate significant differences between D0 and D30 in each group and between treatments.

Results. The statistical analysis for comparison between the data of each group in the different evaluation periods showed that there was no statistical significant difference in Dimensions D1, D4, D5, D6 and D7 ($p > 0.05$). In the Dimensions D2 (physical pain), significant improve was observed at D30 in relation to baseline in Control ($p=0.02$) and PBM ($p=0.005$) groups. In the Dimension D3 (Psychological discomfort), only control group was associated with a significant difference between baseline and D30 ($p=0.02$). No difference was observed between groups in relation to the total OHIP score.

Conclusion. The study shows that both treatments were able to improve the quality of life of OLP patients regarding pain but only the conventional treatment with corticoid was associated with an improve in the psychological discomfort related with the disease.

Ethics and dissemination: This protocol was approved (#2.375.410) by the Nove de Julho University (UNINOVE) Research Ethics Committee. **Registration details:** NCT03320460.

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The Photodynamic Efficacy of Phenothiazinium Photosensitizers is directly proportional to the oxidative stress photoinduced.

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The Phenothiazinium dyes methylene blue, Azure A and Azure B were evaluated in terms of intracellular reactive oxygen species production and their photodynamic effect was directly related to the production of reactive oxygen species.

Introduction:

The action of photodynamic therapy on cells is associated with the production of reactive oxygen species (ROS). These oxidant species have cytotoxic effects through action on biomolecules, which are essential for cellular function and homeostasis. The oxidative stress measurement can be performed by quantifying the oxidation of the 2', 7'-dichlorodihydrofluorescein diacetate (DCFDA) probe. This non-fluorescent probe crosses cell membranes, is de-esterified intracellularly to 2', 7'-dichlorohydrofluorescein (DCFH) and is transformed into 2', 7'-dichlorofluorescein (DCF), highly fluorescent, shortly after oxidation. A large diversity of ROSs can oxidize DCFH into DCF.

Objective: The objective of this work is to quantify the production of ROS in HeLa cells treated by Photodynamic Therapy with the phenothiazinium photosensitizers (PS) Methylene Blue (MB), Azure A (AA) and Azure B (AB).

Method: HeLa cells were seeded in 6-well plates at the density of (2×10^5) cells / well. Cells were treated with the PSs 10 $\mu\text{mol} / \text{L}$ in 1% FBS DMEM, during

3 hours in the dark. After 2.5 hours of incubation with the PSs (ie, 30 minutes before the end of the incubation) DCFDA was added to reach a final concentration of 5.0 $\mu\text{mol} / \text{L}$, kept under incubation for an additional 30 minutes. After completion of the incubation, the supernatants were removed and the cells were washed with Phosphate Buffer Saline. The plate was exposed to radiation over a period of 12 minutes with Biolambda LED system, $660 \pm 10\text{nm}$ (Radiant Exposure 7 J / cm^2 ; Irradiance 9.7 mW/ cm^2). At the end of the irradiation, the cells were trypsinized and analyzed a flow cytometer.

Results:

MB generated higher ROS production when compared to other photosensitizers. AB presented a slightly higher ROS formation than AA. Previous data from the group¹ showed that AM is the most effective PS within the series, followed by AB, while AA is the lowest effective PS. Thus, through the quantification of EROs, it was possible to verify that the PDT efficacy of these PSs is directly proportional to oxidative stress photoinduced.

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Evaluation of chamomile and fig extracts combined to photobiomodulation therapy to minimize the effects of UV-A radiation in keratinocytes.

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The UVA (9.0 J/cm²) radiation decreased keratinocyte viability and the extracts of chamomile and fig, which were expected to protect cells from damage, showed toxicity after UVA exposure. On the other hand, photobiomodulation Therapy (1.0 J/cm²) was able to increase the viability of the cells previously exposed to UVA, even when the extracts were applied.

Introduction: Photoaging is caused by ultraviolet radiation (315-400nm), which are attributed to generation of reactive oxygen species (ROS) by light absorption of endogenous chromophores. The ROS are naturally eliminated by antioxidant system, but prolonged exposure to ultraviolet radiation can result in a redox imbalance. Antioxidant is any substance capable of slowing or preventing damage due to the inactivation of ROS. Plant extracts contain various components that exhibit antioxidant properties. On the other hand the photobiomodulation therapy (PBMT) has been shown to be an efficient tool to aid in cell renewal.¹ On it, the combination of antioxidant therapy with extracts and photobiomodulation seems to bring a possibility important in reducing of UV radiation.

Objective: The objective of this work was to evaluate the combination of plant extracts combined to photobiomodulation therapy in human keratinocytes in culture to reduce the damage effects of UV-A radiation.

Method: Human keratinocytes (HaCaT) were seeded in 48 well plates (60,000 cells/well) and, after attachment were exposed to UVA (366 ± 10 nm, 2.5 mW / cm², 9.0 J/cm², 90 minutes), then treated with Fig or Nutwood extract (0.3% for 24 hours, DMEM 1% FBS) and, finally,

photobiomodulation therapy (LED cluster, 640 ± 12 nm, 2.6 mW/cm², 1.0 J/cm², 7 minutes). At the end of the treatments, cells were kept in the incubator for 48 hours, when the MTT colorimetric assay was performed (2). The isolated controls were also performed (only UVA, only extracts, only PBMT, UVA+extracts, extracts+PBMT).

Results: It was observed that the extracts of chamomile and fig did not exhibit toxicity in the dark, with no statistically significant difference in relation to the dark control. The extracts caused a slight decrease in viability in relation to UVA Control (from 80 to approximately 70%), while in the UVA+Extract+PBMT group, in which PBMT was applied after the UVA irradiation followed by the Extract Treatment, an slight improvement in cellular viability was observed in relation to the UVA group but were not statistically significant for both extracts.

Conclusion: Although extracts initially did not show toxicity at the concentration of 0.3%, a tendency of toxicity was observed when they were applied after exposure to UVA. This effect may be related to cell membrane fragility following UV induced damage, which would increase its permeability to polyphenols, causing toxicity. On the other hand, PBMT seems to be an interesting tool to restore skin cells after UVA damage, however, the detailed mechanism and effects need to be better explored.

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

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Macrophage Light Absorption Spectrum

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Macrophages are essential cells in the tissue repair process, as along with responding to pathogens, they are responsible for the production of important mediators that regulate this process. Macrophages can differentiate into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes according to the signaling pathways found in the lesion's microenvironment. Photobiomodulation (PBM), in red and infrared spectra, has been extensively studied due to its effects on macrophage modulation. For PBM to have an effect, emitted light must be absorbed by cellular chromophores thus triggering an intracellular response called the primary reaction. The emitted light wavelength will determine absorption in the different cellular and tissue types. Thus, knowledge of the absorption spectrum of these cells is important to standardize the parameters used in PBM.

Objective: To evaluate the absorption of different light spectra by M0 and M1 macrophages. For this purpose, peritoneal macrophages were obtained from Balb / c female mice aged 6-7 weeks.

Methodology: Cell suspensions were obtained via aspiration with a syringe and a needle, conditioned in conical tubes and immersed in ice until the tests were carried out. The wash was centrifuged and the resulting pellets resuspended in RPMI medium supplemented with 3% FBS. The cells were plated (1.0×10^6) and maintained in an oven at 37°C and 5% CO₂ for 2h. For activation of macrophages to M1 profile, cell cultures were treated with 1µg / mL Escherichia coli LPS and 0.2µg / mL IFN-γ and cultured in RPMI medium supplemented with 3% FBS for 24 hours. After this

period, a spectral absorption spectrophotometer was used. **Results:** Both M0 macrophages and M1 macrophages preferentially absorb light at wavelengths between 400 to 550 nm.

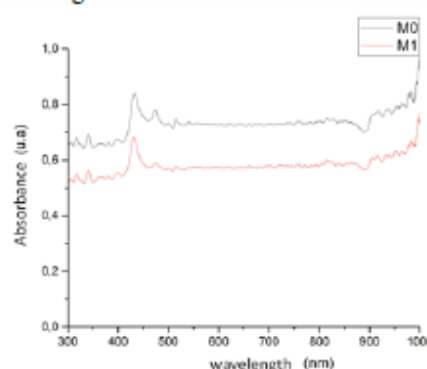


Fig. 1: Graph of variation in light absorption according to wavelength

Conclusion: The absorption spectrum of cells and tissues assists in choosing the most optimal light wavelength for performing PBM.

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EVALUATION OF REMINERALIZATION OF SPOT LESION WITH 808 nm DIODE LASER

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The use of diode laser can promote a remineralization of the oxygen prisms and leave the substrate more responsive to fluoride. Fluid therapy is already well established in the literature, as the protocols for use are extensive and require several sessions, which makes it difficult for patients to adhere to treatment. The association of laser use and treatment can accelerate the therapeutic process of lesions from initial dental healing. The result on the evaluation should be the remineralization of dental enamel after irradiation with diode laser in ferma disease of dental bovine dental. The specimens are prepared for the initial formation of the lesion and irradiation with diode laser and treated with fluorine varnish (Duraphat). In the experimental groups the diode laser with wavelength of 808nm is used. Afterwards, they will be randomly divided into 5 groups (n = 15): treated only with laser (G1); laser and remineralizing solution (G2), laser and fluorine varnish (Duraphat 5%) (G3), only made with fluorine varnish (G4). A remineralizing

solution will be performed at 7, 14, 21 and 28 days. For a characterization of radiation, an Optical Coherence (OCT) and Speckle laser will be used. The data will be analyzed statistically.

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Low Level Laser Therapy (LLLT) attenuates pulmonary inflammation in experimental asthma model induced by House Dust Mite (HDM) - dosimetric study

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Asthma is characterized by chronic inflammation of the airways. It presents high prevalence, economic and social cost. Several models aim to discover new therapies. LLLT is relatively new and effective, very low cost, with no side effects. However, there is still no consensus on the optimal dose to be used.

In this sense, the objective of the present study was to evaluate the best dose in an experimental model of asthma induced by HDM.

We used Balb/c mice, males, divided into 10 experimental groups: Control, asthmatic (HDM), LLLT (1J, 3J, 5J and 7.5J) and HDM + LLLT (1J, 3J, 5J and 7.5J). The asthmatic animals received administration of 100ug/animal HDM and LLLT applications (diode laser: 660nm, 100mW and four different energies 1J, 3J, 5J and 7.5J) for 16 days. After 24 hours, we studied inflammatory parameters in BALF, cytokines and histological analysis.

The results showed that LLLT was able to modulate the pulmonary inflammation observed by reducing the number of cells in Bronchoalveolar Lavage Fluid (BALF) ($p < 0.01$) as well as reducing the percentage of neutrophils ($p < 0.01$), eosinophils ($p < 0.05$) and lymphocytes ($p < 0.05$). On the other hand, LLLT increased the level of IL-10 ($p < 0.01$) and reduced levels of IL-4, IL-5 and IL-13 in BALF ($p < 0.001$). LLLT was able to reduce the production of mucus ($p < 0.001$) and peribronchial eosinophils ($p < 0.001$).

It is important to emphasize that LLLT has several benefits in different pulmonary diseases ¹⁻³. Specifically, in clinical bronchial asthma, recent studies have demonstrated its benefits in reducing side effects, improving spirometric parameters, as well as reducing drug use ^{4,5}.

We concluded that the use of LLLT in the treatment of chronic inflammation of the airways attenuated the inflammatory process. We emphasize, in general, that the 3J laser presented better results. Thus, photobiomodulation may be considered a promising tool for the treatment of chronic pulmonary allergic inflammation observed in asthma.

Funding: Financial support of the Amparo Foundation for Research in the State of São Paulo (FAPESP). Rigonato-Oliveira, NC (Process 2015/23152-6) and Ligeiro-Oliveira (Process 2012/16498-5).

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Low Level Laser Therapy (LLLT) attenuates pulmonary inflammation in experimental asthma model induced by House Dust Mite (HDM) - dosimetric study

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Photodynamic Therapy for the Endodontic Treatment of Primary Teeth: A Randomized Controlled Clinical Trial

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Introduction. Endodontic treatment in primary teeth is a complex procedure due to the instrumentation, complexity of the apical delta, the biological cycle of primary teeth, physiological root resorption and long treatment sessions, during which children are not always cooperative[1]. The success of endodontic treatment depends on the effective decontamination of the root canal systems. The premature loss of primary teeth can compromise the development of the stomatognathic system and installation of the permanent dentition as well as have consequences that can affect one's social life[1]. Antimicrobial PDT is based on the interaction of three factors: a light source at a specific wavelength, a photo-activated dye or photosensitizing agent, such as methylene blue or toluidine blue, and oxygen. When the laser irradiates the photosensitizing agent, energy transference takes place between the light, photosensitizing agent and substrate, giving rise to singlet oxygen and free radicals[2]. These substances alter the metabolism of the bacterial cell wall by affecting lipids, proteins and nucleic acids, leading to the death of bacterial cells through the process of apoptosis[3]. The interest in the use of aPDT in endodontic treatment is related mainly due to its proven antimicrobial effect, the fact that this method does not promote microbial resistance, the easy painless administration and the beneficial effects of laser therapy[4]. The aim of the present study was to compare the effectiveness of conventional endodontic treatment with and without aPDT through microbiological, clinical and radiographic analyses.

Methods and analysis. Thirty single-root primary anterior teeth were selected. The teeth were in healthy male and female children aged two to five years with a diagnosis of pulp necrosis treated at the pediatric dentistry clinic of University Nove de Julho and received approval from the Human Research Ethics Committee of University Nove de Julho (São Paulo, Brazil) under process number 832.657.

Inclusion Criteria: Children aged two to five years with at least one primary anterior tooth with irreversible pulpitis or pulp necrosis due to caries or trauma with at least 2/3 of the root remaining and who had not been submitted to antibiotic therapy in the previous three months were included in the study.

Exclusion Criteria: Children with compromised health, those with primary anterior teeth with resorption of 2/3 or more of the root, cryptic involvement and those having been

submitted to antibiotic therapy in the previous three months were excluded from the study.

The patients were randomly allocated to two groups: Group I – conventional root canal therapy (n = 15); Group II – conventional root canal therapy combined with antimicrobial PDT (n = 15). For PDT, methylene blue at a concentration of 0.005% was used as the photosensitizing agent, which was applied to the interior of the canal with a sterile paper cone for three minutes, followed by the administration of laser light for 40 seconds (wavelength: 660 nm; energy density: 4 J/cm²; power: 100 mW). The laser was delivered in direct contact at the entrance to the root canal. Two microbiological samples of the intra-canal content were taken (one before and one after immediately treatment in both groups) using paper cones. Radiography was used to evaluate the repair process. The clinical evaluation involved the investigation of fistulas and mobility. These evaluations were performed one and three months after treatment. The data were submitted to statistical analysis (Mann-Whitney test) with a 5% significance level.

Results. The bacterial counts (CFUs/ml) demonstrated reductions in both groups: 99% (SD: 3%) in Group II and 93% (SD: 13%) in Group I.

Conclusion. The present findings demonstrated that the clinical, radiographic and microbiological results achieved with conventional endodontic treatment combined with antimicrobial photodynamic therapy using the parameters employed in this study on primary teeth with a diagnosis of pulp necrosis.

Trial registration: NCT02485210.

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Effects of low intensity laser on skin graft surgery

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Objective. To report the results obtained in studies with application of photobiomodulation in skin graft surgery.

Methods. Survey of clinical studies published in the last five years on the use of low intensity laser in skin graft surgery. Excluded studies that didn't evaluate the healing of skin graft surgery by using low intensity laser.

Results. It was selected 3 studies in the total. One study used red and infrared laser after the excision of the burned skin and after the graft. Another study used red laser, infrared laser and intravenous laser on burn and after graft surgery. In the last study, the red laser was used in the skin graft donor area.

Tabela 1. Results of laser in graft skin surgery

Authors	Methods	Results
¹ Nooshafarin Kazemikhoi et al	Nine patients with burns on both hands or both feet; undergoing excision and grafting. The limbs (hand or foot) of the laser group were irradiated after excision of the burned skin and after grafting (in this case, 7 sessions). Used red laser in the wound bed and infrared in the margins; both groups (hands or feet) treated with vaseline and gauze.	The rate of wound dehiscence after skin graft surgery was significantly lower in the laser treated group compared to the control group receiving only classic dressing.
² Mostafa Dahmardehei et al	Six type 2 diabetic patients, total 13 burn ulcers grade 3. They used red laser to the wound bed, infrared laser to the margins and intravenous therapy with red laser, before (7-10 sessions until the granulation tissue cover the area completely) and after grafting surgery (3-5 sessions), to avoid dehiscence of the grafted area.	The grafted areas became fully healed after 5 days and there was no relapse or other complications during an average of about 6 months.
³ Reza Vaghardoost et al	Eleven patients with total thickness burn underwent early excision and grafting.	Donor site size decreased in both groups on day 7

After grafting of the burned area, 18 skin donor sites were selected and divided into two parts randomly, one as a laser group (Mepitel and laser dressing) and one as a control (Mepitel dressing). There were 4 sessions of red laser after grafting, with irradiation in the wound bed.	and this reduction was significantly greater in the laser group.
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Discussion: Red laser was used in all studies. The patient with Diabetes Mellitus may present with tissue perfusion deficit and the low intensity laser can contribute to the healing of wounds due to burns and graft donor areas. The skin graft donor area also becomes a wound, needs to be treated and can be benefited by the use of the laser.

Conclusion. In all studies, low intensity laser wounds (burns, graft sites, and donor areas) treated with a low intensity laser had a significantly greater surface reduction compared to those wounds treated with classic dressing (control group) such as dehiscence, which is a complication of grafting surgeries was significantly lower in the laser treated groups.

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**EFFECTS OF PHOTOBIMODULATION PLUS VITAMIN C IN THE
 INFLAMMATORY AND FIBROTIC PARAMETERS IN EXPERIMENTAL
 MODEL OF LUNG FIBROSIS**

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Abstract: Pulmonary fibrosis (PF) is a chronic and progressive disease characterized by progressive lesion of the pulmonary parenchyma, inflammatory infiltrate and interstitial fibrosis. Treatment of PF is still a clinical health problem, so new therapies are needed. In this context, photobiomodulation has been showing good results for several inflammatory diseases, including lung diseases, and here we studied the effects of LED on PF.

cm²; Density of energy: 5,8 J/cm²; Issuance: Continuous (cw); Total Radiant Emission: 24 J. Treatment specifications: Exposure time: 152 s; Irradiated points: 1 point; Irradiation Method: Direct skin contact; Anatomical location: trachea and lungs; Irradiation rhythm: punctual; Number of treatments: 1 day, seven applications; Optical properties of tissue: Healthy tissue; Animals not shaved. These parameters were measured.

Introduction: Paraquat (PQ) is one of the most herbicides used by several countries, although of their toxic effects in humans and animals. PQ exposition induces oxidative stress and can cause pulmonary fibrosis. Pulmonary fibrosis (PF) is a chronic and progressive lung disease characterized by progressive lesion of the pulmonary parenchyma, inflammatory infiltrate and interstitial fibrosis. Due to the absence of effective treatment, we aimed to investigate the role of photobiomodulation

Results: Our data showed that LED plus Vit C reduced the level of IL-6, IL-17A, TNF- α , TGF- β in the lung homogenates as well as reduced the collagen deposition into the lung. However, did not alter the level of MMP-9 in the lung homogenates.

Conclusions: Our data showed that LED plus vitamin C might be an important tool to treat lung fibrosis. Thus, these results open the possibility for new studies and alternatives more efficient to treat lung fibrosis.

Methods: Adult male C57BL6 mice were submitted to the induction of PF by the administration of Paraquat (10mg / kg, ip) and after 7 days of induction, the mice were treated during 7 days with Photobiomodulation (LED) and vitamin C (150mg/kg, ip). Device specifications: BioLambda Apparatus LEDsabr, São Paulo, Brazil; Probe Design, Single Probe; Wavelength: 660 nm; Radiant Power: 160 mW; Power Density: 38,5 mW/cm²; spot area: 4,15

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ADMINISTRATION OF LOW-LEVEL LASER ON MUSCLES OF MASTICATION FOLLOWING THE INDUCTION OF INITIAL FATIGUE: PROTOCOL FOR A RANDOMIZED, CONTROLLED

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Background: Orofacial pain encompasses painful conditions, such as temporomandibular disorder (TMD). Multidisciplinary health teams seek to control such musculoskeletal disorders to improve the quality and functional capacity of the muscles of mastication. The aim of the proposed study is to evaluate the effect of low-level laser therapy as a form of treatment for the prevention of initial fatigue of the muscles of mastication (masseter and anterior temporal muscles) as well as the recovery of these muscles after induced exhaustion (caused by isometric contraction) in young adults.

Methods: The participants will be 78 healthy male and female volunteers between 18 and 34 years of age. The volunteers will be randomly allocated to a laser group (n = 26), sham group (n = 26), and control group (n = 26). All participants will be submitted to a clinical evaluation to record mandibular movements, bite force, muscle sensitivity to palpation, and initial muscle fatigue. Initial fatigue will be induced by isometric contraction of the jaws. Maximum voluntary contraction will be performed to record the time until initial exhaustion of the masseter

muscle (determined by electromyography). The groups will then be submitted to the interventions: active laser therapy (wavelength: 780 nm; fluence: 134 J/cm²; power: 50 mW; irradiance: 1.675 W/cm²; exposure time: 80 seconds per point) on 3 points of the masseter and 1 point on the anterior temporal muscles on each side; sham laser (placebo effect); or no intervention (control). Maximum voluntary contraction will be performed again after the interventions to record the time until initial exhaustion of the masseter muscle (determined by electromyography). Differences in individual time until exhaustion between the pre- and postintervention evaluations will be measured to determine the effect of low-level laser therapy.

Discussion: Although studies have been made with the use of low-level laser therapy in TMDs and on the effect of photobiomodulation on fatigue, this is the first study to test this therapy in the prevention of fatigue in this region. The clinical relevance lies in the fact that longer dental procedures could take place if the patients are less prone to fatigue.

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