UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MARCELA PINTO MONTEIRO DE OLIVEIRA

ESTUDO DA AÇÃO ANTIMICROBIANA DA TERAPIA FOTODINÂMICA SOBRE LESÕES DE CÁRIE PRODUZIDAS *IN VITRO* NA DENTINA DE DENTES BOVINOS

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Mestre em Odontologia, Área de Concentração em Odontopediatria.

Orientadora: Prof[a]. Dr[a]. Marinês Nobre dos Santos Uchôa

Piracicaba
2010

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecária: Marilene Girello – CRB-8ª. / 6159

OL4e

Oliveira, Marcela Pinto Monteiro de.
Estudo da ação antimicrobiana da terapia fotodinâmica sobre lesões de cárie produzidas *in vitro* na dentina de dentes bovinos. / Marcela Pinto Monteiro de Oliveira. -- Piracicaba, SP: [s.n.], 2010.

Orientador: Marinês Nobre dos Santos Uchôa.
Dissertação (Mestrado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. *Streptococcus mutans*. I. Nobre dos Santos, Marinês. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

(mg/fop)

Título em Inglês: Photodynamic therapy effect in carious bovine dentin - An *in vitro* study

Palavras-chave em Inglês (Keywords): 1. *Streptococcus mutans*

Área de Concentração: Odontopediatria

Titulação: Mestre em Odontologia

Banca Examinadora: Marinês Nobre dos Santos Uchôa, Wanessa Christine de Souza Zaroni, Érico Barbosa Lima

Data da Defesa: 25-02-2010

Programa de Pós-Graduação em Odontologia

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Dissertação de Mestrado, em sessão pública realizada em 25 de Fevereiro de 2010, considerou a candidata MARCELA PINTO MONTEIRO DE OLIVEIRA aprovada.

Profa. Dra. MARINÊS NOBRE DOS SANTOS UCHOA

Profa. Dra. WANESSA CHRISTINE DE SOUZA ZARONI

Prof. Dr. ÉRICO BARBOSA LIMA

DEDICATÓRIA

Aos meus pais ***Inês*** e ***Fernando (in memorian)*** por tanta dedicação e por tudo que me ensinaram até hoje. Sem o amor e incentivo de vocês, durante cada momento da minha vida, eu não teria forças e estrutura para nada. Ao meu pai, por deixar tanta saudade e lindas lembranças. À minha mãe, por ser exemplo de força e de vida para mim. Amo demais vocês!!!

Aos meus irmãos ***Marcos e Gustavo*** por serem acima de tudo amigos e parceiros para qualquer momento. Me orgulho muito dos dois e amo demais vocês!

Ao meu noivo **Wagner Filho**, pela confiança e carinho. Pela compreensão durante esse tempo distante, sempre me apoiando e torcendo pelo meu sucesso. Você é um homem maravilhoso! Te amo muito!

AGRADECIMENTOS

À ***DEUS,*** pela vida. Por me guiar pelos melhores caminhos, com saúde, amor, paz e sempre acompanhada de pessoas maravilhosas!

À minha mãe **Inês**, por toda dedicação e força. Sem o seu amor e seu incentivo, nada disso seria possível! Obrigada pela educação e por todo carinho, que servem de alicerce para tudo em minha vida. Obrigada por toda preocupação e torcida pelo meu sucesso. Agradeço também tanta paciência e sabedoria, que me fazem te admirar cada dia mais! Mãe, você é exemplo de vida para mim! Amo você mais que tudo!

Ao meu pai **Fernando (in memorian)**, pelo carinho, por todos os ensinamentos deixados e por ter sido sempre exemplo de determinação e força para enfrentar os obstáculos da vida. Sei que você me acompanha a cada passo e continua vibrando com as minhas conquistas. Obrigada por ter deixado tantas boas lembranças e muita saudade! Te amo!

Aos meus irmãos **Marcos** e **Gustavo**, pessoas de coração sem tamanho, que proporcionam tantos bons momentos na minha vida! Sem vocês, minha caminhada não seria tão feliz! Amo muito os dois!

À minha tia **Regina**, pelos ensinamentos de vida. Sempre aprendi muito com você! Obrigada pelos bons e sensatos conselhos e por todo apoio e carinho!

Ao meu noivo ***Wagner Filho***, que sempre acreditou em mim e torceu para que tudo desse certo, mesmo de longe. Obrigada por suportar a distância e a ausência, sempre com tanto amor, e ao mesmo tempo ser tão presente e

carinhoso. Obrigada por ser esse homem maravilhoso, que sempre apóia minhas decisões e torna minha vida mais feliz! Amo você!

À ***Profa. Dra. Marinês Nobre dos Santos Uchôa***, amiga e orientadora, pela oportunidade e confiança. Seus ensinamentos e experiência me fizeram crescer muito. Obrigada por acreditar em mim e ajudar-me a concluir este trabalho.

À **Profa. Dra**. **Lidiany Karla Azevedo Rodrigues**. Agradeço todos os momentos que esteve comigo, me auxiliando nos experimentos e na conclusão desse trabalho. Admiro sua competência e determinação! Obrigada por tudo!

Aos meus tios **Silvia, João, Nilva, Marlene** e **Zéito**. Aos meus primos **Rodrigo, Alexandre, Nina, Ana, Lucas** e **Elisa**. Obrigada por tanto carinho e pela força que me transmitem sempre! Vocês transformam os simples momentos em muita alegria! Amo vocês!

Aos meus tios **Amiltom, Adilson, Gabriela** e **Sueli**, e aos primos **Marília, Luís, Pedro, Bento, Diego, Marcello, Cristina, Mariana** e **Guilherme**. Agradeço pela torcida pelo meu sucesso!

Aos primos **Gó, Eliana, Marina** e **Felipe** pelo carinho e incentivo.

Aos tios e primos **Wilma, Tadeu, Laís, Mariana, Mari, Vinicius, Tia Tele, Ciça** e **Popi**. Obrigada por torcerem pelo meu sucesso e vibrarem a cada conquista!

Às amigas e cunhadas **Natália** e **Juliana**, por tornarem a nossa casa mais bonita e alegre. Obrigada pelos bons momentos! Gosto muito de vocês!

À **Maíra, D. Graça**, **Dr. Wagner, Fabrício, Maraísa** e **Gabriela**. Muito obrigada pela torcida! Obrigada pelos bons momentos juntos, pelo carinho de sempre e por toda hospitalidade durante esses anos de estadias em Teresina.

À Universidade Estadual de Campinas, na pessoa do seu Magnífico Reitor Prof. Dr. Fernando Ferreira Costa; à Faculdade de Odontologia de Piracicaba, na pessoa do seu diretor Prof. Dr. Francisco Haiter Neto, do Coordenador Geral da Pós-Graduação da FOP – UNICAMP Prof. Dr. Jacks Jorge Júnior, da Coordenadora do programa de Pós-Graduação em Odontologia da FOP-UNICAMP Prof. Dra. Maria Beatriz Duarte Gavião, pela oportunidade de crescimento científico e profissional nesta conceituada instituição de ensino. Agradeço à estrutura oferecida para esta etapa da minha formação profissional e aos funcionários dessa instituição que, com dedicação e profissionalismo, trabalham para manter tudo em ordem.

Às Prof^{as} Dr^{as} da área de Odontopediatria **Regina Maria Puppin Rontani** e **Maria Beatriz Duarte Gavião**. Agradeço toda seriedade e competência, que contribuíram muito para o meu crescimento pessoal e profissional.

Às Prof^{as} Dr^{as} **Carolina Patrícia Aires** e **Eliana Rodrigues** pela colaboração como banca de qualificação desse trabalho e pelo aprimoramento do mesmo.

Às minhas amigas de turma de mestrado, **Éfani, Fernanda, Larissa** e **Marina**. Nossa união e companheirismo foram essenciais para tornar a caminhada menos árdua. Obrigada pela amizade e pelos bons momentos. Torço muito pelo sucesso de vocês!

À amiga **Karlla Almeida Vieira**, pela ajuda na realização dos experimentos. Meus sinceros agradecimentos.

À **Profa. Dra. Cristiane Duque**, que me acompanhou e ensinou a realizar os experimentos microbiológicos iniciais. Obrigada pela paciência e pelo tempo que dedicou para me auxiliar!

As colegas de doutorado **Annicele Andrade, Tais Barbosa, Renata Cerezetti, Patricia Sacramento** e **Thais Parisotto** pelo companheirismo.

Às amigas **Carolina Steiner, Fernanda Pascon, Kamila Kantovitz, Renata Rocha, Marcia Serra, Flávia Gambarelli** pelos bons momentos.

Ao técnico do laboratório de Odontopediatria, **Marcelo Corrêa Maistro**, pela paciência e ajuda durante os experimentos.

Ao laboratório de microbiologia e à **Profa. Dra. Renata de Oliveira Mattos Graner**, pela estrutura que me foi oferecida para a realização deste trabalho.

À **CAPES e CNPq**, pelo auxílio financeiro indispensável ao desenvolvimento de todas as atividades durante meu curso de mestrado.

À **FAPESP** pelo auxílio à pesquisa, que viabilizou a aquisição dos materiais e equipamentos utilizados.

Á todos que indiretamente tiveram grande importância para a realização de mais essa etapa da minha formação.

Meus sinceros agradecimentos

RESUMO

Durante o processo conhecido como terapia fotodinâmica, a aplicação de fotossensibilizadores associados a uma fonte de luz de comprimento de onda complementar, gera produtos que podem danificar componentes essenciais das células, e causar a morte celular. Dentro desse contexto, a aplicação dessa terapia sobre microrganismos presentes em lesões de cárie é de grande valia, uma vez que poderá reduzir a quantidade de tecido dental a ser removido no tratamento da cárie, diminuir as chances de progressão da doença bem como os riscos de acometimento pulpar do elemento dentário. Assim, o objetivo deste estudo *in vitro* foi determinar parâmetros para o uso de um diodo emissor de luz (LED) associado ao corante azul de orto toluidina (TBO) na redução da contagem de *Streptococcus mutans* presentes em lesões de cárie dentinária. Para isto, 72 espécimes de dentina coronária de dentes bovinos foram imersos em cultura contendo *Streptococcus mutans* para produzir lesões de cárie. Tais espécimes foram divididos aleatoriamente em 6 grupos (n=12): Controle (exposição a NaCl a 0,9% por 5 min); TBO (exposição ao TBO a 0,01% por 5 min); LEDA (exposição ao LED por 4,2 min); LEDB (exposição ao LED por 6,5 min); PDTA (exposição ao corante associado ao LED por 4,2 min) e PDTB (exposição ao corante associado ao LED por 6,5 min). As densidades de energia utilizadas para os tempos de 4,2 e 6,5 min, foram de 166 e 249 J/cm^2, respectivamente. Antes e após os tratamentos, amostras de tecido dentinário cariado foram coletadas e analisadas microbiologicamente, por meio da contagem das unidades formadoras de colônia (UFC) de S. *mutans*. A profundidade das lesões de cárie produzidas pelo modelo microbiológico utilizado foi determinada por meio da microscopia de luz polarizada. Foram utilizados os testes ANOVA/Tukey para comparar os valores de log redução dos grupos (α=5%). Observou-se redução significativa de S. *mutans* nos grupos em que aplicou-se TBO associado ao LED, com as duas densidades de energia utilizadas. Entretanto, nenhuma diferença significativa foi encontrada para os diferentes tempos de irradiação. Concluiu-se que os parâmetros utilizados no

presente estudo, para o emprego do LED associado ao TBO, foram efetivos em reduzir a contagem de S. mutans presentes em lesões de cárie dentinária.

Palavras-chave: LED, cárie, dente bovino, *Streptococcus mutans,* estudo *in vitro,* dentina.

ABSTRACT

Photodynamic therapy (PDT) is a technique that consists in the activation of certain photosensitizers by light in the presence of tissue oxygen, resulting in the production of reactive radicals capable of inducing cell death. In this context, this therapy may become a suitable approach to disinfect the dentin tissue during the caries treatment, and reduce the tissue removal, minimizing the probability of caries progression and pulp involvement.

This randomized *in vitro* study determined parameters for using a light-emitting diode (LED) with toluidine blue O (TBO) for reduction of Streptococcus mutans counts inside dentin caries. Seventy two bovine coronary dentin slabs were immersed in *Streptococcus mutans* culture for demineralization production. Dentin slabs were allocated to 6 groups (n=12) as follows: Control (treated with 0.9% NaCl solution for 5 min); TBO (treated with 0.1 mg/ml TBO for 5 min); LEDA (submitted to irradiation for 4.2 min); LEDB (submitted to irradiation for 6.5 min); PDTA (treated with TBO plus irradiation for 4.2 min) and PDTB (treated with TBO plus irradiation for 6.5 min). The energy densities used for 4.2 and 6.5 min correspond at 166 and 249 J/cm^2, respectively. Before and after treatments, dentin samples were analyzed with regard to *S. mutans* counts. The caries lesion depth produced by the microbiological model was analyzed by polarized light microscopy. ANOVA/Tukey tests were utilized to compare log reductions among groups (α=5%). Bacterial reduction was observed when dentin was exposed to both TBO and LED at both irradiation times. However, no difference in *S. mutans* reduction was found between the two energy densities. Concluding, although the use of LED combined with TBO was effective in reducing the Streptococcus mutans counts in carious dentin, this effect may not have clinical significance.

Keywords: LED, caries, bovine tooth, *Streptococcus mutans, in vitro* study, dentin.

SUMÁRIO

INTRODUÇÃO

Durante o processo conhecido como terapia fotodinâmica ou fotossensibilização letal, componentes fotossensíveis endógenos ou exógenos passam para um estado excitado quando expostos a uma luz de comprimento de onda específico, o que é promovido pela passagem dos elétrons para níveis de energia superiores. Neste estado excitado, o fotossensibilizador pode interagir com o oxigênio molecular iniciando a formação de oxigênio singleto altamente reativo (fotoprocesso Tipo II) ou interagir com outras moléculas como aceptores de elétrons resultando na produção de hidroxila e outros radicais orgânicos (fotoprocesso do Tipo I) (MACROBERT *et al*., 1989). Os produtos dessas reações fotoquímicas podem então danificar componentes essenciais das células como a membrana citoplasmática por disrupção ou alterar as atividades metabólicas de maneira irreversível resultando na morte bacteriana (SPIKES & JORI, 1987; MALIK *et al*., 1990; BHATTI *et al*., 1997).

Como a maioria das espécies bacterianas não apresenta componentes especiais com capacidade de absorção significativa pela luz, a utilização de um fotossensibilizador que atraia para si a luz e inicie a formação de radicais livres é importante (WILSON *et al*., 1992). Assim, células desprovidas de componentes fotossensíveis endógenos podem tornar- se sensíveis à luz se forem coradas com fotossensibilizadores ou agentes cromóforos exógenos como o azul de metileno, azul de toluidina, rosa bengal, eosina e hematoporfirinas (WILSON, 1993). No entanto, a habilidade de um componente em absorver uma luz incidente não significa necessariamente que ele possa atuar como um fotossensibilizador. Para produzir efeito antimicrobiano, os fotossensibilizadores devem apresentar picos de absorção próximos ao comprimento de onda da luz emitida pelo laser (WILSON *et al*., 1992) como ocorre com o azul de orto toluidina (TBO). Este corante apresenta pico de absorção máximo em 632 nm (WILSON *et al*., 1995).

Dessa forma, a terapia fotodinâmica mostra-se uma técnica promissora durante o tratamento da cárie, pois seu efeito antibacteriano poderá promover a

redução da contaminação da dentina (WILLIAMS *et al.*, 2004; GIUSTI et al., 2008; LIMA et al., 2009; MELO et al., 2010), e assim permitir a diminuição da quantidade de tecido dental a ser removido no tratamento da cárie (BURNS *et al.*, 1994; BURNS *et al.*, 1995).

O objetivo atual da escavação de dentina cariada, seguindo os princípios da odontologia moderna minimamente invasiva, é remover somente a camada mais superficial da dentina altamente infectada (MASSLER, 1967). A camada mais interna da dentina afetada por cárie, ainda contaminada, porém passível de remineralização (WEI *et al.*, 1968), deve ser mantida, prevenindo assim o envolvimento pulpar do elemento dentário (MAGNUSSON & SUNDELL, 1977; LEKSELL *et al.*, 1994). Porém, clinicamente a diferenciação entre essas zonas é extremamente crítica, de maneira que grande quantidade de tecido desmineralizado, ainda passível de remineralização, pode ser removida durante o preparo cavitário (BURNS *et al.*, 1994). Além disso, a discussão sobre a quantidade de dentina afetada pela cárie ou o número de microrganismos que podem ser mantidos sem haver risco de progressão da lesão ainda não foram estabelecidos (BJØRNDAL *et al.*, 1997; JERONIMUS *et al.*, 1975; GOING *et al.*, 1978; WEERHEIJM *et al.*, 1993; EIDELMAN, 1993). Embora estudos sobre remoção parcial de tecido cariado mostrem uma redução do crescimento bacteriano (BESIC, 1943; BJORNDAL *et al.*, 1997), a remoção de todo o tecido cariado durante o tratamento restaurador ainda é considerada essencial por outros (KREULEN *et al.*, 1997; WEERHEIJM *et al.*, 1999).

Com base no exposto, torna-se necessário o desenvolvimento de uma terapia capaz de inviabilizar as bactérias presentes no tecido dentinário cariado, e dessa forma reduzir a quantidade de tecido dental a ser removido (BURNS *et al.*, 1994; BURNS *et al.*, 1995) e conseqüentemente diminuir o risco de progressão da lesão e o acometimento pulpar.

A ação antimicrobiana de lasers diodos ou LEDs associados a fotossensibilizadores específicos sobre bactérias crescidas em caldo de cultura está bem documentada na literatura (DOBSON & WILSON, 1992; BURNS *et al,.*

1994; WILSON & YIANNI, 1995; BURNS *et al.*, 1995; GRIFFITHS *et al.*, 1997; ZANIN *et al.*, 2002; CHABRIER-ROSELLO *et al.*, 2005). Da mesma forma, os efeitos dessa terapia sobre biofilmes bacterianos já foram estabelecidos (WOOD *et al.*, 1999, O'NEILL *et al.*, 2002; ZANIN *et al.*, 2005; CHABRIER-ROSELLO *et al.*, 2005; ZANIN *et al.*, 2006; WOOD *et al.*, 2006). No entanto, ainda são escassos os estudos que tenham demonstrado o efeito da terapia fotodinâmica em lesões de cárie (WILLIAMS *et al.*, 2004; LIMA *et al.*, 2009; GIUSTI *et al.*, 2008; MELO *et al.*, 2010). De acordo com a densidade do tecido irradiado, o efeito da terapia fotodinâmica sobre microrganismos orais presentes na dentina cariada pode ser reduzido devido à menor quantidade de luz que alcança as bactérias (BURNS *et al.*, 1995). Dessa forma, parâmetros mais efetivos devem ser investigados.

As pesquisas que verificaram o efeito da terapia fotodinâmica em lesões de cárie, que utilizaram diodos emissores de luz, apresentam algumas limitações. A aplicação de fórmulas inovadoras para o cálculo da eficácia da terapia, e ausência de grupo controle (GIUSTI *et al.*, 2008) ou tempos de irradiação extremamente extensos (LIMA *et al.*, 2009; MELO *et al.*, 2010), são fatores que dificultam a avaliação do efeito da terapia fotodinâmica sobre as lesões cariosas, e assim, inviabilizam a aplicação da mesma clinicamente.

Assim, faz-se necessário a realização de pesquisas que objetivem investigar diferentes parâmetros de irradiação, que possibilitem o emprego de um menor tempo para irradiação do tecido cariado. Dessa forma será possível padronizar técnicas realmente efetivas para posterior aplicação clínica da terapia fotodinâmica em tecido cariado.

Dessa forma, o presente trabalho teve como objetivo determinar parâmetros para o uso de um diodo emissor de luz (LED), associado ao corante azul de orto toluidina (TBO), para desinfecção de lesões de cárie dentinária produzidas *in vitro* na dentina coronária de dentes bovinos.

Capítulo 1

"Photodynamic therapy effect in carious bovine dentin – An *in vitro* study"

Marcela Pinto Monteiro-Oliveira, DDS, MS

- *Department of Pediatric Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, BR*

Lidiany Karla Azevedo Rodrigues, DDS, MS, PhD

- Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, CE, Brazil

Mary Anne Sampaio de Melo, DDS, MS

- Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, CE, Brazil

Marinês Nobre-dos-Santos, DDS, MS, PhD

- *Professor, Department of Pediatric Dentistry, Piracicaba Dental School, State University of Campinas, Avenida Limeira 901, CEP 13414-903, Piracicaba, SP, BR*

ABSTRACT

This randomized *in vitro* study determined parameters for using a light-emitting diode (LED) with toluidine blue O (TBO) for reduction of Streptococcus mutans counts inside dentin caries. Seventy two bovine coronary dentin slabs were immersed in *Streptococcus mutans* culture for demineralization production. Dentin slabs were allocated to 6 groups (n=12) as follows: Control (treated with 0.9% NaCl solution for 5 min); TBO (treated with 0.1 mg.ml-1 TBO for 5 min); LEDA (submitted to irradiation for 4.2 min); LEDB (submitted to irradiation for 6.5 min); PDTA (treated with TBO plus irradiation for 4.2 min) and PDTB (treated with TBO plus irradiation for 6.5 min). The energy densities used for 4.2 and 6.5 min correspond at 166 and 249 J/cm^2, respectively. Before and after treatments, dentin samples were analyzed with regard to *S. mutans* counts. The caries lesion depth produced by the microbiological model was analyzed by polarized light microscopy. ANOVA/Tukey tests were utilized to compare log reductions among groups (α=5%). Bacterial reduction was observed when dentin was exposed to both TBO and LED at both irradiation times. However, no difference in *S. mutans* reduction was found between the two energy densities. Concluding, although the use of LED combined with TBO was effective in reducing the Streptococcus mutans counts in carious dentin, this effect may not have clinical significance.

Keywords: LED, caries, bovine tooth, *Streptococcus mutans, in vitro study, dentin*.

INTRODUCTION

Photodynamic therapy is based on the use of photosensitising agents, for instance, toluidine blue O (TBO), which are activated by irradiation with light at a specific wavelength to generate oxygen reactive species, including singlet oxygen and free radicals. These products are capable of damaging essential components of the cells or modifying metabolic activities in ways that may result in cell death (1; 2).

In this context, photodynamic therapy (PDT) may be a potential alternative approach for dentin disinfection of deep dentinal caries. The dentin carious lesion consists of two distinct areas (3): an outer layer characterised by a softened, wet, highly contaminated dentin and an inner layer that tends to be contaminated with a low number of microorganisms. This inner layer is thought to be susceptible to remineralisation and may be preserved during cavity preparation (4), which enables the removal of less carious tissue and decreases the risk of pulp exposure (5; 6). However, distinguishing these layers in clinical settings is very difficult, and current methods of treating dentin lesions involve the removal of both dentin layers (7). The amount of dentin tissue that must be removed or the number of microorganisms that can be left in the cavity without lesion progression has not been established (8-12). Thus, the elimination of bacteria through application of photodynamic therapy inside the tubules and away from the remaining demineralised dentin (3) might contribute to the development of a more conservative approach to treat deep carious lesions (13).

The antibacterial effect of PDT against oral microorganisms associated with dental caries has been previously shown (7; 14-17). This process achieved high bacterial kill of *Streptococcus mutans* in planktonic cells or biofilms, which demonstrated that PDT is a promising technique (18-22), although the efficacy of PDT has not been shown when applied to multispecies biofilm (17). However, studies of induced decontamination of different substrates are relevant because the penetration of a photosensitiser and light scattering are dependent on the

illuminated medium. Consequently, the effect of PDT on oral microorganisms located in demineralised dentin may be reduced due to decreased penetration depth of the photosensitiser, diminished binding to bacterial cells, or attenuated light penetration for photoactivating the dye (13, 23).

In addition, few studies have investigated the antimicrobial effects of photodynamic therapy on dentin carious tissue (23-26). There are several limitations related to these studies, including the lack of control groups and innovative calculations to present the data (24), as well as long irradiation times (25; 26) that result in difficulties assessing the real effect of photodynamic therapy on dentin tissue and posterior application in clinical settings.

This study aimed to determine the necessary light-emitting diode (LED = 636 nm) parameters in combination with toluidine blue O (TBO) at two energy densities to reduce *Streptococcus mutans* counts in dentin caries.

MATERIALS AND METHODS

Experimental design

For this study, 72 bovine incisors lacking previous lesions were used, and 10 bovine incisors were used for polarized light microscopy analysis.

For microbiological analysis, teeth were randomly allocated using the lottery method to six test groups with 12 experimental units per group. This study involved six set conditions that were denoted as follows: Control (carious dentin exposed to 0.9% NaCl solution for 5 min), TBO (carious dentin exposed to TBO for 5 min), LEDA (carious dentin exposed to LED for 4.2 min with an energy dose of 166 J/cm^2), LEDB (carious dentin exposed to LED for 6.5 min with an energy dose of 249 J/cm^2), PDTA (carious dentin exposed to TBO and LED for 4.2 min with an energy dose of 166 J/cm^2), and PDTB (carious dentin exposed to TBO and LED for 6.5 min with an energy dose of 249 J/cm^2).

UV-vis spectroscopy analysis

Ultraviolet visible (UV–vis) optical absorption spectrometry was performed in TBO solution before and after irradiation using a HP 8453 system spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) to characterise the TBO absorption spectrum and correlate this absorption with LED emission spectrum and TBO photodegradation. The temperature was maintained at 25ºC, and 0.01% TBO (w/v) was diluted with distilled, deionised water (pH 7.2) into a quartz cell with a 1 mm light path. Fractionated irradiation was performed for 5 min, and spectra were obtained at 0, 5, and 10 min. The spectra were analysed with Origin Lab 8.0 software (Origin Lab Corporation, Northampton, MA, USA).

Specimen preparation

Teeth were stored in 0.01% (v/v) thymol solution at 4°C prior to use. From each tooth, one slab of bovine coronal dentin (4 x 4 x 2 mm^3) was obtained using a water-cooled diamond saw and a cutting machine (IsoMet Low Speed Saw, Buehler; Lake Bluff, IL, USA). The buccal dentin face was used in this study, and the remaining slab surfaces were covered with an acid-resistant nail varnish, resulting in a dentin surface area of 16 mm^2 that served as a microbial surface on which carious lesions were produced. The dentin slabs were fixed in the piston of 3-ml syringes that were attached to the lids of glass containers, immersed in sterile distilled water, and then sterilised by gamma radiation (Gammacell 220 Excel, GC-220E, MDS Nordion; Ottawa, Canada) with 14.5 kGy for 30 hours (27).

Microbiological caries model

The microorganism *S. mutans* UA 159 was used a model in these studies (20). To prepare the inoculum, *S. mutans* was first grown in an overnight culture of BHI (Bacto Brain Heart Infusion, BD; Franklin Lakes, NJ, USA) using candle-extinguishing jars with a 5–10% carbon dioxide atmosphere. After sterilization, the dental slabs were removed from distilled water and immersed in sterile BHI containing 5% sucrose (w/v). All BHI-containing recipients were

inoculated with 80 µl of 1-2 x 10^8 CFU/ml overnight cultures of *S. mutans*. A specific optical density was determined using a spectrophotometer and used for all samples to adjust the inoculum to the same cell number. Inoculation of each BHI-containing recipient was performed on the first day, and the dentin specimens were transferred daily to fresh medium for 5 days. Figure 1 is a schematic representation of these stages. In addition, each BHI-containing recipient was streaked daily onto a fresh BHI agar plate that had been incubated at 37°C in an atmosphere of 10% CO_2 for 24 h to check for purity.

After lesion production, 10 dentin slabs were cut with a Series 1,000 Deluxe Silverstone-Taylor hard tissue microtome (Sci Fab; Littleton, CO, USA) in the middle of the exposed dentin window to obtain 200 µm sections. These sections were then polished with 600- and 1200-grit abrasive paper to obtain sections of 100 ± 20 µm thickness. The sections were imbibed with water and observed with a Leica DMLP polarised-light microscope (Leica Microsystems, Wetzlar, Germany) coupled to a Leica FFC 280 digital system. Standard photomicrographs at 10 X magnification were taken.

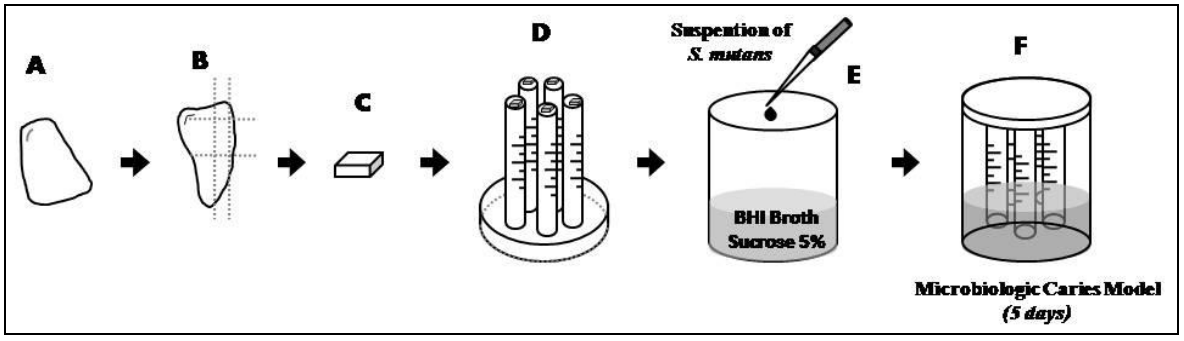


Fig. 1. Schematic diagram illustrating preparation of dentin slabs, and representation of the devices used in the model to produce carious lesions *in vitro*. (**A**) sound bovine incisor; (**B**) cutting the slabs; (**C**) slab preparation; (**D**) fixation of the slabs in the device; (**E**) inoculation of the culture broth; (**F**) microbiological caries model (5 days).

Treatments application

After 5 days, the biofilm formed over the slabs was removed with a #15 scalpel blade, and the carious dentin was exposed. A sample of carious dentin was then collected to obtain a baseline value for tissue contamination, and the treatment for each group was performed. The TBO, PDTA, and PDTB groups were incubated with 5 µl of TBO in the dark for 5 min (pre-irradiation time) without light exposure (28; 29). The control, LEDA, and LEDB groups were incubated with an equal volume of sterile 0.9% NaCl solution instead of TBO during the same period of time.

The photosensitiser TBO (Sigma-Aldrich Company Ltd. Poole, St. Louis, USA) was dissolved in deionized water to obtain a final concentration of 0.1 mg/ml and subsequently kept in the dark. Toluidine blue O (TBO) is a well known blue dye. The light source used was a red light-emitting diode (MMO, São Carlos, SP, Brasil), with a predominant wavelength of 636 nm in the spectrum of emission. A spot with a 3.5 mm cylindrical tip distributed the light. Irradiation was performed in non-contact mode with a diffused beam at a working distance of 3.0 mm. A power meter (Lasermate Coherent ; Santa Clara, CA, USA) was used to measure the peak power, and a maximum output power of 80 mW was determined.

For the LEDA and PDTA groups, slabs were irradiated under stable irradiation power (80 mW) for 4.2 min. Irradiation time was 6.5 min for the LEDB and PDTB groups. Incident energy doses of 166 and 249 J/cm^2 were obtained for groups A and B, respectively. The LED equipment presented no possibility of varying the output power. Figure 2 is a schematic representation of the stages of treatment application and microbiological analysis.

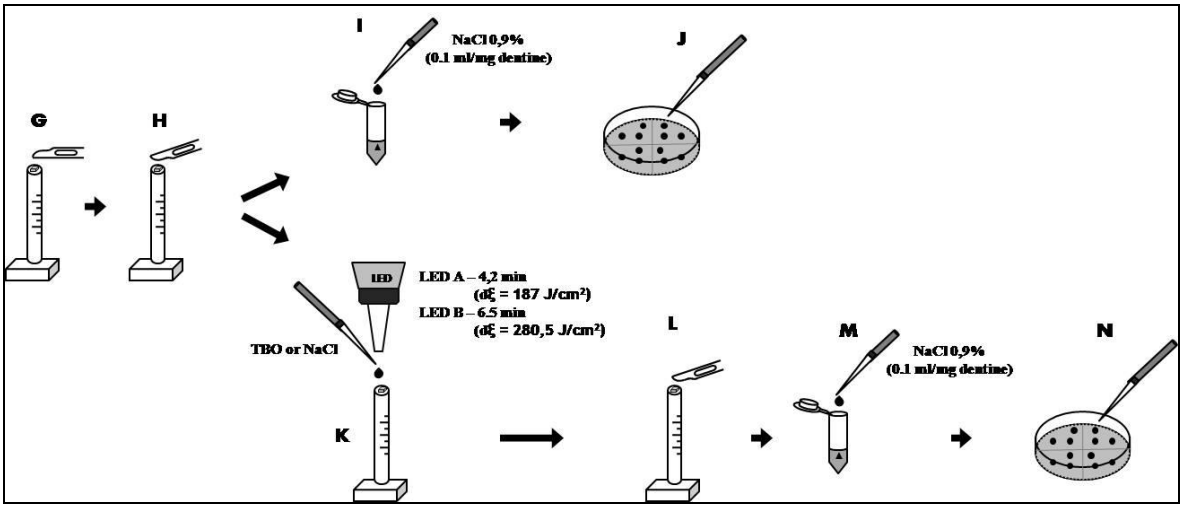


Fig. 2. Schematic diagram illustrating collection of carious dentin, application of treatments, and microbiological analysis. (**G**) biofilm removal; (**H**) baseline sample collection; (**I**) adding NaCl solution to baseline dentin collected and weighed; (**J**) microbiological analysis; (**K**) treatments; (**L**) collection of post-treatment sample; (**M**) adding NaCl solution to post-treatment dentin collected and weighed; (**N**) post-treatment microbiological analysis.

Microbiological analysis

For microbiological analysis before and after the treatments, carious dentin was collected from different portions of each slab using a #15 scalpel blade. The dentin samples were weighed in microcentrifuge tubes, and 0.9% NaCl solution was added to the sample (0.1 ml/mg dentin). The tubes were sonicated for 15 s in an Ultrasonic Processor (Hielscher, UP400S: Ringwood, NJ, USA) to detach the bacterial cells. Subsequently, the suspension was serially diluted (1:10 to 1:100,000) with 0.9% NaCl solution. Samples were plated in triplicate on Mitis Salivarius plus bacitracin (Difco Mitis Salivarius Agar, BD) and incubated for 48 h at 37°C using candle-extinguishing jars under a 5 to 10% carbon dioxide atmosphere. Representative colonies with the typical morphology of *S. mutans* were counted using a colony counter (Phoenix, CP600 Plus; Araraquara, SP, Brasil). The log reduction results were calculated by subtraction of the initial from the final CFU/mg values after being $\log_{10}$ transformed.

Statistical analysis

For assessment of treatment effects, the dependent variable log reduction was analysed, and the equality of variance assumptions (Levene Test) and normal distribution of errors (Shapiro Wilks test) were verified. The data were analysed with one-way ANOVA followed by the Tukey-Kramer test. The significance level was set at 5%. The software BioEstat 5.0 2007 (Instituto de Desenvolvimento Sustentável Mamirauá: Belém, PA, Brasil) was used.

RESULTS

UV-vis Spectroscopy

Figure 3 illustrates the emission spectrum of red LED, and Figure 4 illustrates the aqueous TBO absorption spectrum. A strong absorption band of TBO between 550 to 680 nm and an absorption peak at 632±8 nm were observed. Additionally, Figure 5 shows a decrease in TBO absorption as a function of irradiation time.

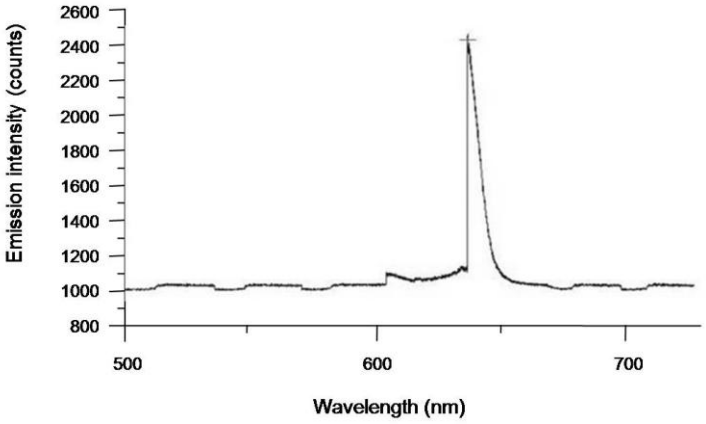


Fig. 3. Emission spectrum of the red LED.

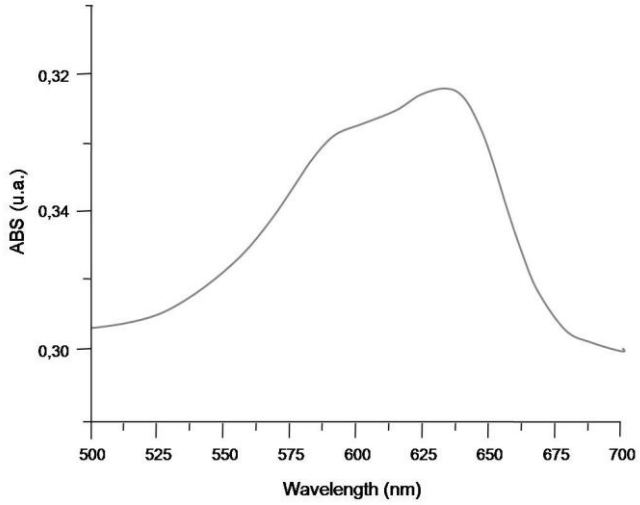


Fig. 4. Absorbance spectrum of the TBO-water solution.

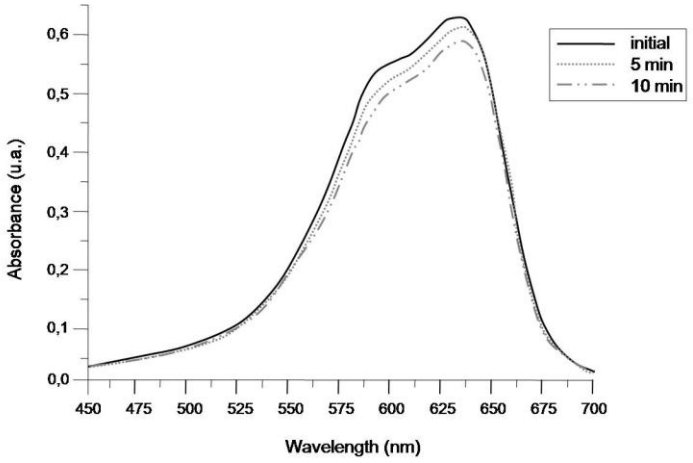


Fig. 5. Absorption spectra after irradiation with the red LED (λ= 636 nm).

Spectroscopic measurements of LED irradiation were collected every 5 minutes.

Microbiological analysis

The weight of dentinal tissue collected from the slabs was 3.19 ± 0.97 mg (mean ± SD).

The mean lesion depth produced by the microbiological model used in this experiment was 253.7 ± 40.66 µm. Figure 6 shows the carious lesion image captured by polarized light microscope.

Fig. 6. Cross section of representative artificial bovine dentin carious lesion in bovine dentin viewed under polarized light in water.

Table 1 shows the log reduction (mean ± standard deviation) achieved for each treatment and the statistical difference among groups. The log reduction values ranged from -1.63 to 2.43. The TBO group presented the lowest value, while the PDTB group exhibited the highest log reduction.

Table 1. Log reduction of *S. mutans* (mean ± standard) achieved by each treatment.

Groups	Log Reduction
Control	-0.04 ± 0.4^{c}
TBO	-0.17 ± 0.76^{c}
LEDA	0.07 ± 0.94^{c}
LEDB	0.29 ± 0.92^{bc}
PDTA	1.17 ± 0.64^{ab}
PDTB	1.12 ± 0.97^{ab}

Means followed by different superscript letters differ significantly (one-way ANOVA and Tukey-Kramer test ,α=0.05).

PDTA and PDTB groups presented statistically significant log reductions compared to the Control, TBO ($p < 0.01$), and LEDA ($p < 0.05$) groups but did not differ from each other. No difference was found among the Control, TBO, and LEDA Groups.

In contrast, LEDB treatment had an effect on bacterial reduction in dentin caries even in the absence of sensitizer, because log reduction data obtained with this group did not statistically differ from the PDTA and PDTB groups.

DISCUSSION

As shown in the UV-vis spectroscopy analyses, there is a match of emission and absorption spectra between LED and TBO that follows the same pattern as the majority of other light sources that generate singlet oxygen. Therefore, the use of an LED source is advantageous considering that the best previous results were obtained using conventional lasers to perform this therapy, although the power output can still be a limiting factor in their widespread application (30; 31). The use of this experimental methodology may result in technological simplification and lower treatment cost in comparison to complex laser systems. Moreover, LED sources provide more than a monochromatic form of irradiation, which is a special characteristic of these light sources compared to lasers that increase the overlay of the spectrum of LED irradiation and the light absorption by TBO. Parameters must still be standardized to perform dentin disinfection by photodynamic therapy using LED sources, which may contribute to a more conservative approach for dealing with deep caries lesions.

According to our results, the PDT at 166 and 249 J/cm^2 (PDTA and PDTB, respectively) was capable of reducing *S. mutans* in carious dentin. These results confirm several studies demonstrating that cariogenic bacteria in culture baths and biofilms are susceptible to PDT (7; 20; 22; 28; 32). However, few studies

have investigated the use of this approach on different substrates. Recent investigations have evaluated the anti-bacterial effectiveness of photodynamic therapy for dentin decay (23-26). Although these studies have demonstrated promising results for using photodynamic therapy to treat dentin, there have been limitations to these investigations, such as the absence of required control groups, utilization of different calculations to verify efficacy (24), and long irradiation times (25; 26).

Our research demonstrated that *S. mutans* hosted in carious bovine dentin is susceptible to photodynamic therapy using TBO and a LED source at energy densities of 166 and 249 J/cm^2. These energy densities were achieved using irradiation times of 4.2 and 6.5 min, respectively, while the previous studies used 10 min to reach 94 J/cm^2 (25) or 15 min to reach 144 J/cm^2 (26). Considering the penetration of the photosensitiser and light scattering into dentin tissue, parameters must be standardised to reduce the irradiation times and increase the energy densities emitted by the LED sources.

The results of the present study agree with those of Giusti *et al*. (24), Lima *et al.* (25), Melo *et al*. (26), and Williams *et al.* (23) which demonstrated an antibacterial effect of PDT against oral bacteria in dentin carious lesions. However, our results showed a lower log reduction of *S. mutans* when compared to recent *in situ* and *in vitro* studies performed with human dentin (25; 26). The decreased effectiveness of PDT in the present study might be due to the different methods used for dentine collection because a scalpel blade was used to collect dentinal tissue, while previous studies used carbide burs in a low-speed drill. Accordingly, a deeper dentine layer may have been removed in this study, which would be less contaminated with bacteria compared to the outer layer removed by Lima *et al.* (25) or Melo *et al*. (26).

Log reduction analysis was performed to compare the efficacy of photodynamic therapy in reducing *S. mutans* counts among different treated groups and confirmed that a treatment of 6.5 min irradiation (LEDB group) was able to reduce *S. mutans* in carious dentin. This photocytotoxicity effect may be

partially explained by the fact that bacteria hosted in dentin were not protected from dryness by the presence of the polysaccharide matrix and higher amounts of water. These results agree with those found in previous studies (25; 26) because a cytotoxic light effect was also found when the LED was used without TBO at 94 (25; 26) or 144 J/cm^2 (26), which demonstrates that the isolated effect of red light on *S. mutans* should be further investigated.

A surprising result of the current study was that the increase in energy density did not exhibit any beneficial effect in reducing the S. *mutans* count after PDT; no numerical or statically significant difference was found between the PDTA and PDTB groups. These results are supported by a recent *in situ* study (25). Giusti *et al.* (24) found a higher level of *S. mutans* inhibition under the highest energy density. However, the latter study was performed using 48 J/cm^2. Therefore, PDT in the present study may have achieved a threshold antibacterial effect because very high energy densities were used. Further investigation must be performed considering that the light source and the photosensitiser were applied under the same characteristics for the two groups. Consequently, only the irradiation time changed the energy densities. The *number of S. mutans* CFU before irradiation was approximately 2.43 x 10^7. Although PDT reduced the bacterial number, the CFU still remained high, as the caries formation process could not be controlled.

CONCLUSION

The use of LED combined with TBO was effective in reducing the Sm counts in carious dentin, but this effect may not have clinical significance.

REFERENCES

1. Castano AP, Demidova TN and Hamblin MR. Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. Photodiagn Photodyn Ther 2004:1:279–293.
2. Akilov OE, O'Riordan K, Kosaka S and Hasan T. Photodynamic therapy against intracellular pathogens: problems and potentials. Med Laser Appl 2006:21:251–260.
3. Kidd EA, Joyston-Bechal S. In: Essentials of dental caries: the disease and its management. Oxford: Oxford University Press, 1997:73-77.
4. Massler M. Changing concepts in the treatment of carious lesions. Br Dent J 1967:123:547-548.
5. Magnusson BO, Sundell SO. Stepwise excavation of deep carious lesions in primary molars. J Int Ass Child 1977:8:36-40.
6. Leksell E, Ridell K, Cvek M, Mejáre I. Pulp exposure after stepwise excavation of deep carious lesions in young posterior permanent teeth. Endod Dent Traumatol 1994:12:192-96.
7. Burns T, Wilson M, Pearson G J. Killing of cariogenic bacteria by light from gallium arsenide diode laser. J Dent 1994:22(5):273-8.
8. Bjørndal L, Larsen T, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res 1997:31:411–7.

9. Jeronimus D J, Till M J, Sveen O B. Reduced viability of microorganisms under dental sealants. J Dent Child 1975:42:275–80.
10. Going RE, Loesche WJ, Grainger DA, Syed SA. The viability of microorganisms in carious lesions five years after covering with a issure sealant. J Am Dent Assoc 1978:97:455–62.
11. Weerheijm KL, de Soet JJ, van Amerongen WE, de Graaff J. The effect of glass-ionomer cement on carious dentine: an in vivo study. Caries Res 1993:27:417–23.
12. Eidelman E. Intentional sealing of occlusal dentin caries: a controversial issue. Pediatric Dent 1993:15:321.
13. Burns T, Wilson M, Pearson GJ. Effect of dentine and collagen on the lethal photosensitization of Streptococcus mutans. Caries Res 1995:29(3):192-7.
14. Dobson J, Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser. Archs Oral Biol 1992:37(11):883-7.
15. Stringer GJ, Bird PS, Walsh LJ. Lethal laser photosensitization of Streptococcus mutans with a visible red diode laser. Aust Dent J 2000:45:94-99.
16. Zanin IC, Brugnera Jr A, Gonçalves RB. In vitro study of bactericidal effect of low level laser therapy in the presence of photosensitizer on cariogenic bacteria. Proceedings of SPIE 2002:Lasers in Dentistry VIII:4610:154–161.
17. Müller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur J Oral Sci 2007:115:77–80.

18. Williams JA, Pearson GJ, Colles MJ, Wilson M. The effect of variable energy input from a novel light source on the photoactivated bactericidal action of toluidine blue O on Streptococcus mutans. Caries Res 2003:37:190–193.
19. Paulino TP, Ribeiro KF, Thedei Jr G, Tedesco AC, Ciancaglini P. Use of hand held photopolymerizer to photoinactivate Streptococcus mutans. Arch Oral Biol 2005:50:353–359.
20. Zanin IC, Lobo MM, Rodrigues LK, Pimenta LA, Hofling JF, Gonçalves RB. Photosensitization of in vitro biofilms by toluidine blue O combined with a light-emitting diode. Eur J Oral Sci 2006:114:64–69.
21. Bevilacqua IM, Nicolau RA, Khouri S, Brugnera Jr A, Teodoro GR, Zângaro RA, Pacheco MT. The impact of photodynamic therapy on the viability of Streptococcus mutans in a planktonic culture. Photomed Laser Surg 2007:25:513–518.
22. Konopka K, Goslinski T. Photodynamic Therapy in Dentistry. J Dent Res 2007:86(8):694-707.
23. Williams JA, Pearson GJ, Colles MJ, Wilson M. The photo-activated antibacterial action of toluidine blue O in a collagen matrix and in carious dentine. Caries Res 2004:38:530–536.
24. Giusti JS, Santos-Pinto L,Pizzolito AC, Helmerson K, Carvalho-Filho E, Kurachi C, Bagnato VC. Antimicrobial photodynamic action on dentine using a light-emitting diode light source. Photomed Laser Surg 2008:26:279–285.
25. Lima JP, Sampaio de Melo MA, Borges FM, Teixeira AH, Steiner-Oliveira C, Nobre Dos Santos M, Rodrigues LK, Zanin IC. Evaluation of the

antimicrobial effect of photodynamic antimicrobial therapy in an in situ model of dentin caries. Eur J Oral Sci 2009:117(5):568-74.

26. Melo MAS, de-Paula DM, Lima JPM, Borges FMC, Steiner-Oliveira C, Nobre-dos-Santos M, Zanin ICJ, Barros EB, Rodrigues LKA. In vitro photodynamic antimicrobial chemotherapy in dentin contaminated by cariogenic bacteria. Laser Physics 2010:(6) (accepted).
27. Rodrigues LKA, Cury JA, Nobre-dos-Santos M. The effect of gamma radiation on enamel hardness and its resistance to demineralization in vitro. J Oral Sci 2004:46(4):215-220.
28. O'Neill JF, Hope C, Wilson M. Oral bacteria in multi-species biofilms can be killed by red light in the presence of toluidine blue. Lasers Surg Med 2002:31:86–90.
29. Zanin IC, Goncalves RB, Brugnera Jr A, Hope CK, Pratten J. Susceptibility of Streptococcus mutans biofilms to photodynamic therapy: an in vitro study. J Antimicrob Chemother 2005:56:324–330.
30. Brancaleon L, Moseley H. Lasers and non-lasers light sources for photodynamic therapy. Lasers Med Sci 2002:17:173-186.
31. O'Neill JF, Wilson M, Wainwright M. Comparative antistreptococcal activity of photobactericidal agents. J Chemother 2003:15:329–334.
32. Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. J Antimicrob Chemother 2006:57(4):680-4.

REFERÊNCIAS *

Besic FC. The fate of bacteria sealed in dental caries. J Dent Res. 1943; 22: 349–54.

Bhatti M, MacRobert A, Meghji S, Henderson B, Wilson M. Effect of dosimetric and physiological factors on the lethal photosensitization of Porphyromonas gingivalis *in vitro*. Photochem Photobiol. 1997; 65(6): 1026-31.

Bjørndal L, Larsen T, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res. 1997; 31: 411–7.

Burns T, Wilson M, Pearson GJ. Effect of dentin and collagen on the lethal photosensitization of Streptococcus mutans. Caries Res. 1995; 29(3): 192-7.

Burns T, Wilson M, Pearson GJ. Killing of cariogenic bacteria by light from gallium arsenide diode laser. J Dent. 1994 Oct; 22(5): 273-8.

Chabrier-Roselló Y, Foster TH, Pérez-Nazario N, Mitra S, Haidaris CG. Sensitivity of Candida albicans germ tubes and biofilms to photofrin-mediated phototoxicity. Antimicrobial Agents and chemotherapy. 2005; 49(10): 4288-95.

Dobson J, Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser. Archs Oral Biol. 1992; 37(11): 883-7.

Eidelman E. Intentional sealing of occlusal dentin caries: a controversial issue. Pediatric Dent. 1993; 15: 321.

Giusti JS, Santos-Pinto L, Pizzolito AC, Helmerson K, Carvalho-Filho E, Kurachi C, Bagnato VC. Antimicrobial photodynamic action on dentin using a light-emitting diode light source. Photomed Laser Surg. 2008; 26: 279–285.

---

* De acordo com a norma da UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

Going RE, Loesche WJ, Grainger DA, Syed SA. The viability of microorganisms in carious lesions five years after covering with a issure sealant. J Am Dent Assoc. 1978; 97: 455–62.

Griffiths MA, Wren BW, Wilson M. Killing of methicillin-resistant Staphylococcus aureus *in vitro* using aluminium disulphonated phthalocyanine, a light-activated antimicrobial agent. J Antimicrobial Chemother. 1997; 40(6): 873-876.

Jeronimus DJ, Till MJ, Sveen OB. Reduced viability of microorganisms under dental sealants. J Dent Child. 1975; 42: 275–80.

Kidd EAM, Bechal JS. Essentials of dental caries: the disease and its management. Bristol Wright, 1987.

Kreulen CM, de Soet JJ, Weerheijm KL, van Amerongen WE. In vivo cariostatic effect of resin modified glass ionomer cement and amalgam on dentin. Caries Res. 1997; 31: 384–9.

Leksell E, Ridell K, Cvek M, Mejáre I. Pulp exposure after stepwise excavation of deep carious lesions in young posterior permanent teeth. Endod Dent Traumatol. 1994; 12: 192-96.

Lima JP, Sampaio de Melo MA, Borges FM, Teixeira AH, Steiner-Oliveira C, Nobre Dos Santos M, Rodrigues LK, Zanin IC. Evaluation of the antimicrobial effect of photodynamic antimicrobial therapy in an *in situ* model of dentin caries. Eur J Oral Sci. 2009; 117(5): 568-74.

Macrobert AJ, Bown SG, Phillips D. Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use. Chichester: Wiley; 1989. p. 4-16.

Magnusson BO, Sundell SO. Stepwise excavation of deep carious lesions in primary molars. J Int Ass Child. 1977; 8: 36-40.

Malik Z, Hanania J, Nitzan Y. Bactericidal effects of photoactivated porphyrins - an alternative approach to antimicrobial drugs. J Photochern Photobiol. 1990; 5(3-4): 281-93.

Massler M. Changing concepts in the treatment of carious lesions. Br Dent J. 1967; 123: 547-548.

Melo MAS, de-Paula DM, Lima JPM, Borges FMC, Steiner-Oliveira C, Nobre-dos-Santos M, Zanin ICJ, Barros EB, Rodrigues LKA. In vitro photodynamic antimicrobial chemotherapy in dentin contaminated by cariogenic bacteria. Laser Physics; no prelo 2010.

O'Neill JF, Hope CK, Wilson M. Oral bacteria in multi-species biofilms can be killed by red light in the presence of toluidine blue. Lasers Surg Med. 2002; 31(2): 86-90.

Spikes JD, Jori G. Photodynamic therapy of tumours and others diseases using porphyrins. Lasers in Medical Science. 1987; 2: 3-15.

Weerheijm KL, de Soet JJ, van Amerongen WE, de Graaff J. The effect of glass-ionomer cement on carious dentin: an in vivo study. Caries Res. 1993; 27: 417–23.

Weerheijm Kl, Groen HJ. The residual caries dilemma. Community Dent Oral Epidemiol. 1999; 27(6): 436-41.

Wei SH, Kaqueller JC, Massler M. Remineralization of carious dentin. J Dent Res. 1968; 47: 381-391.

Williams JA, Pearson GJ, Colles MJ, Wilson M. The photo-activated antibacterial action of toluidine blue O in a collagen matrix and in carious dentin. Caries Res. 2004; 38: 530–6.

Wilson M, Dobson J, Harvey W. Sensitization of oral bacteria to killing by low-power laser radiation. Current Microbiol. 1992; 25(2): 77-81.

Wilson M, Mia N. Sensitisation of Candida albicans to killing by low-power laser light. J Oral Pathol Med. 1993; 22(8): 354-357.

Wilson M, Yianni C. Killing of methicillin-resistant Staphylococcus aureus by low-power laser light. Antimicrobial agents. 1995; 42(1): 62-66.

Wilson M, Yianni C. Killing of methicillin-resistant Staphylococcus aureus by low-power laser light. Antimicrobial agents. 1995; 42(1): 62-66.

Wilson M, Burns T, Pratten J, Pearson GJ. Bacteria in supragingival plaque samples can be killed by low-power laser light in the presence of a photosensitizer. J Appl Bacteriol. 1995 ;78(5): 569-574.

Wood S, Nattress B, Kirkham J, Shore R, Brookes S, Griffiths J, Robinson C. An *in vitro* study of the use of photodynamic therapy for the treatment of natural oral plaque biofilmes formed in vivo. J Photochem Photobiol B. 1999; 50(1): 1-7.

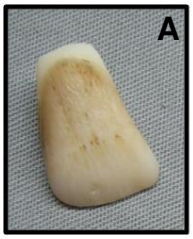
Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. J Antimicrob Chemother. 2006; 57(4): 680-4.

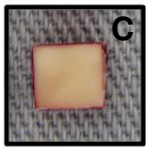
Zanin IC, Lobo MM, Rodrigues LK, Pimenta LA, Hofling JF, Gonçalves RB. Photosensitization of *in vitro* biofilms by toluidine blue O combined with a light-emitting diode. Eur J Oral Sci. 2006; 114(1): 64-9.

Zanin ICJ, Brugnera Jr A, Gonçalves RB. Antimicrobial activity of low level laser in presence of photosensitizer. Journal Dental Research. 2002; 81:446.

Zanin ICJ, Goncalves RB, Brugnera-Jr A, Hope CK, Pratten J. Susceptibility of Streptococcus mutans biofilms to photodynamic therapy: an *in vitro* study. J Antimicrob Chemother. 2005; 56(2): 324-330.

APÊNDICE





A - Coroa de incisivo bovino hígido

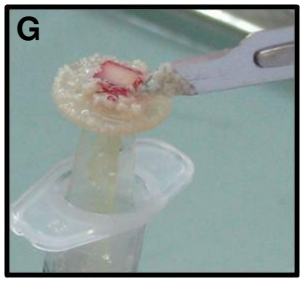
B - Corte dos espécimes

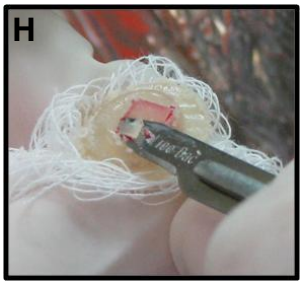
C - Espécimes com dimensões de 4x4x2 mm^2

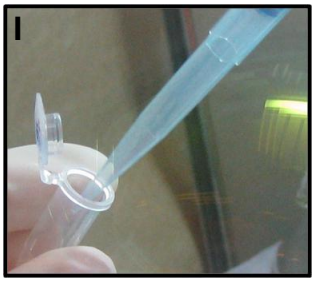
D - Fixação dos espécimes ao dispositivo

E - Modelo microbiológico de cárie composto por 80 µL de cultura de S. *mutans,* BHI caldo e sacarose a 5%, dentro do qual os espécimes foram mantidos durante 5 dias.

F - LED vermelho (λ = 636 nm) utilizado para irradiar os espécimes





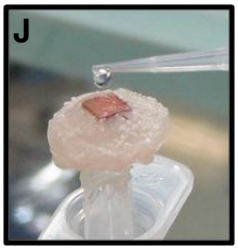


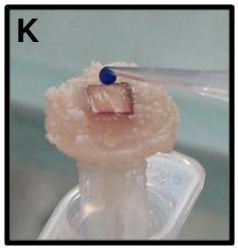
G - Remoção do biofilme sobre os espécimes

H - Coleta do tecido cariado

I - Adição de solução salina na concentração de 0,1 mL/mg de dentina cariada

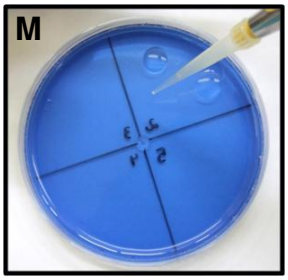
APÊNDICE





J - Aplicação de 5 µL de NaCl (0,9%)
K - Aplicação de 5 µL de TBO (0,01%)

L - Dispositivo utilizado para manter as amostras tratadas com TBO em ambiente protegido da luz durante 5 min (período de pré-irradiação)



M - Semeadura de alíquotas de 25 µL das culturas contendo S. mutans, em triplicata, em MSB (Mitis Salivarius Bacitracina).

N - Jarra de anaerobiose que permitia o crescimento bacteriano em ambiente de microaerofilia.

ANEXO

Journal of ORAL LASER APPLICATIONS

<< Overview | Log out

Download/view manuscript
Pdf manuscript file not yet uploaded by the editorial office

Progress

Progress Report

Help for this page

Manuscript title: Photodynamic therapy effect in bovine carious dentin – In vitro study
Manuscript type: Original Article
All Authors: Marinês Nobre-dos-Santos, Lidiany Karla Azevedo Rodrigues, Mary Anne Sampaio Melo, Marcela Pinto Monteiro-Oliveira,

Status: **New Submission**
Submission number: 1
Date Received: 2010-01-20
Weeks under review: 0
Requests sent: 0
Reviewers agreed: 0
Reviews completed: 0

Top

- Quintessenz Verlags-GmbH - Komturstr. 18 - Berlin - 12099 - Germany
E-mail: wintonowycz@quintessenz.de - Tel: +49 (0)30 761 80 617

Powered by Manuscript Manager