



UNESP - Universidade Estadual Paulista
“Júlio de Mesquita Filho”
Faculdade de Odontologia de Araraquara



Eran Nair Mesquita de Almeida

**Avaliação *in vitro* dos efeitos do clareamento dentário com
fotossensibilização em dentes não vitais**

Araraquara

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Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia de Araraquara para obtenção do título de Doutora em Ciências Odontológicas, na Área de Dentística Restauradora

Orientador: Prof. Dr. Marcelo Ferrarezi de Andrade

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Eran Nair Mesquita de Almeida

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fotossensibilização em dentes não vitais**

Comissão julgadora

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Dedico este trabalho aos meus país e em especial à minha avó, Iracema Figueiredo de Almeida, pelo amor e apoio incondicional.

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“Que a força e benção de cada geração alcancem sempre e inundem a geração seguinte.”
Bert Hellinger*

* Hellinger B. Oração aos antepassados.

Almeida ENM. Avaliação *in vitro* dos efeitos do clareamento dentário com fotossensibilização em dentes não vitais [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2023.

RESUMO

O objetivo deste estudo *in vitro* foi avaliar os efeitos da técnica de clareamento utilizando Led Violeta sobre a resistência à fratura, formação da camada híbrida, microdureza e estabilidade de cor em diferentes períodos de tempo (T1: 7 dias, T2: 14 dias, T3: 21 dias, T30: 30 dias e T9M: 9 meses após a última sessão clareadora). Para avaliação da resistência a fratura e formação da camada híbrida os grupos foram: C: Sem tratamento (grupo controle); HP: Whiteness HP Maxx 35% (FGM); HP-BL: Whiteness HP Maxx 35% (FGM) + Led Azul Twin Flex Evolution (MMOptics); HP-VL: Whiteness HP Maxx 35% (FGM) + Led Violeta – Bright Max Whitening (MMOptics). Para o teste de microdureza e cor, diferentes protocolos foram testados: C: Sem tratamento (grupo controle); HP: Whiteness HP 35% (FGM); HP-BL: Whiteness HP 35% (FGM) + Led Azul Twin Flex Evolution (MMOptics); HP-VL: Whiteness HP 35% + Led Violeta – Bright Max Whitening (MMOptics); VL: Led Violeta – Bright Max Whitening (MMOptics), que foi adicionado para avaliação de cor. Na resistência a fratura foram utilizados (N = 40) espécimes, formação da camada híbrida (N = 20), microdureza (N = 40) e estabilidade de cor (N = 50). Os protocolos clareadores foram aplicados de acordo com a recomendação dos fabricantes dos produtos e dos equipamentos, totalizando 3 sessões de clareamento, com intervalo de 7 dias entre elas. Os espécimes permaneceram em saliva artificial a 37°C durante todo o período referente aos experimentos, sendo substituída a cada 7 dias. Os dados obtidos foram analisados por análise de variância (ANOVA one-way, teste de kruskal-Wallis e ANOVA two-way, respectivamente) e pós teste ao nível de 5% de significância. Não foram encontradas diferenças significativas entre os protocolos de clareamento quanto ao efeito sobre a resistência à fratura ($p > 0,05$). Na avaliação da formação camada híbrida, todos os grupos com HP interferiram em algum nível. Na microdureza HP e C demonstraram respectivamente a maior e menor redução da microdureza dentinária após o uso dos protocolos de clareamento dental ($p < 0.05$). No entanto, não houve diferença entre os protocolos HP-BL e HP-VL ($p > 0.05$). Na avaliação de cor os valores médios (DP) de ΔE e ΔWID , os grupos HP, HP BL e HP VL apresentam maior alteração de cor quando comparados a C e VL. HP, HP BL e HP VL não apresentam diferenças significativas entre si após 9 meses da realização do protocolo de clareamento.

Palavras chave: Clareamento dental. Dente não vital. Peróxido de hidrogênio.

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ABSTRACT

The objective of this in vitro study was to evaluate the effects of the bleaching technique using Led Violet on the resistance to fracture, formation of the hybrid layer, microhardness and color stability in different periods of time (T1: 7 days, T2: 14 days, T3: 21 days, T30: 30 days and T9M: 9 months after the last whitening session). For the evaluation of resistance to fracture and formation of the hybrid layer, the groups were: C: No treatment (control group); HP: Whiteness HP Maxx 35% (FGM); HP-BL: Whiteness HP Maxx 35% (FGM) + Twin Flex Evolution Blue Led (MMOptics); HP-VL: Whiteness HP Maxx 35% (FGM) + Violet Led – Bright Max Whitening (MMOptics). For the microhardness and color test, different protocols were tested: C: No treatment (control group); HP: Whiteness HP 35% (FGM); HP-BL: Whiteness HP 35% (FGM) + Twin Flex Evolution Blue Led (MMOptics); HP-VL: Whiteness HP 35% + Violet Led – Bright Max Whitening (MMOptics); VL: Led Violet – Bright Max Whitening (MMOptics), which was added for color evaluation. The fracture resistance (N = 40) specimens, hybrid layer formation (N = 20), microhardness (N = 40) and color stability (N = 50) were used. The bleaching protocols were applied according to the recommendations of the product and equipment manufacturers, totaling 3 bleaching sessions, with an interval of 7 days between them. The specimens remained in artificial saliva at 37°C throughout the period referring to the experiments, being replaced every 7 days. The data obtained were analyzed by analysis of variance (one-way ANOVA, Kruskal-Wallis test and two-way ANOVA, respectively) and post-test at a 5% significance level. No significant differences were found between bleaching protocols regarding the effect on fracture toughness ($p > 0.05$). In evaluating the hybrid layer formation, all groups with HP interfered at some level. In terms of microhardness, HP and C showed, respectively, the greatest and smallest reduction in dentin microhardness after the use of dental bleaching protocols ($p < 0.05$). However, there was no difference between the HP-BL and HP-VL protocols ($p > 0.05$). In the color evaluation, the mean values (SD) of ΔE and ΔWID , the HP, HP BL and HP VL groups present greater color change when compared to C and VL. HP, HP BL and HP VL do not present significant differences among themselves after 9 months of the bleaching protocol.

Keywords: Tooth bleaching. Tooth, nonvital. Hydrogen peroxide.

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1 INTRODUÇÃO

A alteração da cor dos dentes é uma preocupação constante entre os pacientes, podendo afetar a autoestima e o convívio social. O processo de escurecimento dentário em dentes não vitais ocorre geralmente devido a trauma na coroa dentária e a necrose pulpar, onde o extravasamento do sangue proveniente da polpa é responsável pelo manchamento progressivo da estrutura. Assim, quanto menor o tempo de exposição ao agente pigmentante e menor grau de escurecimento, maior a probabilidade de sucesso no tratamento clareador¹⁻⁴.

Para o clareamento de dentes não vitais preconiza-se a técnica imediata. Essa técnica é realizada apenas em consultório, e consiste na aplicação de géis clareadores em altas concentrações como o peróxido de hidrogênio (PH) 35% que é posicionado na câmara pulpar e face vestibular do dente a ser clareado. Pode-se associar a utilização de uma fonte de energia LED azul e laser, que promove a aceleração na reação clareadora, proporcionando assim ganho no tempo de realização do procedimento^{5,6}.

O (PH) tem a capacidade de liberar substâncias altamente reativas com o poder de penetrar no esmalte e na dentina, possibilitando que cadeias moleculares longas de pigmento sejam segmentação, gerando cadeias moleculares menores e cromaticamente menos escurecidas⁷. Esta reação química que ocorre quando utilizado um gel clareador é conhecida como oxirredução^{8,9}.

No que se refere aos riscos associados a técnica clareadora em dentes não vitais, o mais preocupante é a reabsorção radicular externa. Este tipo de reabsorção ocorre na região cervical da coroa dentária. Para evitar este dano é essencial a realização do tampão com material ionômero na região cervical intracoronal¹⁰.

Embora a técnica de clareamento dentário seja uma alternativa conservadora, menos dispendiosa e eficaz, relatos na literatura associam os protocolos utilizados a possíveis alterações no dente, como redução da microdureza o que pode influenciar na resistência à fratura. Ainda neste contexto, é importante ressaltar que o oxigênio residual proveniente do gel clareador continua na estrutura dentária após a última sessão, o que causa a diminuição na resistência adesiva imediatamente após aos protocolos clareadores. Desta forma, preconiza-se aguardar de 7 a 14 dias para realização de futuras restaurações¹¹⁻¹⁴.

Recentemente, surgiu o clareamento com fonte de luz violeta, tratando-se de uma luz visível e não ionizante. Sua ação ocorre por mecanismos físicos, possibilitando a quebra das moléculas de pigmento dos dentes¹⁵⁻¹⁷. A técnica para dentes não vitais deve ser realizada em consultório, podendo associar à utilização de gel clareador à base de peróxido de hidrogênio (PH), dessa maneira buscando a longevidade na estabilidade da cor. O grande diferencial é que a luz pode ser utilizada isoladamente, mesmo com ausência do gel clareador, o que a torna uma tecnologia inovadora^{18,19}.

Considerando a ausência de estudos sobre a técnica com a utilização de luz violeta como alternativa clareadora em dentes não vitais, torna-se importante a realização de pesquisas que avaliem seu potencial clareador e seu efeito sobre diferentes características da estrutura dentária.

2 PROPOSIÇÃO

Comparar o efeito e a longevidade de diferentes protocolos de clareamento dental para dentes despolpados.

2.1 Objetivos Específicos

Avaliação da resistência à fratura, após término dos protocolos para as diferentes técnicas de clareamento;

Avaliação da formação da camada híbrida, após término dos protocolos para as diferentes técnicas de clareamento;

Avaliação da microdureza dentinária, após término dos protocolos para as diferentes técnicas de clareamento;

Avaliação da efetividade na alteração de cor, 7 dias após cada sessão de clareamento dentário;

Avaliação da longevidade na alteração de cor, 30 dias e 9 meses após término dos protocolos de clareamento dentário.

3 PUBLICAÇÕES

A partir desta pesquisa de doutorado produzimos três artigos que são apresentados a seguir.

3.1 Publicação 1*

Efeito do protocolo de clareamento com LED violeta sobre a resistência a fratura e formação da camada híbrida de dentes tratados endodonticamente

Resumo

Objetivo: Avaliar *in vitro* o efeito do protocolo de clareamento com LED violeta associado a alta concentração de peróxido de hidrogênio (HP) sobre a resistência a fratura e formação da camada híbrida.

Métodos: Quarenta dentes bovinos foram tratados endodonticamente e aleatorizados em quatro grupos (n=10): C - Controle, HP - Peróxido de hidrogênio 35%, HP-BL - Peróxido de hidrogênio 35%, + blue LED, HP-VL - Peróxido de hidrogênio 35%, + violet LED. Foram realizadas 3 sessões de clareamento dental com intervalo de 7 dias entre elas. As coroas foram restauradas 10 dias após a última sessão e submetidas ao teste de resistência a fratura. Cinco espécimes de cada grupo foram preparados para avaliação da formação da camada híbrida por meio de imagens de microscopia eletrônica de varredura (MEV). Os resultados de resistência a fratura foram obtidos a partir dos testes de ANOVA one-way e os de MEV a partir do teste Kruskal-Wallis considerando o nível de significância de 5%.

Resultados: Não houve diferença significativa no teste de resistência a fratura ($p > 0,05$). HP e HP-BL apresentaram maior alteração na formação da camada híbrida que o grupo C ($p < 0,05$). HP-VL foi similar aos grupos C, HP e HP-BL ($p > 0,05$).

* O artigo segue as normas do periódico *Photodiagnosis and Photodynamic Therapy* ao qual será submetido.

Conclusão: Não foram observados efeitos na redução da resistência a fratura para todos os grupos avaliados. Independente do protocolo realizado, a presença de HP interferiu em algum nível na formação da camada híbrida quando realizada restauração 10 dias após a última sessão de clareamento.

Palavras-Chave: Clareamento dental; Peróxido de hidrogênio; Endodontia; Fratura; LED violeta.

Introdução

O clareamento dental é um tratamento estético conservador, eficaz e que demanda menor tempo clínico [1]. Em dentes tratados endodonticamente, pode ser utilizada a técnica interna e externa, onde o componente mais utilizado é o gel de peróxido de hidrogênio (HP)[2–4]. O HP atua através de mecanismos químicos, onde há liberação de substâncias altamente reativas chamadas de radicais livres, que são capazes de penetrar na estrutura dental e quebrar as moléculas de pigmento dos dentes [1,5].

A técnica de clareamento com HP pode ser associada a utilização de fontes de luz. O LED violeta (405 ± 10 nm) surgiu como uma tecnologia inovadora que promove o clareamento através de mecanismos físicos, diferentemente do LED azul (450 ± 10 nm), que é capaz de acelerar o processo clareador apenas na presença de um gel clareador [6]. Segundo Panhoca et al. [7], a luz violeta utilizada isoladamente pode produzir energia suficiente para quebrar os pigmentos no esmalte. Klaric et al. [8], afirma que ela tem a capacidade de quebrar as moléculas de pigmento da dentina, que por serem fotorreceptoras, são reduzidas sem causar prejuízos térmicos ao dente.

Em revisão sistemática realizada por Rossi et al. [9] os autores relatam que o LED violeta utilizado sozinho parece ter potencial para clarear os dentes, embora com efeito menor

quando comparado a utilização de peróxidos. O HP em alta concentração associado a luz violeta parece potencializar o efeito do clareamento. Infelizmente, a utilização de altas concentrações de HP sozinho já foi associada a alterações na estrutura dental, como alteração da microdureza, morfologia da superfície, composição química e formação da camada híbrida [8,10].

Com base na literatura correlata, segue-se em busca de novos protocolos que mantenham a eficácia clareadora dos géis de alta concentração (padrão ouro) e reduzam possíveis efeitos adversos. Sabe-se que a dentina é a maior parte da estrutura dentária e qualquer alteração em suas propriedades biomecânicas causa impacto na resistência dental [11–13]. Além disso, a formação de uma camada híbrida íntegra e estável é fundamental para propiciar uma adesão satisfatória ao longo do tempo [14,15]. No entanto, ainda não se sabe como o LED violeta associado ao HP age sobre a resistência e formação da camada híbrida.

Portanto, o presente estudo teve como objetivo avaliar os efeitos de protocolos de clareamento dental utilizando HP 35% associado ou não a irradiação com LED violeta ou LED azul sobre a resistência à fratura e formação da camada híbrida de dentes tratados endodonticamente. A hipótese nula testada foi que os protocolos clareadores não influenciam na resistência à fratura e formação da camada híbrida.

Materiais e Métodos

Preparação dos Espécimes

Inicialmente o projeto foi submetido à Comissão de Ética em Pesquisa em Animais. Devido à perda de dentes no momento da seleção dos espécimes que fariam parte da pesquisa, foram solicitados (n = 80) dentes bovinos. Os dentes foram armazenados em solução de timol 1%, a 4°C. No momento do uso, foram lavados em água corrente por 12 horas. Primeiro os dentes foram polidos com pasta de pedra-pomes mais água com auxílio de escova Robinson em

baixa rotação. Imediatamente após foram avaliados com lupa estereoscópica, e somente fizeram parte aqueles que não apresentaram trincas, fraturas ou qualquer desgaste na face vestibular.

Avaliação da resistência a fratura

Foi realizado acesso coronário com ponta diamantada esférica 1014 e 1016 (KG Sorensen, São Paulo, Brasil) em quarenta dentes incisivos bovinos e preparo químico e mecânico para obturação do canal radicular e restauração provisória segundo técnica descrita por Garrido et al. [16].

As raízes de todos os espécimes foram incluídas em um plástico matriz (largura interna de 16,5 mm x comprimento de 20,0 mm) com resina acrílica autopolimerizável (Classic Jet, São Paulo, SP, Brasil) até a junção cimento- esmalte, de acordo com adaptação da técnica proposta por Kuga et al [12].

Os espécimes foram deixados intocados por 24 horas até a polimerização da resina estar completa. Então, foi realizada a remoção de 3 mm de guta-percha e confeccionado o tampão cervical com um ionômero de vidro autopolimerizável (Vidrion R; SS White, Rio de Janeiro, RJ, Brasil) que foi colocado como uma barreira cervical até a junção cimento - esmalte. Em seguida, um pellet de algodão foi colocado na câmara pulpar e a cavidade foi vedada com material restaurador temporário (RT) (Villevie; Dentalville do Brasil Ltda., Joinville, SC, Brasil).

Na sessão seguinte, os dentes então foram aleatorizados em quatro grupos (n= 10). Os materiais utilizados estão descritos na tabela 1 e os protocolos clareadores dos grupos seguem abaixo:

Tabela 1. Grupos investigados, protocolos de clareamento e marcas comerciais utilizadas neste estudo.

Groups	Protocolo de clareamento (marca registrada, fabricante, endereço)
C	Grupo controle
HP	Peróxido de hidrogênio 35% (Whiteness HP MAXX- FGM, Joinville, SC, Brazil)
HP-BL	Peróxido de hidrogênio 35% (Whiteness HP MAXX- FGM, Joinville, SC, Brazil) + blue LED (Twin Flex Evolution - MMOptics, São Carlos, SP, Brazil)
HP-VL	Peróxido de hidrogênio 35% (Whiteness HP MAXX - FGM, Joinville, SC, Brazil) + violet LED (Bright Max Whitening - MMOptics, São Carlos, SP, Brazil)

- C: Sem protocolo de clareamento. Os espécimes foram armazenados em água destilada a 37°C. O meio foi trocado a cada 7 dias.
- HP: A manipulação do gel Whiteness HP MAXX (FGM, Joinville, SC, Brasil) foi realizada de acordo com as recomendações do fabricante. A proporção 3:1 de HP e espessante foi manipulada por 10 segundos até sua homogeneização. Em seguida, uma camada de aproximadamente 1 mm de espessura do gel foi aplicada. Foram realizadas duas aplicações de 15 min, totalizando 30 min.
- HP-BL: A manipulação do gel Whiteness HP MAXX (FGM, Joinville, SC, Brasil) foi realizada como descrita anteriormente e o gel foi fotoativado por LED azul (Twin Flex Evolution - MMOptics, São Carlos, SP, Brasil) por 9 minutos, de acordo com as instruções do fabricante. Duas aplicações de gel clareador e irradiação com LED azul foram realizadas em cada sessão. Cada sessão teve 18 min de duração.
- HP-VL: A manipulação do gel Whiteness HP MAXX (FGM, Joinville, SC, Brasil) foi

realizada como descrita anteriormente. O gel foi aplicado e o LED violeta foi ativado logo em seguida. A irradiação consistiu em vinte ciclos de 60 segundos, com pausa de 30 segundos, de acordo com as instruções do fabricante.

Foram realizadas três sessões em cada grupo, com intervalo de 7 dias entre cada uma. Nos grupos HP, HP-BL e HP-VL, o gel clareador foi aplicado na superfície vestibular e dentro da câmara pulpar. Os espécimes foram imersos em saliva artificial durante todo o período experimental e foi realizada a troca da solução a cada 7 dias.

Após cada sessão de clareamento, todo o gel clareador foi aspirado e a coroa dentária irrigada com água destilada. O acesso coronário foi temporariamente protegido com algodão e RT (Villevie; Dentalville do Brasil Ltda., Joinville, SC, Brasil). No C (controle), após o tratamento endodôntico, a coroa dentária foi restaurada com resina composta.

As coroas dentárias dos grupos foram restauradas após 10 dias da última sessão de clareamento. A dentina foi condicionada com ácido fósfórico a 37% (Codac 37, FGM, Joinville, SC, Brasil), o sistema adesivo etch-and-rinse foi aplicado (Adper Scotchbond Multipurpose, 3M ESPE, Sumaré, SP, Brazil) e a restauração foi realizada com resina (Charisma, Heraeus Kulzer, São Paulo, SP, Brazil).

Os dentes foram mantidos em saliva artificial a 37°C e após 7 dias foram submetidos ao teste de resistência à fratura em uma máquina de teste eletromecânica (EMIC DL 2000; São José dos Pinhais, PR, Brasil). Foi utilizada carga de compressão a uma velocidade constante de 0,5 mm/minuto, com célula de carga de 5 kN. Para adaptação da amostra ao aparelho de ensaio, foi utilizado um dispositivo cilíndrico com ponta cônica [12]. A carga final necessária para fraturar a coroa foi registrada. Os dados obtidos na resistência a fratura foram submetidos a

análise de Variância ANOVA one-way e pós teste de Tukey, considerando o nível de significância de 5%.

Análise de microscopia eletrônica de varredura

Os espécimes para a avaliação da formação da camada híbrida (N = 24) entre o sistema adesivo e a dentina coronária foram obtidos a partir de corte transversal, realizado em espécimes que passaram pelo teste de resistência a fratura. Foi utilizado disco diamantado montado em máquina de cortes Isomet 1000 (Buhler, Lake Bluff Illinois USA), separando a coroa da raiz na região cervical. Após, foi realizado seccionamento no longo eixo da coroa dental, assim obtendo duas secções. A secção inicialmente foi polida com lixa na granulação #1200 montada em politriz DP-10 (Panambra Struers) e lavada em ultrassom por 3 minutos, para a remoção de eventuais resíduos.

Na sequência, foram submersas em ácido clorídrico 6N, por 30 segundos, lavadas em água destilada e imersas em solução de hipoclorito de sódio a 2,5%, por 10 minutos e finalmente lavadas também em água destilada por outros 10 minutos. Após os espécimes estarem secos, foram obtidas impressões individualizadas com vinil polissiloxano (Express XT, 3M ESPE, St. Paul, MN, USA), da superfície preparada e obtidas réplicas em resina epóxi (Buehler, São Paulo, SP, Brasil). Estas foram metalizadas e preparadas para análise em microscópio eletrônico de varredura (JMS 5310, JEOL, Tóquio, Japão), utilizando 20kV.

Foi obtida uma imagem de cada espécime (500x de magnificação) Fig. 1. Posteriormente, as imagens foram avaliadas na região na interface dente – restauração, foi utilizada uma medida descritiva de 0 a 3 para avaliar a continuidade da camada híbrida. Assim, "0" representando a continuidade da camada híbrida em todos os terços da imagem capturada, ou seja, ausência de fissuras em todos os terços; "1" representando uma continuidade em dois terços; "2" representando uma continuidade em pelo menos um terço; e "3" indicando que todos os terços

apresentaram uma descontinuidade na camada ou fissuras híbridas [17]. Os dados obtidos foram submetidos ao teste Kruskal-Wallis considerando o nível de significância de 5%.

Resultados

Resistência à fratura

Não foram encontradas diferenças significativas entre os protocolos de clareamento ($p > 0,05$).

Tabela 2. Média, desvio padrão e intervalo de confiança da resistência à fratura de dentes tratados endodonticamente de acordo com diferentes protocolos de clareamento dentário.

Grupos	Média (DP)	Intervalo de Confiança (IC)
C	926,89 (149,56) ^a	(834,19-1019,58)
HP	690,35 (232,64) ^a	(546,16-834,54)
HP-BL	722,35 (221,05) ^a	(585,35-859,36)
HP-VL	778,65 (206,14) ^a	(650,88-906,42)

^a Letras iguais indicam que não houve diferença estatisticamente significativa entre os grupos ($p > 0,05$). C: Controle; HP: Peróxido de hidrogênio 35%; HP-BL: Peróxido de hidrogênio 35% e luz LED azul; HP-VL: Peróxido de hidrogênio 35% e luz LED violeta.

Microscopia eletrônica de varredura

HP e HP-BL demonstraram maior alteração na formação da camada híbrida quando comparados ao Controle ($P > 0.05$). Por outro lado, não houve diferença entre HP-VL e os protocolos C, HP e HP-BL ($P < 0.05$). A tabela 3 demonstra a média e desvio padrão da continuidade na formação da camada híbrida após os protocolos de clareamento dental.

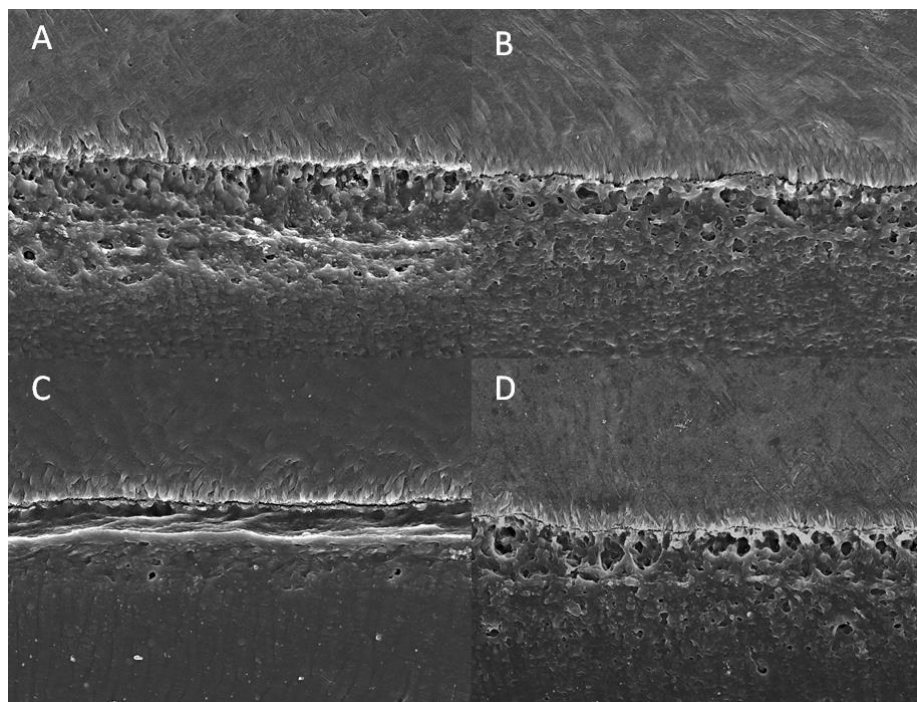


Figura 1. (A) - C: Controle; (B) - HP: Peróxido de hidrogênio 35%; (C) - HP-BL: Peróxido de hidrogênio 35% e luz LED azul; (D) - HP-VL: Peróxido de hidrogênio 35% e luz LED violeta.

Tabela 3. Valores médios (desvio padrão) de acordo com os grupos e tempo de avaliação em relação ao número de lacunas e a continuidade da camada híbrida. Pontuações de medida descritiva de 0 a 3.

Groups	Avaliação da camada híbrida	
	Mediana	1Q – 3Q
C	0 ^a	0 – 0.5
HP	3 ^b	2,5 - 3
HP-BL	3 ^b	3 - 3
HP-VL	2 ^{ab}	2 - 3

^{ab} Letras diferentes indicam diferença estatisticamente significativa entre os grupos (Teste de Kruskal-Wallis $p < 0.05$). C: Controle; HP: Peróxido de hidrogênio 35%; HP-BL: Peróxido de hidrogênio 35% e luz LED azul; HP-VL: Peróxido de hidrogênio 35% e luz LED violeta.

Discussão

O protocolo de clareamento em dentes tratados endodonticamente utilizando LED violeta associado HP 35% não reduziu significativamente a resistência a fratura, o mesmo foi observado nos grupos C, HP e HP-BL. Em relação a formação da camada híbrida, HP-VL foi similar aos demais grupos avaliados. No entanto, HP e HP-BL apresentaram alteração significativa em comparação ao grupo C. Portanto, a hipótese nula foi parcialmente rejeitada.

Dentes sem vitalidade possuem características diferentes de dentes vitais. Essa diferença morfológica pode ser atribuída à cárie, fraturas e menor quantidade de remanescente dental, podendo apresentar ainda um maior grau de pigmentação dos dentes [11,18]. Devido à essa maior pigmentação, o HP em alta concentração é normalmente utilizado [2,4,19]. No entanto, seu uso está associado a efeitos deletérios sobre os componentes orgânicos e inorgânicos da estrutura dental [8].

Considerando os últimos achados acerca da luz violeta no clareamento de dentes tratados endodonticamente [2,4], tornou-se pertinente a realização de estudos para avaliar seu efeito e segurança sobre a resistência a fratura e a formação da camada híbrida. Segundo Ribeiro et al. [20] e Manzoli et al. [21] o protocolo HP associado ao LED violeta apresenta efeito similar ao HP utilizado sozinho. Em nosso estudo avaliamos os efeitos do LED violeta com o HP, considerando a capacidade de diminuir o tempo de realização da técnica de clareamento sem causar efeitos deletérios a estrutura dental [22].

A realização de três sessões de clareamento não afetou a resistência a fratura nos grupos experimentais. Os resultados observados neste estudo diferiram dos achados por Galloza et al. [3], que verificou maior redução da resistência a fratura após três sessões de clareamento. No entanto, nesse estudo os autores utilizaram o peróxido de hidrogênio em uma proporção diferente e associado a pigmento, o que pode ter gerado uma maior catálise de HP e ocasionado redução da resistência a fratura.

Outra questão a ser considerada, é que mesmo não existindo diferença significativa entre o grupo HP-VL e o C, ainda há a possibilidade de interferência na formação da camada híbrida quando a restauração é realizada 10 dias após a última sessão clareadora. Acredita-se que a presença do gel HP 35%, mesmo que por um menor período de tempo possa afetar a formação da camada híbrida [23].

A literatura mostra que o HP causa alterações no substrato e a presença de radicais oxidativos (ROS) promove dano a adesão das restaurações quando realizadas logo após o clareamento, pois afeta a polimerização dos materiais resinosos [23]. Somado a isso, o uso da luz gera calor, aumentando a liberação de ROS e acelerando a reação clareadora [24]. Segundo Manzoli et al. [21], o LED violeta que atua em 405 nm associado ao HP não afeta negativamente a temperatura, o que pode justificar os resultados encontrados neste estudo.

Apesar disso, vale considerar que algum aterfado técnico durante a manipulação dos espécimes excepcionalmente pode ter desencadeado fissuras na interface de adesão dos dentes clareados, sendo interessante a realização de estudos complementares por meio da desmineralização e desproteínização do substrato dentinário para confirmar o efeito durante procedimentos adesivos.

Conclusão

O uso de HP 35% associado à irradiação com LED violeta ou LED azul não interferiu na resistência a fratura de dentes tratados endodonticamente. No entanto, alterações na formação da camada híbrida de dentes restaurados após 10 dias da última sessão de clareamento foram observadas para os grupos HP e HP-BL em comparação ao grupo controle, diferentemente do grupo HP-VL.

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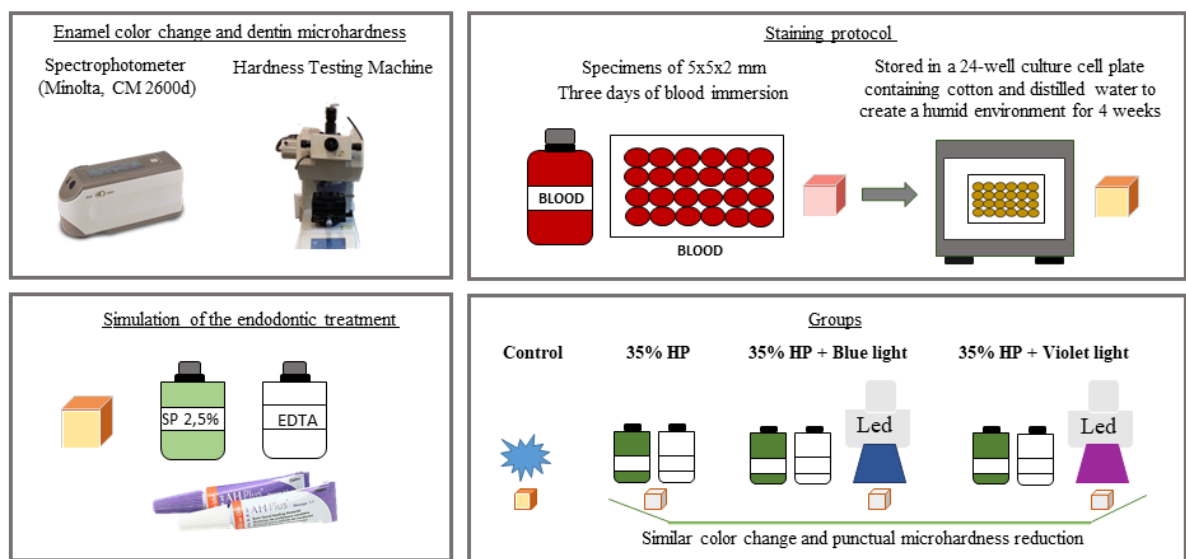
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3.2 Publicação 2*

Effect of non-vital tooth bleaching photoactivated with blue or violet LED on color and microhardness

Graphical Abstract

Effect of non-vital tooth bleaching photoactivated with blue or violet LED on color and microhardness



* O artigo segue as normas do periódico *Photodiagnosis and Photodynamic Therapy* ao qual foi submetido.

Abstract

Background: To evaluate the efficacy of non-vital dental bleaching protocols using 35% hydrogen peroxide photoactivated with violet LED on color and microhardness of endodontically treated teeth.

Methods: Forty specimens were selected and randomized into 4 groups (n = 10): C - Control, HP - 35% hydrogen peroxide, HP + BL - 35% hydrogen peroxide + blue LED, HP + VL - 35% hydrogen peroxide + violet LED. Three bleaching sessions were performed for each group. Color analysis was performed 7 days after each bleaching session. Two-way repeated measure ANOVA and Bonferroni test were used to evaluate the effect of different bleaching protocols and evaluation times on the dependent variables (ΔE and ΔL). Dentin microhardness was measured 24 hours after the third bleaching session. Data were evaluated by ANOVA and Tukey's test at a significance level of 5%.

Results: Differences on ΔE and ΔL were verified after the first and second bleaching sessions ($p < 0.05$) and showed stability after the third one, for all the groups. No differences were observed among HP, HP + BL, and HP + VL groups, regardless of the evaluation time ($p > 0.05$). HP and C showed the greatest and smallest reduction in dentin microhardness ($p < 0.05$), respectively. No difference between HP + BL and HP + VL protocols ($P > 0.05$) was observed.

Conclusions: High concentration hydrogen peroxide (35%) photoactivated with violet LED bleached endodontically treated teeth effectively. However, the same protocol negatively affected the dentin microhardness, but not in the same level of 35% HP solely used.

Keywords: Endodontics, Dental bleaching, Hydrogen peroxide, LED, Microhardness.

1. Introduction

Dental bleaching is a safe, minimally invasive, and effective aesthetic treatment, showing satisfactory outcomes when well indicated [1–4]. To achieve adequate color change in a short time, high concentration hydrogen peroxide (HP) gels combined with longer exposure time have been proposed [5]. However, the biological and morphological safety of this procedure may be compromised since self-reported tooth sensitivity, and negative effects on the elastic modulus, microhardness, and toughness of the enamel and dentin can be observed [5–7].

The bleaching process occurs when free oxygen radicals are released during the decomposition of HP. These free radicals diffuse through the enamel and dentin breaking the pigmented macromolecules that give yellowish appearance to teeth. However, this process is slow, and the use of light sources such as halogen light, light emitting diodes (LEDs), diode lasers, argon lasers and plasma arc lamps have been proposed to accelerate the decomposition of HP [2,8].

The combined use of HP and violet LED has been emerged since the wavelength between 405 – 410 nm coincides with the wavelength of pigmented molecules, which allows that the photons break long carbon chains into smaller ones and increase the generation of free radicals from the HP [9–13]. Previous studies have shown positive effects when combining violet LED irradiation and concentration hydrogen peroxide on vital teeth [12,14].

Teodosio et al. [20], in vitro study were one of the first to report in the literature on the efficacy of bleaching non-vital tooth using the violet LED associated with 35% hydrogen peroxide, however, there is only one other case report study where the whitening effect on non-vital teeth was evidenced [4]. Despite these studies, there are no comparative evaluations on bleaching with violet LED and blue LED protocol or and the effects on the morphological

alterations. More studies are needed to establish the appropriate irradiation time and bleaching protocols [20].

This *in vitro* study aimed to evaluate the efficacy of bleaching protocols using high concentration hydrogen peroxide photoactivated or not with violet LED and blue LED on the color change and dentin microhardness of non-vital tooth. The null hypothesis tested was the absence of effects on color change and dentin microhardness.

2. Materials and methods

2.1. Specimens' preparation

Bovine teeth were obtained and stored in 0.1% thymol solution at 4°C. At the moment of use, the teeth were washed for 12 hours to remove residues. After, the teeth were cleaned using pumice stone and Robinson brush (KG Sorensen Ind. E Com. Ltd, São Paulo, Brazil) at low-speed rotation. All teeth were evaluated in a stereomicroscope (20x magnification) and only those without enamel cracks or defects were included.

2.2. Experimental groups

C: Control group;

HP: 35% Hydrogen peroxide (Whiteness HP - FGM, Joinville, SC, Brazil);

HP + BL: 35% Hydrogen peroxide (Whiteness HP - FGM, Joinville, SC, Brazil) + blue LED (Twin Flex Evolution - MMOptics, São Carlos, SP, Brazil);

HP + VL: Hydrogen peroxide 35% (Whiteness HP - FGM, Joinville, SC, Brazil) + Violet LED (Bright Max Whitening - MMOptics, São Carlos, SP, Brazil).

2.3. Color change evaluation

The color was evaluated at: T0 – initial color assessment (after staining); T1 – 7 days after the first bleaching session; T2 – 7 days after the second bleaching session; T3 – 7 days after the third bleaching session.

Seventy-five bovine teeth were selected and transversally sectioned at the cervical region using a diamond-coated disk coupled in a precision cutting machine (IsoMet 1000, Buhler, Lake Bluff, IL, USA) to separate the crown from the root. After that, enamel/dentin specimens were obtained from the buccal surface of the crown (5 x 5 x 2 mm). The specimens were planned with silicon carbide sandpapers (#600, #1200, #1500) coupled in a politrax (DP-10, Panambra Struers) and then polished with diamond paste and felt discs. After that, the specimens were submitted to ultrasonic bath in distilled water for 5 min.

The color assessment was performed using a digital spectrophotometer (CM-2600d, Konica Minolta) in the same room and under similar illumination conditions. Three color readings enamel for each specimen were performed using the CIELab color system (L^* a^* b^*). After, the specimens were stained with defibrinated sheep blood (NewProv; Pinhais, PR, Brazil) according to Freccia et al. [15]. After the blood staining, the specimens were washed in running water and stored in a 24-well plate with only the buccal surface in contact with artificial saliva. The specimens were stored for 30 days, with the artificial saliva being systematically changed every 7 days. After staining, the simulation of the endodontic treatment was performed according to the adaptation of the technique described by Janiboni et al. [17].

To select the included specimens, a new color assessment was performed after the blood staining and the ΔE values were calculated. The forty included specimens were randomized into the experimental groups (Table 1). This selection was performed to ensure the initial color standardization of the specimens.

Table 1. Distribution of groups according to the bleaching protocol used.

Groups	Bleaching protocols
C	No bleaching protocol. Buccal face immersed in distilled water, changing the distilled water every 7 days.
HP	HP 35% hydrogen peroxide applied to the buccal surface (enamel) and pulp chamber (dentin) of the specimens. Two 15-minute applications of the whitening gel in the same session. Three sessions were performed at 7-day intervals.
HP + BL	35% hydrogen peroxide applied to the buccal surface (enamel) and pulp chamber (dentin) of the specimens and activation of the blue LED for 9 minutes. Two applications of whitening gel and use of the blue LED in the same session. Three sessions were performed at 7-day intervals.
HP + VL	35% hydrogen peroxide was applied to the buccal surface (enamel) and pulp chamber (dentin) of the specimens. The gel was applied and the violet light was allowed to activate for 2 minutes (5 cycles of 60 seconds, with a 30-second pause). Two applications of whitening gel and activation of the violet led in the same session. In total 10 light activation cycles. Three sessions were performed at 7-day intervals.

Table 1 shows the bleaching protocols performed. A 1-mm thin layer of the bleaching gel was applied over the buccal and palatal surfaces of the specimens. To change the bleaching gel during the session, a sterilized gauze was used. At the end of the session, the gel was removed with air/water jets for 30 seconds and the teeth were stored at 37°C with the buccal surface submerged in water, changing the medium every 7 days.

To determine the color change after the treatment protocols at different evaluation times, ΔE (according to the CIELab system) was calculated [16].

Data normality and sphericity were verified by Shapiro-Wilk ($p \geq 0.059$) and Mauchly ($p < 0.001$) tests, respectively. The Greenhouse-Geisser correction was used due to the sphericity assumption was not proven. Data were analyzed using the PASW Statistics software (version 22.0; SPSS Inc., Chicago, IL, USA), with a significance level of 5%. Two-way ANOVA for repeated measures and Bonferroni test were used to evaluate the effect of different treatments (C, HP, HP + BL, HP + VL) and evaluation times (T1, T2, T3) on the dependent variables (ΔE_{ab} , and ΔL).

2.4. Microhardness evaluation

To evaluate the microhardness, enamel/dentin specimens ($n = 75$) were obtained from the buccal surface ($4 \times 4 \times 2$ mm). The simulation of the endodontic treatment was performed [17]. Then, the pulp chamber dentin surface was prepared for the microhardness test according to the protocol performed by Aranda-Garcia et al. [18]. Surface planning was performed with #300 to #1200 sandpaper (Norton, Lorena, SP, Brazil) and polishing with aluminum oxide (Profill; SS White, Rio de Janeiro, Brazil). Afterwards, the specimens were washed with distilled water.

Microhardness measurements were obtained with a Hardness Testing Machine (Buehler, Japan) and a Knoop indenter with a load of 10 g for 20 sec. Initial measurements of

dentin microhardness were performed in the palatal surface of the dental crown 7 days after the endodontic treatment. Three indentations were performed for each specimen with a distance of 200 μm between them. The mean microhardness was calculated for each specimen and only those that presented values close to the general mean were included ($n = 40$). After that, the included specimens were randomized ($n = 10$) into the four experimental groups. The final microhardness evaluation was performed 24 hours after the 3rd bleaching session. The difference between the initial and final values was interpreted as the effect on dentin microhardness. The value was expressed as percentages. Data were evaluated using ANOVA and Tukey's test at a significance level of 5%.

3. Results

3.1. Color change evaluation

Table 2 shows the mean and standard deviation of ΔE values. Color changes after the first and second bleaching sessions in the HP, HP + BL and HP + VL groups ($P < 0.05$) were observed. However, no differences were observed between the protocols ($p > 0.05$) regardless of the evaluation time. Group C showed no color change.

ΔL results behaved similarly. The HP, HP + BL and HP + VL groups showed color change until the second bleaching session and remained stable after the third one. The inter-group comparisons showed that HP + VL was similar to HP and HP + BL groups ($p > 0.05$) (Table 3).

Table 2. Mean (standard deviation) of ΔE values according to the experimental groups and evaluation times.

Group	T1	T2	T3
C	3.04 (1.41) ^{Aa}	4.17 (1.67) ^{Aab}	3.71 (1.46) ^{Aa}
HP	9.64 (1.68) ^{Ba}	11.63 (1.36) ^{Bb}	11.96 (0.95) ^{Bb}
HP + BL	10.15 (3.07) ^{Ba}	12.75 (2.48) ^{Ba}	12.81 (3.62) ^{Bab}
HP + VL	8.69 (2.52) ^{Ba}	11.96 (2.93) ^{Bb}	11.56 (2.09) ^{Bb}

^{A-a} Different uppercase letters at the same column ($p \leq 0.001$) or different lowercase letter at the same row ($p \leq 0.021$) denote statistically significant difference within the same evaluation time or group, respectively. C, control; HP, Whiteness HP 35%; HP + BL, Whiteness HP 35% (FGM) + Blue Twin Flex Evolution LED; HP + VL, Whiteness HP 35% + Violet Led – Bright Max Whitening.

Table 3. Mean (standard deviation) of ΔL values according to the experimental groups and evaluation times.

Groups	T1	T2	T3
C	- 0.18 (2.32) ^{Aa}	0.71 (2.82) ^{Aab}	- 0.07 (2.88) ^{Aa}
HP	8.94 (1.68) ^{Ba}	11.02 (1.41) ^{Bb}	11.13 (1.13) ^{Bb}
HP + BL	9.48 (2.87) ^{Ba}	12.18 (2.23) ^{Bb}	12.02 (3.88) ^{Bab}
HP + VL	7.82 (2.79) ^{Ba}	11.39 (2.73) ^{Bb}	10.87 (1.99) ^{Bb}

^{A-a} Different uppercase letters at the same column ($p \leq 0.001$) or different lowercase letters at the same row ($p \leq 0.044$) denote statistically significant difference within the same evaluation time or group, respectively. C, control; HP, Whiteness HP 35%; HP + BL, Whiteness HP 35% (FGM) + Blue Twin Flex Evolution LED; HP + VL, Whiteness HP 35% + Violet Led – Bright Max Whitening.

3.2. Microhardness evaluation

HP and C showed the greatest and smallest reduction in dentin microhardness after the tooth whitening, respectively ($P < 0.05$) (Fig.1). On the other hand, no differences between the HP + BL and HP + VL were observed ($P > 0.05$). Table 4 shows the arithmetic mean and standard deviation of the percentage of dentin microhardness reduction after the tooth whitening protocols.

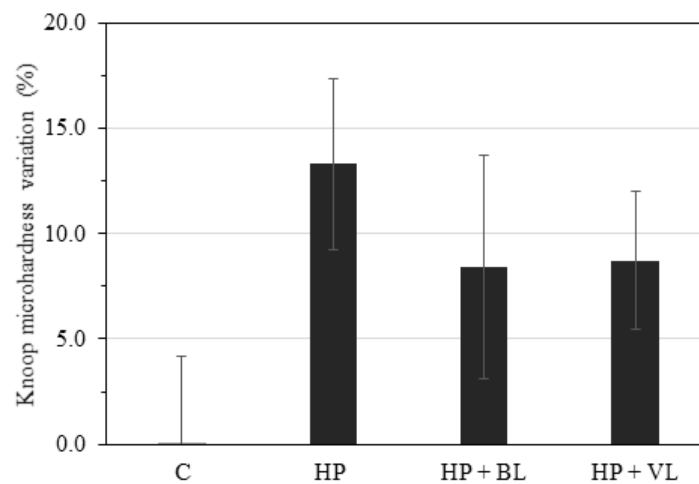


Figure 1. Mean and confidence interval of the reduction in microhardness (%) according to the experimental groups.

Table 4. Arithmetic mean, standard deviation and percentage of dentin microhardness reduction (in Knoop) according to the experimental groups.

Groups	Mean (SD)	Percentage reduction
C	35.63 (0.0082) ^a	0.01
HP	30.55 (1.84) ^c	13.31
HP + BL	32.53 (3.73) ^b	8.40
HP + VL	32.45 (3.12) ^b	8.73

^{abc} Different letters indicate significant differences between the evaluated groups ($P < 0.05$). C, control; HP, Whiteness HP 35%; HP + BL, Whiteness HP 35% (FGM) + Blue Twin Flex Evolution LED; HP + VL, Whiteness HP 35% + Violet Led – Bright Max Whitening.

4. Discussion

This study evaluated bleaching efficacy and the microstructural effect of dental bleaching using 35% HP and violet LED irradiation in non-vital tooth. The values of ΔE and

ΔL in HP + Violet LED were significantly higher than the control group (no treatment) and were the same as in the HP and HP + Blue LED groups. These data show that the dental bleaching was effective.

Our results indicate that the whitening protocol with two applications per session of the gel (HP 35%) for 2 min, followed by 5 cycles of 60 sec of light activation and pause time of 30 seconds, contributed positively to the color change. On the other hand, Gallinari et al. [7] showed that the greatest whitening effect with HP 35% occurs regardless of the use of violet light after three cycles with 7 light activations each, totaling 21 light activation cycles. However, in our study, 10 light activation cycles were performed per session, so it is believed that the main benefit of using the violet LED may be the reduction in the time of the whitening session.

Kury et al. [11] verified through a randomized controlled clinical trial that the violet LED associated with HP 35% resulted in a greater color change compared to the group with HP 35% gel alone. In this context, it is important to emphasize that the duration of the application of the bleaching gel may interfere with the effectiveness of dental bleaching.

In our study, the HP group consisted of two 15-minute applications of the whitening gel, totalizing 30 minutes. This time was estimated so that there was better standardization between the groups evaluated. The HP group showed no significant differences in the values of ΔE and ΔL compared to HP+BL and HP+VL.

Despite recent findings about the effectiveness of violet light when associated with different concentrations of peroxides [19–22], there are still few studies reporting its effect on the mechanical properties of dentin. Therefore, this study aimed to evaluate the effect of the bleaching protocols with violet LED and HP on the dentin microhardness.

Regarding microhardness, HP + VL showed less decrease compared to HP group. However, in all the bleaching protocols that used high concentration hydrogen peroxide some

level of alteration in dentin microhardness was observed. Thus, our null hypothesis was rejected. HP + BL was used in this study to compare their effect with violet LED. Group C, without treatment, was chosen as a negative control, to allow comparison with the other groups. HP, on the other hand, represents the gold standard within whitening procedures, therefore, comparison is essential when new whitening protocols are tested [2,7,9,17,23,24].

Microhardness analysis can evaluate after the bleaching protocols the structural damage in dentin [25], and the greater reduction of microhardness can lead to a lower resistance to tooth fracture. For this reason, the evaluated area of dentin was standardized, since the different diameters of the dentinal tubules in the region could result in bias [26,27].

Possibly, the shorter contact time of the gel with the enamel/dentin contributed to the lower reduction of microhardness in HP + BL and HP + VL (Fig.1). Therefore, it is suggested that shorter contact of HP time promoted by the use of LED resulted in a smaller reduction of the organic components of the dentin and alterations in the morphology of the dental structure that influence the mechanical properties [28].

Our study describes the color and microstructural effect of combining violet LED or blue LED photoactivation with 35% hydrogen peroxide as an innovative alternative protocol for non-vital tooth bleaching. Based on the limitations of this *in vitro* study, it can be concluded that the photoactivation with violet LED combined with 35% hydrogen peroxide is an interesting conservative approach. However, further studies must be performed to evaluate this protocols under a clinical setting.

5. Conclusion

Within the limitations of this *in vitro* study, the bleaching of non-vital tooth using high concentration hydrogen peroxide and violet LED photoactivation was effective in color change. All the bleaching protocols with HP decreased the dentin surface of the specimens microhardness; however, the solely use of hydrogen peroxide showed the greatest reduction.

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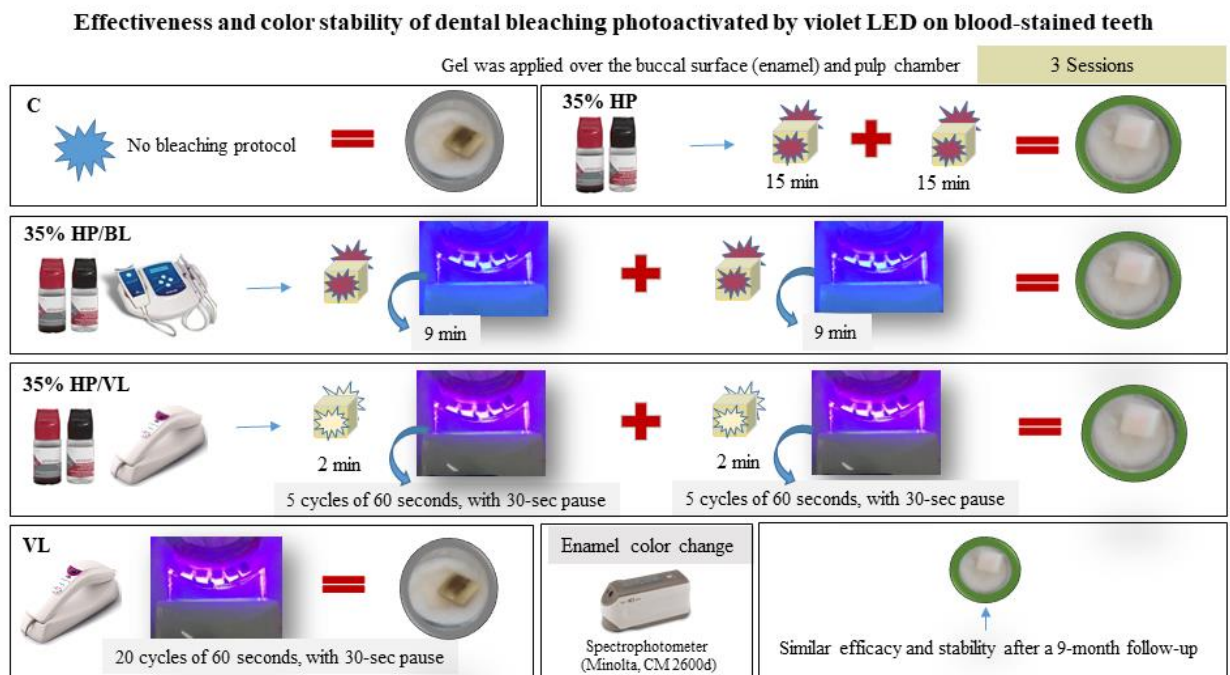
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3.3 Publicação 3*

Effectiveness and color stability of dental bleaching photoactivated by violet LED on blood-stained teeth

Graphical Abstract



* O artigo segue as normas do periódico *Photodiagnosis and Photodynamic Therapy* ao qual foi submetido.

Abstract

Background: Few studies have investigated the effect of violet LED irradiation associated or not with bleaching agents on blood-stained teeth. This *in vitro* study aimed to evaluate the whitening efficacy and color stability of non-vital dental bleaching using 35% hydrogen peroxide (HP) photoactivated with violet LED (VL) compared to 35% HP alone and 35% HP photoactivated with blue LED (BL).

Methods: Fifty bovine dental crowns were used to obtain specimens of 5x5x2 mm. After selection based on a previous colorimetric analysis, the specimens were blood-stained and randomly assigned into five groups (n = 10): control (no treatment); 35% HP, 35% HP/BL; 35% HP/VL; and VL. Three bleaching sessions were performed and the colorimetric analysis (ΔE_{ab} , ΔL , and ΔWI_D) was recorded after 7 days, 30 days, and 9 months of the last bleaching session. Two-way repeated measures ANOVA followed by Bonferroni post-hoc test was used at a significance level of 5%.

Results: 35% HP, 35% HP/BL, and 35% HP/VL showed the higher values of ΔE_{ab} , ΔL , e ΔWI_D ($P < 0.05$), without intra- and intergroup differences ($P > 0.05$). C and VL were similar in all the evaluation times ($P > 0.05$), showing lower values of ΔE_{ab} , ΔL , and ΔWI_D ($P < 0.05$).

Conclusions: 35% HP/VL can be a viable alternative for dental bleaching in endodontically-treated teeth, showing whitening efficacy similar to 35% HP solely used, even after a 9-month follow-up. VL used alone was not effective to bleach blood-stained teeth.

Keywords: Dental bleaching; Hydrogen peroxide; Color change; Blue LED; Violet LED.

1. Introduction

Violet light-emitting diode (LED) is an innovative light source in dental bleaching working by physical mechanisms [1]. Its wavelength (405 – 410 nm) is capable to bleach the dental structure, even when solely used [2]. The conventional bleaching protocols are based on chemical mechanisms in which a hydrogen peroxide(HP)-based agent can be associated or not with a light source [3–6] .

HP is responsible to bleach the dental structure by the release of highly reactive molecules that penetrate into the enamel and dentin and break complex pigmented molecules (chromophores). This chemical reaction is so-called oxi-reduction in which long carbon chains are transformed into smaller ones, making the appearance of teeth visually lighter [7–9]. Besides, light sources such as diode lasers, LED units, and halogen lamps can be used as photocatalysts to accelerate the bleaching reaction by the generated heat, improving the bleaching efficacy [10].

However, Maran et al. [11], in a systematic review, pointed out that the use of light sources did not improve the bleaching efficacy of the in-office technique using HP. With the upcoming of violet LED, novel studies have been conducted to validate its bleaching efficacy when associated with HP, or even alone, in teeth stained with various pigments [12–20]. In terms of endodontically-treated teeth, the literature is scarce regarding the clinical viability of violet LED photoactivation [21,22].

Color change in endodontically-treated teeth can occur due to the presence of sealer residues in the coronal region, necrosis, or blood leakage after trauma. The tooth discoloration progressively occurs; thus, the exposure to the pigment must be reduced or eliminated to achieve a better prognosis of color change after bleaching [23]. In addition, tooth discoloration may impair the self-esteem and social living of the patient, as well as the quality of life. It is

important to emphasize that dental bleaching is a conservative, effective, and time-saving approach compared to prosthetic rehabilitation [10,24].

The use of violet LED has demonstrated promising results when associated with HP-based agents [18,25]. Bearing in mind the few studies in endodontically-treated teeth, this study aimed to long-term evaluate the bleaching efficacy of in-office dental bleaching using violet LED associated or not with 35% HP agents compared to 35% HP alone or associated with blue LED. The null hypothesis tested was that the different bleaching protocols have the same efficacy in terms of color change over the evaluation times.

2. Materials and methods

2.1. Specimens' preparation

The sample size was determined using the G*Power software [26] considering ANOVA for repeated measures, an effect size of 0.6, [12,16,27] a significance level of 5% ($\alpha = 0.05$), and a power test of 95% ($1 - \beta$ error). After the calculation, the sample size was defined as 8 specimens per group.

Eighty-five bovine incisors without enamel cracks or defects were previously selected. All teeth were stored in 0.1% thymol solution at 4°C until the beginning of the experiments. Specimens of 5x5x2 mm were obtained from the central region of the buccal surface of the crown using a precision cutting machine (IsoMet 1000; Buehler Inc., Lake Bluff, IL, USA). After that, the enamel surface of the specimens was planned with silicon carbide sandpapers in decreasing granulation (#600, #1200, #1500) and polished with diamond past and felt discs. After polishing, the specimens were submitted to ultrasonic bath in distilled water for 5 minutes (Cristófoli, Campo Mourão, PR, Brazil), and then stored at 37°C ($\pm 1^\circ\text{C}$) until the first colorimetric analysis.

Tooth staining was performed after the first colorimetric assessment. The staining protocol used defibrinated sheep blood (NewProv; Pinhais, PR, Brazil), according to an adaptation of the staining technique proposed by Freccia et al. [28] and Guimarães et al. [29]. This protocol consisted of three days of blood immersion. After that, the specimens were cleaned in running water, and then dried and stored in a 24-well culture cell plate containing cotton and distilled water to create a humid environment for 4 weeks.

After staining, the simulation of the endodontic treatment was performed according to the adaptation of the technique described by Janiboni et al. [30,31] and Del Carpio-Perochena et al. [32] by immersing the specimens in a 2.5% sodium hypochlorite solution (Bioquímica, São José do Rio Preto, SP, Brazil) for 30 minutes, refreshing the solution every 3 minutes. 17% EDTA (Asfer, São Caetano do Sul, SP, Brazil) was applied for 3 minutes and then an epoxy resin-based cement containing calcium hydroxide (Sealer Plus; MK Life, Porto Alegre, RS, Brazil) was impregnated on the dentin surface using a microbrush (KG Sorensen, São Paulo, SP, Brazil). After 15 min, the cement was removed from the surface with a sterilized gauze and 95% ethanol. Then, the specimens were strategically placed to guarantee that only the enamel surface was in contact with the artificial saliva, in order to simulate the oral conditions.

2.2. Specimens' allocation

A blinded operator randomized the specimens into the five experimental groups only after the staining protocol. To standardize and avoid sample bias, all the specimens were submitted to colorimetric analysis using a digital spectrophotometer (CM 2600d - Konica Minolta, Petrópolis, RJ, Brazil) to ensure similar ΔE_{ab} values among them.

2.3. Experimental groups

The materials evaluated are shown in Table 1. The bleaching protocols were performed as follows:

Table 1. Investigated groups, bleaching protocols, and trademarks used in this study.

Groups	Bleaching protocol (Trademark, manufacturer, address)
C	Control group
35% HP	35% Hydrogen peroxide (Whiteness HP - FGM, Joinville, SC, Brazil)
35% HP/BL	35% Hydrogen peroxide (Whiteness HP - FGM, Joinville, SC, Brazil) + blue LED (Twin Flex Evolution - MMOptics, São Carlos, SP, Brazil)
35% HP/VL	Hydrogen peroxide 35% (Whiteness HP - FGM, Joinville, SC, Brazil) + violet LED (Bright Max Whitening - MMOptics, São Carlos, SP, Brazil)
VL	Violet LED (Bright Max Whitening - MMOptics, São Carlos, SP, Brazil)

- **C:** No bleaching protocol. Specimens were stored with the buccal surface immersed in distilled water at 37°C. The medium was changed every 7 days.
- **35% HP:** Three bleaching sessions of 30 min using the Whiteness HP gel (FGM, Joinville, SC, Brazil) were performed with an interval of 7 days. The handling of the gel was performed according to the manufacturer recommendations. The proportion 3:1 of HP and thickener was handled for 10 sec until its homogenization. After that, an approximately 1-mm thickness layer of the gel was applied over the buccal surface (enamel) and pulp chamber of the specimens with two applications of 15 min, totalizing 30 min.
- **35% HP/BL:** Three bleaching sessions using the Whiteness HP gel (FGM, Joinville, SC, Brazil) photoactivated by blue LED (Twin Flex Evolution - MMOptics, São

Carlos, SP, Brazil) were performed with an interval of 7 days. Each session had 18 min of duration. The gel was handled as previously described above. The gel was applied over the buccal surface (enamel) and pulp chamber (dentin) of the specimens and immediately photoactivated by blue LED irradiation for 9 min, according to the manufacturer's instructions. Two applications of bleaching gel and blue LED irradiation was performed in each session.

- 35% HP/VL: Three bleaching sessions using the Whiteness HP gel (FGM, Joinville, SC, Brazil) photoactivated by violet LED (Twin Flex Evolution - MMOptics, São Carlos, SP, Brazil) were performed with an interval of 7 days. Each session had 18 min of duration. The gel was handled as previously described above. The gel was applied over the buccal surface (enamel) and pulp chamber (dentin) of the specimens and after 2 min they were photoactivated by violet LED. The irradiation consisted of five cycles of 60 seconds, with 30-sec pause, according to the manufacturer's instructions. Two applications of bleaching gel and violet LED irradiation was performed in each session.
- VL: Three bleaching sessions using only violet LED irradiation were performed with an interval of 7 days. Each session had 29 min and 30 sec of duration. The violet LED source was applied on the buccal surface (enamel) and pulp chamber (dentin) of the specimens. The irradiation consisted of twenty cycles of 60 sec with a 30-sec pause, according to the manufacturer's instructions.

After the end of each bleaching session, the gel was removed with air/water-jet for 30 sec and the specimens were stored in a 24-well culture cell plate with the buccal surface immersed in artificial saliva at 37°C.

2.4 Colorimetric analysis

To evaluate the color change, a digital spectrophotometer was used (CM 2600d - Konica Minolta, Petrópolis, RJ, Brazil). The colorimetric analysis was performed before and after the staining protocols, and 7 days after the final bleaching session. To long-term evaluate the bleaching efficacy, the colorimetric analysis was also performed after 30 days and 9 months after the final bleaching session.

All the colorimetric analysis was performed in the same room with similar illumination conditions by the same operator. Three color readings were performed for each specimen in each period of evaluation.

The color change was evaluated by the colorimetric systems: CIELab (ΔE^* and ΔL^*) [16] and ΔWI_D (CIELAB-based Whiteness Index for Dentistry) [15,33].

2.5 Statistical analysis

After verifying the normality and sphericity of the data by Shapiro-Wilk ($p \geq 0.059$) and Mauchly tests ($p < 0.001$), respectively, the Greenhouse-Geisser correction was used. Two-way repeated measures ANOVA followed by Bonferroni post-test was used for multiple comparisons. The PASW statistical software (version 22.0; SPSS Inc., Chicago, IL, USA) was used at a significance level of 5%.

3. Results

Table 2 shows the mean and standard deviation values for ΔE . 35% HP, 35% HP/BL and 35% HP/VL showed higher values than C and VL, regardless of the evaluation time ($p < 0.05$). No differences among the groups that used 35% HP were observed after 30 days and 9 months of the final bleaching session ($p > 0.05$).

Table 2. Mean (standard deviation) values for ΔE_{ab} according to the groups and evaluation time.

Groups	Evaluation time		
	T1	T2	T3
C	3,59 (1,42) ^{Aa}	3,62 (1,49) ^{Aab}	6,03 (2,72) ^{Aab}
35% HP	11,87 (0,89) ^{Ba}	11,32 (3,06) ^{Ba}	12,66 (2,88) ^{Ba}
35% HP/BL	12,76 (3,58) ^{Ba}	12,64 (3,73) ^{Ba}	14,06 (2,94) ^{Ba}
35% HP/VL	11,47 (2,11) ^{Ba}	10,83 (3,91) ^{Ba}	13,23 (3,03) ^{Ba}
VL	3,31 (1,47) ^{Aa}	3,94 (2,16) ^{Aab}	6,24 (1,53) ^{Ab}

^{A-a} Different uppercase letters in the same column ($p \leq 0.001$) or lowercase letter in the same row ($p \leq 0.021$) denote statistically significant difference within the same evaluation time and group, respectively. C, no treatment (negative control); 35% HP, Whiteness HP 35% (positive control); 35% HP/BL, Whiteness HP 35% + Blue LED; 35% HP/VL, Whiteness HP 35% + Violet LED; VL, Violet LED. T1, 7 days after the 3rd bleaching session; T2, 30 days after the 3rd bleaching session; T3, 9 months after the 3rd bleaching session.

Similarly, the ΔL results showed higher values for 35% HP, 35% HP/BL, and 35% HP/VL compared to C and VL, regardless of the evaluation time ($p > 0.05$) (Table 3).

Table 3. Mean (standard deviation) values for ΔL according to the groups and evaluation time.

Group	Evaluation time		
	T1	T2	T3
C	- 0,05 (2,79) ^{Aa}	0,36 (1,66) ^{Aa}	4,67 (2,64) ^{Ab}
35% HP	11,10 (1,18) ^{Ba}	10,32 (3,66) ^{Ba}	12,07 (2,83) ^{Ba}
35% HP/BL	12,00 (3,76) ^{Ba}	11,79 (3,82) ^{Ba}	13,43 (2,67) ^{Ba}
35% HP/VL	10,84 (1,96) ^{Ba}	9,85 (3,06) ^{Ba}	12,55 (2,86) ^{Ba}
VL	0,55 (2,24) ^{Aa}	- 0,36 (1,44) ^{Aab}	4,97 (1,75) ^{Ac}

^{A-a} Different uppercase letters in the same column ($p \leq 0.001$) or lowercase letter in the same row ($p \leq 0.021$) denote statistically significant difference within the same evaluation time and group, respectively. C, no treatment (negative control); 35% HP, Whiteness HP 35% (positive control); 35% HP/BL, Whiteness HP 35% + Blue LED; 35% HP/VL, Whiteness HP 35% + Violet LED; VL, Violet LED. T1, 7 days after the 3rd bleaching session; T2, 30 days after the 3rd bleaching session; T3, 9 months after the 3rd bleaching session.

Regarding the ΔWI_D , the groups 35% HP, 35% HP/BL, and 35% HP/VL showed higher values than C and VL ($p < 0.05$) in T1, T2, and T3. For 35% HP/BL and 35% HP/VL a decrease in the color change was observed after 30 days. No differences were observed among the groups that used hydrogen peroxide (35% HP, 35% HP/BL, and 35% HP/VL), regardless of the evaluation time ($p > 0.05$). VL showed lower ΔWI_D values, similar to the control group in all evaluation periods.

Table 4. Mean (standard deviation) values for ΔWI_D according to the groups and evaluation time.

Group	Evaluation time		
	T1	T2	T3
C	4,26 (3,31) ^{Aa}	4,05 (2,25) ^{Aab}	5,71 (3,65) ^{Ab}
35% HP	15,76 (2,96) ^{Ba}	14,61 (2,89) ^{Ba}	12,75 (3,93) ^{Ba}
35% HP/BL	14,21 (5,52) ^{Ba}	12,79 (5,63) ^{Bab}	12,49 (5,37) ^{Ba}
35% HP/VL	12,99 (5,57) ^{Ba}	11,61 (6,14) ^{Bb}	11,22 (5,82) ^{ABb}
VL	6,46 (2,83) ^{Aab}	5,92 (2,47) ^{Aab}	7,07 (2,87) ^{Aac}

^{A-a} Different uppercase letters in the same column ($p \leq 0.001$) or lowercase letter in the same row ($p \leq 0.021$) denote statistically significant difference within the same evaluation time and group, respectively. C, no treatment (negative control); 35% HP, Whiteness HP 35% (positive control); 35% HP/BL, Whiteness HP 35% + Blue LED; 35% HP/VL, Whiteness HP 35% + Violet LED; VL, Violet LED. T1, 7 days after the 3rd bleaching session; T2, 30 days after the 3rd bleaching session; T3, 9 months after the 3rd bleaching session.

4. Discussion

This study evaluated the effectiveness of non-vital dental bleaching with or without 35% HP associated or not with violet LED as an alternative protocol for blood-stained teeth compared to the conventional 35% HP and 35% HP + Blue LED. The null hypothesis was rejected, since the groups behaved differently after 7 days, 30 days and 9 months of the last bleaching session.

The dimension of the specimens was standardized in 5 x 5 x 2 mm to allow the initial color reading in a Minolta Spectrophotometer. Our methodology was chosen to reproduce the tooth discoloration classified as an intrinsic post-eruptive stain and simulate the dental bleaching in a non-vital tooth that suffers dental trauma, culminating in blood overflowing into

the dentin tubules and progressive staining of the tooth [34]. Thus, we performed the staining of the specimens by blood immersion and the endodontic treatment after 4 weeks in order to reproduce the clinical scenario in a more reliable manner. As described by Teodósio et al. [22], first, we performed the tooth staining and then the endodontic treatment. The staining protocol was performed according to the technique proposed by Freccia et al. [28], and Guimarães et al. [29], which has been used to stain extracted teeth after blood pigmentation.

The existence of different bleaching and staining protocols increases the risk of bias [11,12,18,22,35,36]. Because of this, we chosen to perform three sessions using the HP for a maximum of 30 minutes [17], ensuring a more accurate intergroup comparison by the standardization of the bleaching protocols. The HP/BL and HP/VL groups consisted of sessions of 18 minutes, while the duration of the sessions for VL group was 29 minutes and 30 sec.

The HP/BL and HP/VL protocols followed manufacturer's recommendations. Regarding the protocol using violet LED alone, we used the same protocol indicated for vital teeth (twenty cycles of 60 sec with a 30-sec pause). Although the bleaching technique with violet light alone can bleach the tooth, the color change is not comparable to that promoted by HP [18].

Considering the dimension of the specimens, it was easily possible to irradiate the enamel and dentin simultaneously, by positioning the LED perpendicular to the surface of the specimen. Moreover, we ensure that the distance between the acrylic tip of the violet LED source and the dental surface not exceed 2.0 cm, otherwise, in this case, a large portion of the delivered light would be lost, reducing the necessary light intensity for the photobleaching, according to the manufacturer's instructions.

The colorimetric analysis was carried out only 7 days after the last bleaching session, preventing misinterpretation of the results due to dehydration of the dental surface, and the presence of residual free radicals in the structure that could change the tooth color [35]. To

ensure greater accuracy in color readings, the coordinates of the CIELab system (ΔE and ΔL) and Whiteness Index for Dentistry (ΔWI_D), which represents the evaluation of whiteness in dentistry [33], were used.

Our results showed that blue and violet LEDs associated with HP 35%, in high concentration, can achieve the same bleaching efficacy of HP used alone, which is in accordance with a systematic review carried out by Maran. et al. [11], that stated that the bleaching efficacy is not influenced by the use of light. However, it is important to emphasize the possibility of reducing the clinical time without affecting the long-term efficacy of the bleaching when high concentration HP is photoactivated by LED units.

In our study ΔE_{ab} , ΔL , and ΔWI_D showed similar behavior for the groups that used HP with or without light irradiation regardless of the evaluation time (T1, T2 and T3). On the other hand, the violet led used alone did not change the tooth color significantly in all evaluation times (Tables 2, 3, and 4). VL showed no statistically significant differences compared to the control group. Despite the differences within the same group according to the evaluation time, we can observe that there was a pattern for all the values. It is possible to infer that the use of high concentration hydrogen peroxide associated with light or not, directly influences the effectiveness and potential of whitening in non-vital teeth. An interesting outcome we found was to verify that the efficacy and color stability of bleaching using violet LED and HP was similar to the gold standard protocol (35% HP).

In a previous study carried out by Ribeiro et al. [37] the authors claim that violet LED photoactivation increased the color change of enamel and dentin when associated with 35% HP, regardless of the irradiation time (15 min, 30 min or 45 min). Galinnari et al. [12] showed that 35% HP used alone on teeth stained with black tea, has a greater whitening effect, regardless of the use of violet light. Teodósio et al. [22], observed that the HP photoactivated

or not with a violet LED presents greater color change when compared to the violet LED alone in endodontically treated teeth.

It is claimed that the violet LED photoactivation appears to enhance the bleaching efficacy of peroxides [20,36]. However, a systematic review highlighted that the clinical efficacy of violet LED photoactivation are still questionable due to a high heterogeneity of related studies, limited number of randomized clinical trials, lack of standardization of the bleaching protocols, and the quality of *in vitro* studies [18].

The violet LED irradiation can be a peroxide-free alternative in dental bleaching, but with lower bleaching efficacy than the association with peroxides [38,39]. Therefore, it justifies the need for longer periods of time and even a possible increase in the number of sessions in order to achieve a satisfactory whitening result. In our study, to allow comparison of groups, we standardized the performance of 3 sessions for each protocol.

It has been demonstrated that violet light has limited penetration into the dental structure. Thus, it can be inferred that dental bleaching using only violet LED irradiation would be restricted to enamel since the photobleaching is hindered by the poor penetration of the light [40]. Moreover, the light may not achieve the pigmented molecules located in deeper areas close to the enamel/dentin junction. Particularly in non-vital teeth after dental trauma, the dentin tends to be high pigmented due to the blood overflow into the dentin tubules [23,34]. In this context, the use of violet LED irradiation alone may not achieve successful bleaching, and longer exposure times can be used as an attempt to overcome these limitations.

To the best of knowledge, the main purpose of this *in vitro* study was to evaluate novel bleaching protocols using violet LED photoactivation in blood-stained teeth. Despite the promising results of using violet LED associated with high concentration hydrogen peroxide, it must be considered that *in vitro* studies cannot accurately reproduce the *in vivo* conditions.

Thus, in vivo studies are crucial to evaluate the clinical efficacy of bleaching protocols using violet LED.

5. Conclusion

Within the limitations of this in vitro study, it can be concluded that 35% hydrogen peroxide photoactivated by violet LED is effective to bleach blood-stained non-vital teeth, showing a similar color change to hydrogen peroxide used alone or photoactivated by blue LED after a 9-month follow-up. However, the sole use of violet LED irradiation did not promote successful bleaching, being comparable to the group with no treatment.

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4 CONCLUSÃO

O uso de HP 35% associado à irradiação com LED violeta é eficaz na alteração de cor de dentes tratados endodonticamente e não interfere na resistência a fratura. Embora a luz tenha efeitos benéficos quanto a efetividade, causa alteração na microdureza da dentina e na formação da camada híbrida de dentes restaurados após 10 dias da última sessão de clareamento.

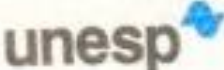
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* De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: <http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf>

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ANEXO A – COMITÊ DE ÉTICA



UNIVERSIDADE ESTADUAL PAULISTA
"JULIO DE MESQUITA FILHO"
Câmpus de Araraquara
FACULDADE DE ODONTOLOGIA



CERTIFICADO

Certificamos que a proposta intitulada **"AVALIAÇÃO IN VITRO DOS EFEITOS DO CLAREAMENTO DENTÁRIO COM FOTOSSENSIBILIZAÇÃO EM DENTES NÃO VITAIS"**, registrada com o nº 21/2019, sob a responsabilidade do(a) Prof(a). Dr(a). **Milton Carlos Kuga** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA em reunião de 18/10/2019.

Finalidade	☐ Ensino ☒ Pesquisa Científica
Vigência do Projeto	Março/2022
Espécie/linhagem	gado Nelore / Carneiro desfibrimado
Nº de animais	280 dentes bovinos / 50ml
Peso/idade	-
Sexo	-
Origem	Mondelli Ind. de Alimentos S.A. / Brasil Científica



Prof. Dr. PAULO SÉRGIO CERRI
Vice Coordenador da CEUA

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**Não autorizo a publicação deste trabalho pelo prazo de 2 anos após a data de
defesa**

(Direitos de publicação reservado ao autor)

Araraquara, 24 de Fevereiro de 2023.