UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MATHEUS KURY RODRIGUES

O EFEITO DO LED VIOLETA E PLASMA DE ARGÔNIO NO CLAREAMENTO DENTAL DE CONSULTÓRIO

THE EFFECT OF VIOLET LED AND ARGON PLASMA ON IN-OFFICE TOOTH BLEACHING

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestre em Clínica Odontológica, na Área de concentração em Dentística.

Dissertation presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Dental Clinics, in Operative Dentistry Area.

Orientadora: Prof.ª Dr.ª Vanessa Cavalli Gobbo

Este exemplar corresponde à versão final da dissertação defendida pelo aluno Matheus Kury Rodrigues e orientada pela Prof[a] Dr[a] Vanessa Cavalli Gobbo.

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UNIVERSIDADE ESTADUAL DE CAMPINAS
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PROFª. DRª. VANESSA CAVALLI GOBBO

PROFª. DRª. MARIA CAROLINA GUILHERME ERHARDT

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A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

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RESUMO

O objetivo deste trabalho foi avaliar o efeito do LED violeta (LED) e plasma de argônio (NATP), associados ou não ao peróxido de hidrogênio 35% (HP) e carbamida 37% (CP), no clareamento dental de consultório. No primeiro estudo, 90 coroas bovinas, pigmentadas com chá preto, foram submetidas aos tratamentos (n=10): LED, LED/HP, LED/CP, NATP, NATP/PH, NATP/CP, HP, CP e controle (C). LED foi irradiado vinte vezes por 1min com intervalos consecutivos de 30s (10 sessões) e NTAP foi aplicado por 10min (6 sessões). HP e CP foram aplicados por 30min (3 sessões). Quando associados a HP e CP, LED foi irradiado durante toda aplicação dos géis e NTAP foi aplicado nos 10min finais. Os espécimes foram mantidos em saliva artificial entre sessões e C permaneceu em saliva. Valores de L*a*b* foram obtidos com espectrofotômetro após pigmentação (T_0), clareamento (T_b) e 14 dias (T_{14}). Alteração de cor (ΔE), ΔL, Δa e Δb foram determinadas nos intervalos T_b-T_0(Δ1) e T_{14}-T_0(Δ2). Concentração intrapulpar de PH foi quantificada (μL/mL) na útlima sessão. Após 14 dias, a morfologia dos espécimes (n=3) foi observada em microscópico eletrônico de varredura. ΔE, ΔL e concentração intrapulpar de HP foram submetidos à ANOVA dois fatores e Tukey e Δa e Δb, à Kruskal-Wallis e Mann-Whitney (α=5%). Em ambos intervalos, LED e NATP promoveram ΔE e ΔL>C. ΔE foi semelhante para LED/HP e LED/CP. LED/CP promoveu maior ΔE que CP. LED/HP e LED/CP promoveram aumento de ΔL e diminuição de Δb, comparado a LED. Maior concentração intrapulpar de peróxido foi observada para HP, independentemente da ativação. LED e NATP não promoveram alterações morfológicas do esmalte; todavia, mudanças topográficas foram observadas com a aplicação de HP e CP. O estudo II, um ensaio clínico controlado, tratou pacientes aleatorizados pelo fator tratamentos (n=18): LED, LED/CP, CP, LED/HP, HP. A cor foi mensurada conforme estudo I e escala de cor visual (SGU). Risco absoluto e intensidade da sensibilidade dental (TS) foram avaliados usando escala analógica visual. Concentrações de cálcio (Ca) e fósforo (P) do esmalte foram avaliadas após microbiópsia (T_0,T_b,T_{14}). One-way ANOVA e teste de Tukey avaliaram ΔE, ΔL, Δa e Δb e ANOVA de medidas repetidas e teste de Tukey, Ca/P. Risco absoluto de TS foi submetido a Chi-quadrado, e ΔSGU e intensidade da TS a Kruskal-Wallis e Mann-Whitney. Em ambos intervalos, LED/HP promoveu maior ΔE/ΔSGU. LED/CP foi maior que CP apenas para ΔE/ΔSGU1. LED mostrou menor ΔE entre grupos. ΔL e Δa 1 e 2 não foram influenciados por LED. Δb1 foi maior para LED/CP que CP, e Δb2 foi maior para LED/HP que HP. TS foi mais prevalente para LED/CP(61,1%) ou HP(94,4%). Maior TS foi reportada para LED/HP apenas na 3ª sessão e no 1º intervalo. Ca/P manteve-se para cada grupo após 14 dias. Concluiu-se que, LED e NTAP foram eficazes, e associação de LED e CP foi benéfica para alteração de cor, *in vitro*. Clinicamente, LED mostrou-se mais eficaz associado a géis.

Palavras-chave: Clareamento Dental, Peróxido de Hidrogênio, Diodos Emissores de Luz

ABSTRACT

The objective of this study was to evaluate the effect of LED and NATP associated or not with 35% hydrogen peroxide (HP) and 37% carbamide peroxide (CP). Firstly, 90 incisor bovine crowns stained with black tea were submitted to the following treatments (n=10): LED, LED/HP, LED/CP, NATP, NATP/PH, NATP /CP, HP, CP and C - control. LED was irradiated twenty times for 1 min with consecutive 1-min intervals (10 sessions) and NTAP was applied for 10 minutes (6 sessions). HP and CP were applied for 30 min (3 sessions). When associated to HP and CP, LED was irradiated during all gel application and NTAP for the last 10 minutes of gel exposure. Specimens were maintained in artificial saliva (AS) among intervals and C was stored in AS throughout the experiment. L*a*b* values were obtained by means of spectrophotometry after staning (T_0), last bleaching session (T_b) and 14 days. Color change (ΔE), luminosity (ΔL), red*green (Δa) and yellow*blue axis (Δb) were determined among time points T_b-T_0 (Δ1) and T_{14}-T_0 (Δ2). Intrachamber hydrogen peroxide concentration (μL/mL) was quantified during last session. After 14 days, specimens (n=3) were prepared for surface analysis under scanning electron microscope (SEM). ΔE, ΔL and HP concentration values were submitted to two-way ANOVA and Tukey test. Δa and Δb was tested under Kruskal-Wallis and Mann-Whitney (α=5%). At both intervals, LED and NTAP promoted ΔE higher than C ($p<0.05$). ΔE was similar for LED/HP and LED/CP ($p>0.05$). ΔE for LED/CP was superior than CP ($p<0,05$). LED/HP and LED/CP promoted increase of Δl and decrease of Δb, in comparison to LED ($p<0.05$). Higher peroxide concentration was observed for HP, regardless of the activation mode ($p<0.05$). Although LED and NTAP mantained enamel morphology, topographical alterations were observed after HP and CP application. Secondly, a randomized controlled clinical treated patients randomly (n=18) distributed into: LED, LED/CP, CP, LED/HP, HP. Color evaluation was performed likewise the first study and visual shade guide (ΔSGU). Absolute risk (AR) and intensity (ITS) of TS were determined using a visual analogue scale (VAS). Calcium (Ca) and phosphorous (P) concentrations were evaluated after enamel microbiopsies at T_0, T_b, T_{14}. One-way ANOVA and Tukey tests were used for ΔE, ΔL, Δa Δb, and repetead measures ANOVA and Tukey test for Ca/P. AR was tested with Chi-Square, and ΔSGU and TS intensity with Kruskal-Wallis and Mann-Whitney. At both intervals, LED/HP promoted higher ΔE/ΔSGU, but LED presented the lowest ΔE ($p<0.05$). ΔE/ΔSGU1 was higher for LED/CP than CP, and ΔL and Δa 1,2 were not influenced by LED. Δb1 was higher for LED/CP than CP, although Δb2 was superior for LED/HP in comparison to HP. TS was more prevalent for LED/CP (61.1%) or HP (94.4%). Higher TS intensity was reported for LED/HP only during 3rd session and 1st interval. Ca/P ratio was mantained for each group after 14-day follow-up. Therefore, LED and NTAP alone were efficient, and association of LED with CP was *in vitro* benefical for color change. Clinically, efficacy of LED was demonstrated with higher color change when associated with bleaching gels.

Key words: Tooth Bleaching, Hydrogen Peroxide, Light-emitting Diodes

SUMÁRIO

1. INTRODUÇÃO

O agente clareador mais comumente utilizado no clareamento dental de consultório é o peróxido de hidrogênio (HP) em concentrações de 20 a 35% (Kwon, 2016). Emprega-se também o peróxido de carbamida (CP) em concentrações de 35 a 37% (Peixoto et al., 2018); porém, menor eficácia clareadora é reportada para esse agente. No peróxido de carbamida, a uréia associada ao peróxido de hidrogênio será dissociada quando em contato com o dente para então fornecer água e oxigênio (Joiner et al., 2006). Dessa maneira, a concentração de peróxido de hidrogênio em géis de PC é reduzida a 1/3 em relação a géis de PH. Ainda, a adição de outros componentes, como espessantes, pode tornar a liberação de oxigênio mais lenta (Kwon et al., 2016).

Relatos indicam que baixas e altas concentrações de PH podem causar alterações na estrutura e na composição mineral do esmalte, diminuindo a resistência intrínseca deste tecido (da Silva et al., 2005, Cavalli et al., 2004), e que maiores concentrações de PH modificam a proporção cálcio/fosfato do esmalte (Cavalli et al., 2004, Cavalli et al., 2004, Tezel et al., 2007). Análises *in vitro* mostraram que as alterações morfológicas e aumento da rugosidade do esmalte podem ocorrer devido à perda mineral (Berger et al., 2008, Liporoni et al., 2010), sendo que o pH do gel clareador é possivelmente responsável por tais mudanças no esmalte (Torres et al., 2014). Contudo, estudos demostraram que géis clareadores não acarretaram em mudanças no perfil de superfície e no conteúdo mineral do esmalte, independentemente do pH e, também, da concentração do agente clareador (Sa et al., 2013, Pinto et al., 2017).

Alternativas para aumentar o efeito clareador de agentes clareadores de uso em consultório por meio de luz halógena, LED/laser e LED em comprimentos de onda distintos, demonstraram resultados controversos na efetividade dessa associação (He et al., 2012, Maran et al., 2018). Foi observado que a utilização de luz halógena pode agir como possível termocatalisador do HP, aumentando a eficácia do agente clareador (Ontiveros & Paravina, 2009, Kwon et al., 2013). Todavia, foram relatados resultados contrários demonstrando ausência de efeito benéfico da utilização de luz (Lima et al., 2009; de Freitas et al., 2016). Mesmo que estudos mostrem que haja sinergia na ativação por luz de géis de HP em baixa concentração, uma recente revisão sistemática mostrou que as fontes de luz halógena, LED/laser e LED não aumentam a eficácia clareadora de HP tanto em altas quanto em baixas concentrações (Maran et al., 2018). Embora os resultados relacionados à eficácia da utilização de diferentes fontes de luz associadas ao clareamento sejam conflitantes, foi observado que essa associação não exacerbou negativamente os efeitos causados no conteúdo mineral do esmalte (Berger et al., 2010). Todavia, não há relatos clínicos das

consequências do uso da luz associada a géis clareadores de HP ou CP no conteúdo mineral do esmalte.

Outro aspecto que gera preocupação está relacionado à potencialização da difusão intrapulpar do HP quando associado à fontes de luz (Briso et al., 2016, Cavalli et al., 2016). Foi relatado que a aplicação de luz potencializa a presença de peróxido na câmara pulpar devido ao aumento na decomposição do peróxido (Camargo et al., 2009), embora também foi reportado que o uso de LED, LED/laser de diodo e luz halógena não influenciaram a penetração intra-pulpar do HP em dentes hígidos e com restaurações adesivas (Cavalli et al. 2016).

Clinicamente, a presença de peróxido de hidrogênio na polpa pode desencadear a sensibilidade dental durante a aplicação do gel, por um período médio de até 4 dias após a aplicação (Dahl e Pallesen, 2003, Markowitz, 2010), sendo esse um importante motivo pelo qual o paciente interrompe o tratamento ou opta por um tratamento menos conservador (Briso et al., 2016). Ensaios clínicos randomizados apontam que o uso de CP em alta concentração gera menor sensibilidade em relação ao gel de HP (Peixoto et al., 2016). Ainda, há dados que suportam que o uso da luz em clareamento de consultório não aumenta a sensibilidade dental para HP em altas concentrações. Entretanto, a intensidade da sensibilidade pode aumentar quando géis de HP em baixa concentração são associados à luz (Maran et al., 2018).

Com o intuito de diminuir a sensibilidade durante o clareamento, recentemente foi introduzido no mercado odontológico uma nova geração de LED violeta (405 nm) para clareamento de consultório sem a utilização do peróxido (Zanin, 2016). Estudos preliminares *in vitro* mostraram que a luz violeta foi capaz de alterar favoravelmente a cor de dentes artificialmente pigmentados (Panhoca et al., 2016; Kury et al., 2018; Kury et al., 2018). Hipotetiza-se que a luz violeta exerça papel físico no clareamento, quebrando as moléculas pigmentantes aderidas à superfície do esmalte dental pela possível compatibilidade entre o comprimento de onda da luz e o pico de absorção dos pigmentos (Rastelli et al., 2018). Ainda de acordo com o fabricante, o LED violeta pode ser associado ao CP e HP em altas concentrações para intensificar o efeito clareador em pacientes que relatam pouca ou nenhuma sensibilidade (Sugestão de Protocolos de Utilização, MMOptics, São Carlos, SP, Brasil). Devido à escassez de pesquisas *in vitro* e *in vivo* e estudos clínicos randomizados, não há, até o momento, indícios que demonstrem a eficácia desses protocolos e o efeito dos mesmos na concentração intra-pulpar de HP, sensibilidade dental e superfície do esmalte (Lago et al., 2017; Panhoca et al., 2017).

Além do LED violeta, tem sido investigado *in vitro* o efeito que o plasma de argônio (NTAP) exerce no clareamento dentário quando associado a peróxidos (Claiborne, 2014, Santak et al., 2014). O plasma, é o quarto estado da matéria e está indicado ao tratamento

clareador em sua forma não-térmica, por transferir íons em temperatura ambiente ao dente e, por consequência, não alterar propriedades dentais (Kong et al., 2009). Seu mecanismo de ação pode ser explicado pela produção de radicais livres do peróxido de hidrogênio (Kim et al., 2010, Lee et al., 2009) ou pela modificação da energia de superfície em conjunto à aplicação de gel de peróxido (Pan et al., 2013; Sun et al., 2010).

Relatos indicam que o uso do plasma associado a CP 15% produziu melhor efeito que apenas CP, e que o plasma não aumentou a temperatura pulpar acima de 37^{o}C (Nam et al.,2013). Outros estudos utilizam longos períodos de aplicação (20 min) do plasma para avaliar seu efeito no clareamento (Lee et al., 2009; Sun et al., 2010), o que torna a técnica inviável clinicamente. Entretanto, um estudo *in vitro* reduziu o tempo de aplicação do plasma para 2 min previamente ao HP, e apesar da eficácia clareadora não ser aumentada nesse caso, foi relatado melhora na rugosidade de superfície para os grupos tratados com plasma (Ruivo, 2015). Sendo assim, presume-se ser viável um protocolo clareador com tempo reduzido da aplicação do plasma para avaliar a eficácia da técnica, e análises para verificar se o plasma não eleva a concentração intrapulpar de peróxido de hidrogênio.

Diante do exposto, o objetivo desse trabalho, inicialmente, foi investigar *in vitro* a eficácia do LED violeta e plasma atmosférico, como protocolos associados ou não a géis de HP e CP em altas concentrações no clareamento dental, e o efeito dos mesmos na concentração intra-pulpar de peróxido de hidrogênio. Em um segundo momento, esse trabalho avaliou *in vivo,* o efeito dos protocolos clareadores com LED violeta na alteração de cor, sensibilidade dental e conteúdo mineral do esmalte por meio de um ensaio clínico controlado randomizado.

2. ARTIGOS

2.1. ***Artigo 1:*** *In Vitro* Effect of Violet LED and Non-Thermal Atmospheric Plasma on In-Office Bleaching Gels: an study.*

Short Title: In-office Bleaching Activated by Violet LED and Non-Thermal Atmospheric Plasma

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SUMMARY

The study evaluated the effect of violet LED (LED) and non-thermal atmospheric plasma (NTAP) associated with 35% hydrogen peroxide (HP) and 37% carbamide peroxide (CP) or the efficacy of LED or NTAP alone on in-office bleaching. Ninety bovine incisor crowns stained with black tea were divided according to bleaching groups (n=10): LED, LED/HP, LED/CP, NTAP, NTAP/HP, NTAP/CP, HP, CP and control (C). The CIE L* a* b* parameters were evaluated using a spectrophotometer at the baseline (T_0), after bleaching (T_b) and 14 days elapsed from bleaching (T_{14}). Color change (ΔE), lightness (ΔL), redness (Δa) and yellowness (Δb) were evaluated at intervals T_b-T_0 (ΔE1, ΔL1, Δa1 Δb1) and T_{14}-T_0 (ΔE2, ΔL2, Δa1 Δb2). The intrapulpal concentration of hydrogen peroxide was quantified by means of spectrophotometry analysis (μL/mL). SEM images were obtained to evaluate the enamel surface's morphology after bleaching. ΔE, ΔL and HP intrapulpal concentration data were submitted to two-way ANOVA and Tukey tests, and Δa and Δb were evaluated by the Kruskal-Wallis and Mann-Whitney tests (α=5%). At both intervals, LED and NTAP resulted in ΔE and ΔL values higher than C. ΔE was similar for LED/HP and LED/CP, even though the ΔE for LED/CP was superior than CP ($p<0.05$). LED/HP and LED/CP increased ΔL and decreased Δb compared with LED alone. Δa for LED was equal to LED/CP ($p>0.05$) and different from NTAP ($p<0.05$). The Δb for NTAP/CP and NTAP/HP differed only after 14 days of storage in artificial saliva. Higher peroxide intrapulpal concentration was determined for the HP-treated groups, regardless of activation mode ($p<0.05$). LED and NTAP did not cause alterations in the enamel's morphology. However, topographical changes were observed for SEM after the application of HP and CP. Therefore, LED and NTAP alone promoted color change, and the use of LED produced an additional effect on the color change produced by CP without enhancing intrapulpal hydrogen peroxide concentration and the changes on enamel surface.

Clinical significance: Patients with moderate-intense tooth sensitivity could be benefited with the application of LED and NTAP on in-office bleaching protocols, especially with high-concentrated carbamide peroxide, if their efficacy and safety are verified in further studies.

INTRODUCTION

As an alternative to enhance in-office bleaching efficacy, different activation modes such as heat, light and addition of catalysts in the gel have been used in the past years.[1] Although no consensus exists on the efficacy of light activation,[2-4] a recent systematic review showed that bleaching with hydrogen peroxide (HP) associated with halogen lamps, LED/laser and LED under different wavelengths did not improve bleaching.[5] Thus, clarifying the role of light activation in different in-office bleaching agents is extremely important for dentists.

In addition, concern exists regarding safety in the use of light due to the trans-enamel and trans-dentin diffusion of hydrogen peroxide into the pulp chamber.[6-8] Theoretically, the free radicals decomposed from hydrogen peroxide break the cromophores in dentin down into smaller molecules, promoting bleaching.[1] However, peroxide radicals reach the pulp chamber due to low molecular weight.[9] Although studies report that the light activation of in-office bleaching agents does not increase intra-chamber concentration,[6-7] there is evidence that the diffusion of HP into the pulp chamber depends on concentration, viscosity and presence of additives in the bleaching agent.[10-11] Clinically, the presence of HP in the pulp chamber promotes tooth sensitivity. A randomized clinical trial indicated that high concentrations of carbamide peroxide (CP) promoted lower intensity of tooth sensitivity compared to HP gel,[12] but data suggest that the use of light under low-concentrated HP may increase the intensity of tooth sensitivity.[5]

To overcome HP's limitations, a novel generation of violet LED as light source for in-office bleaching was introduced with the purpose of not using bleaching agents on patients with moderate to severe tooth sensitivity.[13] As the wavelength of violet light (approximately 405 nm)[13] differs from that of blue light (peak at 467 nm),[5] which is commonly employed in in-office bleaching, the light emitted from the new device could match the absorbance peak of the staining molecules adhered to the enamel's surface.[14] In addition, the violet LED's manufacturer indicates the association of this source with high-concentrated HP or CP for patients without or with low intensity of tooth sensitivity,[14-15] to increase the decomposition of HP into free radicals by possibly elevating the agents' temperature.[16] Although insufficient information is available concerning the efficacy of violet LED protocols associated or not with peroxides and their impact on intrapulpal HP concentration, this light is currently used by both dentists and patients.[14-15,17-18]

In the literature, the use of non-thermal atmospheric plasma (NTAP) for activating high and low-concentrated bleaching gels has been *in vitro* investigated.[19] As plasma, the so-called fourth state of matter, generates OH radicals,[20] it could provide a synergic effect when bleaching with peroxide gels. According to observations, NTAP could be more efficient than LED under blue wavelength to enhance the color alteration promoted by HP and CP,[19] and

may not change the enamel's topography when associated with agents under low concentration.[20] Researchers have already demonstrated that the morphology of the enamel surface is altered due to the application of either HP or CP,[21] even though these changes were not influenced by the light activation.[22] Furthermore, the removal of proteins from the enamel surface after application of NTAP[23] could result in the alteration of the teeth's color in the absence of gels, being an option to overcome HP's limitations.

Therefore, the aim of this study was to evaluate the *in vitro* efficacy of violet LED and non-thermal atmospheric plasma associated or not with high-concentrated HP and CP agents. Furthermore, the effects of these protocols on the concentration of hydrogen peroxide in the pulp chamber and on the enamel surface's morphology were evaluated. The null hypotheses were that LED and NTAP would (1) not increase the efficacy of the bleaching agents, (2) not bleach without the association with HP or CP, (3) not increase the concentration of hydrogen peroxide in the pulp chamber, and (4) not promote morphological alterations.

MATERIAL AND METHODS

Experimental Design

The experimental units (ninety bovine incisors) were submitted to the following factors (n=10):

1. Bleaching agent (three levels):
    - HP: 35% hydrogen peroxide (Whiteness HP, FGM Dental Products, Joinville, SC, Brazil),
    - CP: 37% carbamide peroxide (SuperEndo, FGM)
    - C: control (no application of bleaching agent).
2. Activation modes (three levels):
    - LED: violet LED light (Bright Max Whitening, MMOptics, São Carlos, SP, Brazil)
    - NATP: non-thermal atmospheric argon plasma (Surface Plasma Tool Model SAP; Surface–Engineering and Plasma Solution, Campinas, Brazil)
    - C: control (no activation).

The International Comission on Illumination's (CIE) L* a* b* parameters for color were measured after the staining protocol (T_0), after bleaching (T_b) and 14 days elapsed from bleaching (T_{14}). Color change (ΔE), lightness (ΔL), redness (Δa) and yellowness (Δb) were evaluated at intervals T_b-T_0 ($\Delta E1$, $\Delta L1$, $\Delta b1$) and T_{14}-T_0 ($\Delta E2$, $\Delta L2$, $\Delta b2$). The hydrogen peroxide's intra-chamber concentration (µg/mL) was determined spectrophotometrically, and the enamel surface morphology was observed under scanning electron microscopy (SEM) fourteen days after bleaching (T_{14}).

Specimens' Preparation

Extracted bovine incisors were cleaned and stored in a 0.1% thymol solution at 4^0C for no more than 30 days. Bovine incisors without cracks and defects in the enamel were selected. The roots were cut with diamond discs (DhPro, Paranaguá, PR, Brazil) coupled to a handpiece (Kavo, Joinville, SC, Brazil) 2 mm below the cementoenamel junction. The pulp chamber was thoroughly cleaned and the pulp tissue was discarded (Hedstrom files, Maillefer Dentsply, Ballaigues, Switzerland). Ninety crowns with the same buccal wall thickness (3.5 mm) were selected.[6] The crowns were completely immersed in an infusion prepared with 2 g of black tea (Leão, São Paulo, SP, Brazil) boiled in 100 ml of distilled water for 24 h and continuously stirred.[24] An adhesive label (6x6 mm) was placed on the enamel's buccal surface, and the entire crown was isolated with nail polish (Impala, Guarulhos, SP

Brazil). The unpainted enamel area was submitted to prophylaxis with a mixture of pumice (SS White, São Paulo, SP, Brazil) and distilled water before the first color reading and bleaching.

Group Division and Bleaching Procedures

The specimens were randomly divided into groups (n=10) according to the factors *bleaching agents* and *activation mode*:

- **(LED):** Violet LED light irradiation without bleaching agent
- **(LED/HP):** 35% hydrogen peroxide light-activated with violet LED light
- **(LED/CP):** 37% hydrogen carbamide light-activated with violet LED light
- **(NATP):** Non-thermal argon plasma without bleaching agent
- **(NTAP/HP):** 35% hydrogen peroxide activated with non-thermal argon plasma
- **(NTAP/CP):** 37% hydrogen carbamide activated with non-thermal argon plasma
- **(HP):** 35% hydrogen peroxide without activation
- **(CP):** 37% hydrogen carbamide without activation
- **(C):** Without bleaching agent and without activation

L*, a* and b* values were statistically similar between the groups before the bleaching procedures. Specifications of materials, equipment and bleaching protocols are described in Table 1 and 2, respectively. The specimens were stored in artificial saliva [1.5 mM calcium ($CaCl_2$), 0.9 mM phosphate (NaH_2PO_4), 0.15 mM KCl, pH 7.0] among intervals at 37 ^{0}C, and the solution was renewed every two days.[25]

Table 1. Commercial name, specification and basic composition and manufacturer's instructions of activation modes and bleaching gels

Commercial name (Manufacturer, Address)	Specification/Composition	Manufacturer's instruction
LED: Bright Max Whitening – BMW (MMOptics, São Carlos, SP, Brazil)	Four light emitting diode lamps in violet wavelength (405 nm) positioned in a curved acrylic tip. The manufacturer provided the following specifications: Illumination area of the tip = 10.7 cm^2; maximum power = 22VA; optical power = 1.2 W.	Applied for 30 min (20 consecutive 1-min irradiations at 30-s intervals) associated or not with high-concentrated bleaching agents. When no agent was applied, 4 to up to 10 appointments are indicated with 4 to 7-day intervals. Three sessions with a 1-week interval are the limit indicated when associated with peroxide agents.
NTAP: Non-thermal Atmospheric Plasma (Surface Plasma Tool Model SAP; Surface-Engineering and Plasma Solution, Campinas, SP, Brazil)	Two metal electrodes separated by a 0.5 mm dielectric layer, with 0.8 mm diameter openings. 5 KHz frequency and 7 KV amplitude. The compressed argon gas was used at a 0.8 to 1 SLM ratio. The voltage is around 5000v, with an operational 5 mA current.	No manufacturer's instructions were available for bleaching purposes. The literature reported the *in vitro* association of argon plasma and bleaching gels with application of the nozzle in varying distances from the enamel's surface and gels.[19-20]
CP: Whiteness Super Endo (FGM, Joinville, SC, Brazil)	37% carbamide peroxide, neutralized carbopol, inert filler, glycol and deionized water. pH was informed by the manufacturer as = 7.0.	Even though this study used this material for external bleaching, the manufacturer recommends its use in the walking bleaching technique in non-vital teeth bleaching. The bleaching agent is placed into the pulp chamber and must be evaluated and replaced if necessary every 3 to 4 days, up to eight times.
HP: Whiteness HP (FGM, Joinville, SC, Brazil)	35% hydrogen peroxide, dyes, glycol, inert filler, deionized water and thickener. pH was informed by the manufacturer as = 6.0.	Indicated for vital teeth bleaching with three 15-min applications. A seven-day interval is required between up to four sessions.

Table 2. Bleaching protocols

	LED	NTAP	NO ACTIVATION
HP	A single 30-min 35% HP gel application light-activated with 20 consecutive 1-min irradiations of violet LED light in 30-s intervals. The light was positioned 8-mm away from the specimens for all LED groups. **Number of sessions:** 3 **Intervals**: 7 days	A single 30-min 35% HP gel application associated with NTAP application over the final 10 min of exposure to the gel. The specimens were placed 2 cm away from the exit nozzle of the device and in the middle of the bleaching area. **Number of sessions:** 3 **Intervals**: 7 days	A single 30-min 35% HP gel application without activation. All protocols using HP had 0.01 g of this gel applied over the bleaching area. **Number of sessions:** 3 **Intervals**: 7 days
CP	A single 30-min 37% CP gel application light-activated with 20 consecutive 1-min irradiations of violet LED in 30-s intervals. **Number of sessions:** 3 **Intervals**: 7 days	A single 30-min 37% CP gel application was associated with NTAP application over the final 10 min of exposure to the gel. **Number of sessions:** 3 **Intervals**: 7 days	A single 30-min 37% CP gel application without activation. All protocols using CP had 0.01 g of this gel applied over the bleaching area. **Number of sessions:** 3 **Intervals**: 7 days
Without bleaching agents	Twenty 1-min irradiations of the violet LED light in consecutive 30-s intervals. A moist gauze was applied over the surface of the specimens between intervals. A mouth-like shaped template was obtained to simulate the teeth's alignment during violet light irradiation for all LED groups. **Number of sessions:** 10 **Intervals**: 4 days	The NTAP nozzle was applied on the enamel's surface free of any bleaching gel for 10 minutes. Plasma was kept static during all applications. **Number of sessions:** 6 **Intervals**: 4 days	The specimens were stored in artificial saliva between T_0 and T_b. The solution was refreshed every two days.

LED= light-emitting diode
NTAP= non-thermal atmospheric argon plasma
HP=hydrogen peroxide
CP=carbamide peroxide

Colorimetric Evaluation

Color evaluation was performed at the baseline – after staining and prophylaxis (T_0), after bleaching and thoroughly rinsing with distilled water (T_B) and 14 days elapsed from bleaching and of immersion in artificial saliva (T_{14}). Four measurements were performed on the enamel using a digital spectrophotometer (EasyShade, Vita Zahnfabrik, Bad Säckingen, Germany) and the mean values were obtained for each specimen. Data were obtained according to the CIE system: L*(black-white), a*(green-red), b*(blue-yellow). These parameters determined color change ($\underline{\Delta}E$), according to the formulae $\underline{\Delta}E= [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$. Delta values were obtained at two intervals: ΔE1, ΔL1, Δa1 and Δb1 (T_b-T_0) and ΔE2, ΔL2, Δa2 Δb2 (T_{14}-T_0).

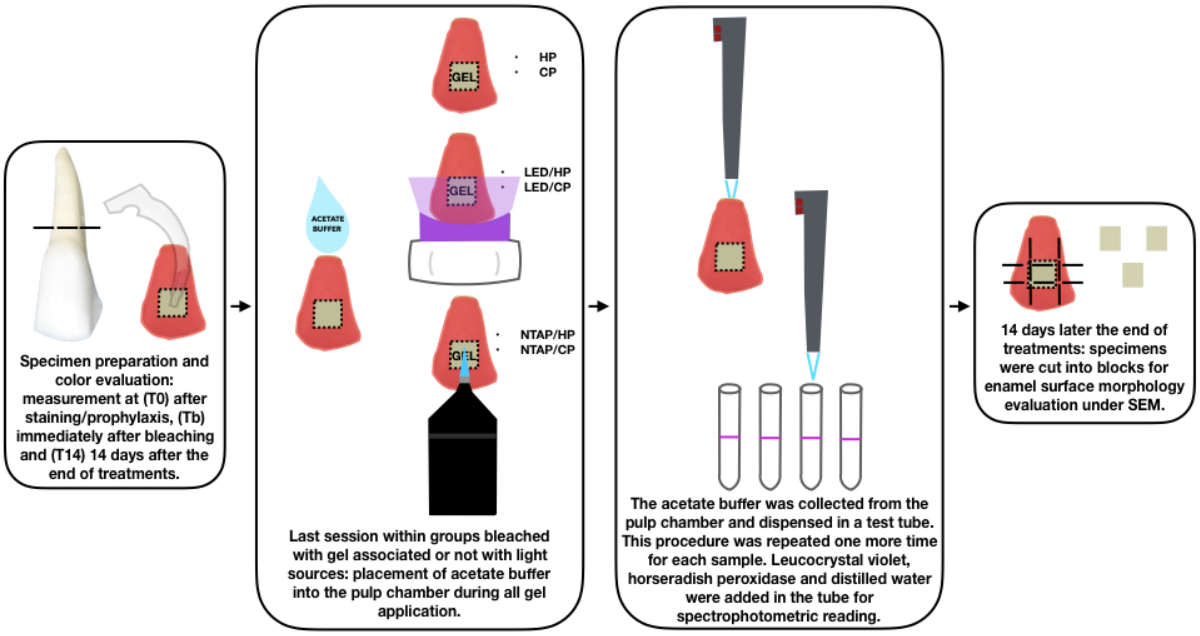
Concentration of Hydrogen Peroxide in the Pulp Chamber

The pulp chambers of groups LED/HP, NTAP/HP, HP, LED/CP, NTAP/CP and CP (excluding the LED and PL groups) were filled with 150 µL of 2M acetate buffer (4.5 pH) at the beginning of the last bleaching session. At the end of bleaching, the buffer was collected and transferred to a test tube and the procedure was repeated with other 150 µL of buffer to completely remove the remaining HP. One hundred µL of leuco-crystal violet (0.5 mg/µL; Sigma-Aldrich, St Louis, MO, USA), 50 µL of horseradish peroxidase (1 mg/mL; Sigma-Aldrich, St. Louis, MO, United States) and 2650 µL of distilled water were added to the tube to spectrophotometrically determine (DU 800, Beckman Coulter Inc, Brea, CA, USA) the concentration of hydrogen peroxide. The wavelength used for the reading of optical density was 596 nm.[6]

Morphology of the Enamel's Surface

Fourteen days after the bleaching therapies and storage in artificial saliva, the enamel-treated surfaces (n=3) were cut into blocks using a diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and the blocks were coupled to stubs for gold sputtering (MED 010, Balzers, Balzer, Liechtenstein). The enamel surface's morphology within different bleaching protocols was observed under SEM (JEOL-JSM, 6460LV, Tokyo, Japan) with 1000 magnification. The schematic representation of the experiment is presented in Figure 2.

Figure 1. Summarized methodology flow



Abbreviations: HP, hydrogen peroxide; CP, carbamide peroxide; LED, light-emitting argon plasma; NTAP, non-thermal atmospheric argon plasma; SEM, scanning electron microscope

Statistical analysis

The data were submitted to exploratory analysis (SPSS Statistics Version 23, IBM Corp, Armonk, NY, USA). Normal distribution and homoscedasticity were verified for ΔL. ΔE and concentration of HP values were considered normal after Log transformation. These data were submitted to two-way analysis of variance (ANOVA, according to factors *bleaching agents* and *activation modes*) and Tukey test. The Δa and Δb values were subjected to Kruskal-Wallis and *post-hoc* Mann-Whitney and tests were performed at 5% significance level.

RESULTS

Color change

Color change (ΔE) at different intervals (ΔE1: T_b-T_0) and (ΔE2: T_{14}-T_0) are described in Table 3. The "bleaching agents" (p=0.000) and the "activation modes" (p=0.000) factors and the interaction between "bleaching agents" and "activation modes" (p=0.001 for ΔE1; p=0.000 for ΔE2) significantly affected the ΔE results.

LED or NTAP did not enhance color change for HP at both observation times (p>0.05). Violet LED light increased CP ΔE values at both intervals (p<0.05), and NTAP did not affect color change for CP (p>0.05). LED/HP and LED/CP exhibited similar ΔE for both evaluated times (p>0.05). HP without activation promoted higher ΔE than CP and C (p<0.05). LED and NTAP without bleaching resulted in a similar color change and were significantly higher than the control group (p<0.05).

Table 3. Mean ΔE1 (T_b-T_0) and ΔE2 (T_{14}-T_0) values and standard deviations, according to bleaching agent or activation mode.

Bleaching agents	Activation mode					
	ΔE1			ΔE2		
	LED	**NTAP**	**No activation**	**LED**	**NTAP**	**No activation**
HP	31.5 (6.6) Aa	25.1 (6.2) Aa	25.2 (8.1) Aa	32.18 (5.8) Aa	28.0 (7.1) Aa	28.3 (8.2) Aa
CP	27.1 (8.7) Aa	22.2 (5.7) Ab	18.9 (5.8) Bb	28.6 (6.8) Aa	19.4 (5.6) Bb	20.6 (3.7) Bb
Without agent	13.1 (3.5) Ba	10.7 (3.5) Ba	4.3 (0.8) Cb	14.8 (4.2) Ba	10.4 (3.8) Ca	4.7 (1.0) Cb

Means followed by different letters differ statistically at 5% significance level, according to two-way ANOVA and Tukey test. Uppercase letters compare the bleaching agents within each activation mode and lowercase letters compare the activation mode for each bleaching agent and interval.

Table 4 reports the mean ΔL 1 and 2 values. Two-way ANOVA demonstrated that the "bleaching agent" (p=0.000) and "activation mode" (p=0.000 for ΔL 1; p=0.001 for ΔL 2) factors significantly influenced the alteration in luminosity. Interaction between factors was detected for both times (p=0.001 for ΔL 1; p=0.005 for ΔL 2). The activation modes did not increase the luminosity (ΔL) of the teeth for each bleaching gel (p>0.05). LED/HP and LED/CP were statistically different at ΔL1 (p<0.05) and similar at ΔL2 (p=0.216). At both intervals, LED and NTAP without the bleaching agents promoted a higher ΔL than C (p<0.05).

Table 4. Mean ΔL1 (T_b-T_0) and ΔL2 values (T_{14}-T_0) and standard deviation, according to bleaching agent or activation mode.

Bleaching agents	Activation mode					
	ΔL1			ΔL2		
	LED	**NTAP**	**No activation**	**LED**	**NTAP**	**No activation**
HP	26.3 (9.2) Aa	19.9 (6.5) Aa	20.5 (6.9) Aa	23.8 (8.5) Aa	21.5 (6.5) Aa	21.3 (6.9) Aa
CP	19.8 (8.5) Ba	17.8 (5.5) Aab	15.2 (7.7) Bb	18.3 (7.4) Aa	15.9 (5.8) ABa	14.2 (7.2) Ba
Without agent	8.2 (3.5) Ca	10.7 (3.5) Ba	0.5 (1.6) Cb	9.0 (3.7) Ba	8.4 (3.1) Ba	0.6 (2.5) Cb

Means followed by different letters differ statistically at 5% significance level, according to two-way ANOVA and Tukey test. Uppercase letters compare the bleaching agents within each activation mode and lowercase letters compare the activation mode for each bleaching agent and interval.

Δa values are displayed in Table 5, and the changes in the red*green axis for HP and CP were not affected by the activation modes ($p>0.05$). LED/CP promoted a Δa that was similar to the one promoted by LED and LED/HP ($p>0.05$), but LED and LED/HP were different ($p<0.05$) at both times. Δa1 and 2 were similar for NTAP/HP and NTAP/CP ($p>0.05$). LED resulted in statistically higher Δa1 and 2 than NTAP and C.

At both intervals, the association of LED with HP and CP decreased the teeth's yellowness (Δb) (Table 6) compared to the group without the agent ($p<0.05$). LED and NTAP did not increase Δb for HP, regardless of the time of evaluation ($p>0.05$). At both intervals, LED/CP exhibited higher median Δb values than CP ($p=0.0001$), and NTAP/CP did not affect the Δb of CP ($p>0.05$). Finally, LED without bleaching significantly decreased the teeth's yellowness as opposed to NTAP and C.

Table 5. Median Δa1 (T_b-T_0) and Δa2 (T_{14}-T_0) and maximum and minimum values, according to bleaching agent or activation mode.

Bleaching agents	Activation mode					
	Δa1			Δa2		
	LED	**NTAP**	**No activation**	**LED**	**NTAP**	**No activation**
HP	-7.8 (-8.6, -6,1) Aa	-6.2 (-9.4, -4.7) Aa	-7.6 (-10.8, -5.5) Aa	-9.9 (-10.6,-7.9) Aa	-8.2 (12.3, -7.2) Aa	-10.6 (-13.2,-8.3) Aa
CP	-7.8 (-8.5, -4.3) ABa	-7.2 (-9.6, -3.6) Aa	-5.9 (-8.3, -4.1) Aa	-8.4 (-10.6, -6.8) ABa	-8.7 (-6.7, -3.7) Aa	-7.9 (-9.0,-7.5) Aa
Without agent	-4.8 (-6.5, -4.2) Ba	-2.5 (-3.7, -2.2) Bb	-2.3 (-2.5, -1,7) Bb	-6.1 (-7.8, -5.5) Ba	-2.5 (-3.5, -1.6) Bb	-2.3 (-3.0,-2.0) Bb

Medians followed by different letters differ statistically at 5% significance level, according to Kruskal-Wallis and Mann-Whitney tests. Uppercase letters compare the bleaching agents within each activation mode and lowercase letters compare the activation mode for each bleaching agent and interval.

Table 6. Median Δb1 (T_b-T_0) and Δb2 (T_{14}-T_0) and maximum and minimum values, according to bleaching agent or activation mode.

Bleaching agents	Activation mode					
	Δb1			Δb2		
	LED	**NTAP**	**No activation**	**LED**	**NTAP**	**No activation**
HP	-12.2 (16.9, -8.0) Aa	-13.7 (-15.0, -8.1) Aa	-12.3 (-15.8, -3.9) Aa	-17.5 (-21.6, -16.8) Aa	-15.4 (-17.7, -14.2) Aa	-16.0 (-18.6, -10.9) Aa
CP	-15.9 (-20.0, -12.0) Aa	-9.2 (-9.6, -5.8) Ab	-5.2 (-8.3, -3.5) ABb	-19.4 (-21.3, -17.3) Aa	-8.7 (-9.4, -8.3) Bb	-10.8 (-13.4, -3.8) Ab
Without agent	-8.3 (-11.0, -5.4) Ba	-2.7 (-6.9, -1.2) Bb	-2.8 (-3.4, -1.8) Bb	-10.7 (-11.6, -5.7) Ba	-2.8 (-4.6, -1.3) Bb	-1.9 (-3.8, -1.0) Bb

Medians followed by different letters differ statistically at 5% significance level, according to Kruskal-Wallis and Mann-Whitney tests. Uppercase letters compare the bleaching agents within each activation mode and lowercase letters compare the activation mode for each bleaching agent and intervals.

Concentration of Hydrogen Peroxide in the Pulp Chamber

The mean values of intra-chamber hydrogen peroxide concentration are represented in µg/mL (Table 7). The hydrogen peroxide concentration in the pulp chamber of the HP-treated groups was significantly higher than that of the CP groups (p=0.000), regardless of activation mode. HP led to an intrapulpal concentration of oxygen radicals that was four times greater than the one obtained by CP. Moreover, the intra-chamber concentration of hydrogen peroxide was not altered by LED or NTAP activation (p=0.580), and no interaction was detected between the type of gel and activation mode (p=0.983).

Table 7. Mean values and standard deviations of hydrogen peroxide concentration (µg/mL) according to bleaching agent or activation mode.

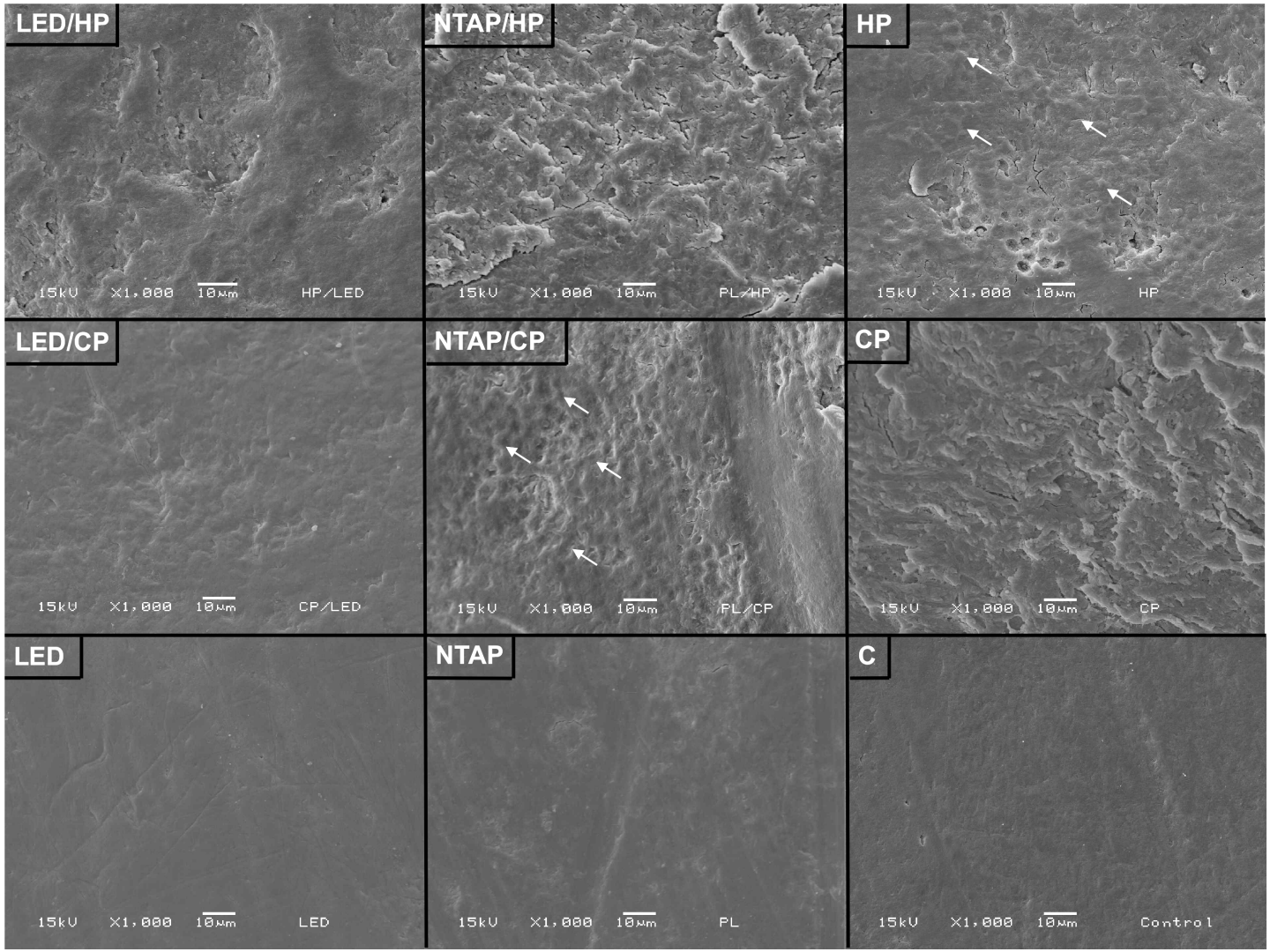
Bleaching agents	Activation mode		
	LED	NTAP	No activation
HP	1.69 (1.36) Aa	1.29 (0.80) Aa	1.62 (1.38) Aa
CP	0.43 (0.16) Ba	0.34 (0.20) Ba	0.41 (0.27) Ba

Means followed by different letters differ statistically at 5% significance level, according to two-way ANOVA and Tukey test. Uppercase letters compare the bleaching agents within each activation mode and lowercase letters compare the activation mode for each bleaching agent.

SEM Analysis

Representative SEM images of the enamel's morphology, obtained fourteen days elapsed from bleaching and of storage in artificial saliva, are displayed in Figure 3. The HP treatment caused alterations in the enamel surface such as loss of smoothness regardless of activation mode of bleaching. Even though the arrows in the HP group indicate that the inter-prismatic spaces were affected only in this group, NTAP/HP and LED/HP also showed noticeable changes in the enamel's topography, e.g., porosities and depressions. The CP groups also demonstrated differences between surfaces in the comparison with the control group, showing that CP was predominantly more irregular and that the inter-prismatic spaces (arrows) were affected in the NTAP/CP group. The images suggest that changes in the enamel's smoothness were slightly less pronounced in the LED/CP group than in the CP group; the application of LED and NTAP without the agent preserved the enamel, and maintained the surface's smoothness, keeping it flat and free of irregularities

Figure 2. SEM representative images obtained 14 days elapsed from bleaching.



Affected interprismatic areas area pointed by arrows in representative images of HP and NTAP/CP groups.

DISCUSSION

Light activation of in-office bleaching has been extensively investigated over the past few decades.[1] Several studies have demonstrated the impact of the association of bleaching with halogen lamps, laser or LED irradiations on color change, and controversial results have been reported.[2-4] Recently, a systematic review stated that the efficacy of bleaching was not affected by the use of light in either low or high-concentrated hydrogen peroxide.[5] Although the HP-treated groups were not affected by activation mode, the violet LED light significantly enhanced the color (ΔE) of CP. Therefore, the first null hypothesis was rejected. The CP's composition and lower hydrogen peroxide concentration[1] may have played an important role in this association. Due to the thickener's presence,[26] carbamide peroxide has a slower decomposition rate. Therefore, the violet LED could have accelerated carbamide breakdown by increasing the gel's temperature and consequently the number of molecular collisions, leading to the disruption of molecular bonds in the bleaching agent.[1]

To corroborate these findings, a greater variation of Δb* values was observed for LED/CP in comparison to the CP and NTAP/CP groups, at both intervals. As the b* coordinate represents the yellow*blue axis,[16] the reduction in b values implies a decrease in the teeth's yellowness, suggesting that this protocol, LED/CP, increased the amount of hydrogen peroxide by-products interacting with intrinsic organic compounds.[1]

It is known that dentin plays a major role in the tooth's color due to the enamel's translucency.[27] Ubaldini et al. (2013) showed that hydrogen peroxide barely interacts with the enamel, probably due to the lack of organic chromophores, but reported that hydrogen peroxide interaction occurs in dentin.[28] Researchers have shown that LED under a blue wavelength (465nm peak) resulted in greater color change than bleaching assisted with a halogen lamp and diode lasers or even without light activation.[29,30] Notwithstanding, violet wavelength peaks at 405 nm could indicate it has lower capacity to penetrate dental tissues.[31] Therefore, the assumption that LED irradiation could excite the organic molecules in dentin and make them more reactive with hydrogen peroxide by-products[32] is unlikely for violet light. Therefore, we credit the color enhancement instigated by LED/CP to the ability of LED to accelerate CP breakdown rather than to it interacting with organic molecules in dentin.

Kishi et al. (2011) demonstrated that LED containing a violet component was more efficient for activating a bleaching gel with titanium dioxide than LED with only blue units. Although the most suitable light for activating this photocatalyst is the ultraviolet light, it is still not suitable for clinical use.[33] Therefore, we might assume that violet LED could be applied with specific bleaching gels.

Additionally, the activation modes associated with CP and HP did not increase the teeth's luminosity even after 14 days of storage in artificial saliva, which rules out the

possibility of dehydration as responsible for a misleading enhancement in LED/CP bleaching.[34] Hahn et al. (2013) reported that light-assisted bleaching with blue LED, halogen lamp and laser maintained the color shift for 3 months.[29] Moreover, a clinical trial pointed out that the color was stable for 14 days after light-assisted (blue LED) 35% CP bleaching therapy.[30] However, a long-term evaluation either *in vitro* or *in vivo* could enlighten whether the outcomes of violet LED bleaching are influenced by time.

Violet LED alone was capable of changing the teeth's color to a lower extent than HP and CP, thus rejecting the second null hypothesis. This protocol was performed since the manufacturers' instructions suggested applying LED in up to 10 sessions in the absence of gel in patients experiencing severe tooth sensitivity.[35] According to the results, the effect of this light on extrinsic stains cannot be discarded.[13] Rastelli et al. (2018) showed that LED could excite the organic compound adhered to the enamel surface and break it down into smaller compounds.[14] It is noteworthy that the ΔE values reached by not only LED alone, but also by all protocols, were remarkably high and perceptible,[36] which could not be extrapolated for clinical settings, once artificial tea staining could have favored their action on the enamel surface. While both activation modes alone promoted ΔL values that were higher than the control at both times, LED resulted in Δa and Δb values that were superior to NTAP. Thus, LED was more efficient for removing red and yellow polyphenolic chromophores present in tea.[24] In addition, a* and b* changes indicated that the ΔE promoted in the C group could have been caused by the detachment of these staining molecules during the storage in artificial saliva.

The application of non-thermal argon plasma without bleaching also resulted in a perceivable color change notably due to the increase of luminosity, considering the 3.3 unit threshold for ΔE.[37] Plasma has the ability of producing electrons and a range of ions and molecules. The temperature of the produced components defines whether plasma is "thermal" or "non-thermal". While particles in thermal plasma are equilibrated, the non-thermal plasma ions and electrons are colder and hotter, respectively.[38] The production of OH^- radicals made NTAP a feasible alternative for tooth bleaching. According to Lee et al. (2009), non-thermal plasma associated with bleaching remarkably removed protein from the enamel's outer surface.[23] Even though the authors could not confirm whether this action was a result of plasma, our study showed that NTAP itself promoted color change. As the specimens were submitted to prophylaxis after black tea staining, the loose pigment was removed before plasma was applied on the enamel, which suggests that the color change was caused by the OH^- radicals released from NTAP. Although one might say that the clinical application of plasma results in long-term bleaching outcomes due to the increased number of appointments, 10 LED sessions promoted results that were similar to those

obtained with 6 NTAP sessions. Indeed, efforts could be made to provide, in the future, an adequate device to improve the clinical applicability of plasma.

Nevertheless, NTAP did not enhance efficacy of the bleaching agents. The color coordinates were similar to those of the bleaching-only groups. Conversely, authors have reported that the use of high-concentrated CP and HP with plasma for the full-length application of gel enhanced color change.[23,39] These divergences in results may have occurred due to differences between the times of exposure to NTAP and the bleaching gel. Based on pilot studies, we decided to attempt a clinically viable alternative by reducing NTAP and increasing the gel's application times. However, further studies should define suitable options of protocols for bleaching assisted with argon plasma.

The concentration of hydrogen peroxide in the pulp chamber of both plasma and violet LED associated with bleaching has not yet been described. Our results are the first to indicate that activation modes did not increase the intra-chamber peroxide concentration for high-concentrated carbamide and hydrogen gels ($p>0.05$). Thus, the third null hypothesis was accepted. Since there is a lack of studies that aim to detect the NTAP's effect on hydrogen peroxide concentration, we suggest that this association is at least as safe as the use of HP or CP without activation for this parameter. However, some studies disagree in relation to light activation and peroxide diffusion. While Park et al. (2016) pointed out that the association of 25% HP with a blue LED source increased the concentration of hydrogen peroxide in the pulp chamber,[40] Cavalli et al. (2016) verified no significant difference among 35% HP assisted with LED, LED/laser and halogen lamps.[6] The latter showed that a short LED wavelength (blue) did not increase the diffusion of hydrogen peroxide through dentin into the pulp cavity, which is in agreement with our study. If the assumption that LED/CP efficacy was enhanced by greater hydrogen peroxide decomposition is true, the by-products should have interacted with the chromophores and not reached the pulp chamber.

The intrapulpal concentration of HP was approximately four times greater than that of the CP groups. Recently, a study proved that agents with higher viscosity promoted lower concentration of peroxide in the pulp chamber than low and medium-viscosity gels.[11] CP's composition is more viscous than HP's,[9] which could contribute to this difference, along with the discrepancy of the hydrogen peroxide concentration within the agent. Additionally, Mena-Serrano et al. (2015) indicated that differences in composition, such as the presence of ions, could produce contrasting results with same gel concentrations.[10] Hence, the concentration of the bleaching gels is not the only factor to consider when interpreting these results.

Marson et al. (2015) evaluated the intrapulpal concentration and decomposition rate of a 35-38% HP range. The authors reported that 86% of the initial hydrogen peroxide concentration was maintained for all products after 45 minutes. In addition, the study showed that a single 45-min application did not lead to a threefold intra-chamber concentration of

hydrogen peroxide as the protocol of three 15-min application of high-concentrated HP did.[41] This evidence, along with the same bleaching efficacy reported elsewhere[42], supported the decision of not replenishing the HP and CP agents during the 30-min protocol suggested by the violet LED light's manufacturer. This decision aimed to reduce time and to simplify the technique.

The SEM analysis indicated morphological alterations in the enamel surface for both HP and CP, regardless of activation modes. It is known that the higher the pH, the more efficient the bleaching agent is.[43] Nonetheless, some bleaching gels manufactured in syringes are neutralized or even brought to an acidic pH to extend the product's shelf life.[44] The pH of both agents were verified (HP: 7.0 and CP: 6.0), but not throughout the application protocols, which may be a limitation of our study. The detection of affected inter-prismatic spaces in at least one of the HP and CP groups revealed that pH, at some point of the gel's application, could have been critical and promoted changes in the enamel. Sa et al. (2013) conducted an *in vitro* study that showed that the low pH of in-office bleaching was determinant to cause the enamel's demineralization, which could be connected to the loss of minerals.[45] Additionally, Pinto et al. (2017) stated that bleaching agents without calcium resulted in the reduction of the enamel microhardness, regardless of the products' pH.[46] As the manufacturer did not provide information on the concentration of the components present in the gels used herein, composition may also have affected the enamel's structure.

Representative images of the groups treated with bleaching without light activation corroborate other results[21] in which the inter-prismatic area was affected. On the other hand, the similarity between LED, NTAP and C indicates the safety of the activation modes for the enamel surface's morphology; therefore, we accepted the fourth null hypothesis. In the comparison among NTAP and associations of LED with bleaching, NTAP showed morphology changes that were slightly closer to those of the bleached-only groups. Conversely, Nam et al. (2018) found that non-thermal argon plasma had a positive effect on bleaching results and on the surface morphology and roughness of enamel submitted to 15% CP, significantly decreasing biofilm formation.[20] These contrasting results may occur due to the different bleaching composition or to plasma application time. These authors applied the argon plasma in 30-min gel applications, increasing its benefits for the enamel surface. Furthermore, *in situ* observations may indicate that human saliva and adhered pellicle may prevent changes to the enamel surface.[45]

The association of CP with violet LED light was efficient for bleaching, and it could be an alternative to diminish the side effects of HP with lower concentrations of peroxide reaching the pulp. However, CP alone resulted in sufficient color change to be clinically detected. Furthermore, randomized clinical trials and safety analysis, such as pulp temperature and cell viability, are still necessary to verify the efficacy and safety of violet LED

alone or in association with CP and HP agents. Furthermore, the non-thermal argon plasma protocol may require adjustments in application times as NTAP did not demonstrate negative effects on the intrapulpal concentration of peroxides and on the enamel's structure.

CONCLUSION

Within the limitations of this *in vitro* study, the following conclusions can be drawn:

(1) LED increased ΔE, ΔL and Δb values compared to no activation when associated with CP for stained teeth, even after color stabilization.
(2) ΔE, ΔL, Δa and Δb were not affected by LED or NTAP when associated with HP.
(3) LED and NTAP not associated with peroxide agents increased ΔE and ΔL compared to the control group. However, better results were obtained when they were associated with bleaching agents.
(4) LED and NTAP did not increase the intrapulpal HP concentration, and the application of HP resulted in higher concentration than the one obtained by CP.
(5) LED and NTAP did not promote surface alteration when not combined with bleaching agents.
(6) LED could be associated with CP or used alone for patients with no sensitivity tolerance.

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REGULATORY STATEMENT

This research was conducted respecting the rules of the local Ethical and Research Comitee and the policies of the University.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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2.2 Artigo 2: In-office bleaching with violet LED associated or not with 35% hydrogen peroxide or 37% carbamide peroxide: a randomized controlled clinical study.*

Short Title: Randomized controlled clinical trial on in-office bleaching with Violet LED.

*Manuscrito formatado às normas para submissão ao periódico internacional *Clinical Oral Investigations.*

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Title: In-office bleaching with violet LED associated or not with 35% hydrogen peroxide or 37% carbamide peroxide: a randomized controlled clinical study.

SUMMARY

Objective: this study assessed the effect of violet LED associated or not with 35% hydrogen peroxide (HP) and 37% carbamide peroxide (CP) on in-office bleaching. **Methods:** volunteers were divided into groups (n=20): LED, LED/CP, CP, LED/HP and HP. Colorimetric evaluation was performed using a spectrophotometer (ΔE, ΔL, Δa, Δb) and a visual shade guide (ΔSGU) at baseline (T_0), after bleaching (T_b) and in the 14-day follow-up (T_{14}). A visual analogue scale was used to determine absolute risk (AR) and intensity of tooth sensitivity (TS). Spectrophotometry evaluation of enamel's microbiopsies quantified calcium (Ca) and phosphorous (P) concentrations. ΔE, Δa, ΔL and Δb were evaluated with one-way, and Ca/P ratio with two-way repeated measures ANOVA and Tukey's test. ΔSGU and TS intensity were evaluated with Kruskal-Wallis and Mann-Whitney, and AR, with Chi-Squared. **Results:** LED/HP promoted greater color change than HP ($p<0.05$). ΔE and ΔSGU were similar for LED/CP and HP ($p>0.05$). ΔL and Δa were not influenced by LED activation. LED/HP presented the greatest Δb after fourteen days of bleaching ($p<0.05$). Overall, LED did not affect TS intensity, but it increased AR for CP. No differences were observed in Ca/P ratio within each group between T_0 and T_{14}. **Conclusions:** Violet LED only enhanced HP color change. However, LED/CP reached same bleaching efficacy of HP with reduced TS. All protocols did not affect Ca/P ratio. **Clinical Relevance:** Efficacy of high-concentrated HP is enhanced by Violet LED, and the light activation of high-concentrated CP reaches the same efficacy of HP with lower TS and maintenance of enamel mineral content.

Key words: Tooth Bleaching, Hydrogen Peroxide, Carbamide Peroxide, Light, Dentin Sensitivity, Dental Enamel.

INTRODUCTION

Tooth bleaching is a common procedure in the routine of dentists as the aesthetic appeal seems to be a permanent trend among patients [1]. Even though in-office bleaching gels are applied on teeth's surface in a shorter period of a time in comparison to those of at-home therapy [2], several researches have conducted *in vivo* evaluations attesting to the similar efficacy of in-office bleaching over the past decades [3,4]. Nevertheless, light activation of bleaching is a topic under constant discussion since a previous systematic review reported that light does not affect color change outcomes for high-concentrated hydrogen peroxide (HP), but inconclusive results were obtained for lower concentrations [5]. Conversely, Maran et al. (2017) stated that light did not enhance the efficacy of in-office bleaching regardless of the concentration of HP [6].

Other than bleaching efficacy, tooth sensitivity (TS) is a parameter that is largely investigated under several *in vivo* in-office bleaching protocols [6,7]. Light activation does not increase this symptom when high-concentrated hydrogen peroxide gel is used, and the concentration of peroxide itself seems not to affect prevalence and intensity of TS [6]. Nevertheless, a recent randomized clinical trial introduced 37% carbamide peroxide (CP) not associated with light as a feasible alternative to reduce TS caused by bleaching [8].

In addition, even though studies have shown that both low and high-concentrated peroxide gels may affect the enamel's surface's integrity [9,10], others have implied that light activation does not modify the enamel's morphology or the mineral changes caused by bleaching gels [11]. The adequate selection of the bleaching agent associated with light is still fundamental for the procedure's safety [12]. However, the concentrations of calcium and phosphorus were clinically observed to be maintained after at-home and in-office bleaching without light association [13]. Additionally, there is no *in vivo* data on the impact of light-assisted in-office bleaching on the enamel's mineral concentration.

A novel generation of violet LED (LED) light for in-office bleaching [14] has raised concerns over possible side effects promoted by the light as bleaching protocol [15,16], since no *in vitro* or *in vivo* evidences support its efficacy and safety. According to the manufacturer's instructions, violet light should be used without bleaching gels in patients reporting moderate to intense TS, and LED could also be used along with high-concentrated HP or CP in patients with absent or low TS [17]. Under an approximate 405 nm wavelength [14], violet light could have the same absorbance peak of pigments on the enamel's surface [15]. Combined with bleaching agents, it could increase the gels' temperature and, consequently, increase the decomposition of hydrogen peroxide into free radicals [2]. The possible efficacy of LED alone could prevent damages on the enamel's surface and eliminate the presence of HP in the pulp chamber, which may reduce TS.

In view of these facts, this study aimed to assess the treatment's efficacy according to color change in patients submitted to violet LED in-office bleaching associated or not with high-concentrated peroxide gels (35% HP or 37% CP). In addition, tooth sensitivity and the enamel's mineral concentration were also evaluated. The tested null hypotheses were that violet LED would 1) not promote the same color change as peroxide agents, 2) not enhance the efficacy of the bleaching agents, 3) not increase the TS provoked by the peroxide agents, and 4) not cause changes to the enamel's mineral concentration when associated or not with CP or HP.

MATERIAL AND METHODS

Ethical approval and protocol's registration

The clinical trial was approved by the local Ethic and Research Committee (registration number: 2.294.061). The research was registered in the National Clinical Trials Registry (REBEC) under the number RBR-5t6bd9.

Trial design

This was a parallel, randomized, controlled and double-blind clinical trial. Patients were not aware of which treatment group they belonged to. Another research member was responsible for randomizing the patients within the bleaching groups to ensure the allocation concealment mechanism. Even though the clinical operator was informed of which group each volunteer was allocated to, the colorimetric analysis operator was blinded to the procedures.

Recruitment and eligibility criteria

Informative advertisements were used to announce the selection of patients for the present study. All patients selected signed an informed consent form before the first bleaching session. The volunteers included in this clinical trial were over 18 and under 60 years old, and had no carious lesions and gingival health conditions. The eligibility requirements also included patients that had not undergone tooth bleaching over the last three years, and whose upper right canine minimum shade was A2 or darker.

The volunteers were excluded if they had one of the following conditions: enamel cracks, dentin hyper-sensitivity, wide restorations, endodontically treated teeth and edentulous space between maxillary and mandibular premolars. Smokers, pregnant women, nursing mothers and patients who reported allergy to any material used in the sessions were also not selected. Finally, patients that would not be able to attend all the bleaching and follow-up appointments were also excluded.

Sample size calculation

Color change was the primary outcome of this study. A previous research [18] showed that a protocol with 35% HP agent without light activation resulted in 8.3 ± 3.5 bleaching effect (ΔE). Based on that study, a 5% significance level and 80% power were used to calculate the minimum number of patients to detect differences between groups. According to the sample size's calculation (BioStat, AnalystSoft, Walnut, CA, USA), sixteen patients per group would be required, but 20 patients per group were included, so any possible volunteer dropouts would not affect the result.

Randomization, allocation and blinding

Randomization was performed by a research member who was not part of the bleaching and evaluation procedures. This person assigned a code to each participant. Each code was written in an opaque and sealed sheet, and the sheets were distributed randomly among the five intervention groups. The result of this randomization was only revealed to the operator at the beginning of the first bleaching appointment. The participants were blinded to the procedure in terms of agent type (HP or CP) as they did not know which bleaching was applied on the teeth's surface. Although volunteers treated only with LED irradiation could have noticed that no bleaching agent was applied, they were not informed about how their group differed from the others. The operator was aware of the volunteers' bleaching interventions because the agents are visually different. The bleaching agent was not seen by the patients since an assistant helped to blind the procedure. The colorimetric evaluator was blinded to all procedures.

Study intervention

Five different in-office bleaching protocols were defined as the interventions of this study. The materials used are described in Table 1. The groups were established according to each bleaching gel and light activation: (1) LED, (2) LED/CP, (3) CP, (4) LED/HP and (5) HP. The bleaching protocols are detailed in Table 2.

Table 1. Bleaching agents, composition and manufacturer's instructions.

Bleaching Agent (Manufacturer, Address)	Specification/Composition	Manufacturer's instruction
HP – hydrogen peroxide Whiteness HP (FGM, Joinville, SC, Brazil)	35% hydrogen peroxide, thickener, glycol, inert filler, dyes and deionized water. pH was informed by the manufacturer as = 7.0.	Applied on vital teeth. Three changes in every 15 minutes are indicated. A 7-day interval between sessions is required. Treatment must be repeated up to the fourth session.
CP – carbamide peroxide Whiteness Super Endo (FGM, Joinville, SC, Brazil)	37% carbamide peroxide, neutralized carbopol, inert filler, glycol and deionized water. pH was informed by the manufacturer as = 6.0.	Applied on the pulp chamber of non-vital teeth using the walking bleaching technique. Evaluation and changes, if necessary, must be performed in three to four days up to eight times.
Violet LED Bright Max Whitening – BMW (MMOptics, São Carlos, SP, Brazil)	Four light emitting diode light in violet wavelength (405 nm) positioned in a curved acrylic tip. Illumination area of the tip = 10.7 cm^2; maximum power = 22VA; optical power = 1.2 W.	Twenty one-min irradiations of the device with consecutive 30-s intervals without gel application should be used for patients with previous intense tooth sensitivity or be associated with chemical agents for patients with minor or absence of tooth sensitivity. Four to ten sessions with 4-day intervals are indicated when only light is used. The association of bleaching gel limits the number of sessions to 3 with longer one-week intervals.

Abbreviations: LED, light-emitting diode; CP, carbamide peroxide; HP, hydrogen peroxide

Table 2. Detailed bleaching protocols for LED associated or not with peroxide gels and their correspondent groups without light activation

Intervention	Bleaching Protocol Used in This Study
LED	Twenty 1-min irradiations of violet LED light were activated with the light having been permanently positioned 8 mm away from the arches. Thirty-second intervals were performed consecutively between each irradiation. The gingival tissues were protected with a gingival barrier made with flow composite resin (Top Dam, FGM, Joinville, SC, Brazil). The teeth were kept hydrated with a moist gauze during the intervals. The protocol was repeated for 8 sessions with 4-day intervals.
LED/CP and only CP	The same irradiation protocol described above was used to light-activate the 37% CP gel, which was applied with no changes from right to left second premolar in both arches for 30 minutes. Gingiva was protected with barrier, and the gel was applied directly with the syringe provided by the manufacturer on the teeth's entire buccal surface. Three sessions were performed with 7-day intervals. The same procedures were adopted for CP without light activation.
LED/HP and only HP	35% HP gel was light-activated with the same protocol of the LED group. After protection of the gingival tissues, the thickener and peroxide were mixed in a container, and this mixture was applied on the entire buccal surface from premolar to premolar with a brush only one time for 30 minutes. LED was immediately positioned facing the arches. Firstly, the gel showed a reddish color, which changed to transparent within the first minutes. Three sessions and 7-day intervals were also adopted as protocol for this group. The HP group received the same protocol with the exception of light activation.

Abbreviations: LED, light-emitting diode; CP, carbamide peroxide; HP, hydrogen peroxide.

Colorimetric Evaluation

An objective evaluation of color change was conducted with contact-type intraoral spectrophotometer Easy Shade (Vita Zahnfabrik, Bad Säckingen, Germany). An impression with dense silicon (Zhermak, Kouigo, Italy) was preliminary obtained from the upper right arch of the volunteers, and a hole with the same dimension of the spectrophotometer's tip was created in the upper right canine [19]. Thus, the region of color analysis was standardized for all evaluation times. Dental prophylaxis was performed before the baseline color measurement and the patients were requested to not intake dark beverages and food. Before treatment (T_0), the evaluator recorded the values of the CIE L*a*b* coordinates, and this procedure was repeated after the last bleaching session (T_b) and 14 days after the end of the intervention (T_{14}). While L* represents the teeth's luminosity, a* and b* indicate the measurement of the red*green and yellow*blue axes, respectively. Subsequently, the values were used to calculate $\Delta E= [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$ for different intervals: $\Delta E1$ (T_b-T_0) and $\Delta E2$ (T_{14}-T_0). $\Delta L1$, $\Delta L2$, $\Delta a1$, $\Delta a2$, $\Delta b1$ and $\Delta b2$ were also calculated.

Additionally, a subjective color evaluation (ΔSGU) [19] was carried out by the same blind evaluator at the same evaluation times as the objective assessment, using a visual shade guide unit (Vita Zahnfabrik, Bad Säckingen, Germany). The guide tabs were sorted by value from highest (1) to lowest (16), as follows: B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4 and C4. To calculate ΔSGU at T_b and T_{14}, the color registered was subtracted from the initial shades. The evaluator was calibrated to measure color at the middle third of the upper right canine.

Tooth Sensitivity (TS) Analysis

A visual analogue scale (VAS) was handed out to the volunteers at the end of the first bleaching session. The volunteers were asked to indicate the intensity of TS in each session using this 0-10 pain scale, in which 0 was equal to no sensitivity and 10 to the most intense sensitivity [18]. The patients were requested to not use dentifrices for reducing tooth sensitivity. The operator also asked the patients to record TS intensity between sessions. The volunteers who reported absence of TS during the periods of evaluations, indicated it as zero. When the patients reported it as at least level 1, they were considered to be sensitive to the intervention. Thus, the absolute TS risk for the bleaching protocols was measured.

Enamel's Mineral Content

To quantify the calcium (Ca) to phosphorus (P) ratio of teeth submitted to the interventions, the technique "enamel microbiopsy" was used. In this study, the protocol published by Amaral et al. (2012) [13] was adapted for use in the first upper premolar at T_0,

T_b and T_{14}. The biopsy site was isolated by the operator with an adhesive tape (3M Oral Care, St. Louis, MN, United States) with a circular 1.6 mm perforation. Five µL HCl 1.6M in 70% gycerol (v/v) (Sigma-Aldrich, St. Louis, MO, United States) were applied in this region for 20 s with continuous gentle stirring. This solution was collected and dispensed in a test tube with 200 µL of ultra-purified water. Afterwards, 5 µL of 70% glycerol were applied on the same region for 10 s, and also transferred to the same tube.

The Arsenazo III and malachite green methods described by Vogel et al. (1983) [20] were used to determine the Ca and P concentration in µg, respectively, in 25 µL of each sample. The absorbance was read in 96-well plates at 650 nm wavelength in a Multiskan Spectrum (Thermo Scientific, Waltham, MA, United States) microplate reader. The results were expressed in Ca/P ratio.

Statistical Analysis

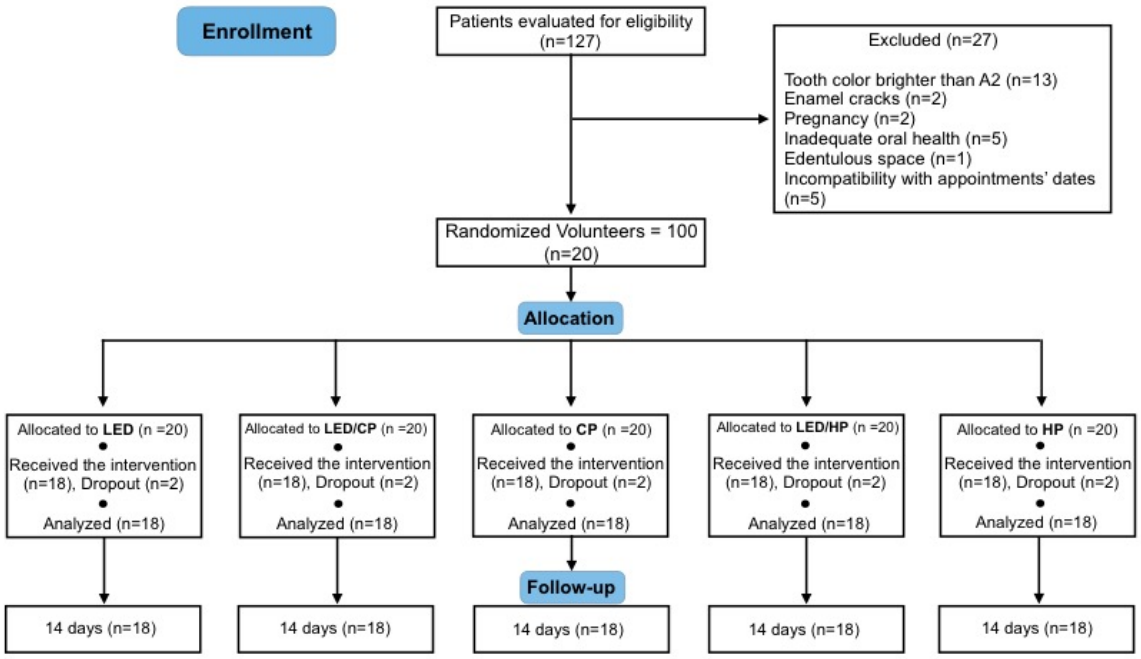
The color change and mineral concentration data were submitted to exploratory analysis for normal distribution and homoscedasticity. The ΔL, Δa and Δb at both intervals met the parameters of normality, and ΔE was transformed into square root values after Levene's test for equal of variances. The Δ values were submitted to one-way ANOVA and Tukey test. ΔSGU and the intensity of the TS values were statistically analyzed using the Kruskal-Wallis and Mann-Whitney tests. The absolute TS risk was tested using the non-parametric Pearson Chi Square test. The confidence interval for the absolut risk was calculated. The Ca and P concentrations were tested using two-way repeated measures ANOVA and Tukey Test (between-subject factor: "treatment"; within-subject factor: "time of evaluation"). The analyses were performed at 5% significance level, using SPSS Statistics Version 23 (IBM Corp, Armonk, NY, United States)

RESULTS

Characteristics of the participants included

The recruitment took place between November and December 2017. After the clinical examination for eligibility of 127 patients, 100 volunteers (n=20) were included in this study (Figure 1). The characteristics and baseline values of the patients are described in Table 3. The bleaching appointments were performed from January 15, 2018 to November 13, 2018. A dropout rate of 2 patients per group was detected. The reasons for dropout were incompatibility with the appointments' dates or the volunteers having moved to distant cities. All patients (n=18) submitted to the intervention were evaluated for the primary outcome (color change) and for the secondary outcomes (TS and enamel's mineral content).

Figure 1. Flowchart of the RCT from the evaluation for eligibility of the volunteers to the 14-day follow-up.



Abbreviations: LED, light-emitting diode; CP, carbamide peroxide; HP, hydrogen peroxide.

Table 3. Baseline values for the CIEL*a*b* coordinates and main descriptive characteristics of the selected volunteers.

Coordinate values (Mean, SD)	**L***	81.84 (4.93)
	a*	5.96 (3.62)
	b*	27.13 (7.03)
Age in years (Mean, SD)		21,46 (4,14)
Gender (%)	**Male**	32.0%
	Female	68.0%
Ethnicity (%)	**White**	89.0%
	Black	5.5%
	American Indian	2.2%
	Yellow	3.3%

Color Change

All protocols produced ΔE values higher than 3.3. The ΔE values (Table 4) of LED/HP-treated patients were significantly higher among the groups at both intervals ($p<0.001$) followed by HP and LED/CP, which exhibited similar color change ($p>0.05$). At both intervals, HP promoted greater color change than CP ($p<0.05$). LED/CP showed higher ΔE values than CP at ΔE1, but 14 days after bleaching (ΔE2), LED/CP was similar to CP. Patients treated with LED only showed the lowest color change means at both intervals ($p<0.05$). After bleaching (ΔSGU1), the LED-treated groups showed the lowest ΔSGU results ($p<0.05$) (Table 4), but 14 days after bleaching, the LED and CP- groups exhibited similar ΔSGU ($p=0.97$). LED/HP produced the highest ΔSGU values at both intervals ($p<0.05$).

Table 4. Mean ΔE ($T_B - T_0$) and ΔE2 ($T_{14} - T_0$) values and standard deviations according to treatments, and medians (minimum and maximum values) of ΔSGU 1 ($T_B - T_0$) and ΔSGU 2 ($T_{14} - T_0$).

Treatments	**ΔE1 ($T_B - T_0$)**	**ΔE2 ($T_{14} - T_0$)**
LED	3.4 (1.3) D	3.7 (1.4) D
LED/CP	7.8 (2.0) B	8.6 (2.1) BC
CP	5.7 (2.5) C	6.6 (3.0) C
LED/HP	12.9 (2.6) A	14.4 (2.2) A
HP	8.8 (3.0) B	10.0 (4.1) B
Treatments	**ΔSGU 1 ($T_B - T_0$)**	**ΔSGU 2 ($T_{14} - T_0$)**
LED	3.5 (0.3;4.0) C	2.5 (1.0;4.0) C
LED/CP	7.0 (6.0;8.8) B	6.0 (4.5;7.0) B
CP	5.5 (3.0;7.0) B	3.0 (3.0;6.0) BC
LED/HP	10.0 (7.0;10.0) A	9.0 (7.0;10.0) A
HP	7.0 (4.5;8.5) B	7.0 (4.5;7.8) B

Uppercase letters compare bleaching protocols (treatments).

LED was the only group that did not enhance the luminosity (ΔL) of the upper right canine after 14 days ($p<0.05$) (Table 5). LED/HP and LED/CP were similar to HP and CP, respectively ($p>0.05$), but LED/HP exhibited higher ΔL values than CP, at both intervals ($p<0.05$). The Δa evaluation revealed that LED and LED/CP were equal ($p>0.05$), and LED/CP, CP and LED/HP also did not statistically differ at both times ($p>0.05$). Moreover, HP was superior to LED and LED/CP ($p<0.05$) for Δa. The Δb evaluation was different between LED/CP and CP after bleaching ($p=0.003$), and between LED/HP and HP 14 days elapsed from bleaching ($p=0.001$) (Table 7). LED/HP, HP, LED/CP showed a similar decrease in tooth yellowness (Δb) ($p>0.05$), which was higher than in the CP and LED-treated groups ($p<0.05$), at Δb1. Fourteen days after bleaching (Δb2), LED/HP showed the most significant decrease in yellowness among the groups, and LED, the lowest ($p<0.05$).

Table 5: Mean and standard deviations of ΔL, Δa and Δb for both intervals 1 ($T_B - T_0$) and 2 ($T_{14} - T_0$) values according to treatments.

Treatments	**ΔL1 ($T_B - T_0$)**	**ΔL2 ($T_{14} - T_0$)**
LED	-0.3 (2.5) C	-1.0 (1.7) D
LED/CP	2.9 (3.2) BC	2.6 (3.6) BC
CP	3.4 (2.8) B	4.5 (3.2) B
LED/HP	8.0 (3.4) A	7.7 (3.3) A
HP	5.0 (3.0) AB	5.6 (3.2) AB
Treatments	**Δa ($T_B - T_0$)**	**Δa ($T_{14} - T_0$)**
LED	0.0 (1.19) A	0.34 (0.7) A
LED/CP	-0.9 (1.8) AB	-1.0 (1.0) AB
CP	-1.6 (1.2) BC	-1.7 (1.6) BC
LED/HP	-2.0 (1.5) BC	-1.9 (1.3) BC
HP	-2.7 (1.0) C	-2.5 (1.6) C
Treatments	**Δb1 ($T_B - T_0$)**	**Δb2 ($T_{14} - T_0$)**
LED	0.0 (2.3) B	-1.1 (2.5) C
LED/CP	-6.0 (2.4) A	-6.3 (2.3) B
CP	-2.3 (2.1) B	-3.6 (2.5) B
LED/HP	-8.2 (4.2) A	-11.1 (5.1) A
HP	-6.2 (2.5) A	-7.2 (2.5) B

Means followed by different letters differ statistically at 5% significance level, according to one-way ANOVA and Tukey test. Uppercase letters compare bleaching protocols (treatments).

Tooth Sensitivity

TS values are presented up to the third session because the LED group's volunteers reported no TS after the third appointment. Regarding the absolute TS risk (Table 6), patients were considered sensitive even if they reported sensitivity in only one bleaching session. The Chi-square test revealed difference between groups ($p<0.001$). The LED group had the lowest percentage of volunteers with TS. CP, LED/CP and LED/HP were similar ($p>0.05$), with 44.4%, 61.1% and 88.8% TS risk, respectively. However, HP showed 94.4% TS risk and was statistically higher than the others ($p<0.05$).

Table 6. Absolute risk of TS expressed in percentage for each bleaching protocol.

Treatments	**Absolute Risk (95% Confidence Interval)**
LED	0.16 (0.00-0.32)
LED/CP	0.61 (0.40-0.81)
CP	0.44 (0.23-0.65)
LED/HP	0.88 (0.74-1.00)
HP	0.94 (0.84-1.00)

Pearson's Chi square test ($p<0.001$)

TS intensity was evaluated for each session and between intervals (Table 7). At the 1st session, LED and LED/CP exhibited lower TS than the LED/HP and HP groups ($p<0.05$) and were similar to the CP groups ($p>0.05$). At the 2nd session, all groups showed the same TS intensity ($p>0.05$). At the 3rd session, violet LED increased the intensity of TS for HP ($p=0.20$). During the 1st interval, LED/HP showed higher TS than HP ($p<0.05$). During the 2nd and 3rd intervals, no significant difference was found between the HP and CP groups and their respective activated groups ($p>0.05$).

Table 7. Median values (minimum and maximum value) of intensity of tooth sensitivity (TS) reported by the patients during the sessions and their intervals.

Treatments	**1st session**	**2nd session**	**3rd session**
LED	0.0 (0.0;4.0) B	0.0 (0.0;4.0) A	0.0 (0.0;2.0) B
LED/CP	0.0 (0.0;3.0) B	0.0 (0.0;3.0) A	0.0 (0.0;7.0) AB
CP	0.0 (0.0;3.0) AB	0.0 (0.0;10.0) A	0.0 (0.0;4.0) B
LED/HP	2.0 (0.0;7.0) A	1.0 (0.0;9.0) A	2.0 (0.0;7.0) A
HP	2.5 (0.0;3.0) A	0.0 (0.0;7.0) A	0.0 (0.0;3.0) B
Treatments	**1st interval**	**2st interval**	**3st interval**
LED	0.0 (0.0;1.0) C	0.0 (0.0;3.0) C	0.0 (0.0;7.0) B
LED/CP	0.0 (0.0;5.0) BC	0.0 (0.0;6.0) B	0.0 (0.0;6.0) AB
CP	0.0 (0.0;5.0) C	0.0 (0.0;6.0) B	0.0 (0.0;3.0) B
LED/HP	5.0 (0.0;9.0) A	3.0 (1.0;7.0) A	3.0 (0.0;7.0) A
HP	0.5 (0.0;10.0) B	2.0 (0.0;10.0) AB	0.0 (0.0;10.0) AB

Medians followed by different letters differ statistically at 5% significance level, according to the Kruskal-Wallis and Mann-Whitney tests. Uppercase letters compare TS according to treatment and to the sessions' intervals. No TS was reported for the LED group after the third session.

Enamel's Mineral Content

Table 8 illustrates the Ca/P ratio results after analysis of calcium and phosphorus. Difference was detected for between-subjects "treatment" ($p=0.009$), while the within-subjects "time" analysis revealed no significant difference ($p=0.654$). Regarding the Ca/P ratio, CP was significantly higher than LED/CP and LED/HP at T_0, and no differences were detected between LED/HP and LED/CP and their respective groups after bleaching (T_B) and 14 after the intervention ($p>0.05$). Within each intervention, the Ca/P ratio did not decrease ($p>0.05$) except for LED/CP at T_B and T_{14}, which were both statistically similar to T_0.

Table 8. Mean values and standard deviations of the Ca/P ratio for each intervention group according to times T_0 (baseline), T_B (after bleaching) and T_{14} (14 days after bleaching)

Treatments	**Ca/P (T_0)**	**Ca/P (T_B)**	**Ca/P (T_{14})**
LED	2.33 (0.88) ABa	1.98 (0.68) Aa	1.96 (0.80) ABa
LED/CP	1.76 (0.98) Bab	1.59 (1.12) ABa	2.30 (1.52) ABb
CP	2.65 (1.38) Aa	2.37 (1.59) Aa	2.49 (1.02) Aa
LED/HP	1.79 (0.94) Ba	1.40 (0.88) Ba	1.68 (1.07) Ba
HP	2.14 (0.71) ABa	1.85 (0.87) ABa	2.19 (1.13) ABa

Means followed by different letters differ statistically at 5% significance level, according to two-way repeated measures ANOVA and Tukey Test. Uppercase letters compare bleaching protocols (treatments) and lowercase letters compare evaluation times.

DISCUSSION

The introduction of a new generation of violet LED for in-office bleaching and the suggestion of its association with peroxide agents raise concerns over this treatment's safety and efficacy. Case reports which applied violet LED without any chemical agent showed color changes according to the visual shade guide after approximately 5 sessions [15,16]. In this study, the color evaluation showed that LED exhibited the lowest ΔE values at both intervals among the groups. However, the LED protocol reached values above the clinically noticeable difference of 3.3 units for ΔE [21]**.** The individual analyses of the L*, a* and b* coordinates revealed that while luminosity (Δa) and redness (Δa) were not affected by violet LED alone, the reduction of yellowness (Δb) at time point T_{14} played a role in color change for the LED protocol.

This could be explained by the violet light's wavelength, which is approximately 405 nm. The emission band of the violet LED could correspond to the absorption peak of the stained particles, which leads to them breaking down into shorter and uncolored molecules [14]. As pigments are reactive to light and the violet light has lower capability of penetration through teeth [22], we believe that the LED mechanism is restricted to the enamel's surface. Dental prophylaxis was performed at the beginning of each session to reduce extrinsic staining, and to not overestimate the color change produced by LED compared to the other groups. Moreover, the decision of interrupting the LED protocol after 8 sessions was based on the fact that no color change was detected after the 6^{th} appointment.

The ΔSGU evaluation indicated similar color change for LED and CP after 14 days of the end of the bleaching protocols. Nevertheless, this was not observed for ΔE of CP, which exhibited higher ΔE than LED ($p<0.05$). Based on these results, the first null hypothesis was accepted. In spite of the fact that the evaluator was calibrated and the illumination conditions were standardized throughout the study, the differences between ΔSGU and ΔE may have been caused by the relevant characteristics of each evaluation method. According to Joiner & Luo (2017), visual shade guides have some drawbacks such as inadequate range of shades and systematic inconsistencies between shade tabs [23]. Some studies showed that spectrophotometers promote more accurate results than visual shade guides [24, 25]. On the other hand, clinical studies verified great accuracy and repeatability of the spectrophotometer, e.g., VITA Easyshade [26].

At time point $T_{b,}$ LED/CP promoted greater color change (ΔE) than CP. This difference might be explained by the decrease in tooth yellowness (Δb) promoted by LED/CP compared to CP at the same interval. This could lead to the assumption that violet LED enhanced the rate of decomposition of carbamide peroxide into free radicals, possibly caused by the heat, and the by-products were able to interact with the organic chromophores

of dentin [27]. However, the Δb* difference between the LED/CP and CP groups was no longer detected 14 days after bleaching. Similarly, Hann et al. (2013) observed that HP without violet light exhibited greater ΔE after one week of treatment than bleaching associated with different light sources [28]. It is suggested that a residual effect may occur, since CP presents prolonged decomposition rate than HP [29].

Although a similar color change was observed between the LED/CP and HP treatments, LED exhibited lower sensitivity levels. In view of this fact, the light activation of CP could be an alternative for patients with moderate-intense tooth sensitivity. In addition, this fact could broaden the clinical indication of CP, which is commonly used in the internal bleaching of non-vital teeth [30]. There is a lack of *in vitro* studies on the efficacy and safety of the application of high-concentrated carbamide peroxide on the enamel's outer surface. Peixoto et al. (2018) reported, in a randomized clinical trial, that the color change resulted from bleaching with 37% CP was significantly lower than that with 35% HP, which is in agreement with our outcomes [8]. Although no randomized clinical trials tested the impact of light on 37% CP, several studies reported different findings in relation to the efficacy of light activation for low-concentrated HP gels, whose hydrogen peroxide concentration is similar to that of 37% CP [2]. Thus, concentration may not be the only factor to affect bleaching efficacy. The long-term follow-up of color change outcomes for LED/CP will help to elucidate whether its efficacy is comparable to bleaching with high-concentrated HP over time.

The use of violet LED significantly enhanced the efficacy of HP even after posttreatment subjective and objective color evaluation, thus invalidating the second null hypothesis. The ability of LED/HP to decrease yellowness (Δb*) was statistically superior to HP's at T_{14} ($p<0.05$), which suggests a residual effect of light-activated HP. Since HP's decomposition rate is faster than CP's [2], the immediate Δb* values for LED/HP and HP were similar. However, the gel's rise in temperature, leading to extended formation of by-products, could have prolonged HP's bleaching action. A systematic review pointed out that the bleaching efficacy of low and high-concentrated HP agents is not influenced by light activation [6]. Nevertheless, only few studies evaluated the use of light sources with a violet light wavelength component, and the activation time of LED, laser and halogen lamps differed remarkably between the studies. For instance, while Kugel et al. (2009) irradiated 25% HP with a non-specified light source during the 60-minute gel application [31], Freitas et al. (2016) applied LED/laser for 3 minutes to activate 35% HP [32]. Moreover, a recent network meta-analysis demonstrated that none light source presented superiority of color change outcomes [33]. Although we acknowledge the contribution of these systematic reviews for this field of study, the generation of violet LED should be considered a new approach to in-office bleaching, and further studies on its efficacy should test its irradiation

protocols, which, up until this moment, have only been established by the manufacturer. Like for LED/CP, the follow-up of LED/HP will be helpful to determine its long-term efficacy.

The suggestion of new clinical protocols also considers tooth sensitivity levels, a meaningful safety parameter reported by patients. Several *in vivo* clinical researches have shown TS as a common side effect of tooth bleaching [6,18,34]. This symptom in moderate or severe levels did not lead to the interruption of bleaching [4, 35], and the long-term evaluation showed no permanent TS [36]. In a previous clinical report, no difference for absolute risk between the light-activated 20% and 35% HP treatments was detected [19]. However, the intensity of TS was significantly superior for the 35% HP groups. Even though our study's sample size was not calculated for the TS evaluation and these data should be evaluated cautiously, this secondary outcome showed that the absolute risk for LED/CP was significantly lower than for HP. Although Basting et al. (2012) reported that the absolute TS risk was higher for patients treated with 20% CP (at-home bleaching) than for those treated with 35% HP (in-office bleaching) [37], another RCT showed that the TS intensity levels for 37% CP were lower than for 35% HP [8]. In our study, the intensity levels at the 1st session were inferior for light-activated CP. A minimum absolute risk difference was detected for HP (94.4%) and LED/HP (88.8%), and difference in intensity was only detected at the 3rd session (LED/HP>HP) and at the 1st one-week interval. Nevertheless, the prevalence of TS for CP increased under LED activation (LED/CP>CP). Thus, the third null hypothesis was rejected, as LED increases TS depending on the type of the gel.

The 30-s intervals during the irradiation cycle of violet LED are performed as an attempt to not overheat the pulp and, consequently, not cause irreversible damages to the tissue. The classic study of Zach & Cohen (1965) demonstrated that a 5.5 ^{0}C increase in pulp temperature resulted in significant pulp necrosis [38]. Although studies on the temperature rise of light-assisted bleaching are controversial in terms of which light source raises temperature the most [27, 39], an *in vivo* research showed that a 60-s polywave LED unit irradiation for light-curing increased pulp temperature over 5.5^{0}C [40]. Yet, no data were found to ensure whether the time interval indicated by the violet LED's manufacturer is adequate or if a longer cooling phase should be adopted to protect pulp tissue even in the absence of bleaching gel. Further investigations should be performed on the protocols' safety such as pulp temperature and cell viability, and as the color change after HP application alone is considered extremely clinically perceptible [41], this should be taken into account in the decision on the light-activation of this gel.

The trans-dentin diffusion of HP into the pulp chamber has been associated with TS, which may last up to 4 days after the appointment [42]. The penetration of hydrogen peroxide into the pulp chamber is richly described [43], and although there is a lack of data verifying the influence of violet LED on the penetration levels of free radicals, some authors showed

that light activation did not increase the intra-chamber concentration of hydrogen peroxide caused by the application of HP gels [43, 44].

Enamel microbiopsy is a technique used to clinically detect the concentration of ions such as fluoride, calcium and phosphorus [45], which analyzes mineral changes after different challenges. Even though the Ca/P ratio was different between some groups throughout the study, this ratio was maintained within each bleaching protocol between T_0 and T_{14}. Therefore, the last null hypothesis was accepted. Berger et al. (2010) demonstrated that 35% HP was responsible for reducing the enamel's mineral content. However, according to the authors, light activation with halogen lamp and LED/laser did not increase mineral loss [11]. The inherent variation of the enamel's mineral content [13] could explain the differences between patients at the baseline. As LED did not alter the Ca/P ratio after the application of HP and CP, the application of violet light seems not to harm the enamel structure's and not to enhance the impact of the gel on the mineral content. Sa et al. (2013), in an *in vitro* study, found that 38% HP without light activation was capable of changing the conformation of the enamel's prisms and interprismatic spaces [46]. Nonetheless, this pattern was not found for the same bleaching protocols tested *in situ*. Therefore, the presence of salivary pellicle may protect the enamel surface against changes promoted by the bleaching gel's application. Furthermore, the same authors stated that pH plays an important role in the enamel's surface's changes *in vitro* [46]. Contrariwise, Pinto et al. (2017) observed that a bleaching agent with acidic pH did not cause changes in the enamel's mineral content [12]. Therefore, the choice of the bleaching agent to be used in association with violet LED should also consider the agent's rheology and composition.

According to the results, violet LED light with or without the bleaching agent was efficient in terms of color change and did not influence the changes in the enamel's mineral content. The decision on which gel is more adequate will depend on the patient's expectancy and initial color measurement, since LED/HP resulted in greater color change. In addition, tooth sensitivity should also be considered since the absolute TS risk indicated that less-concentrated agents activated with violet LED, such as CP, would reach similar results to HP's with less symptoms. The bleaching protocol should be performed after the correct tooth discoloration diagnosis. In addition, the patients must be aware that color change with LED alone will not reach similar efficacy to that of chemical agents, and the treatment regimen is longer.

CONCLUSION

Violet LED light promoted a clinically perceptible color change, but it did not reach the same efficacy as the HP-treated groups. The association of LED with HP enhanced color change, and the bleaching results of the LED/CP group were similar to those of the HP group, with lower tooth sensitivity. No changes in the enamel's mineral content were observed 14 elapsed from the treatments for each bleaching protocol.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

M. Kury declares that he has no conflit of interest. E. E. Wada declares that she has no conflict of interest. D. P. da Silva declares that she has no conflict of interest. C. P. M. Tabchoury declares that she has no conflict of interest. M. Giannini declares that he has no conflict of interest. V. Cavalli declares that she has no conflict of interest.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Piracicaba Dental School Ethical Committee - 2.294.061) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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CONFLICT OF INTEREST

The authors report that they have no financial, professional nor other interest of any nature on the divulgation of the data herein present.

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3. DISCUSSÃO

Apesar do clareamento de consultório com LED violeta ser uma realidade clínica, o plasma de argônio ainda permanece como uma possibilidade para futuras indicações do clareamento dentário. Entretanto, ambos necessitam de evidências que suportem a eficácia e segurança de suas aplicações.

Embora esse estudo tenha demonstrado diferenças entre associações de géis clareadores com LED e PL, é importante ressaltar que ambos diferem em relação ao seu mecanismo de ação. Enquanto a luz violeta provavelmente aumenta a temperatura do gel clareador (Joiner & Luo, 2017), o plasma fornece radicais livres do peróxido de hidrogênio ao gel clareador (Clairborne et al., 2014). Um estudo mostrou que PL foi superior ao LED azul no aumento da eficácia de géis clareadores (Nam et al., 2013). Entretanto, o mesmo não foi verificado nesse estudo, uma vez que a luz violeta foi mais eficaz na associação com CP em alta concentração. Contudo, o tempo de aplicação de PL reduzido desse estudo pode ter comprometido essa associação. Adicionalmente, o mecanismo de ação de ambas as tecnologias pode ter proporcionado a eficácia clareadora quando empregados sozinhos, na superfície dos dentes.

Embora a pesquisa com PL no clareamento dental seja limitada a estudos laboratoriais, o uso do LED violeta em pacientes tornou as pesquisas clínicas imprescindíveis. Similarmente aos resultados do *in vitro,* os resultados do ensaio clínico randomizado demonstraram que o LED aumentou a eficácia clareadora de CP imediatamente após ao clareamento. Entretanto, após 14 dias, o ΔE de LED/CP e CP foram semelhantes. Além disso, enquanto os resultados *in vitro* mostraram que o LED não exacerbou o clareamento para HP em ambos os tempos de avaliação, o estudo *in vivo* demonstrou o oposto.

Tais diferenças podem ser explicadas pela composição heterogênea do substrato dental em estudo. Estudos sugerem que a composição química e morfológica, bem como as propriedades físicas, do dente devem ser consideras ao analisar dados (Yassen et al. 2012). Por exemplo, Hahn et al. (2013) reportou em um estudo *in vitro,* que LED em comprimento de onda azul não potencializou o efeito de gel clareador HP em alta concentração para dentes humanos. Entretanto, Gomes et al. (2009), mostraram efeito imediato positivo na mesma associação para dentes bovinos. Portanto, diferenças na composição e morfologia, como disposição dos prismas do esmalte e morfologia dentinária, podem explicar as diferenças entre os resultados *in vitro* e *in vivo* deste trabalho.

Ademais, enquanto o estudo *in vitro* utilizou protocolo de pigmentação com chá preto, o ensaio clínico randomizado fez uso de unidades experimentais com pigmentações inerentes do paciente selecionado. Young et al. (2012) comprovaram o efeito clareador de

HP sobre os cromógenos presentes no chá preto, demonstrando inclusive que o mesmo é potencializado quando associado ao LED azul e aditivos químicos no gel clareador. Mesmo que seja questionado que a pigmentação do chá preto seja depositada apenas na superfície do dente, e que o efeito do agente clareador esteja restrito à essa pigmentação, a profilaxia prévia aos protocolos clareadores previniu tal ocorrência. Os valores *in vitro* de Δa e Δb revelaram que o LED violeta foi capaz de remover os pigmentos de coloração avermelhada e amarelada presentes no chá preto (Sulieman et al., 2003), e como os valores *in vivo* demonstraram valores de a* e b* com pouca alteração, a presença do pigmento no estudo *in vitro* exacerbou o efeito clareador da luz violeta.

Comparando os resultados clínicos e laboratoriais obtidos para LED violeta sem géis clareadores, ambos resultaram em ΔE superiores ao limiar de clareamento clinicamente perceptível (Joiner & Luo, 2017). Não obstante, a alteração de cor em decorrência do estudo clínico foi expressivamente inferior à relatada no estudo *in vitro*. Nesse caso, a pigmentação com chá preto que permaneceu na superfície dos dentes bovinos após a profilaxia pode ter tornardo o clareamento mais eficaz para LED e todos os grupos do estudo *in vitro*, uma vez que a pigmentação dos dentes dos pacientes pode não ter diminuído, na mesma proporção, os valores iniciais das coordenadas L*a*b*.

Em relação à segurança dos protocolos, LED não aumentou a concentração de peróxido de hidrogênio na câmara pulpar após o clareamento com HP e CP. Embora Camargo et al. (2009) tenha reportado aumento dessa concentração quando da utilização de LED azul, isso não ocorreu em outros estudos (Kwon et al., 2013, Cavalli et al., 2016).

Não há relatos acerca do efeito do plasma de argônio na penetração de peróxido de hidrogênio; entretanto, esses dados mostram que a técnica pode ser segura em razão do fornecimento de radicais livres ao gel clareador não ter aumentado os valores da concentração intra-pulpar de peróxido de hidrogênio. O uso de CP promoveu menor penetração intra-pulpar de peróxido, o que poderia indicar maior segurança das técnicas que fizeram uso desse agente clareador.

De maneira geral, a sensibilidade dental reportada pelos pacientes nesse ensaio clínico randomizado não foi influenciada por LED. Os dados *in vitro* do efeito da luz na concentração intra-pulpar de peróxido de hidrogênio desse estudo e de outros na literatura podem estar associados aos dados de sensibilidade dental apresentados. Há autores que creditam as mudanças no tecido conjuntivo à penetração intra-pulpar de peróxido de hidrogênio, desencadeando a sensibilidade dental (Briso et al., 2016). Soares et al. (2014) demonstraram que reduzindo o tempo de aplicação ou diminuindo pela metade a concentração de peróxido de hidrogênio, há redução da citotoxicidade. Como a concentração de peróxido para CP é menor (Kwon et al., 2016), a sensibilidade para esses grupos pode ter sido menos pronunciada que para HP. Visto que os estudos *in vitro* e *in vivo*

reportaram que a eficácia clareadora de LED/CP foi similar ao protocolo de HP, tal protocolo pode ser indicado para pacientes que reportem sensibilidade dental sem comprometer o clareamento.

Adicionalmente, no estudo *in vitro,* o LED violeta não aumentou as alterações morfológicas promovidas pelos géis clareadores e quando associado a estes, o mesmo não promoveu *in vivo*, mudanças significativas no conteúdo de Ca e P do esmalte após 14 dias de acompanhamento. Tal fato, reforça a segurança da indicação de LED/CP para pacientes com sensibilidade prévia e, também, de LED/HP para pacientes sem relato de sensibilidade dental. Estudos *in vitro* demonstraram que géis clareadores sem ativação por luz resultaram em irregularidades, aumento de porosidade e alterações morfológicas do esmalte (Cavalli et al., 2004, Grazioli et al., 2018). Há relatos de diminuição da microdureza para clareamento de consultório, mesmo que fontes de luz não tenham exacerbado esse perfil de desmineralização (Berger et al., 2010).

Por outro lado, um estudo *in situ* mostrou que a rugosidade de superfície e morfologia do esmalte não foram alteradas após clareamento, mostrando que a presença da película adquirida e saliva humana, podem reverter o quadro apresentado em estudos laboratoriais (Sa et al, 2013). Adicionalmente, Amaral et al. (2012) reportaram que agentes clareadores tanto em baixas como em altas concentrações, sem fotoativação, não alteraram o conteúdo mineral de pacientes submetidos a clareamento caseiro ou de consultório, respectivamente.

Tais achados explicam o motivo pelo qual alterações morfológicas foram encontradas para todos grupos tratados com géis clareadores, embora a análise clínica não tenha demonstrado diferenças significativas. Interessantemente, foi detectado que PL promoveu alterações morfológicas mais próximas das causadas apenas pelo gel clareador. Entretanto, Nam et al. (2018) mostraram que PL promoveu menor alteração topográfica no esmalte em relação ao CP 15% associado com LED azul. Tal cenário pode ser explicado pela diferença entre os tempos estipulados para associação do plasma, uma vez que Nam et al. aplicaram o plasma por 30 minutos da exposição do gel na superfície. Ainda, as diferenças na composição dos géis clareadores podem promover resultados distintos, já que há relatos de que o pH pode influenciar os efeitos dos géis sobre a desmineralização dentária (Torres et al., 2014), ou ainda, cada gel pode influenciar a concentração final de cálcio e fosfato de maneira distinta independentemente de seu pH (Pinto et al., 2017).

Em virtude da aplicação apenas laboratorial permitida para PL, estudos clínicos são eticamente inviáveis para verificar a replicabilidade desses resultados para o parâmetro clínico. Todavia, investigações *in vitro* adicionais acerca do conteúdo mineral após tratamento com PL ou LED podem disponibilizar informações importantes para a compreensão dos resultados apresentados.

4. CONCLUSÃO

De acordo com os dados obtidos no estudo *in vitro* e *in vivo*, foi possível concluir que:

- LED violeta e plasma de argônio alteraram a cor de dentes pigmentados, porém, apenas LED aumentou a eficácia clareadora de CP.
- A concentração intrapulpar de peróxido de hidrogênio foi maior para os grupos tratados com HP que CP, independente da ativação por LED violeta ou plasma de argônio.
- LED e PL não promoveram mudanças morfológicas no esmalte quando aplicados sem géis clareadores.
- Clinicamente, LED violeta promoveu alteração de cor, porém com resultados inferiores aos dos pacientes tratados com peróxido de hidrogênio.
- A associação de LED com CP promoveu alteração de cor *in vivo* semelhante ao HP.
- Em geral, a sensibilidade dental não foi afetada pela ativação de HP e CP com o LED violeta.
- Não houve alteração no conteúdo mineral dos pacientes submetidos aos protocolos clareadores após 14 dias do término dos tratamentos.
- Cada protocolo clareador utilizando LED violeta, associado ou não a géis clareadores, deve levar em consideração a queixa principal do paciente em relação à expectativa do tratamento e experiência prévia de sensibilidade dental.

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* De acordo com as normas da UNICAMP/FOP, baseadas na padronização do International Committee of Medical Journal Editors - Vancouver Group. Abreviatura dos periódicos em conformidade com o PubMed.

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ANEXO 1 - CERTIFICADO DO COMITÊ DE ÉTICA EM PESQUISA

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "Análise in vivo do LED violeta na alteração de cor, sensibilidade e conteúdo mineral do clareamento dental", CAAE 72879717.7.0000.5418, dos pesquisadores Vanessa Cavalli Gobbo, Marcelo Giannini, Maria Carolina Guilherme Erhardt, Daylana Pacheco da Silva, Erika Eiko Waida e Matheus Kury Rodrigues, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 25/09/2017.

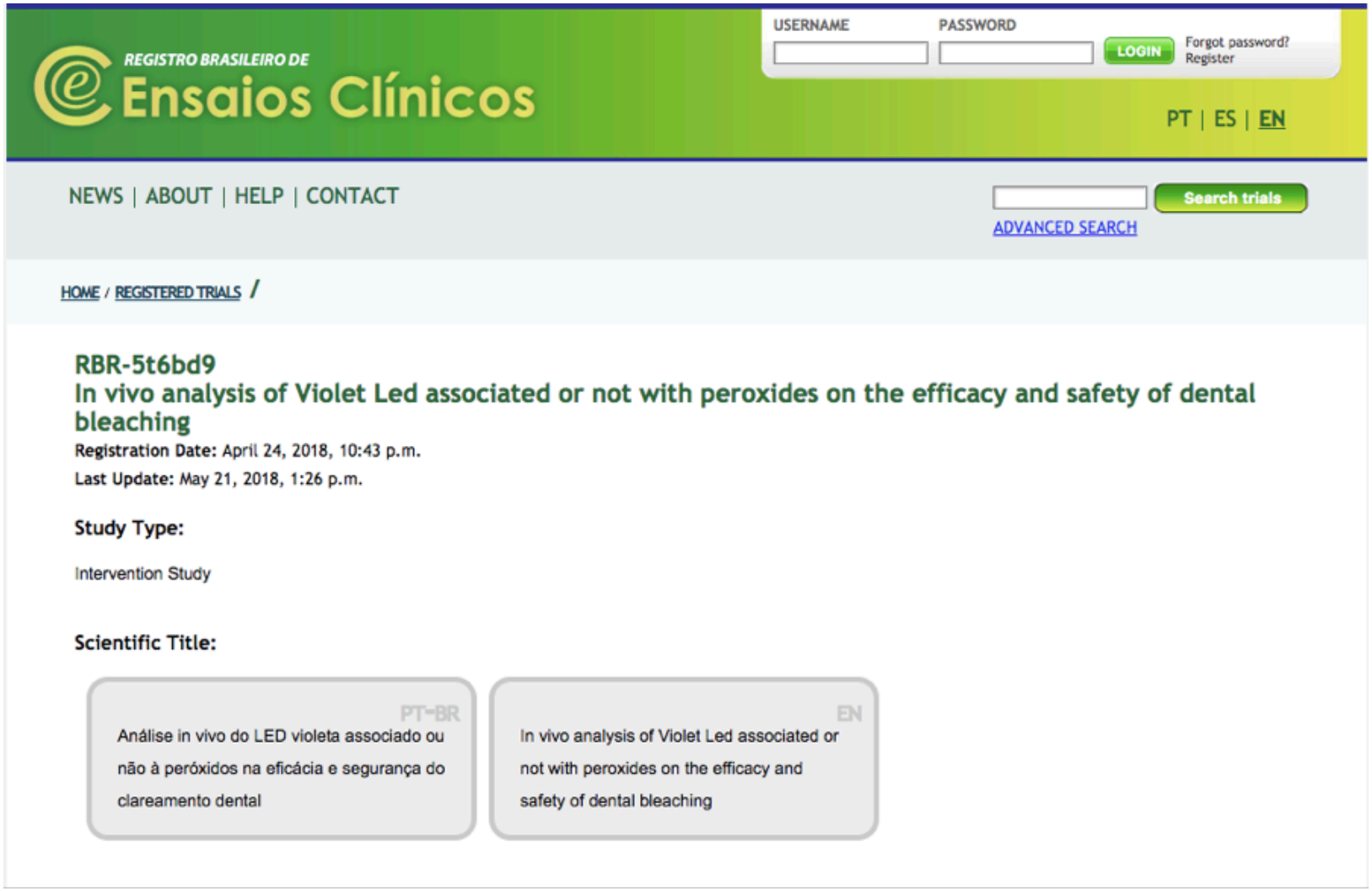
The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "In vivo Purple LED analysis on the color alteration, tooth sensitivity and mineral content of dental bleaching", CAAE 72879717.7.0000.5418, of the researcher's Vanessa Cavalli Gobbo, Marcelo Giannini, Maria Carolina Guilherme Erhardt, Daylana Pacheco da Silva, Erika Eiko Waida and Matheus Kury Rodrigues, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on September, 25 2017.

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

ANEXO 2 - REGISTRO DO ENSAIO CLÍNICO



ANEXO 3 - RELATÓRIO DE ORIGINALIDADE

Dissertação Matheus Kury

RELATÓRIO DE ORIGINALIDADE

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ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

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ANEXO 4 – COMPROVANTE DE SUBMISSÃO DE ARTIGO

OPERATIVE DENTISTRY

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Detailed Status Information

Manuscript #	19-036-L
Current Revision #	0
Submission Date	2019-02-11
Current Stage	Editorial Office Processing
Title	The effect of Violet LED and Non-Thermal Atmospheric Plasma on In-office Bleaching: an in vitro study
Running Title	In-office Bleaching Activated by Violet LED and Non-Thermal Atmospheric Plasma
Manuscript Type	Laboratory Research
Authors	1 Matheus Kury 2 Carolina Perches 3 Daylana Pacheco da Silva 4 Carolina André 5 Cinthia Tabchoury 6 Marcelo Giannini 7 Vanessa Cavalli (corr-auth)
Financial Disclosure	I certify that all financial and material support for this research and work are clearly acknowledged in the manuscript.
Abstract	The study evaluated the effect of violet LED (LED) and non-thermal atmospheric plasma (NTAP) associated with 35% hydrogen peroxide (HP) and 37% carbamide peroxide (CP) or the efficacy of LED or NTAP alone on in-office bleaching. Ninety bovine incisor crowns stained with black tea were divided according to bleaching groups (n=10): LED, LED/HP, LED/CP, NTAP, NTAP/HP, NTAP/CP, HP, CP and control (C). The CIE L* a* b* parameters were evaluated using a spectrophotometer at the baseline (T0), after bleaching (Tb) and 14 days elapsed from bleaching (T14). Color change (ΔE), lightness (ΔL), redness (Δa) and yellowness (Δb) were evaluated at intervals Tb-T0 (ΔE1, ΔL1, Δa1 Δb1) and T14-T0 (ΔE2, ΔL2, Δa1 Δb2). The intrapulpal concentration of hydrogen peroxide was quantified by means of spectrophotometric analysis (μL/mL). SEM images were obtained to evaluate the enamel surface's morphology after bleaching. ΔE, ΔL and HP intrapulpal concentration data were submitted to two-way ANOVA and Tukey tests, and Δa and Δb were evaluated by the Kruskal-Wallis and Mann-Whitney tests (=5%). At both intervals, LED and NTAP resulted in ΔE and ΔL values higher than C. ΔE was similar for LED/HP and LED/CP, even though the ΔE for LED/CP was superior than CP ($p<0.05$). LED/HP and LED/CP increased ΔL and decreased Δb compared with LED alone. Δa for LED was equal to LED/CP ($p>0.05$) and different from NTAP ($p<0.05$). The Δb for NTAP/CP and NTAP/HP differed only after 14 days of storage in artificial saliva. Higher peroxide intrapulpal concentration was determined for the