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"INFLUÊNCIA DA ESPESSURA DO ESMALTE E DA APLICAÇÃO PRÉVIA DE AGENTE DESSENSIBILIZANTE NA EFICÁCIA DO CLAREAMENTO DENTAL"

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"THE INFLUENCE OF ENAMEL THICKNESS AND PRIOR APPLICATION OF A DESENSITIZING AGENT ON DENTAL BLEACHING EFFICACY"

Orientadora: Prof[a] Dr[a] Débora Alves Nunes Leite Lima

Co-orientador: Prof Dr Luis Alexandre Maffei Sartini Paulillo

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Clínica Odontológica da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do título de Mestra em Clínica Ondontológica com área concentração em Dentística.

Master dissertation presented to the Clinical Dentistry Post-Graduate Program of the Piracicaba Dental School of the University of Campinas to obtain the MsC grade in Clinical Dentistry with major in Operative Dentistry.

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL
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ALUNA JULIANA DO CARMO PÚBLIO, E ORIENTADA
PELA PROFA. DRA. DÉBORA ALVES NUNES LEITE LIMA.

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"Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível".

Charles Chaplin

RESUMO

O objetivo deste estudo foi avaliar a influência da espessura do esmalte e a aplicação prévia de dessensibilizante na eficácia do tratamento clareador. O trabalho foi dividido em 2 estudos. No primeiro estudo foi testada a influência da espessura do esmalte (0,5mm de espessura, 1,0mm de espessura planificada, 1,0mm de espessura não planificada e sem esmalte- controle) na eficácia do clareamento em profundidade, variando-se o tipo de agente clareador, peróxido de carbamida (PC) 10% e peróxido de hidrogênio (PH) 35%. No segundo estudo foi avaliada a influência da aplicação prévia de agente dessensibilizante, fluoreto de sódio 2% e nitrato de potássio 5% associado ao fluoreto de sódio 2% e, sem agente dessensibilizante (controle) na eficácia do clareamento dental com PH35%. Nos dois estudos foram usados fragmentos dentais bovinos, pigmentados por chá preto, e distribuídos por esquema inteiramente casual no primeiro estudo e aleatório por sorteio no segundo estudo (n=10) em grupos de acordo com os tratamentos acima**.** As amostras foram armazenadas em saliva artificial durante as 3 semanas de tratamento. As leituras de cor da dentina oposta (1,75mm de espessura) do estudo 1 e as leituras de cor do esmalte (1,0mm de espessura) e dentina oposta (1,75mm de espessura) do estudo 2, foram realizadas após o manchamento (baseline) e após cada semana de tratamento clareador, utilizando o método CIE Lab através de espectrofotômetro (Konica Minolta CM 700d, Japan). Para o estudo 1 os valores de ΔE, ΔL, Δa e Δb datados foram submetidos à análise de variância ANOVA em esquema fatorial e teste de Tukey (α=0,05). Para o estudo 2, a coordenada L* datada (L=100 – lightness; L=0 – darkness) foi submetida por meio de análise de medidas repetidas PROC MIXED e teste de Tukey-Kramer e os valores de ΔE datados foram submetidos à análise de variância ANOVA e teste de Tukey (α=0,05). O esmalte de 2 amostras de cada grupo do estudo 2 foi observado em microscopia eletrônica de varredura (MEV). Nos resultados destes estudos pode-se observar que o clareamento com PC10% foi mais efetivo que o PH35% em profundidade dentinária para todos os

parâmetros de delta, com exceção no terceiro tempo dos deltas. A presença da camada aprismática no esmalte interferiu na eficácia do PC10% somente no primeiro tempo de clareamento em ΔE1, ΔL1 e Δb1, entretanto não interferiu nos tempos de clareamento testado com PH35% (estudo 1). Ainda, o uso de agente dessensibilizante realizado previamente ao clareamento dental não interferiu no mecanismo de ação do PH35% em profundidade (estudo 2).

Palavras chaves: clareamento dental, pigmentação dental, agente dessensibilizante, cor.

ABSTRACT

The aim of this study was to evaluate the influence of enamel thickness and prior application of a desensitizing agent on the effectiveness of bleaching treatment. This project was divided into two studies. Firstly, we tested the influence of enamel thickness (0.5 mm thick, 1.0 mm planned thick, 1.0 mm unplanned thick and absence of enamel - control) on the effectiveness of bleaching, in-depth, according to the type of bleaching agent, as follows: 10% carbamide peroxide and 35% hydrogen peroxide. Secondly, we evaluated the influence of prior application of a desensitizing agent (potassium nitrate associated with 2% sodium fluoride, 2% neutral fluoride, or with no desensitizing agent - control) on the effectiveness of tooth bleaching by using 35% hydrogen peroxide. In both studies we used bovine teeth fragments, stained with black tea, which were allocated into groups according to the aforementioned treatments, by an entirely causal scheme for the first study and by random drawing for the second one (n=10). The specimens were stored in artificial saliva during the 3-week-treatment. Color readings of the underlying dentin (1.75 mm thick) concerning the study 1, and color readings of enamel (1.0 mm thick) and underlying dentin (1.75 mm thick) of the study 2, were performed after staining (baseline) and after each week of bleaching treatment using the CIE Lab method by means of spectrophotometer (Konica Minolta CM 700d, Japan). For the study 1, the values of ΔE, ΔL, Δa and Δb recorded were subjected to factorial analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$). For the study 2, the coordinate L* recorded (L = 100 - lightness, L = 0 - darkness) was submitted to analysis of repeated measures PROC MIXED and Tukey-Kramer's test, and the Δ values registered underwent analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$). The enamel of 2 specimens from each group of the study 2 was observed under scanning electron microscopy. According to the findings, it could be observed that the bleaching with 10% CP was more effective than that with 35% PH as regards dentin depth for all parameters delta, except the third time deltas. The presence of the prismless layer of enamel interfered with the effectiveness of 10% CP just in the first time

of bleaching in ΔE1, ΔL1and Δb1, however it did not affect the times of bleaching when 35% HP was tested (study 1). In addition, the use of a desensitizing agent prior to tooth bleaching did not interfere with the mechanism of action of the 35% hydrogen peroxide concerning tooth depth (study 2).

Key-words: tooth bleaching, dental pigmentation, desensitizing agents, color.

SUMÁRIO

INTRODUÇÃO

Na odontologia foram desenvolvidas técnicas com o objetivo de tornar os dentes mais claros através de métodos mais conservadores, como o clareamento dental. Este tratamento permite a alteração da cor dos dentes através da remoção de pigmentos intrínsecos e extrínsecos presentes na estrutura dental (Sulieman, 2004; Ushigome, *et al*., 2009; Goldberg, *et al*., 2010).

Os dentes humanos são formados por esmalte, dentina e polpa dental. O esmalte dental é um tecido mineralizado, originado das células especializadas- ameloblastos, e suportado pela dentina, que é constituída por tecido conjuntivo denso vital, menos mineralizado e mais resiliente que o esmalte, suportada pela polpa dentária, formada por tecido conjuntivo não-mineralizado (He, *et al*., 2009; Goldberg, *et al.*, 2011). O esmalte é o tecido mais mineralizado do corpo humano, sendo altamente organizado, formado por 87% de conteúdo inorgânico, 11% de água e 2% de matéria orgânica em volume (Risnes, *et al*., 1998; He, *et al*., 2009). A parte inorgânica é constituída por cristais de fosfato de cálcio (hidroxiapatita [Ca_{10} (PO_4) $_6$ (OH) $_2$]) firmemente unidos. Os cristais de apatita se agrupam diferentemente no interior do esmalte criando os prismas de esmalte. A superfície mais externa do esmalte é formada por uma camada aprismática composta por cristais alinhados em ângulos retos com a superfície do dente e paralelos entre si. Abaixo desta camada está localizada a camada prismática, esta possui cristais em angulações difusas e tridimensionais com aspecto irregular (Ripa, 1966; Gwinnett, 1973). A disposição cruzada dos prismas é um constituinte importante para a estrutura de reforço dos dentes, o que confere força e resistência ao tecido (Shimizu, *et al*., 2005; Raue, *et al*., 2012), além de proteção a dentina e a polpa dental.

O esmalte é um tecido permeável, que permite trocas iônicas entre o dente e a cavidade bucal, particularmente a saliva por estar constantemente em contato com o tecido adamantino. Esta permeabilidade facilita a entrada de íons e substâncias que possuem baixo peso molecular na estrutura dental, como os agentes clareadores e substâncias

pigmentantes (Hegedeus, *et al*., 1999; Gökay, *et al*., 2000). Estes pigmentos presentes na cavidade bucal podem penetrar o esmalte pelas estruturas dos cristais e prismas e alcançar a dentina (Garberoglio, *et al*., 1974; Sulieman, *et al*., 2003), causando manchamento dentário que não mais pode ser removido com profilaxia (Viscio, et al., 2000; Azrak, *et al*., 2010).

Por muitos anos o peróxido de hidrogênio tem sido o agente de escolha para a remoção de pigmentos dentais (Viscio, *et al*., 2000; Kwon, *et al*., 2002). O mecanismo de ação do clareamento consiste em uma reação de oxirredução, em que os radicais oxigênios gerados pela quebra do peróxido, oxidam as moléculas pigmentantes, tornando-as menores e passíveis de serem removidas da estrutura dental. Para que isso aconteça, o agente clareador precisa penetrar a estrutura de esmalte, alcançando assim os pigmentos presentes na dentina (Gökay, *et al*., 2000; Sulieman, 2004). As técnicas de clareamento em dentes vitais frequentemente utilizam peróxido de hidrogênio e peróxido de carbamida (Joiner, 2007; Abouassi, *et al*., 2010) e a forma de aplicação depende da concentração do produto. Altas concentrações de peróxidos hidrogênio (20% a 35%) e peróxido de carbamida (35% a 37%) são utilizados na técnica de clareamento de consultório e o intervalo entre as sessões são de 7 dias (Lima, *et al* 2009), e baixas concentrações de peróxido de hidrogênio (4 % a 10%) e peróxido de carbamida (10 %, 16 % e 22%) são utilizadas em clareamento caseiro, realizado pelo paciente sob orientação profissional com uso de moldeiras contendo peróxido (Ushigome, *et al*., 2009; Abouassi, *et al*., 2010; Azrak, *et al*., 2010), estando ambos disponíveis na forma de gel.

A eficácia do clareamento dental está diretamente ligada à concentração e tempo que o peróxido de hidrogênio e/ou íons oxigênio atingem a dentina e os pigmentos nela presentes (Ito, *et al*., 2011; Lima, *et al*., 2011). No entanto, deve existir um limite na quantidade de agente clareador nesta região uma vez que esta substância pode ser tóxica ao tecido pulpar (Joiner, *et al.,* 2004; Goldberg, *et al.*, 2010). Como a passagem desta substância é facilitada dentro da dentina devido aos túbulos dentinários (Brännström, 1963), para os dentes vitais recomenda-se a aplicação do peróxido apenas na superfície de esmalte. O esmalte, por suas características de alto conteúdo mineral, protege a dentina e a

polpa, modulando a passagem do peróxido na estrutura dentária, e assim, protegendo a vitalidade dos dentes (He, *et al.,* 2009).

A ação dos agentes clareadores leva à efeitos colaterais, como a sensibilidade dentinária, alteração gengival e na superfície do esmalte, e consequências decorrentes da liberação de alguns componentes dos materiais restauradores como o mercúrio em restaurações de amálgama, também foram relatados (Dahl, *et al.,* 2003; Tay, *et al*., 2009; Eimar, *et al*., 2012), e são fenômenos importantes a considerar. Dentre os efeitos colaterais, a sensibilidade dentinária é a mais relatada clinicamente pelos pacientes. Os radicais livres podem alterar os lipídios e proteínas que são componentes orgânicos dos tecidos dentários duros (Hegedus, *et al.*, 1999). Devido às alterações nesses tecidos, afim de proporcionar maior conforto ao paciente, buscando prevenir ou diminuir a sensibilidade dentinária podem ser usados agentes dessensibilizantes no tratamento clareador (Leonard, *et al*., 2004; Tay, *et al.*, 2009; Reis, *et al*., 2011; Basting, *et al*., 2012).

Os agentes dessensibilizantes agem de duas formas, uma envolve o efeito de obliteração, impedindo o movimento dos fluidos dos túbulos dentinários e auxilia na remineralização do esmalte; e a outra envolve o bloqueio da atividade nervosa pulpar, alterando a excitabilidade sensorial que causa a sensibilidade dentinária (Pashley, *et al.*, 1986; Tay, *et al*., 2009; Martin, *et al*., 2010; Basting, *et al*., 2012). O tratamento de sensibilidade no consultório incluem o uso de verniz, dessensibilizante, agentes anti-inflamatórios, flúor e resinas restauradoras (Lutins, *et al*., 1984; Trowbridge, *et al.,* 1990;). Os agentes dessensibilizantes tópicos são largamente utilizados por proporcionar maior conveniência e efeito imediato (Leonard, *et al*., 2004; Tay, *et al*., 2009).

Tay *et al*. (2009), avaliaram o efeito do uso de dessensibilizante (nitrato de potássio 5% associado a fluoreto de sódio 2%) e verificaram rápida redução da sensibilidade dentinária comparados à tratamentos que não utilizaram o produto. Já Zielboz *et al.* (2008) mostraram que o uso de dessensibilizantes é recomendado para revestimentos de superfícies de raiz durante a aplicação dos agentes clareadores. No entanto, não é conhecido, se estes dificultam a penetração eficiente dos peróxidos através da dentina subjacente. Segundo Hannig, *et al*. (2011), a difusão do peróxido na dentina depende da

composição e concentração do agente clareador utilizado, além da aplicação de um agente dessensibilizante poder reduzir significativamente a penetração de peróxido através da dentina.

A literatura tem mostrado que o esmalte é permeável ao peróxido de hidrogênio, entretanto, não há estudos que avaliem a influência da espessura de esmalte e da aplicação prévia de agentes dessensibilizantes na eficácia do clareamento dental. Diante disso, o objetivo deste estudo foi avaliar a influência da espessura do esmalte e presença da camada aprismática no clareamento em dentina profunda. Ainda, foi avaliado o efeito da aplicação prévia de nitrato de potássio associado ao fluoreto de sódio 2% na eficácia do clareamento dental em profundidade.

CAPÍTULO 1

The influence of enamel thickness on bleaching efficacy: an in-depth color analysis

Running title: Influence of enamel thickness on bleaching

ABSTRACT

The aim of this study was to evaluate the influence of enamel thickness on tooth bleaching efficacy, in-depth, according to the type of bleaching agent: 10% carbamide peroxide (CP) or 35% hydrogen peroxide (HP). Eighty bovine dental fragments were previously stained in a solution of black tea for 6 days, which were distributed in an entirely casual delineation into 8 groups (n = 10), with 1.75 mm dentin thickness and different enamel thicknesses, as follows: 0.5 mm, 1.0 mm planned, 1.0 mm unplanned (prismless enamel) and absence of enamel. The bleaching gels were applied following the manufacturer's recommendations. The specimens were stored in artificial saliva for 3 weeks. The bleaching on the opposite dentin was evaluated at four times: after staining with tea (baseline) and after each of the 3 weeks of bleaching, by means of the CIE Lab method using reflectance spectrophotometer (Konica Minolta CM 700d). The values of ΔE, ΔL, Δa and Δb recorded were subjected to repeated-measures analysis of variance (ANOVA) and Tukey's test (α=0.05). The results showed an increase in dentin lightness (L*), with decreased redness (a+) and yellowness (b+). In addition, the treatment using 10% CP had higher means in relation to 35% HP, not differing only at the third time. Hence, the bleaching using 10% CP was found to be more effective than that using 35% HP regarding dentin depth, and the presence of the prismless enamel layer did not interfere directly in the efficacy of 35% HP.

Key-words: bleaching agents, tooth discoloration, spectrophotometry.

INTRODUCTION

Tooth bleaching is a conservative aesthetic treatment for dental discoloration, which acts in the removal of pigments present in the tooth structure, making it looks whiter. These dental discolorations are a result of the deposition of intrinsic or extrinsic chromogens pigments. The intrinsic discoloration is caused by the incorporation of chromogens within the tooth structure during odontogenesis or after the eruption. Extrinsic discoloration, in turn, occurs when external tooth chromogens such as coffee, tea and wine, are deposited on the tooth surface after the eruption.[1, 2]

In the last years, carbamide peroxide and hydrogen peroxide have been used for dental bleaching with various techniques and concentrations. High and low concentrations of peroxides are used for in-office and home bleaching treatments, respectively.[3-5] These bleaching have hydrogen peroxide as the active principle, which can be applied directly to the tooth surfaces, or produced from the chemical reaction of sodium perborate or carbamide peroxide.[6, 7]

The penetration and diffusion of bleaching are given by the enamel permeability, which allows the passage of ions and low-molecular-weight substances to the interior of the tooth through the prisms, reaching the dentin.[1] The light scattering and absorption properties of the enamel and dentin, respectively, are linked to tooth coloration, and dentin is responsible for such coloration.[8] According to Wiegand and others[9], tooth color is highly influenced by changes in the color of the subsurface dentin, thus making relevant the studies investigating the in-depth diffusion of bleaching within tooth structures. Dietschi and others[10] showed that the efficacy of bleaching on enamel and dentin is directly related to the mode and time of application as well as to product composition and concentration. During the course of treatment, it may be possible to analyze quantitatively if bleaching is occurring by using a colorimeter or spectrophotometer.[11]These methods allow observing the color change in the teeth.

The literature has shown that enamel is permeable to hydrogen peroxide. Nevertheless, there have been no studies evaluating the influence of enamel thickness and

presence of prismless and prismatic enamel on the efficacy of bleaching, in depth. Thus, the aim of this study was to evaluate by spectrophotometry the influence of different thicknesses of enamel on the efficacy of bleaching, in depth, according to the type of bleaching agent: 10% carbamide peroxide and 35% hydrogen peroxide.

METHODS AND MATERIALS

Eighty bovine incisors were stored in a 0.1% thymol buffered solution and distilled water, after being collected and disinfected. These teeth were examined under a 4X magnifying glass (Carl Zeiss, Santo Amaro, SP, Brazil) in order to check for cracks or staining, which could possibly influence the results of this research. If a flaw was found, the tooth was discarded and replaced. Then the teeth were stored in distilled water under cooling, until the moment of their use.

The crowns were separated from the teeth on the root at the cemento-enamel junction with the use of a double-faced diamond disk (KG Sorensen Ind. Com Ltd, Cotia, SP, Brazil) mounted on a low-speed dental hand piece under constant water irrigation. The blocks were obtained using a diamond cutting disc (Extec Dia, Wafering Blades 102 mm x 0.3mm x 12.7 mm, Enfield, USA) coupled to a metallographic cutter (Isomet 1000, Buehler Ltd., Lake Buff, IL, USA).

Two cuts were performed both in the mesiodistal and cervical-incisal directions, resulting in one block of each tooth originated from the most cervical region of the crown. Thereby, were obtained blocks of the buccal surfaces of the teeth with 4 mm length and 4 mm width.

The enamel and opposite dentine surfaces were abraded with #600 and #1200 grit silicon carbide (SiC) sandpapers, in a polishing machine (Arotec Ind. Com., Cotia, SP, Brazil) under constant water irrigation, until achieving the block height of 1.75 mm dentin and different thicknesses of enamel (0.5 mm planned, 1.0 mm planned, 1.0 mm unplanned – prismless and absence of enamel). In the interval between each application of sandpaper, the specimens were cleaned with distilled water in an ultrasonic tank (T7 Type, CT Model, Thornton-Inpec electronic Ltd, Vinhedo, SP, Brazil).The thickness of each specimen was

standardized by using a digital caliper (Carl Mahr Esslingen GmbH). Each specimen was then marked with a diamond bur #1012 (KG Sorensen Ind. Com Ltd, Cotia, SP, Brazil) on one of the sides to standardize the sample position in the spectrophotometer. The staining of the specimens was performed by immersion in a solution of black tea, which was changed at every 24 hours for 6 days and remained in contact with the enamel and dentin during the whole time. The solution of tea was produced by mixing up 100 mL of distilled water boiled for 5 minutes with 1.6 g of black tea (Leão Junior S.A., Curitiba, PR, Brazil) infused for 5 minutes. After this period of immersion in the solution, the specimens were stored in artificial saliva[12] for 2 weeks and changed daily for color stabilization. Prior to the reading on the spectrophotometer, the black tea dregs formed on the enamel and dentin surfaces were removed by a single operator using a rubber cup with a mixture of pumice and water (2:1 ratio) at low speed for 30 seconds each side.[13]

The color analyses were carried out in the opposite dentin at four times: after staining with black tea (baseline) and after each of the 3 weeks of bleaching. The specimens were placed in a Teflon device (sample-holder) inside a light cabin (GTI Mini Matcher MM1e, GTI Graphic Technology Inc., Newburgh, NY, USA) to standardize the ambient light during the measurement process, and then the samples were subjected to a reading with the spectrophotometer Konica Minolta CM-700d (Konica Minolta Investment Ltd. Sensing Business Division, Shanghai, China) previously calibrated was used in accordance with the manufacturer's instructions. The values obtained were quantified on the CIE Lab system as three coordinates (L*, a*, b*) that define the color of an object within a three-dimensional color space by means of a microcomputer using On Color QC Lite software (Konica Minolta, Japan) to generate spectral measurements as a function of wavelength for data processing and analysis. The coordinate L* represents the degree of lightness ranging from 0 (black) to 100 (white); the coordinate a* evaluates the presence of the pigments red (positive a*) and green (negative a*); and likewise the coordinate b* refers to the pigments yellow (positive b*) and blue (negative b*) present in the specimens[1,8]. The L*a*b* system allows the numeric definition of a color as well as the difference between two colors using the following formula: $\Delta E = [(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2]^{1/2}$. At the first time of the

color analysis stage, there was a selection of the specimens showing similar means and discard of outliers, so there would be a greater standardization of the specimens.

For bleaching procedures, dental blocks were fixed in a device and approximately 1.0 mm of the bleaching was applied on the enamel surface. The gel was applied according to the manufacturer's instructions. The bleaching procedures were performed according to the following protocols:

Groups 1, 2, 3 and 4: 10% carbamide peroxide (10% Whiteness Perfect - FGM). The gel was applied onto the enamel surface, and stored at 37±2 °C for 4 hours. Bleaching was performed daily for 21 days.

Groups 5, 6, 7 and 8: 35% hydrogen peroxide (Whiteness HP MAXX - FGM). The gel was applied onto the enamel surface for 45 minutes. Three bleaching sessions were accomplished, with a 7-day interval.

The carbamide peroxide gel was applied once daily for 4 hours, whereas the hydrogen peroxide gel was applied three times of 15 minutes each for a total time of 45 minutes. At every application, the gel was removed from the specimen surface with the aid of flexible plastic cotton-tipped rods (Swabs, Johnson & Johnson, São José dos Campos, SP, Brazil). At the end of the bleaching session, the specimens were washed thoroughly in running water, dried with absorbing paper and stored in artificial saliva at a temperature of 37±2 °C. After the end of each bleaching session, there was a 24-hour-interval of storage of the specimens in artificial saliva aiming at their rehydration before performing the color readings.

Following exploratory data analysis, the variables ΔE, ΔL, Δa and Δb were subjected to factorial analysis of variance (ANOVA) and Tukey's test, with a significance level of 5%.

RESULTS

Tables 1 to 4 express the means of Delta E, L, a, b (ΔE, ΔL, Δa, Δb), and were organized comparing three different times: baseline x 1st week of bleaching (ΔE1, ΔL1,

Δa1, Δb1); baseline x 2nd week of bleaching (ΔE2, ΔL2, Δa2, Δb2); and baseline x 3rd week of bleaching (ΔE3, ΔL3, Δa3, Δb3).

- **Delta E (ΔE)**

As can be seen in table 1 (ΔE means), at all times and treatments the group "0 mm enamel (D)" had higher ΔE means. For the times ΔE1 and ΔE2, the groups treated with 10% CP showed higher ΔE means in relation to those treated with 35% HP, with no statistical difference for the group "1.0 mm unplanned enamel (E1A)". For the time ΔE3, the group "E1" 10% CP had higher mean than "E1" 35% HP with statistically significant difference.

Table 1: Mean (Standard deviation) of ΔE as a function of treatment, thickness and time.

Treatment	Thickness	Time		
		ΔE1	**ΔE2**	**ΔE3**
10% Carbamide Peroxide	0mm enamel (D)	*24.74(5.22)a	*34.60(5.45)a	41.16(5.95)a
	0.5mm enamel (E 0.5)	*19.03(3.06)b	*27.77(3.97)b	36.80(3.08)ab
	1mm planned enamel (E1)	*16.64(1.63)b	*25.98(3.43)bc	*34.88(3.38)b
	1mm unplanned enamel (prismless) (E1A)	11.19(3.18)c	20.44(5.21)c	31.55(4.35)b
35% Hydrogen Peroxide	0mm enamel (D)	13.39(2.85)a	28.43(3.93)a	37.31(4.63)a
	0.5mm enamel (E 0.5)	6.81(1.68)b	16.49(2.78)b	31.48(3.42)b
	1mm planned enamel (E1)	6.96(2.87)b	16.64(3.85)b	25.14(2.65)bc
	1mm unplanned enamel (prismless) (E1A)	8.03(1.93)b	14.86(3.47)b	28.26(4.12)c

Means followed by different letters (lowercase in vertical comparing thickness within each treatment) indicate a significant difference ($p \leq 0.05$). *It differs from 35% hydrogen peroxide in the same thickness and time ($p \leq 0.05$).

- **Delta L (ΔL)**

The findings in table 2 (ΔL means) showed that for all times and treatments, the group "D" had higher ΔL means, differing from all the other groups. For the times ΔL1 and ΔL2, the groups treated with 10% CP showed higher means in relation to 35% HP; in this respect, only the group "E1A" 35% HP at ΔL1 did not show statistical difference. For the time ΔL3, the group "E1" 10% CP had a higher mean than "E1" 35% HP with statistically significant difference.

Table 2: Mean (Standard deviation) of ΔL as a function of treatment, thickness and time.

Treatment	Thickness	Time		
		ΔL1	**ΔL2**	**ΔL3**
10% Carbamide Peroxide	0mm enamel (D)	*15.65(4.17)a	*23.40(4.23)a	32.24(4.53)a
	0.5mm enamel (E 0.5)	*10.30(2.89)b	*15.89(3.45)b	25.95(2.59)b
	1mm planned enamel (E1)	*9.70(1.53)b	*16.02(3.22)b	*24.55(3.33)b
	1mm unplanned enamel (prismless) (E1A)	5.47(2.15)c	*10.58(3.70)c	19.75(3.21)c
35% Hydrogen Peroxide	0mm enamel (D)	8.54(2.32)a	19.58(3.48)a	28.02(4.32)a
	0.5mm enamel (E 0.5)	4.32(1.24)b	9.62(3.00)b	21.94(2.08)b
	1mm planned enamel (E1)	4.71(2.55)b	9.88(3.50)b	14.84(3.50)c
	1mm unplanned enamel (prismless) (E1A)	4.46(1.63)b	7.16(2.20)c	19.54(2.89)bc

Means followed by different letters (lowercase in vertical comparing thickness within each treatment) indicate a significant difference ($p \leq 0.05$). *It differs from 35% hydrogen peroxide in the same thickness and time ($p \leq 0.05$).

- **Delta a (Δa)**

As seen in table 3 (Δa means), at the time Δa1, 10% CP had the lowest means, with differences between the groups "E0.5" and "E1" 35% HP. For the time Δa2, 10% CP had the lowest means differing from 35% HP, except for the group "D". At the times Δa2 and Δa3, the group "D" showed the highest Δa mean in each treatment, not differing only from the group "E0.5" treated with 35% HP.

Table 3: Mean (Standard deviation) of Δa as a function of treatment, thickness and time.

Treatment	Thickness	Time		
		Δa1	**Δa2**	**Δa3**
10% Carbamide Peroxide	0mm enamel (D)	-5.54 (1.88)a	-4.13 (3.01)b	-0.14 (2.99)b
	0.5mm enamel (E 0.5)	*-7.36 (2.13)a	*-8.49 (2.36)a	-5.89 (2.51)a
	1mm planned enamel (E1)	*-6.08 (1.74)a	*-7.41 (3.09)a	-6.18 (3.51)a
	1mm unplanned enamel (prismless) (E1A)	-5.81 (0.78)a	*-9.03 (1.48)a	-9.61 (2.00)a
35% Hydrogen Peroxide	0mm enamel (D)	-3.80 (1.09)a	-4.33 (2.23)b	-2.00 (3.34)b
	0.5mm enamel (E 0.5)	-2.62 (0.71)a	-5.41 (1.62)a	-5.24 (1.92)ab
	1mm planned enamel (E1)	-2.17 (1.00)a	-5.15 (2.44)a	-6.71 (3.77)a
	1mm unplanned enamel (prismless) (E1A)	-4.02 (0.95)a	-7.07 (2.06)a	-6.59 (2.07)a

Means followed by different letters (lowercase in vertical comparing thickness within each treatment) indicate a significant difference ($p \leq 0.05$). *It differs from 35% hydrogen peroxide in the same thickness and time ($p \leq 0.05$).

- **Delta b (Δb)**

According to the findings expressed in table 4 (Δb means) comparing the treatments at all times, 10% CP showed lower means with statistical differences in relation to 35% HP, except for the group "E1A" at the time Δb1, which had a higher mean, also differing from the other groups treated with 10% CP. At the times Δb1 and Δb2 for each treatment, the group "D" showed a lower mean with differences in relation to the other groups. At the time Δb3, this group differed from "E1A" 10% CP and did not differ from the group "E0.5" 35% HP.

Table 4: Mean (Standard deviation) of Δb as a function of treatment, thickness and time.

Treatment	Thickness	Time		
		Δb1	**Δb2**	**Δb3**
10% Carbamide Peroxide	0mm enamel (D)	*-18.18(3.61)a	*-24.94(3.85)a	*-25.20(5.23)a
	0.5mm enamel (E 0.5)	*-13.87(2.62)b	*-20.84(3.38)b	*-25.18(3.18)a
	1mm planned enamel (E1)	*-11.87(1.65)b	*-18.65(2.99)bc	*-23,59(3.04)ab
	1mm unplanned enamel (prismless) (E1A)	-7.70(2.70)c	*-14.70(4.50)c	*-22.49(3.65)b
35% Hydrogen Peroxide	0mm enamel (D)	-9.46(2.07)a	-19.90(3.13)a	-23.79(5.68)a
	0.5mm enamel (E 0.5)	-4.47(1.33)b	-11.97(2.02)b	-21.86(2.95)ab
	1mm planned enamel (E1)	-4.29(2.07)b	-11.84(3.26)bc	-18.57(2.17)b
	1mm unplanned enamel (prismless) (E1A)	-5.16(1.49)b	-10.59(3.37)c	-19.19(3.11)b

Means followed by different letters (lowercase in vertical comparing thickness within each treatment) indicate a significant difference ($p \leq 0.05$). *It differs from 35% hydrogen peroxide in the same thickness and time ($p \leq 0.05$).

DISCUSSION

This study evaluated the influence of enamel thickness on bleaching efficacy, in-depth, by using bovine dental fragments previously stained with black tea. According to Attia and others[14], human and bovine teeth exhibit similar behaviors during the bleaching. This behavior is due to the morphological similarities and physicochemical characteristics between enamel and dentin substrates of human and bovine teeth, including number and density of dentinal tubules and collagen matrix, making of them an alternative experimental source.[15-17] For dental bleaching, were used gels containing 10% carbamide peroxide and

35% hydrogen peroxide, which were applied directly to the enamel surface of the specimens. Although the byproducts of the reaction involving hydrogen peroxide and carbamide peroxide are the same, i.e., oxygen-free radicals, studies have shown that the mechanism of action of these two agents is different, since the peroxide breakdown reaction resulting in radical-releasing occurs differently. Carbamide peroxide in contact with the tooth surface dissociates into hydrogen peroxide and urea. Subsequently, the urea continues to decompose into carbon dioxide (CO_2) and ammonia (NH_3^+). Hydrogen peroxide, in turn, dissociates into free radicals, which oxidize long-chain organic molecules responsible for coloration of dental tissue cleaving their double bonds.[18, 19]

With the purpose of analyzing the efficacy of bleaching, ΔE values were calculated as they show the numerical value of tooth color change between different times.[11, 20] At the times ΔE1 and ΔE2, the highest ΔE means were found for the groups treated with 10% CP, differing from the groups treated with 35% HP, with the exception of the group "E1A". The presence of ammonia in the carbamide peroxide elevates the pH of the medium making it alkaline, and thus potentiates the action of the bleaching.[21] Furthermore, the presence of Carbopol thickener, a water-soluble polymer, in its composition[22] increases the half-life of carbamide peroxide and thus gradually releases hydrogen peroxide, followed by its dissociation into free-radicals. According to Dietschi and others[23], home bleaching with carbamide peroxide has shown greater efficacy in whitening deeper structures such as dentin, due to the continuous and long release of hydrogen peroxide radicals, corroborating with the findings of this research.

When comparing the groups investigated according to the type of treatment and time, it can be observed that the group with absence of enamel (group “D”) showed the highest ΔE mean, differing from the others. In this regard, enamel acts as a filter in the passage of ions and substances into the tooth due to the presence of prisms, so the bleaching agents applied directly onto the dentin surface have their action facilitated, allowing a greater color change. This finding is in agreement with the study by Eimar and others[7], who observed that the peroxide oxidation reaction is better when tooth structures have higher organic content.

At the time ΔE1, the presence of prismless enamel "E1A" influenced the 10% CP bleaching, showing less color change in comparison with the group lacking the prismless enamel "E1". Enamel is formed by apatite crystals arranged as layers of prisms in different directions.[24,25] So, the prismless layer is found in the most superficial area of the enamel, in which crystals are arranged parallel to each other and perpendicular to the enamel surface. In the prismatic layer, however, crystals are found at different angles in a three-dimensional array and with irregular appearance.[26-28] The prismless layer is more mineralized due to its higher amount of inorganic matter, which makes the surface denser and less permeable[29] when compared to the prismatic enamel. This fact possibly interfered with the penetration of 10% CP into the tooth.

Nevertheless, these same groups did not differ in the treatment with 35% HP. This might be likely due to its mechanism of action and larger amounts of free radicals, so the presence of a prismless layer did not affect bleaching in these groups. The low molecular weight of hydrogen peroxide facilitates its own diffusion into the tooth through the enamel prisms and organic matrix, towards the dentin-enamel junction thereafter reaching the dentine. Moreover, the viscosity of hydrogen peroxide is lower than that of 10% CP, because the last has Carbopol in its composition increasing viscosity.

The fact that there was no statistical difference in the same group "E1A" between treatments may be associated with the time of ion dissociation and hydrogen peroxide concentration in each bleaching agent. Carbamide peroxide takes a longer dissociation of its products until the release of hydrogen peroxide ions, in addition to being found at a lower concentration – around 3.5% in the 10% CP gel. On the other hand, as soon as the 35% HP gel is applied onto the tooth structure it dissociates, and such high concentration of ions will result in a quick action on the tooth surface. However, this does not mean that 35% HP is more effective than 10% CP, because when analyzing the groups within treatments at different times we can see that 10% CP showed higher means in relation to 35% HP, and only at the time ΔE3 the groups did not show statistical differences, except for the group "E1" that had higher mean with statistical difference for the treatment with

10% CP. This finding can be directly associated with the consecutive applications of the bleaching agents, there being saturation between groups in the treatments. At the end of the time ΔE3, this saturation was not reached by the group "E1", but when compared to the other groups within the same treatment, it differed only from the group "D", which allows observing that despite the statistical difference in the treatment with 35% HP, this group showed the same behavior between those means of the bleaching treatment with 10% CP. According to Hanks and others[30], the diffusion of hydrogen peroxide through dentin is related to the consecutive applications, allowing longer exposure of the tooth to peroxide. Bernardon and others[31] observed that the degree of bleaching achieved by the home and office techniques was similar. Also, Dietschi and others[10] verified that after the recommended number of applications, hydrogen peroxide had no greater significance than carbamide peroxide, and that the effect of bleaching on dentin had as the major dependent-factor the time of contact with the tooth surface.

This study also evaluated the three color coordinates (ΔL, Δa and Δb) separately. Among these coordinates, ΔL is the most important parameter to evaluate dental bleaching. The lightness represented by ΔL is perceptible to the human eye, indicating the color change of the tooth between light and dark, which is a feature of great clinical relevance. Considering the human eye, changes in lightness are more easily detected than other color parameters (Δa and Δb) which represent saturation, present on the CIE Lab system.[23,32] According to the results obtained in this research, the parameter ΔL showed greater variance between the color means in the groups, followed by the Δb means.

As regards the lightness evaluated by ΔL ($L_f - L_i$) (table 2), the groups had behaviors very similar to those of ΔE at all times. The groups treated with 10% CP obtained higher means compared to 35% HP at the times ΔL1 and ΔL2, except for the group "E1A" at ΔL1. The group "D" at all times and treatments showed higher ΔL mean differing from the other groups. These behaviors of ΔE and ΔL are directly related one another, since ΔL values are included in the formula of ΔE. Furthermore, the lightness displayed by the groups within each treatment and time allow to observe that the bleaching was effective, taking into account the consecutive applications of the bleaching agents. These products acted

removing the chromogens from the tooth structure, which facilitates their diffusion trough dentin, in-depth, and thus increases the lightness.[8, 33]

Analyzing the saturation of the specimens in the coordinate Δa ($a_f - a_i$) (table 3), it can be seen that when comparing the groups treated with 10% CP and 35% HP, there was a statistical difference at the times Δa1 and Δa2. At the time Δa3, it is observed a lower statistical variation between the means of the groups regarding the treatments. Sulieman and others[1], who performed whitening on enamel surface of teeth previously stained with tea, noted that during bleaching the Δa means decreased, ranging from positive a+ to negative a- values, with greeness; throughout the bleaching sessions, those values tended to approach zero, i.e., for more neutral colors (white, gray) there was a decrease in the Δa values with negative means, as also observed in the study by Wiegand and others[9]. Still, Lenhard[34] observed little change in the means of the coordinate Δa throughout the bleaching sessions, therefore corroborating the findings of the present research.

The findings of this study point to a greater variation in the Δb ($b_f - b_i$) means towards blue, as can be seen in table 4. Sulieman and others[1] affirm that during the bleaching sessions it can be observed a decrease in the Δb means towards the blue (b^-) when the enamel surfaces are analyzed. In this study, the means turned to negative over the bleaching sessions, i.e., there was a decrease in the Δb means, possibly due to the prior staining of the specimens with a solution of black tea. During the bleaching process, the Δb means ranged from a more yelloness (b+) toward a bluness (b-), which is consistent with the study by Sulieman and others[1] that could observe the progress of the means toward the blue. As regards the time Δb, the Δb1 and Δb2 means of the groups and treatments followed the same behavior of ΔL, which is in agreement with Bengel[35], who verified major changes in the values of the coordinates L* and b* after bleaching . The means of all deltas for the group "0.5 mm enamel (E0.5)" at all times and treatments were found to be similar, not differing from the group "E1A". This shows that the difference in the enamel thicknesses is not a factor that influences the success of bleaching, which could be observed in this study.

According to Sulieman and others[1], the staining of teeth by immersion in tea allows a better evaluation of the bleaching technique, since this method reproduces the pattern of intrinsic tooth staining. During processing of the tea it can be found polyphenolic chromogens, colorless theasinensins, and theaflavins that give red-orange color and brightness, in a ddition to thearubigins with brownish-rust.[1,36] The presence of these substances features the red-orange color of the black tea. These are directly related to the saturation observed in the findings reported here.

Even so, the means found for the bleaching treatment with prior staining with tea revealed that besides a significant change in lightness (L*) was identified for dentin, it was also possible to observe a greeness in the Δa means (reaching more neutral colors, such as white and gray), and a bluness in the Δb means. With these findings it was verified that dentin appeared lighter over the weeks of bleaching, and that the treatment with 10% CP had higher delta means. For the groups treated with 35% HP, lower difference between the treatments was found only at the third time.

CONCLUSION

The bleaching agent 10% carbamide peroxide was found to be more effective than 35% hydrogen peroxide as regards dentin depth. The presence of a prismless enamel layer influenced negatively the efficacy of 10% carbamide peroxide only at the first bleaching session. However, it did not interfere with the bleaching efficacy of 35% hydrogen peroxide.

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CAPÍTULO 2

"Efficacy of tooth bleaching with prior application of a desensitizing agent"

Running Title: The influence of desensitizing prior bleaching.

ABSTRACT

AIM: The aim of this study was to evaluate the efficacy of bleaching on enamel and opposite dentin surfaces using 35% hydrogen peroxide (HP) and prior application of a desensitizing agent. **METHODS:** Thirty bovine dental fragments were previously stained in a solution of black tea, and were randomly divided into 3 groups (n = 10), with thicknesses of 1.0 mm enamel and 1.75 mm dentin, according to the following protocols: NF - 2% neutral fluoride + bleaching; D - desensitizing agent + bleaching; WD – without desensitizer + bleaching. The bleaching efficacy was evaluated at four times: after staining with tea (baseline) and after each of the 3 weeks of bleaching, by means of the CIE Lab method using reflectance spectrophotometer. The data coordinate L* was evaluated by analysis of repeated-measures PROC MIXED and Tukey-Kramer's test. The ΔE values were subjected to ANOVA in a split-plot scheme and Tukey's test (α = 0.05). **RESULTS:** The 35% HP showed greater efficacy on deep dentin after removal of enamel stains, with increasing means during all times in all

treatments. **CONCLUSIONS:** The use of a desensitizing agent prior to the bleaching session did not affect the mechanism of action of 35% hydrogen peroxide with regard to tooth depth.

Key-words: Dentin Desensitizing Agent, Teeth Bleaching, Tooth Discoloration, Hydrogen Peroxide and Carbamide Peroxide.

INTRODUCTION

Tooth discoloration is caused by extrinsic stains resulting from the deposit of pigments on the tooth outer surface, and/or by intrinsic stains from chromogenic materials deposited on enamel and dentin[1,2]. In order to remove these stains, the tooth bleaching has been the treatment of choice in dental practice. Several methods, application times, types and concentrations of gels can be applied to vital teeth[3]. Low concentrations of carbamide peroxide (10%, 16% and 22%) and hydrogen peroxide (6% to 7.5%) have been used for home bleaching, and high concentrations of carbamide peroxide (35% to 37%) and hydrogen peroxide (20% to 35%) have been employed for in-office bleaching[4-6].

The bleaching include hydrogen peroxide as the active principle present in their composition, and its mechanism of action is by dissociation into free radicals[7,8]. Due to its low-molecular-weight, hydrogen peroxide diffuses deeply through the tooth structures toward dentin, reaching organic molecules. Hence, it changes the chemical structure of molecules through cleavage of their double bonds, making them smaller[3,5,8-10] and capable

of being removed from the tooth structure by diffusion, or even promotes a lowered light absorption, increasing the lightness of the object[11,12].

The bleaching agent when at high concentrations have dentinal sensitivity as one of the major adverse effects, which is caused by: tooth dehydration during bleaching; increased permeability of enamel and dentin, which facilitates the passage of peroxide toward the pulp resulting in transient inflammation; or even diffusion of peroxides and movements of fluids, which can stimulate the receptors of dentinal tubules by activating sensory nerves causing dentinal sensitivity[13-15]. The sensitivity reported by patients typically lasts four days, on average, after the bleaching sessions[16].

With the purpose of controlling this transient sensitivity, different types of products have been marketed, such as neutral fluoride, acidulated fluoride and desensitizing agents. As these products aim to control the adverse effect of peroxides, studies have evaluated their action along with the bleaching treatment[14,17].

In addition to sensitivity caused by gels at high concentrations, studies have also investigated the possible effects of peroxide on tooth morphology. Qualitative analysis by Scanning Electron Microscopy (SEM) is an effective method that has allowed assessing changes in tooth surface morphology after bleaching[18,19]. These *in vitro* studies contribute to the clinical research aimed at reducing sensitivity during the bleaching treatments[15,20].

The effects caused by the use of bleaching have been the object of research for a many years, so the aim of this study was to evaluate the influence of prior application of a desensitizing agent (5% potassium nitrate associated with 2% sodium fluoride) on the

efficacy of tooth bleaching, in depth, using 35% hydrogen peroxide, as well as to observe the enamel topography by SEM.

MATERIALS AND METHODS

Thirty bovine incisors were stored in a 0.1% thymol buffered solution and distilled water, after being collected and disinfected. These teeth were examined under a 4X magnifying glass (Carl Zeiss, Santo Amaro, SP, Brazil) in order to check for cracks or staining, which could possibly influence the results of this research. If a flaw was found, the tooth was discarded and replaced. Then the teeth were stored in distilled water under cooling, until the moment of their use.

The crowns were separated from the teeth on the root at the cemento-enamel junction with the use of a double-faced diamond disk (KG Sorensen Ind. Com Ltd, Barueri, SP, Brazil) mounted on a low-speed dental hand piece under constant water irrigation. The blocks were obtained using a diamond cutting disc (Extec Dia, Wafer Blade 102 x 0.3 x 12.7 mm) coupled to a metallographic cutter (Isomet 1000, Buehler Ltd., Lake Buff, IL, USA). Two cuts were performed both in the mesiodistal and cervical-incisal directions, resulting in one block of each tooth originated from the most cervical region of the crown. Therefore, were obtained blocks of the buccal surfaces of the teeth with 4 mm length and 4 mm width.

The enamel and opposite dentine surfaces were abraded with #600 and #1200 grit silicon carbide (SiC) sandpapers, in a polishing machine (Arotec Ind. Com., Cotia, SP, Brazil) under constant water irrigation until reaching the block height of 2.75 mm (1.75 mm

dentin and 1 mm enamel). In the interval between each application of sandpaper, the specimens were cleaned with distilled water in an ultrasonic bath (T7 Type, CT Model, Thornton-Inpec electronic Ltd, Vinhedo, SP, Brazil).The thickness of each specimen was standardized by using a digital caliper (Carl Mahr Esslingen GmbH). Each specimen was then marked with a round bur #1012 (KG Sorensen on one of the sides to standardize the sample position in the spectrophotometer.

The staining of the specimens was performed by immersion in a solution of black tea, which was changed at every 24 hours for 6 days and remained in contact with the enamel and dentin during the whole time. The solution of tea was produced by mixing up 100 mL of distilled water boiled for 5 minutes with 1.6 g of black tea (Leão Junior S.A., Curitiba, PR, Brazil) infused for 5 minutes. After this period of immersion in the solution, the specimens were stored in artificial saliva[21] for 2 weeks and changed daily for color stabilization. Prior to the reading on the spectrophotometer, the black tea dregs formed on the enamel and dentin surfaces were removed by a single operator using a rubber cup with a mixture of pumice and water (2:1 ratio) at low speed for 30 seconds each side[22].

The color analyses were carried out in the enamel and opposite dentin at four times: after staining with black tea (baseline) and after each of the 3 weeks of bleaching. The specimens were placed in a Teflon device (sample-holder) inside a light cabin (GTI Mini Matcher MM1e, GTI Graphic Technology Inc., Newburgh, NY, USA) to standardize the ambient light during the measurement process, and then the samples were subjected to a reading with the spectrophotometer Konica Minolta CM-700d (Konica Minolta Investment

Ltd. Sensing Business Division, Shanghai, China) previously calibrated was used in accordance with the manufacturer's instructions. The values obtained were quantified on the CIE Lab system as three coordinates (L*, a*, b*) that define the color of an object within a three-dimensional color space by means of a microcomputer using On Color QC Lite software (Konica Minolta, Japan) to generate spectral measurements as a function of wavelength for data processing and analysis. The coordinate L* represents the degree of lightness ranging from 0 (black) to 100 (white); the coordinate a* evaluates the presence of the pigments red (positive a*) and green (negative a*); and likewise the coordinate b* refers to the pigments yellow (positive b*) and blue (negative b*) present in the specimens[1,8]. The L*a*b* system allows the numeric definition of a color as well as the difference between two colors using the following formula: $\Delta E = [(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2]^{1/2}$. At the first time of the color analysis stage, there was a selection of the specimens showing similar means and discard of outliers, so there would be a greater standardization of the specimens.

For the procedures using 2% neutral fluoride and the desensitizing agent and for the bleaching treatment, the specimens were fixed properly and the subsequent steps were performed following the manufacturers' protocols:

Group 1: prior application of 2% neutral fluoride (Flugel - transparent neutral fluoride gel - 2% Sodium Fluoride, DFL), and 35% hydrogen peroxide bleaching gel (Whiteness HP MAXX, FGM). Fluoride was applied for 4 minutes on the enamel surface,

followed immediately by the application of hydrogen peroxide for 45 minutes. Three sessions of bleaching were held, with a 7-day interval.

Group 2: application of 5% potassium nitrate associated with 2% sodium fluoride (Desensibilize KF 2%, FGM), and 35% hydrogen peroxide bleaching gel (Whiteness HP MAXX, FGM). The desensitizer was applied for 10 minutes on the enamel surface, followed immediately by the application of hydrogen peroxide for 45 minutes. Three sessions of bleaching were held, with a 7-day interval.

Group 3: application of 35% hydrogen peroxide bleaching gel (Whiteness HP MAXX - FGM). Hydrogen peroxide was applied on the enamel surface for 45 minutes. Three sessions of bleaching were held, with a 7-day interval.

The applications of 2% neutral fluoride and the desensitizer were performed only once for each session, maintaining a thickness of about 1 mm on the enamel. The hydrogen peroxide gel was applied 3 times for 15 min each, with a total time of 45 minutes. The 2% neutral fluoride and desensitizer were removed from the specimen surface with the aid of flexible plastic cotton-tipped rods (Swabs, Johnson & Johnson, Brazil). The specimens were washed thoroughly under running water, dried with absorbing paper, and then underwent application of hydrogen peroxide. The bleaching gel was removed using flexible plastic cotton-tipped rods (Swabs, Johnson & Johnson, Brazil), and after 45 minutes the specimens were washed thoroughly in running water, dried with absorbing paper and stored in artificial saliva at a temperature of 37±2 °C. After the end of each bleaching session,

there was a 24-hour-interval of storage of the specimens in artificial saliva aiming at their rehydration before performing the color readings.

Following exploratory data analysis and selection of the best covariance structure, the variable L* was analyzed by mixed models for repeated-measures (PROC MIXED) and Tukey-Kramer's test at a significance level of 5%. The variable ΔE was recorded and data were subjected to analysis of variance (ANOVA) in a Split-plot scheme and Tukey's test at a significance level of 5%.

For analysis under scanning electron microscopy (SEM), after planning with silicon carbide sandpapers and before treatments on the surfaces, the specimens were polished on felt (TOP, RAM and SUPRA - Arotec, Cotia, São Paulo, SP, Brazil), associated with metallographic diamond paste (6 µm - TOP, 3 µm - RAM, 1 µm – SUPRA - Arotec, Cotia, SP, Brazil) and lubricating oil (Arotec - metallographic preparation, Cotia, SP, Brazil), in order to obtain larger smoothness of their surfaces. At the end of the weeks of treatment, the specimens were prepared by dehydration in absolute alcohol and coated with approximately 20Ëm golden alloy, and could be observed under SEM thereafter. Two of the specimens that did not undergo treatment were also analyzed. In addition, specimens receiving only 2% neutral fluoride and the desensitizer without hydrogen peroxide were prepared for SEM analysis. Photomicrographs were obtained with the aid of a scanning electron microscope (JEOL JSM-5600 LV, Tokyo, Japan) with magnification of 2000x, being elected the most representative areas of each specimen. To assist in the interpretation

of SEM images, it was measured the pH of the products (table 1) using the Orion 290A+ pH meter (Thermo Electron Corporation).

Table 1. Composition and pH of the products used in the treatments.

Materials	Composition	pH
2% neutral fluoride	2% neutral fluoride	7.27
Desensitizer	5% potassium nitrate; 2% neutral fluoride	6.54
HP MAXX 1	30-35% hydrogen peroxide	1.57
HP MAXX 2	thickener, mixture of dyes, glycol, inorganic filler and deionized water	8.68
HP MAXX 1 + HP MAXX 2	35% hydrogen peroxide + thickener	*5.45
Artificial saliva	0.062% potassium chloride, 0.085% sodium chloride, 0.005% magnesium chloride, 0.016% calcium chloride, 0.08% anhydrous dibasic potassium phosphate, 0.2% nipagin; 0.4% hydroxypropylmethylcellulose, purified water 100% q.s., and 6% sorbitol.	7.34

*pH after mixing up the bleaching products

RESULTS

- **L* values (L= 100 - light; L= 0 – dark)**

According to the findings presented in tables 2 and 3 (L* means), there was no statistical difference between the groups analyzed for all times and surfaces. In the first week of treatment, all groups had higher L* means in relation to baseline. This behavior regarding the treatments was repeated over the subsequent times, with increasingly means and statistical differences when compared to the previous time.

Table 2. Means of L* (standard deviation) of the enamel as a function of the treatment and time.

Surface	Treatment	Time			
		Baseline	1st week	2nd week	3dr week
Enamel	2% Neutral Fluoride (NF)	62.75 (3.12)Da	73.04 (2.33)Ca	75.58 (2.03)Ba	76.98 (2.13)Aa
	Desensitizer (D)	64.06 (2.59)Da	72.53 (1.56)Ca	75.47 (1.20)Ba	77.22 (1.09)Aa
	Without desensitizer (WD)	64.50 (2.45)Da	72.50 (2.12)Ca	74.90 (1.88)Ba	73.70 (2.00)Aa

Means followed by different letters (uppercase letters in the lines and lowercase letters in the columns) indicate statistical differences ($p \leq 0.05$).

Table 3. Means of L* (standard deviation) of the underlying dentin as a function of the treatment and time.

Surface	Treatment	Time			
		Baseline	1st week	2nd week	3rd week
Dentin	2% Neutral Fluoride (NF)	39.85 (5.92)Da	48.40 (6.44)Ca	53.52 (6.46)Ba	55.98 (6.20)Aa
	Desensitizer (D)	39.35 (5.39)Da	47.27 (5.63)Ca	51.86 (5.03)Ba	55.62 (5.05)Aa
	Without desensitizer (WD)	39.52 (5.62)Da	49.56 (7.15)Ca	53.44 (6.63)Ba	56.30 (6.07)Aa

Means followed by different letters (uppercase letters in the lines and lowercase letters in the columns) indicate statistical differences ($p \leq 0.05$).

- **Delta E (ΔE)**

Tables 4 and 5 bring the Delta E (ΔE) means comparing three different times: baseline x 1st week of bleaching (ΔE1); baseline x 2nd week of bleaching (ΔE2); and baseline x 3rd week of bleaching (ΔE3).

As seen in tables 4 and 5 (ΔE Means) for enamel and dentin surfaces, respectively, comparing treatments within each time, there was no statistical difference between the groups; and over the times there was an increasingly increment in the means of the groups in relation to the previous time.

Table 4. Means (standard deviation) of ΔE as a function of the treatment and time with regard to the enamel surface.

Treatment	Time		
	ΔE1	ΔE2	ΔE3
2% Neutral Fluoride (NF)	11.54 (1.14)a	17.83 (1.79)a	20.99 (1.44)a
Desensitizer (D)	10.51 (2.24)a	16.52 (3.45)a	21.33 (4.04)a
Without desensitizer (WD)	13.05 (3.66)a	17.86 (3.35)a	21.62 (3.74)a

Means followed by the same letters (lowercase letters in the columns) do not differ (p>0.05).

Table 5. Means (standard deviation) of ΔE as a function of the treatment and time with regard to the dentin surface.

Treatment	Time		
	ΔE1	ΔE2	ΔE3
2% Neutral Fluoride (NF)	10.70 (3.29)a	13.35 (3.58)a	14.76 (3.80)a
Desensitizer (D)	8.65 (1.97)a	11.64 (2.26)a	13.39 (2.51)a
Without desensitizer (WD)	8.25 (2.18)a	10.65 (2.64)a	12.52 (2.64)a

Means followed by the same letters (lowercase letters in the columns) do not differ (p>0.05).

Scanning Electron Microscopy (SEM)

A photomicrograph of the surface treated with 2% neutral fluoride and 35% hydrogen peroxide (NF) showed changes in the enamel morphology such as pores and

erosions (Figure 1a) when compared to the untreated group (Figure 2c). The group treated with the desensitizing agent and 35% hydrogen peroxide (D) showed surfaces with pores and depressions, in addition to more apparent enamel prisms (Figure 1b) when compared to NF. The group without desensitizer (WD) and with application of 35% hydrogen peroxide was found to show higher demineralization of the enamel surface with pores, depressions and more apparent enamel prisms (Figure 1c) when compared to the previous groups.

The surfaces treated with 2% neutral fluoride, desensitizer and no bleaching treatment (without 35% HP), had less surface destruction. The surfaces treated with 2% neutral fluoride showed a smooth and homogeneous pattern with the presence of micropores and few pores, in addition to less apparent enamel prisms (Figure 2a). The surface treated with the desensitizing agent (Figure 2b) showed a homogeneous pattern with few pores, however, with an increased number of micropores, and more apparent prisms when compared to the surfaces treated with 2% neutral fluoride. The surfaces stored in artificial saliva did not undergo any treatment, therefore showed few changes: micropores on the surface, very few pores, in addition to almost undetectable enamel prisms (Figure 2c).

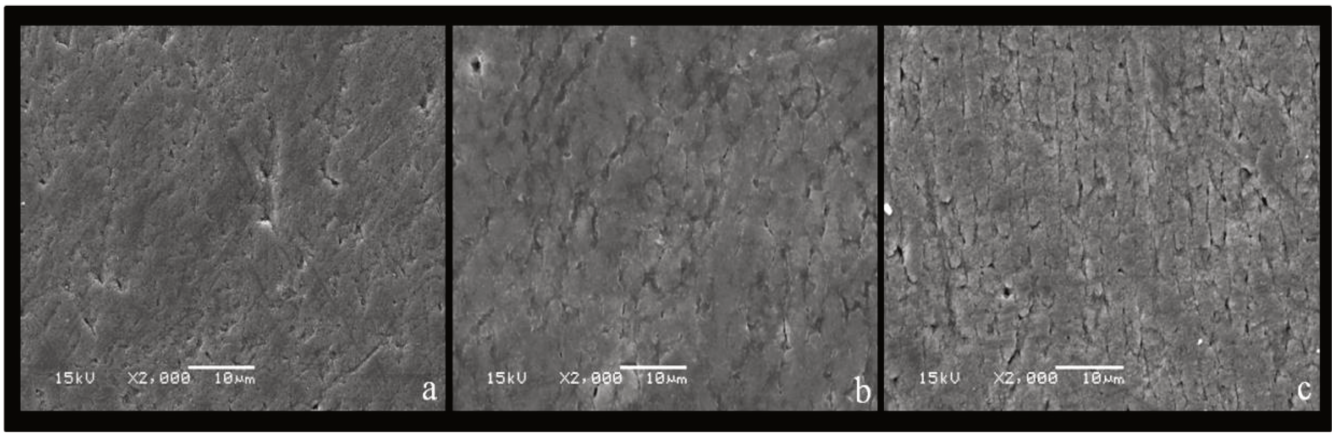

Fig 1. SEM analysis of the enamel surfaces after being subjected to the following treatments: a) NF + 35% HP; b) D + 35% HP; c) WD + 35% HP (control), with magnification of 2000x.

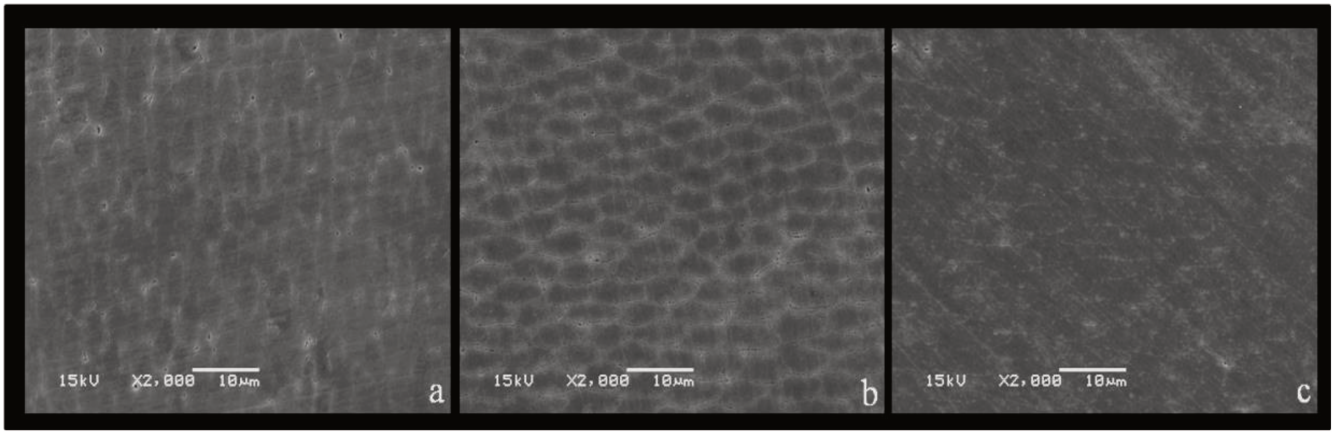

Fig 2. SEM analysis of the enamel surfaces after being subjected to the following treatments: a) 2% neutral fluoride; b) desensitizer; c) without desensitizer, with magnification of 2000x.

DISCUSSION

This study evaluated the efficacy of tooth bleaching using 35% hydrogen peroxide with prior application of a desensitizing agent (5% potassium nitrate associated with 2% sodium fluoride). Hydrogen peroxide at high concentration penetrates through the enamel pores and diffuses through dentin, in-depth, reaching the pulp tissue[24-26] and causing tooth sensitivity. The desensitizing agent have been used previously to tooth bleaching in order to avoid or minimize this transient sensitivity during the treatment[17,20,27].

Potassium nitrate acts directly in the pulp altering the sensorial excitability during transmission of nerve impulses, decreasing the ability of repolarization after the initial depolarization occurred in the pain transmission. Therefore, it blocks the nerve activity, causing an effect of analgesia[15,17,28]. The 2% neutral fluoride, in turn, acts in the occlusion of dentinal tubules, with precipitation of crystals of calcium fluoride on dentin, and thereby reduces the radius of dentinal tubules blocking the movement of fluids toward the pulp. Also, it assists in the enamel remineralization when applied after bleaching[29], leading to formation of calcium fluoride on enamel, increasing its resistance, which was altered due to the demineralization process caused by the bleaching agent.

The findings of the present research showed that the prior application of 2% neutral fluoride and desensitizer did not influence the bleaching efficacy, since the L* means found did not differ statistically between the treatments for each surface analyzed, with increasing means during the 3 weeks of evaluation. Studies have evaluated the application of the potassium nitrate desensitizing agent associated with 2% sodium fluoride prior to hydrogen

peroxide on the enamel surface, noting that there was no interference in the success of tooth bleaching[15,20] corroborating with the findings of this study. Two percent neutral fluoride was applied on the enamel surface for 4 minutes, which was washed in running water and then received hydrogen peroxide. The specimens were immersed in artificial saliva only at the end of the bleaching session.

Saliva is a remineralizing solution able to repair damages to tooth surfaces when its saturation with respect to calcium and phosphate ions is larger than that of enamel. The fluoride ions are capable of guiding this deposition, and promote tissue remineralization[30,31]. Hence, although the artificial saliva (pH 7.34) was used in this study as a storage medium for the specimens, in order to simulate the oral environment, the protocol followed herein did not allow the availability of calcium and phosphate ions to remineralize the enamel surface immediately after application of fluoride, because after that, the specimens were washed in running water and did not come into contact with saliva, thereby not allowing enamel to undergo remineralization.

In this study, the color analyses were undertaken for enamel and opposite dentin surfaces, in which it was evaluated the bleaching efficacy on the tooth structure, in depth. During the weeks of treatment there was an increase in the lightness of the surfaces. This finding demonstrates that hydrogen peroxide was able to diffuse through the enamel and toward the dentin, in depth, and that its action was not compromised when 2% neutral fluoride and the desensitizer were applied prior to bleaching.

Over the treatment bleaching, the enamel and dentin surfaces showed increasing L* means. This occurred due to the continuous application of hydrogen peroxide on the enamel structure, allowing a continuous removal of pigments from the tooth throughout the time. Despite the L* means of the enamel surface to be higher as compared to the L* means of the opposite dentin, this does not mean that the enamel surfaces lightened more during treatment, since when analyzing the increasing variances of the means in the groups during the weeks of treatment, it was found that the opposite dentin surfaces had a greater increase between the means in relation to those L* means of the enamel. This finding is in agreement with the study by Eimar and others[12], who reported that the removal of pigments from the dental structure with larger amounts of organic matter in the composition is facilitated.

In this study, it was also evaluated the ΔE, which shows the numeric value of the change in tooth color between different times; however, this value does not indicate the direction of the change in the three coordinates on the CIE Lab system[32,33]. Comparing groups within each time on different surfaces, it can be seen that there was no difference between them, and the means remained increasing. Comparing the color change of the specimens between baseline and the 1st week of bleaching, it was found that treatments showed no significant differences in the ΔE1 means (table 3) between the two sorts of surfaces evaluated, as well as when comparing the treatments in each surface.

As regards the values obtained for the subsequent weeks of treatment at the times ΔE2 and ΔE3 (tables 4 and 5), there were increasing ΔE means for the enamel and dentin

surfaces, respectively, in relation to the previous week of bleaching. The results of this study indicated that hydrogen peroxide is effective on dentin, in depth, after removing firstly the pigments from the enamel surface. Thus, the greater contact time allowed a higher concentration of free radicals, which first oxidized pigments in the enamel, before deep dentin underwent bleaching.

The effects of peroxide on hard tissues have been investigated[1,18,19], in order to analyze the possible morphological changes in enamel that are clinically undetectable[34]. Hence, the present study analyzed qualitatively by scanning electron microscopy (SEM) the enamel surfaces after undergoing the aforementioned treatments (figure 1), as well as the surfaces treated only with 2% neutral fluoride and desensitizer, and the untreated surfaces (figure 2).

The enamel surface when treated only with hydrogen peroxide, as in the group "without desensitizer (WD)" (fig. 1c), showed apparent erosions with larger number of pores (small orifices) and exposure of prisms. These images show the action of hydrogen peroxide on the enamel surface, which can be directly related to its acidic pH (pH 5.4) along with the process of oxidation by free radicals upon the enamel organic and inorganic matter[16], that may have caused this morphological alteration.

The application of 2% neutral fluoride as above-mentioned did not affect the mechanism of action of 35% hydrogen peroxide, since L* values remained increasing during the weeks of bleaching. Nevertheless, the enamel surface treated with 2% neutral fluoride analyzed under SEM (figure 1a) showed pores, depressions and other irregularities,

but with less surface change when compared to the group "WD". Martin *et al*. (2010) observed by SEM that when the enamel surfaces were treated with 2% neutral fluoride and 35% hydrogen peroxide in different protocols, they showed less morphological changes when compared to the group without fluoride application. In another study, Ferreira and others[18] confirmed that the application 2% neutral fluoride after 35% hydrogen peroxide exhibited smaller changes in the enamel surface when compared to the acidulated fluoride. In that study, the specimens were stored in artificial saliva for 24 hours after the treatments, differing from the present study reported herein, in which after application of fluoride the specimens did not come into contact with saliva but at the end of each bleaching session. In addition, these studies pointed out that the presence of neutral fluoride assists in minor alterations of the enamel surface when treated with 35% hydrogen peroxide, which is consistent with the findings of this study. With regard to the treatment with the desensitizer prior to applying the bleaching agent (fig. 1b), pores and depressions were observed in smaller numbers when compared to aforementioned treatment, in addition to apparent enamel prisms scattered between homogeneous areas on the enamel surface. Nonetheless, no study in the literature has showed so far the characteristics of the desensitizing agent (5% potassium nitrate associated with 2% sodium fluoride) on the enamel surface by SEM analysis.

The surfaces of the groups that did not receive hydrogen peroxide (figure 2) were found to be smoother, with small numbers of pores, enamel prisms, and no depressions. However, there were micropores (too small pores) over the whole surface, which may be

related to the pH of the products (table 1). The untreated group (figure 2c), whose specimens were stored only in artificial saliva, showed smoother surfaces with few pores and micropores, with almost undetectable prisms. On the surface treated with 2% neutral fluoride (pH 7.2), it was observed micropores around barely detectable enamel prisms. The group treated with the desensitizer (pH 6.5) had a greater number of micropores between the prisms over the entire surface (fig. 2b) when compared with the surfaces treated with 2% neutral fluoride. This presence of micropores can be associated with the acidic pH of the desensitizer. The morphological changes in the enamel were seen by microscopy, and indicated that the application of hydrogen peroxide (fig. 1c) had a significant effect on the enamel surface when compared to the other treatments, especially with regard to the surface of the untreated enamel (figure 2c).

According to the findings of this study, 35% hydrogen peroxide showed greater efficacy on deep dentin after removal of enamel pigments. In addition, the prior application of the desensitizing agent did not compromise the success of tooth bleaching, which is of great clinical importance for dentistry since these products prevent or minimize discomforts caused by bleaching.

CONCLUSION

In the tooth bleaching process, the prior application of the desensitizing agent (5% potassium nitrate associated with 2% sodium fluoride) did not affect the mechanism of

action of 35% hydrogen peroxide, in depth. More apparent topographical changes on the enamel surface were observed when hydrogen peroxide was applied.

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CONCLUSÃO

De acordo com os resultados obtidos nos estudos realizados, pode-se concluir que:

1. O clareamento com PC 10% demonstrou ser mais efetivo em profundidade dentinária nas diferentes espessuras de esmalte em relação ao PH 35%, com exceção no terceiro tempo.

2. A presença da camada aprismática no esmalte interferiu na eficácia do PC10% somente no primeiro tempo de clareamento, entretanto, não interferiu nos tempos de clareamento testado com PH35%.

3. Durante o processo de clareamento dental, a aplicação prévia de dessensibilizante (nitrato de potássio 5% associado ao fluoreto de sódio 2%) não interferiu no mecanismo de ação do peróxido de hidrogênio em profundidade dental.

4. Alterações topográficas mais evidentes na superfície do esmalte foram observadas quando aplicado o peróxido de hidrogênio 35% .

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APÊNDICE

DETALHAMENTO DAS METODOLOGIAS

1. Delineamento Experimental

Artigo 1

Unidades experimentais: 80 fragmentos de dentes bovinos

Fatores em estudo:

- Agentes clareadores (2 níveis):
  - → peróxido de carbamida 10 %
  - → peróxido de hidrogênio 35 %
- Espessura do esmalte (4 níveis):
  - → sem esmalte (controle)
  - → 0,5 mm de espessura
  - → 1,0 mm de espessura planificado
  - → 1,0 mm de espessura não planificado (aprismático)
- Tempo (4 níveis):
  - → após o manchamento (baseline)
  - → após a primeira semana do clareamento
  - → após a segunda semana do clareamento
  - → após a terceira semana do clareamento

Variável de resposta:

→Mudança de cor da dentina subjacente por espectrofotometria.

Forma de designar o tratamento das unidades experimentais: Delineamento inteiramente casual, em esquema fatorial.

Divisão dos Grupos:

As amostras foram divididas em 8 grupos (n=10), de acordo com o agente clareador e a espessura do fragmento dental (Tabela 1).

Tabela 1: Grupos de estudo.

GRUPOS	AGENTE CLAREADOR	ESPESSURA DE ESMALTE
Grupo 1	**Peróxido de Carbamida 10%**	**0 mm**
Grupo 2	**Peróxido de Carbamida 10%**	**0,5mm**
Grupo 3	**Peróxido de Carbamida 10%**	**1,0mm planificado**
Grupo 4	**Peróxido de Carbamida 10%**	**1,0mm não planificado**
Grupo 5	**Peróxido de Hidrogênio 35%**	**0 mm**
Grupo 6	**Peróxido de Hidrogênio 35%**	**0,5mm**
Grupo 7	**Peróxido de Hidrogênio 35%**	**1,0mm planificado**
Grupo 8	**Peróxido de Hidrogênio 35%**	**1,0mm não planificado**

Artigo 2

Unidades experimentais: 30 fragmentos de dentes bovinos

Fatores em estudo:

- Tratamentos (3 níveis):
  - → Flúor neutro 2%
  - → Nitrato de potássio associado ao fluoreto de sódio 2%
  - → Sem tratamento (controle)

-Tempo (4 níveis):

- → após o manchamento (baseline)
- → após a primeira semana do clareamento
- → após a segunda semana do clareamento
- → após a terceira semana do clareamento

Variável de resposta:

→Mudança de cor do esmalte e dentina subjacente por espectrofotometria.

Forma de designar o tratamento das unidades experimentais: Delineamento aleatório por meio de sorteio.

Divisão dos Grupos:

As amostras foram divididas em 3 grupos (n=10), de acordo com o tratamento de superfície aplicado anteriormente ao clareamento (Tabela 2).

Tabela 2: Grupos de estudo.

GRUPOS	TRATAMENTO NA SUPERFÍCIE	AGENTE CLAREADOR
Grupo 1	**Flúor Neutro 2%**	**Peróxido de Hidrogênio 35%**
Grupo 2	**Nitrato de Potássio associado ao fluoreto de sódio 2%**	**Peróxido de Hidrogênio 35%**
Grupo 3	**Sem tratamento**	**Peróxido de Hidrogênio a 35%**

2. Preparo dos Espécimes

Para a realização deste estudo, foram utilizados dentes bovinos que, após a coleta, foram armazenados em solução aquosa (água destilada) de timol 0,1% (Dinâmica, Piracicaba, São Paulo, Brasil) tamponado. Após a desinfecção, os dentes foram submetidos à raspagem manual com cureta periodontal para remoção de debris orgânicos e profilaxia com taças de borracha e pasta de pedra-pomes (Maquira Dental Products, Maringá, PR, Brasil) e água. Os dentes foram examinados sob lupa (Zeiss- Carl Zeiss do Brasil) com aumento de quatro vezes para verificar presença de trincas ou manchamento, que

eventualmente poderiam influenciar nos resultados deste estudo. Em seguida, esses dentes foram armazenados, em água destilada sob refrigeração, até o momento da sua utilização. Após a seleção dos dentes, a coroa foi separada da raiz com uso de disco diamantado dupla face (KG Sorensen, Ind. Com. Ltda, Barueri, SP, Brasil) montado em peça reta de mão em baixa rotação sob irrigação constante, à 1mm da junção cemento-esmalte (Figura 1).

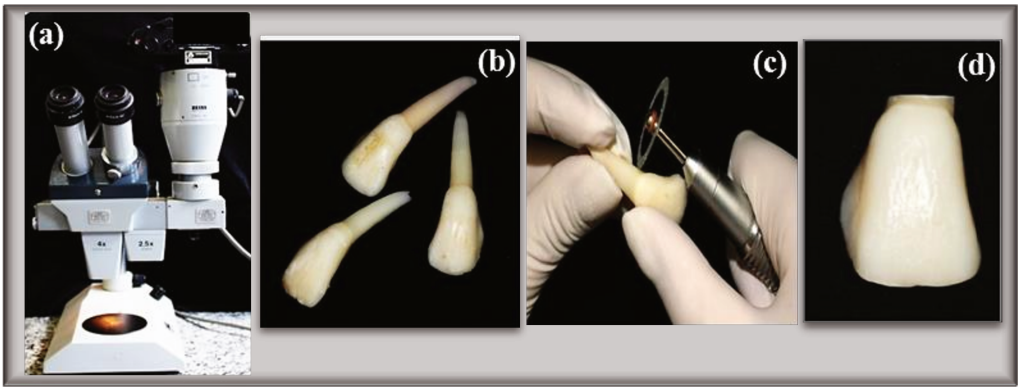

Figura 1: Análise de superfície e corte dos dentes: (a) lupa, (b) dentes selecionados; (c) divisão das porções coroa e raiz; (d) porção coronária após a separação da porção radicular.

Para obtenção das amostras, a porção coronária foi fixada na placa de acrílico com cera utilidade e cola quente, e foram obtidos blocos de dentes através de um disco de corte diamantado (Extec Dia. Wafer Blade 102 x 0,3 x 12,7mm) acoplado em uma Cortadeira Metalográfica (Isomet 1000, Buehler Ltda. Lake Buff, IL, USA) (Figura 2). As amostras foram seccionadas em dois cortes no sentido mésio-distal e dois cortes no sentido cérvico-incisal, obtendo-se 1 bloco de cada dente da região mais cervical da coroa. Assim, foram obtidos blocos das superfícies vestibulares dos dentes com 16 mm^2 (Artigos 1 e 2).

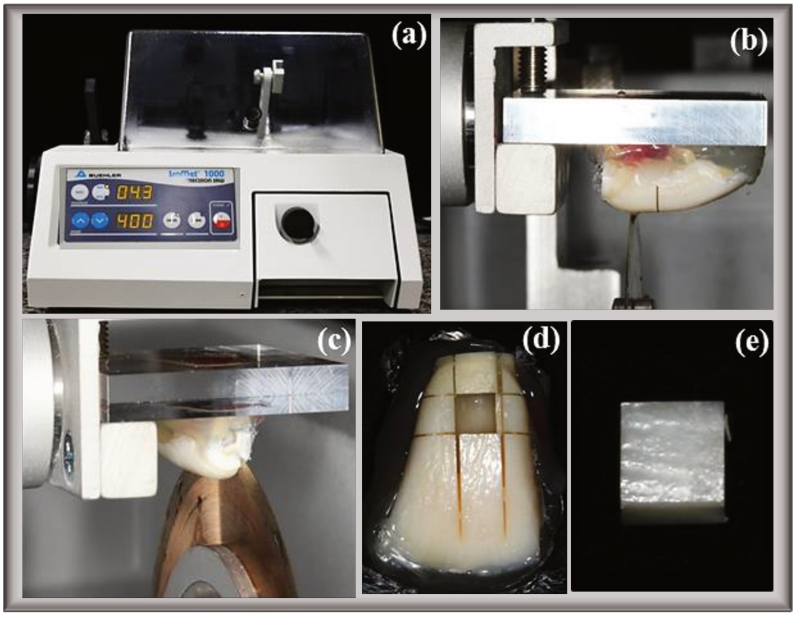

Figura 2: Obtenção de blocos de esmalte: (a) cortadeira metalográfica; (b) corte mesio-distal da coroa; (c) corte cervico-incisal da coroa; (d) coroa seccionada; (e) bloco de dente.

Em discos de acrílico, as amostras foram fixadas com cera pegajosa e posicionadas paralelas em relação à base dos discos permitindo um conjunto de paralelismo (amostra, disco acrílico e lixas) para a planificação e regularização das superfícies do esmalte e dentina subjacente. Uma das faces das amostras ficou livre de cera, permitindo o acompanhamento das espessuras com o auxílio da região posterior de um paquímetro digital (Carl Mahr Esslingen GmbH) (Figura 3), em 1,75 mm (1,75 mm de dentina sem esmalte), 2,25 mm (1,75 mm de dentina e 0,5 mm de esmalte) e 2,75mm (1,75 mm de dentina e 1,0 mm de esmalte) do Artigo1, e 2,75 mm (1,75 mm de dentina e 1 mm de esmalte planificado) para os grupos do Artigo 2 (Figura 4 a, b).

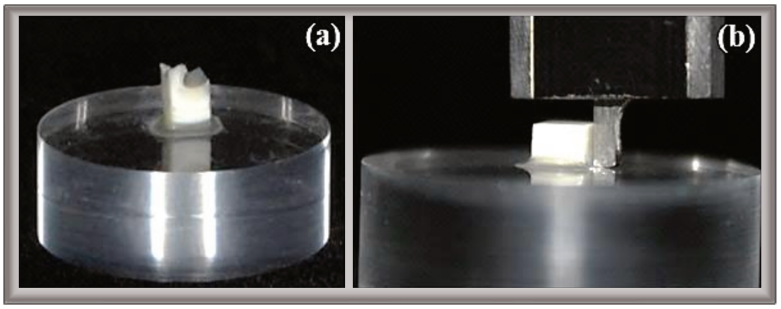

Figura 3: Materiais utilizados para padronização do tamanho dos espécimes: (a) posicionamento e fixação dos espécimes sobre o disco de acrílico; (b) medição das amostras com paquímetro.

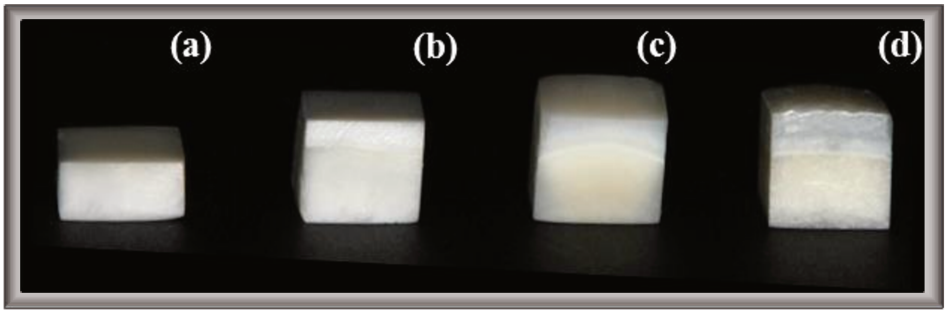

Figura 4: Amostras finalizadas: (a) 0mm esmalte; (b) 0,5mm de esmalte; (c) 1,0mm de esmalte planificado, (d) 1,0mm de esmalte não planificado.

Nessa etapa, foram utilizadas lixas de carbeto de silício de granulação #600 para planificação, e em seguida, #1200 para refinamento das amostras (Carborundum Abrasivos, São Paulo, SP, Brasil) em politriz (Arotec Ind. Com., Cotia, SP, Brasil) em constante refrigeração com água, até se obter a altura do bloco. Entre cada lixa as amostras foram submetidas à limpeza em cuba ultrassônica (Tipo T7 Modelo CT, Thornton- Inpec eletrônica LTDA Vinhedo-SP,Brasil) com água destilada. Finalizadas as amostras, cada espécime recebeu uma marcação com broca esférica nº 1012 (KG Sorensen) em uma das faces laterais com a finalidade de padronizar o posicionamento da amostra durante a leitura com o espectrofotômetro (Figura 5).

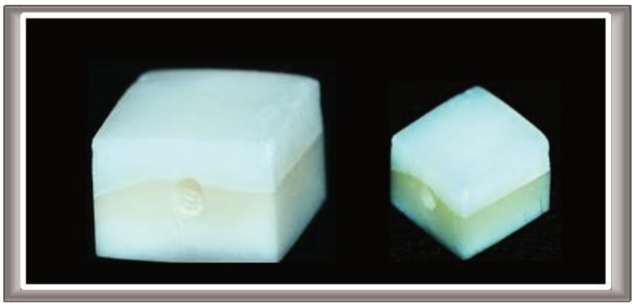

Figura 5: Amostras com marcação em umas das faces laterais com ponta diamantada esférica nº 1012.

3. Protocolo de Manchamento Dental

O manchamento das amostras foi realizado através de imersão em solução de chá preto, a qual era trocada a cada 24 horas, por 6 dias, permanecendo em contato com o esmalte e a dentina todo o tempo. A solução de chá foi produzida a partir de 100 ml de água destilada fervida por 5 minutos, em seguida, misturada a 1,6g de chá preto (Leão Junior S.A., Curitiba, PR, Brasil) em infusão por 5 minutos. Após os 6 dias de imersão na solução, as amostras foram armazenadas em saliva artificial (Figura 6).

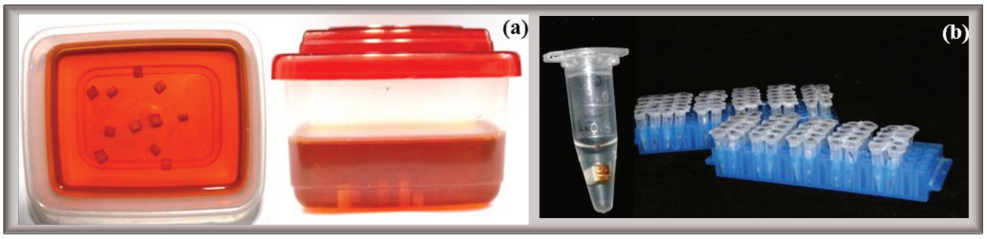

Figura 6: Imersão em chá e armazenamento para estabilização de cor das amostras: (a) imersão na solução de chá preto (b) armazenamento em saliva artificial.

A remoção da borra de chá preto formada sobre o esmalte e a dentina foi removida através do uso de uma taça de borracha com uma mistura de pedra pomes e água (proporção 2:1), em baixa rotação em cada face (Figura 7).

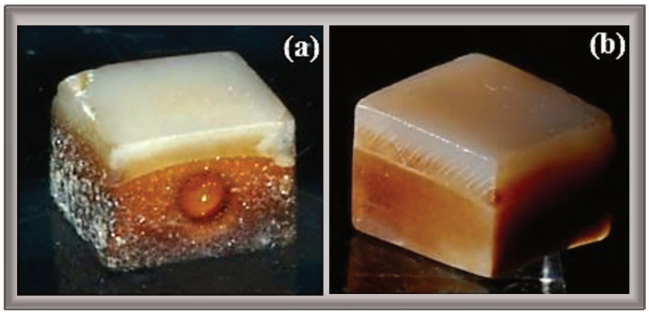

Figura 7: Amostras pigmentadas: (a) antes da remoção da borra (borra nas laterais); (c) após a remoção da borra.

4. Tratamentos de Superfície (Artigo 2)

4.1. Aplicação Prévia do Flúor Neutro 2% e Agente Dessensibilizante

4.1.1. Flúor Neutro 2%

O flúor neutro (Flugel- flúor gel neutro transparente- Fluoreto de sódio 2% -DFL) foi aplicado sobre a superfície do esmalte por 4 minutos (Figura 8).

Figura 8: flúor neutro 2%.

4.1.2. Nitrato de Potássio associado ao fluoreto de sódio 2%

O Nitrato de potássio associado ao fluoreto de sódio 2% (Desensibilize KF 2% - FGM) foi aplicado sobre a superfície do esmalte por 10 minutos (Figura 9).

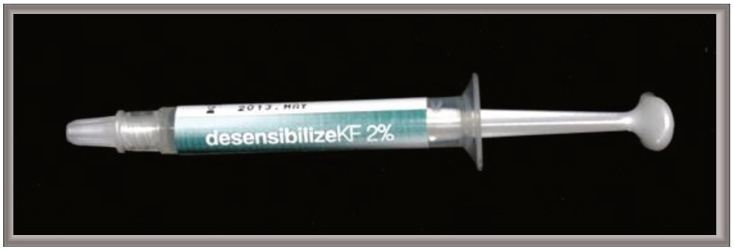

Figura 9: nitrato de potássio associado ao fluoreto de sódio 2%.

O modo de aplicação do flúor neutro 2% e agentes dessensibilizantes nas amostras presas em fita dupla face sobre uma placa de vidro, foi feita com o auxílio de microbrush (Brush Fine KG Sorensen (Figura 10), de forma homogênea com espessura aproximada de 1mm por toda a superfície do esmalte, tendo-se o cuidado para que gel não entrasse em contato com as superfícies laterais dos dentes, uma vez estas não estavam protegidas com qualquer tipo de material. Estes procedimentos se repetiram em todas as sessões de clareamento.

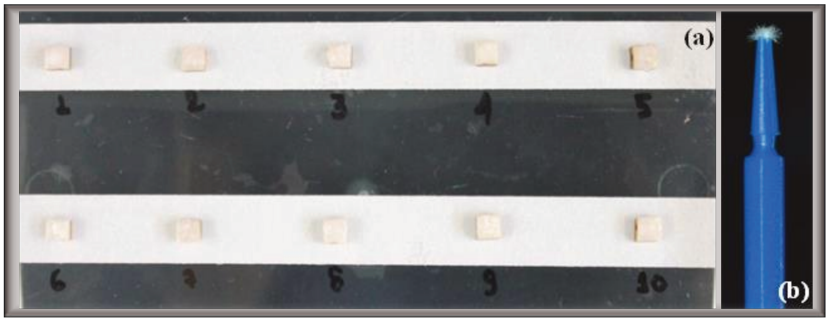

Figura 10: Modo de aplicação dos produtos sobre a superfície do esmalte: (a) posicionamento das amostras sobre a placa de vidro; (b) microbrush.

Os agentes dessensibilizantes foram removidos da superfície do esmalte com hastes flexíveis de plástico com algodão em suas pontas (Cotonetes- Johnson & Johnson, Brasil),

lavados em água corrente e secados com papel absorvente (Kleenex – Kimberly-Clark, Brasil) (Figura 11).

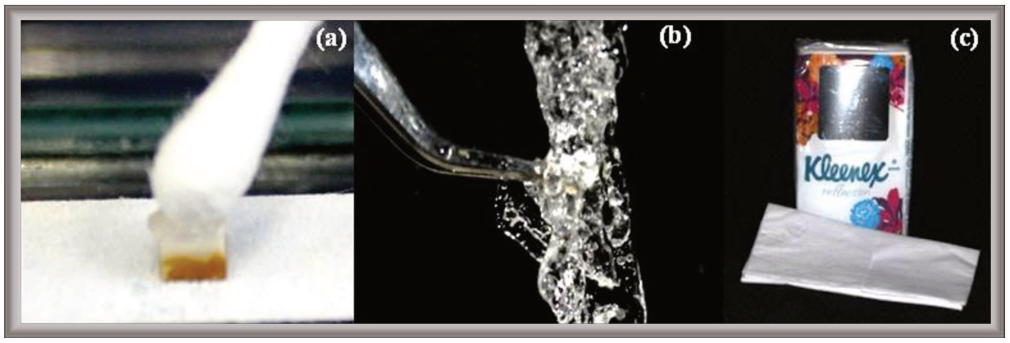

Figura 11: Materiais utilizados para lavagem das amostras: (a) remoção do gel clareador; (b) lavagem em água corrente; (c) secagem em papel absorvente.

5. Protocolo de Clareamento Dental

5.1. Peróxido de Carbamida 10% (Artigo 1)

Dentro de um aparato contendo água destilada e fechado com tampa, sem entrar em contato com os dentes e a superfície do gel, o peróxido de carbamida 10% (Whiteness Perfect 10 % - FGM) foi aplicado sobre a superfície do esmalte por 21 dias consecutivos, permanecendo em contato com o espécime por 4 horas em estufa a 37 ^{o}C ± 2 (Figura 12).

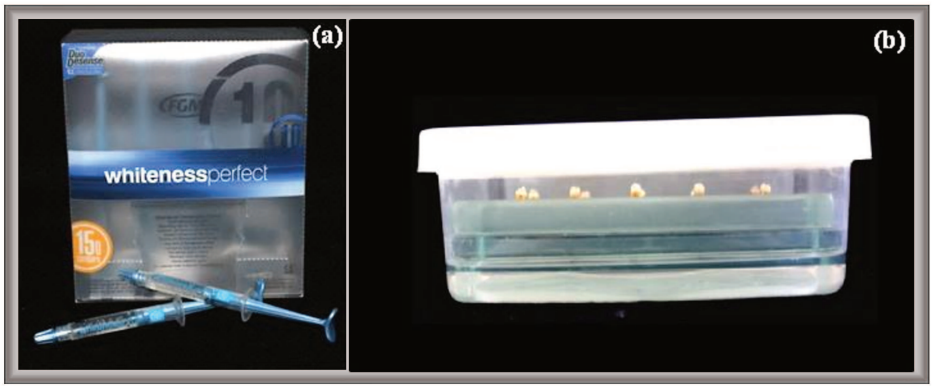

Figura 12: Utilização do peróxido de carbamida 10%: (a) gel clareador; (b) forma de armazenamento durante o tratamento do produto sobre os espécimes.

5.2. Peróxido de Hidrogênio 35% (Artigos 1 e 2)

Em um aparato contendo água destilada e fechado com tampa, e sem entrar em contato com os dentes e a superfície do gel, o peróxido de hidrogênio a 35 % (Whiteness HP MAXX - FGM) foi aplicado na superfície do esmalte, constituindo de 3 aplicações de 15 minutos, em um total de 3 sessões de tratamento com intervalo de 7 dias entre cada sessão. Durante as sessões, os espécimes permaneceram expostos à temperatura ambiente (Figura 13).

Figura 13: Utilização do peróxido de hidrogênio 35%: (a) gel clareador; (b) forma de armazenamento durante o tratamento do produto sobre os espécimes.

O modo de aplicação do gel foi feita da mesma forma que o flúor neutro 2% e o agente dessensibilizante (Figura 10), e a remoção do gel também foi feita com hastes flexíveis de plástico com algodão em suas pontas (Cotonetes- Johnson & Johnson, Brasil) (Figura 14)**,** em seguida, os espécimes foram lavados abundantemente em água corrente, secados com papel absorvente (Kleenex – Kimberly-Clark, Brasil) e armazenados em saliva artificial a uma temperatura de 37 ºC ± 2.

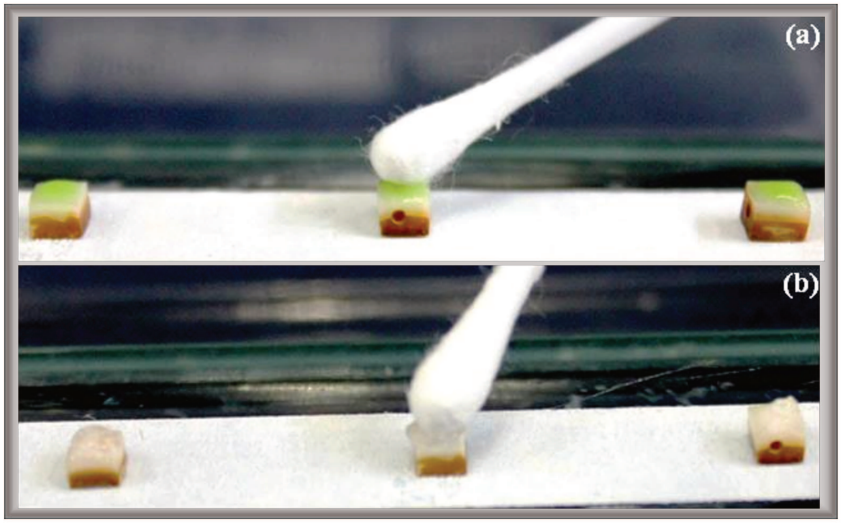

Figura 14: Remoção de gel com hastes flexíveis com algodão nas pontas: (a) peróxido de hidrogênio 35%; (b) peróxido de carbamida 10%.

6. Análise de Cor

Para a realização das leituras de cor, as amostras foram posicionadas em um dispositivo de teflon (porta amostra) dentro de uma Câmara de luz (GTI, Newburgh, NY, USA) para padronização do ambiente. Foi utilizado um espectrofotômetro Konica Minolta CM-700d (Processo FAPESP 10/50336-7) previamente calibrado (Figura 15).

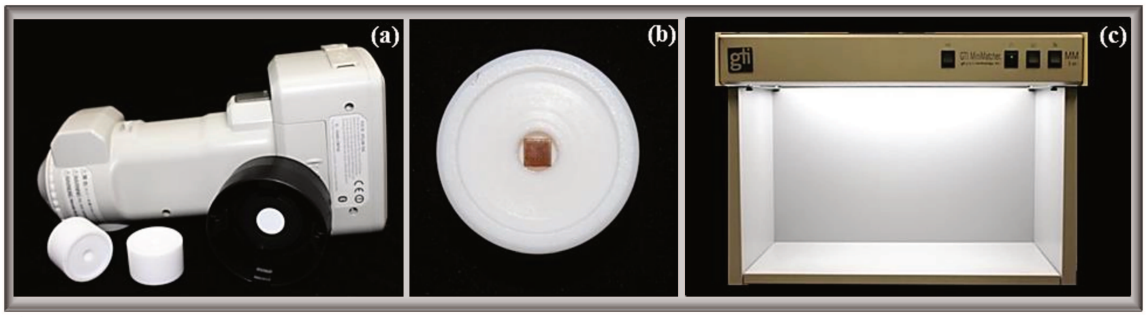

Figura 15: Posicionamento das amostras durante as leituras de cor: (a) espectrofotômetro; (b) porta-amostras; (c) câmara de luz.

7. Microscopia Eletrônica de Varredura (MEV) (Artigo 2)

Para análise em microscopia eletrônica de varredura, apenas as amostras levadas ao MEV após a planificação e refinamento foram polidas em feltros (TOP, RAM e SUPRA - Arotec, Cotia; São Paulo, SP, Brasil), associados à pasta diamantada metalográfica (6 μm – TOP, 3 μm – RAM, 1 μm – SUPRA - Arotec, Cotia, SP, Brasil) e óleo lubrificante (Arotec-Preparação Metalográfica, Cotia, SP , Brasil) (Figura 16), acoplados a politriz giratória (Aropol E, Arotec, Cotia; São Paulo, SP, Brasil) (Figura 4-a). Entre cada etapa e ao final de cada feltro as amostras foram lavadas com água destilada em cuba ultrassônica para remoção de quaisquer debris presentes na superfície de esmalte (Figura 4-d) e em seguida receberam os mesmos tratamentos e clareamento.

Figura 16: Materiais utilizados para o polimento: feltros, pasta de diamante e óleo lubrificante.

Os espécimes foram preparados e metalizados (Metalizador Balt-tec 5CD050 Sputter Coater, Balzers, Liechtenstein) com aproximadamente 20Ëm de liga áurea. E no MEV (JEOL- JSM 5600 LV- Tókio, Japão) foram feitas fotomicrografias de áreas representativas de cada espécime estudado (Figura 17).

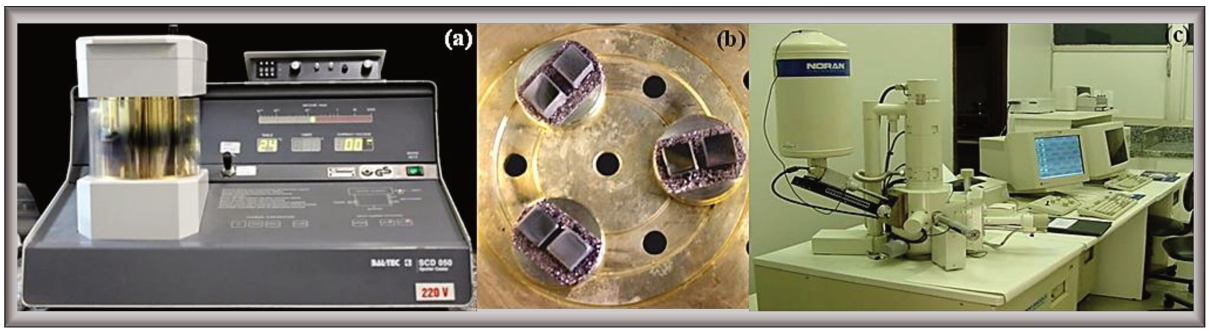

Figura 17: MEV: (a) metalizador; (b) amostras metalizadas; (c) microscópio eletrônico de varredura.

ANEXO 1

Submissão para a revista: Operative Dentistry

Artigo 1

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Manuscript Number: 13-047-L

Manuscript Title: The influence of enamel thickness on bleaching efficacy: an in-depth color analysis

Abstract: The aim of this study was to evaluate the influence of enamel thickness on tooth bleaching efficacy, in-depth, according to the type of bleaching agent: 10% carbamide peroxide (CP) or 35% hydrogen peroxide (HP). Eighty bovine dental fragments were previously stained in a solution of black tea for 6 days, which were distributed in an entirely casual delineation into 8 groups (n = 10), with 1.75 mm dentin thickness and different enamel thicknesses, as follows: 0.5 mm, 1.0 mm planned, 1.0 mm unplanned (prismless enamel) and absence of enamel. The bleaching gels were applied following the manufacturer's recommendations. The specimens were stored in artificial saliva for 3 weeks. The bleaching on the opposite dentin was evaluated at four times: after staining with tea (baseline) and after each of the 3 weeks of bleaching, by means of the CIE Lab method using reflectance spectrophotometer (Konica Minolta CM 700d). The values of ΔE, ΔL, Δa and Δb recorded were subjected to repeated-measures analysis of variance (ANOVA) and Tukey's test (α=0.05). The results showed an increase in dentin lightness (L*), with decreased redness (a+) and yellowness (b+). In addition, the treatment using 10% CP had higher means in relation to 35% HP, not differing only at the third time. Hence, the bleaching using 10% CP was found to be more effective than that using 35% HP regarding dentin depth, and the presence of the prismless enamel layer did not interfere directly in the efficacy of 35% HP.

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ANEXO 2

Submissão para a revista: Journal Investigative and Clinical Dentistry

Artigo 2

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