SANDRINE BITTENCOURT BERGER

EFEITO DE GÉIS CLAREADORES NA DESMINERALIZAÇÃO DO ESMALTE E MÓDULO DE ELASTICIDADE DA MATRIZ ORGÂNICA DA DENTINA BOVINA

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Materiais Dentários.

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RESUMO

O objetivo geral deste estudo foi avaliar o efeito de géis clareadores contendo cálcio ou fosfato de cálcio amorfo (ACP) no módulo de elasticidade da dentina bovina desmineralizada e desmineralização do esmalte hígido ou com lesão inicial de cárie artificial. Capítulo 1: Este estudo avaliou o efeito de agentes clareadores no módulo de elasticidade (ME) da matriz orgânica da dentina bovina. Oitenta e cinco fatias foram obtidas de dentes bovinos e separadas em 5 grupos: grupo controle – sem tratamento (GC), peróxido de hidrogênio 4% (PH4), PH4+0,05% Ca (HP4/Ca), PH 7,5% + ACP (PH7,5) e peróxido de carbamida 10% (PC10). Os grupos PH4, PH4/Ca e PC10 foram tratados com os géis clareadores por 8 horas / dia durante 14 dias, enquanto as amostras do grupo PH7,5 foi submetida ao clareador por 30 minutos, 2 vezes ao dia, durante 14 dias. O esmalte das amostras foi removido e foram preparados 17 espécimes (0,5 x 1,7 x 7,0 mm) por grupo. Em seguida, estas foram desmineralizados em solução de ácido fosfórico 10% por 5 horas e o ME foi mensurado: 24 horas, 7 e 14 dias após o clareamento, utilizando o teste de micro-flexão de 3 pontos. Os dados foram submetidos a ANOVA e teste de Fisher ($p<0,05$). As amostras clareadas após 24 horas e 7 dias mostram menor ME que o GC. Os grupos clareados foram similares ao GC após 14 dias, exceto o grupo PH7,5. O uso de peróxidos pode promover diminuição do módulo de elasticidade da matriz orgânica da dentina bovina. Capítulo 2: O propósito nesta parte da tese foi avaliar o efeito de um clareador experimental e um comercial no esmalte sadio (ES) ou esmalte com lesão inicial de cárie artificial (LC), utilizando microscópio confocal laser de varredura (CLSM). Oitenta blocos (4 x 5 x 5 mm) de esmalte bovino foram usados, sendo que quarenta destes foram desmineralizados com ciclagem de pH para induzir a lesão inicial de cárie artificial. Oito grupos experimentais foram formados a partir dos produtos clareadores e a condição do esmalte dental (ES ou LC), com n=10: Grupos ES: G1 – sem tratamento (controle); G2 – peróxido de hidrogênio 4% (PH4); G3: PH4 contendo 0,05%Ca (Ca); G4 – peróxido de hidrogênio 7,5% (PH7,5) contendo fosfato de cálcio amorfo (ACP). Grupos LC: G5 – não clareado; G6 – PH4; G7 – PH4 contendo Ca; G8 – PH7,5 contendo ACP. Os grupos G2, G3,

G6 e G7 foram tratados com o gel clareador por 8 horas/dia durante 14 dias, enquanto as amostras dos grupos G4 e G8 foram submetidas ao clareador por 30 minutos/duas vezes ao dia, durante 14 dias. Os blocos de esmalte foram corados com solução de rodamina e área fluorescente de desmineralização foi quantificada utilizando CLSM. Os dados foram submetidos a ANOVA e teste de Fisher ($p<0,05$). Para ES, os tratamentos clareadores aumentaram significativamente a área de desmineralização quanto comparado com os grupos não clareados, entretanto, para LC não foi observado diferença estatística significante entre os grupos. A adição de ACP e Ca na composição dos géis clareadores não resultou em redução da desmineralização promovida pelos tratamentos clareadores.

Palavras chave: esmalte bovino, clareamento, microscópio confocal laser de varredura, módulo de elasticidade

ABSTRACT

The aim of this study was to evaluate the effect of bleaching agents containing calcium or amorphous calcium phosphate (ACP) on the elastic modulus of demineralized bovine dentin and demineralization in sound enamel or early artificial caries lesion. Chapter 1: This study evaluated the effect of tooth whitening agents on the elastic modulus (EM) of bovine dentin organic matrix. Eighty-five slices were obtained from bovine teeth and divided into five groups: unbleached control group (CG), 4% hydrogen peroxide (4HP), 4HP+0.05% Ca (4HP/Ca), 7.5% HP (7.5HP) and 10% carbamide peroxide (10CP). The 4HP, 4HP/Ca and 10CP groups were treated with the whitening agents for 8 hours/day during 14 days, while the samples of 7.5HP group were exposed to peroxides for 30 minutes twice a day during 14 days. The enamel of the samples was removed and 17 dentin specimens (0.5 x 1.7 x 7.0 mm) were prepared per group. The specimens were demineralized in 10% phosphoric acid solution for 5 hours and E was assessed using a micro-flexural three-point bend method at 24 hours, 7 and 14 days post-bleaching. The mean values of EM for each group were statistically analyzed using ANOVA and Fisher's test ($p<0.05$). Bleached specimens tested after 24 hours and 7 days showed lower EM than CG. The bleached groups were similar to CG after 14 days, except for the 7.5HP group. The use of peroxides can promoted decrease of EM of bovine dentin organic matrix. Whitening agents can significantly affect of the dentin organic matrix and irreversible damage is observed for selected agents. Chapter 2: The purpose of this study was evaluated to effect of experimental and one commercial bleaching agents on the sound enamel (SE) and with early artificial caries lesions (CL) enamel using confocal laser scanning microscopy (CLSM). Eighty blocks (4 mm thickness x 5 wide x 5 length) of bovine enamel were used and half of them were demineralized with a pH cycling to induce artificial caries lesions. Eight experimental groups were investigated following the bleaching treatments or not and SE or CL (n=10): SE groups: G1 – unbleached (control); G2 - 4% hydrogen peroxide (4HP); G3 - 4HP containing 0.05% Ca (Ca); G4 - 7.5% hydrogen peroxide (7.5HP) containing amorphous calcium phosphate (ACP). CL groups: G5 –

unbleached; G6 – 4HP; G7 - 4HP containing Ca; G8 - 7.5HP containing ACP. The G2, G3, G6, G7 groups were treated with the bleaching agents for 8 hours/day during 14 days, while the samples of G4 and G8 groups were exposed to bleaching agent for 30 minutes twice a day during 14 days. The enamel blocks were stained with rhodamine solution and the quantification of fluorescence demineralization areas of the samples were evaluated using a CLSM. Data were analyzed using ANOVA and Fisher's tests ($p<0.05$). For the SE, the bleaching treatments significantly increased the demineralization area when compared to unbleached group, however, in CL no statistically significant difference was observed among the groups. The addition of ACP or Ca in the composition of the whitening products did not result in the decreasing of the enamel demineralization promoted by bleaching treatments.

Keywords: bovine enamel, whitening, confocal laser scanning microscopy, elastic modulus

SUMÁRIO

INTRODUÇÃO

O clareamento foi inicialmente referido como uma opção de tratamento estético há cem anos, embora o aumento na busca por este tratamento tenha se consolidado a partir do final da década de 80. Em 1868, dentes com vitalidade pulpar foram clareados com ácido oxálico e posteriormente com peróxido de hidrogênio (H_2O_2) (Darnell & Moore, 1990). No entanto, conforme cita Novais & Toledo, 1999, o clareamento para dentes com vitalidade pulpar foi sugerido a partir do trabalho de Ames (1937), que relatou o clareamento de dentes manchados por fluorose, usando uma técnica termocatalítica. Entretanto, o clareamento para dentes com vitalidade pulpar apresentou uma grande revolução quando Haywood & Heymann, em 1989, apresentaram uma técnica com o uso do peróxido de carbamida 10%, na forma de gel, em que o paciente poderia clarear seus dentes utilizando o produto em sua própria residência.

O mecanismo de ação dos agentes clareadores é atribuído à oxidação de moléculas que produzem as alterações de coloração ou escurecimento da dentina e do esmalte (Haywood & Heymann, 1989; Albers, 1991; Goldstein & Garber, 1995). Entretanto, um agente clareador ideal deveria possuir ação rápida e seletiva, sem causar danos aos tecidos dentários e nem a outros tecidos bucais (Yurdukoru *et al*., 2003). Enquanto alguns autores consideram a utilização de agentes clareadores um procedimento seguro (Yurdukoru *et al*., 2003), outros acreditam que o peróxido de hidrogênio possa provocar irritação do tecido pulpar (Li, 1998) ou alterações na estrutura dentária (Spalding *et al*., 2003).

O clareamento dental é possível devido à permeabilidade dos tecidos duros e do baixo peso molecular de alguns componentes químicos ativos dos agentes clareadores, tal como o peróxido de hidrogênio (Arwill *et al*. 1996, Hanks *et al*. 1993). Desta forma, no clareamento de dentes com vitalidade pulpar, uma das grandes preocupações refere-se à penetração de peróxidos para o interior do esmalte e dentina, atingindo a câmara pulpar,

entretanto, os efeitos desta passagem do peróxido de hidrogênio ainda são contraditórios. Alguns autores têm relatado que, mesmo em baixas concentrações, o peróxido de hidrogênio penetra facilmente nas porosidades do esmalte e é capaz de difundir-se até a dentina, alcançando o tecido pulpar (Bowles & Ugwuneri 1987, Cooper *et al*., 1992, Hanks *et al*. 1993, Gokay 2000a,b 2004). Benetti *et al* (2004) avaliou *in vitro* a capacidade de difusão do peróxido de hidrogênio a 10 ou 35% no tecido dental bovino duro após a exposição à géis clareadores. Os referidos autores notaram que as altas concentrações de agentes clareadores produzem altos níveis de peróxido de hidrogênio na câmara pulpar, após a difusão através do esmalte e dentina.

A avaliação dos efeitos de agentes clareadores no esmalte mostram que o tratamento branqueador causam alterações morfológicas superficiais, com perda mineral e consequente redução da microdureza (Moreno & Zahradnik, 1974; McGuckin *et al.*, 1992; Pinheiro Jr. *et al*., 1996; McCraken & Haywood, 1996; Lopes *et al*., 2002; Cimilli & Pameijer, 2001; Pinto, 2004). Estudos que avaliaram a profundidade de desmineralização após o tratamento clareador, observaram que o gel foi capaz de penetrar em 250 μm em profundidade, quando este foi avaliado através de tomografia (Efeoglu *et al*. 2006) e 700 μm quando avaliado através de microdureza Knoop (Attin *et al*. 2005).

Estudos que avaliaram a redução da resistência e união e da microdureza refletem as alterações na superfície, e não caracteriza as alterações na estrutura dentinária. Assim, a integridade estrutural do dente é melhor determinada por estudos de resistência e tenacidade à fratura. Foi observado que a resistência à flexão e o módulo de elasticidade da dentina bovina diminuíram após aplicação direta e diária do peróxido de carbamida (Tam *et al*. 2005a). Reduções significativas na resistência à tração e micro cisalhamento da dentina foram relatados após a aplicação intra-coronária do peróxido de hidrogênio a 30% (Chng *et al*. 2002). Adicionalmente, Rotstein *et al*. (1996) encontraram redução significativa da razão cálcio/fósforo da dentina após o tratamento com peróxido de carbamida a 10% ou peróxido de hidrogênio a 30%.

Com o objetivo de minimizar alguns efeitos adversos do peróxido de hidrogênio na superfície do esmalte, determinados agentes clareadores contêm aditivos, como flúor e outros íons. Estando presente no meio oral, na forma iônica, o flúor potencializa a capacidade remineralizante da saliva em até 90%. Considerando que a dissolução do esmalte depende da concentração inorgânica do meio salivar e do pH da placa (Thylstrup & Fejerskov, 1995), pressupõe-se que se houver concentrações suficientes de flúor (F) e cálcio (Ca) nos agentes clareadores, a perda de cálcio e fosfato do substrato não irá ocorrer mesmo com pH baixo, pois a solução estará supersaturada de íons e o esmalte seria resistente a uma possível desmineralização (Attin *et al*., 1997; Giannini *et al*., 2006). Assim, espera-se que os efeitos do peróxido de hidrogênio na superfície do esmalte sejam minimizados com a adição de fluoretos, cálcio e outros íons. Além disso, recentemente o fosfato de cálcio amorfo (ACP) tem sido adicionado aos produtos clareadores, entretanto, até o momento os efeitos do ACP na remineralização do esmalte têm sido pouco estudados.

Especula-se que géis contendo cálcio poderia promover deposição de mineral apatita nos túbulos dentinários, obliterando-os e desta forma reduzindo a sensibilidade (Schemehorn & Novak, 2007). Os depósitos podem também ocorrer nos túbulos dentinários expostos e esses poderiam bloquear o fluxo do fluido tissular. Isso reduz, ou até mesmo elimina a hipersensibilidade dentinária, de acordo com a teoria hidrodinâmica aplicada à dentina (Brannstrom, 1986) e anteriormente observada em estudos avaliando o fosfato de cálcio em ambiente com pH elevado (Larsen & Jensen, 1980).

Deste modo, este estudo foi dividido em dois capítulos com o objetivo de investigar: (1) o efeito adição de cálcio (Ca) e fosfato de cálcio amorfo (ACP) no módulo de elasticidade da matriz orgânica da dentina bovina depois de 24 horas, 7 dias e 14 dias do tratamento clareador; (2) avaliar através de microscopia confocal laser de varredura a desmineralização após o tratamento clareador no esmalte sadio e no esmalte submetido a

indução de lesão inicial de cárie artificial com a utilização de géis com diferentes formulações.

O presente trabalho é apresentado no formato alternativo de tese de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas. O artigo referente ao Capítulo 1 desta tese foi submetido ao periódico Journal of Materials Science: Materials and Medicine.

Capítulo 1*

Changes in the stiffness of demineralized dentin following application of tooth whitening agents

Abstract

Objectives: The purpose of this study was to evaluate the effect of the bleaching agents on the elastic modulus (EM) of bovine demineralized dentin matrix. Methods: Eighty-five slices were obtained from seventeen bovine teeth. The slices were divided randomly into five experimental groups (n=17): unbleached control group (CG), 4% hydrogen peroxide (HP4), 4% hydrogen peroxide + 0.05% Ca (HP4+Ca), 7.5% hydrogen peroxide + ACP (HP7.5) and 10% carbamide peroxide (CP10). The HP4, HP4+Ca and CP10 groups were treated with the bleaching agents for 8 hours/day during 14 days, while the samples of HP7.5 group were exposed to bleaching agent for 30 minutes twice a day during 14 days. The CG was kept in 100% humidity. After bleaching treatments, the enamel of the samples was removed and 85 dentin beams (0.5 mm thickness, 1.7 mm width and 7.0 length) were prepared from samples. Afterwards, the beams were immersed in 10% phosphoric acid solution for 5 hours and thoroughly rinsed with distilled water for 10 minutes. The beams were tested after 24 hours, 7 and 14 days of storage in distilled water, using three-point bend method. The mean values of EM for each group were statistically analyzed using ANOVA and Fisher's test ($p<0.05$). Results: All bleaching treatments reduced the EM of dentin matrix ($p=0.0008$). After 14 days post-bleaching, the EM of dentin matrix increased for HP4 and HP4+Ca groups ($p=0.0001$). Conclusion: The use of bleaching agents promoted decrease in EM of demineralized dentin, which indicates that the bleaching treatment interacts with the dentin organic matrix. After 14 days post-bleaching

* Trabalho submetido ao periódico Journal of Materiais Science: Materials and Medicine (Anexo 1)

immersed in water, only 7,5% hydrogen peroxide groups did not recovered the EM values when compared the control group.

Keywords: Elastic modulus, Dentin, Whitening

INTRODUCTION

Since its introduction, the nightguard whitening with low concentration of peroxides has been accepted as an efficient and simple aesthetic procedure for removing intrinsic and extrinsic tooth stain and to treat the discolored teeth [1]. Moreover, the nightguard tooth whitening has gained popularity in recent years because it is an affordable and conservative technique to bleach the teeth [2,3].

Changes in the mechanical properties of dental tissues have been reported after whitening treatment [4-8] and alterations to the enamel surfaces such as increased porosities, erosion and demineralization [9,10] has been reported in *in vitro* scanning electron microscopy (SEM) studies. The mechanism by which bleaching agents affect dentin is not well understood [11], however studies reported that hydrogen peroxide may cause dissolution of inorganic content with decreased calcium-phosphorus ratio and reduction in the organic components of dentin by protein oxidation or dentin denaturation [5,13,14].

While a negative effect of bleaching agents on the mechanical properties of undemineralized dentin has been reported using tensile strength tests, the influence of theses agents in the mechanical properties of the organic matrix has not been reported. This study evaluated the effect of four whitening products used at different peroxides concentrations and compositions on the elastic modulus of demineralized coronal bovine dentin at 24 hours, 7 and 14 days post-bleaching. The hypothesis tested in this study was that the whitening would not affect the elastic properties of demineralized dentin when

compared to an untreated group, regardless of the type of bleaching agent and post-bleaching evaluation time.

MATERIAL AND METHODS

Specimen preparation and bleaching

Seventeen extracted bovine incisor teeth were used in this study. After pumicing, they were stored in 0.1% thymol solution at 4º C for 30 days. The root portion was sectioned 1 mm below the cement-enamel-junction and discarded (Figure 1A). The crowns were serially sectioned into 0.5 mm ± 0.1 mm thick in the mesio-distal direction with a diamond blade saw (Series 15HC Diamond, Buehler, Lake Bluff, IL, USA) under constant water irrigation (Figure 1B and 1C) to obtain eighty-five slices (5 slices per tooth). The five slices from each tooth were allocated to one of the five experimental groups (n = 17). Except the buccal enamel surfaces, other surfaces of dental slices were coated with two layers of nail varnish (Revlon Inc., New York, NY, USA).

The composition of the whitening products and the application regimens for the bleached groups were described in Table 1. The slices of the control group were kept in water and were not subjected to any whitening treatment. The remained slices were daily exposed to a mixture of 0.1 mL of a bleaching agent (Figure 1D) with 0.05 mL of artificial saliva and covered with an individual tray. During the treatment period, the slices were placed in 100% humidity at 37 ^{o}C. After daily treatment, the slices were thoroughly rinsed with deionized and distilled water and stored in artificial saliva at 37 ^{o}C.

Immediately after whitening, the enamel was removed and the slices were further trimmed using a cylindrical diamond bur (#557D, Brasseler, Savannah, GA, USA) in a high speed handpiece (Impact Air 45tm, Palidases Dental, Englewood, NJ, USA) to a final rectangular dimension of 0.5 mm thickness x 1.7 mm width x 7.0 mm length (Figure 1E). A dimple was made at one end of the surfaces to allow for repeated measurements to be performed on the same surface. Dentin specimens were immersed in 10% phosphoric acid

solution (LabChem, Pittsburgh, PA, USA) for a period of 5 hours (Figure 1F) and thoroughly rinsed with distilled water for 10 min [15].

Three-point bend microflexural test

Specimens were tested in compression while immersed in distilled water using a universal testing machine (EZ Graph, Shimadzu, Tokyo, Japan), with a 1N load cell at crosshead speed of 0.5 mm/min (Figure 1G and 1H). Load-displacement curves were converted to stress–strain curves and the apparent elastic modulus calculated at 3% strain. Displacement (D) during compression was displayed in millimeters and calculated at a maximum strain of 3% using the following formula [16]:

$$D= \varepsilon L^2/6T$$

where ε is strain, L is support span, and T is thickness of the specimen. Then the modulus of elasticity (E) of the specimens was expressed in MPa (Mega Pascal) and calculated using the following formula:

$$E=PL^3/4DbT$$

where P is the maximum load, L is the support span, D is the displacement, b width of the specimen, and T is the thickness of the specimen.

The specimens were tested at three post-bleaching times: 24 hours, 7 days and 14 days. Between the measurements the samples remained in distilled water at 37 ^{o}C. The data were collected and statistically analyzed using two-way ANOVA (evaluation time x treatments) repeated measurements at a 95% confidence interval and Fisher's PLSD test.

RESULTS

The elastic modulus mean values and standard deviations (MPa ± SD) are shown in Table 2. Two-way ANOVA revealed that there were statistically significant differences for the factor "treatments" (p=0.0008), factor "evaluation time" (p=0.0059) and for the interaction between factors (p=0.0092).

Fisher's PLSD test showed higher elastic modulus for control group when compared to the bleached groups tested at 24 hours, 7 and 14 days post-bleaching (p<0.05). At 24 hours and 7 days post-bleaching, no statistically significant differences were observed among the bleached groups (p>0.05). After 14 days of water storage, only the specimens treated with 7.5% hydrogen peroxide presented lower elastic modulus when compared to the control group (p<0.05). No statistically significant differences were observed among the bleached groups at 14 days post-bleaching (p>0.05).

The control group, bleached with 7.5% hydrogen peroxide and 10% carbamide peroxide had their elastic modulus constant after 24 hours and 14 days (p<0.05). Conversely, the dentine elastic modulus for the groups bleached using 4% hydrogen peroxide with or without 0.05% calcium increased in 14 days of water storage (p>0.05).

DISCUSSION

Studies have shown that whitening treatments with peroxides can reduce the flexural strength and modulus of mineralized dentin. In those studies, mineralized and sound bovine dentin presented approximately 140 to 220 MPa of flexural strength, while the flexural modulus was 12 to 14 GPa [8,17,18]. Maciel et al. [19] and Bedran-Russo et al. [15] indicate that the elastic modulus of decalcified human dentin matrix in water can vary between 5 and 7 MPa. This current study used bovine teeth and the elastic modulus of untreated demineralized dentin (control group) was approximately 3 MPa, which represented half of that for human dentin value. The results may partially reflect the compositional and morphological differences between human and bovine teeth. In this study, all bleaching agents reduced the dentin elastic modulus until 7 days post-bleaching

when compared to an unbleached control group, however, at 14 days post-bleaching, the bleaching agents did not differ from the control group, except for the group treated with 7.5% hydrogen peroxide. Since the elastic properties of demineralized dentin changed after whitening for the four tested materials and most groups recovered or had similar to the values of control group at 14 days of water storage, the hypothesis tested in this study was rejected.

Studies have suggested that bleaching agents are capable of diffusing through enamel and dentin [20,22]. This study confirms the ability of peroxides to diffuse and generate free radicals, which interact with organic structures, since the enamel was kept intact during whitening regimens. Free radical reactions are not specific and can potentially react with other organic structures [4]. The peroxides may cause alteration in the chemical structure of the dentin and reduction in potassium levels and in the Ca/P ratio [13,14]. Moreover, it has been reported compromised mechanical properties [5,23] and increased dentine permeability [24] following whitening treatment.

Although the peroxides compositions and concentrations differed among the whitening products, the elastic modulus of dentin at 24 hours, 7 and 14 days post-bleaching were similar among bleached groups. The two materials that contained calcium (7.5% hydrogen peroxide + ACP (1 to 5% amorphous calcium phosphate) and 4% hydrogen peroxide + 0.05% calcium) had different application modes, but presented similar results. The elastic modulus for the group treated with the bleaching agent containing 7.5% hydrogen peroxide plus ACP was lower than the control group 14 days post-bleaching. The higher peroxide concentration in the gel composition and application (two times/daily) may be accountable for the effect observed in demineralized dentin.

The addition of calcium and fluoride to bleaching agents have been proposed to reduce the mineral loss during whitening with little influence on the organic components of dental tissues [25,26]. The application of 10% carbamide peroxide releases 3% hydrogen peroxide and approximately 7% urea. This bleaching agent contains the lowest peroxide concentration among the materials and the urea released may provide

beneficial effects due to the raise of pH that reduces the demineralization level [4,27]. Urea is a protein destabilizer with ability to breaks covalent and hydrogen bonds [17,18,28], leading proteoglycans and type I collagen structural changes and alterations on the physical properties of dental structures. In this study, the urea-containing carbamide peroxide did not have greater deleterious effects on elastic modulus than only hydrogen peroxide bleaching.

It has been reported that the color of teeth comes from the organic content of dentin [29,20], which can be attacked by components of the bleaching products. The results of this study showed that the changes on elasticity modulus of dentin post-bleaching are due to the effects on the organic matrix, as a secondary or adverse effect produced by the whitening products. The weakening of dental structures has been considered as one of the main causes of bond strength reduction reported immediately after bleaching [31.32]. The recurrence of stains or tooth discoloration may occur along the time as a function of the reversal effect on dentin organic matrix promoted by bleaching agent, as well as the residual oxygen from the peroxides may impair the recovery on the elasticity modulus values of dentin until 7 days post bleaching. After 14 days of storage in water, the concentration of oxygen was reduced or eliminated, which could favor the reversal of compromised elastic modulus values of dentin. This study evaluated a mechanical property of dentin post-bleaching, however, biochemistry analysis would be necessary to confirm the “reversible” effect.

While studies [8,17,18] have analyzed the effect of whitening on the flexural modulus of mineralized and intact dentin, the present study removed the mineral content and evaluated the effects of different bleaching agents on the elastic modulus of only decalcified dentin matrix. The present studies highlight the importance of the mechanical properties of the organic dentin matrix on the overall properties of dentin.

CONCLUSIONS

In conclusion, when the specimens were tested 24 hours after whitening, it was observed a reduction of the elastic modulus of demineralized dentin matrices. However, after 14 days storage in water, most of the bleached groups had similar elastic modulus of an unbleached control group, demonstrating that the effects produced by some bleaching agents can be reversible by storage in water.

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Table 1 – Material used in the study

Bleaching Agent	Main ingredient and concentration	Application time	Manufacturer
Experimental bleaching	4% Hydrogen Peroxide	8 hours daily (14 days)	FGM Prod. Odont., Joinville, SC, Brazil
Experimental bleaching	4% Hydrogen Peroxide (+ 0.05% Ca)	8 hours daily (14 days)	FGM Prod. Odont., Joinville, SC, Brazil
DayWhite ACP	7.5% Hydrogen Peroxide (+ ACP)	30 min - Twice daily (14 days)	Discus Dental, Culver City, CA, USA.
Opalescence	10% Carbamide Peroxide	8 hours daily (14 days)	Ultradent Products Inc, South Jordan, UT, USA
No Bleaching	_____	_____	_____

Table 2 - Means (standard deviation) of the elastic modulus (MPa) of bovine demineralized dentin matrix.

Treatments	Time		
	24 hours	7 days	14 days
4% Hydrogen Peroxide	2.0 (0.5) $^{B\,b}$	2.0 (0.4) $^{B\,b}$	2.5 (0.6) $^{AB\,a}$
7.5% Hydrogen Peroxide +ACP	2.3 (0.8) $^{B\,a}$	2.2 (0.7) $^{B\,a}$	2.3 (1.0) $^{B\,a}$
4% Hydrogen Peroxide + 0.05%Ca	2.5 (0.7) $^{B\,b}$	2.1 (0.4) $^{B\,c}$	2.7 (0.6) $^{AB\,a}$
10% Carbamide Peroxide	2.5 (0.9) $^{B\,a}$	2.5 (0.7) $^{B\,a}$	2.5 (0.6) $^{AB\,a}$
Control Group	3.3 (0.8) $^{A\,a}$	3.0 (0.8) $^{A\,a}$	3.0 (1.0) $^{A\,a}$

Means followed by different letters, capital letters in columns and lower case letters in rows are statistically different (Fisher test, $p<0.05$).

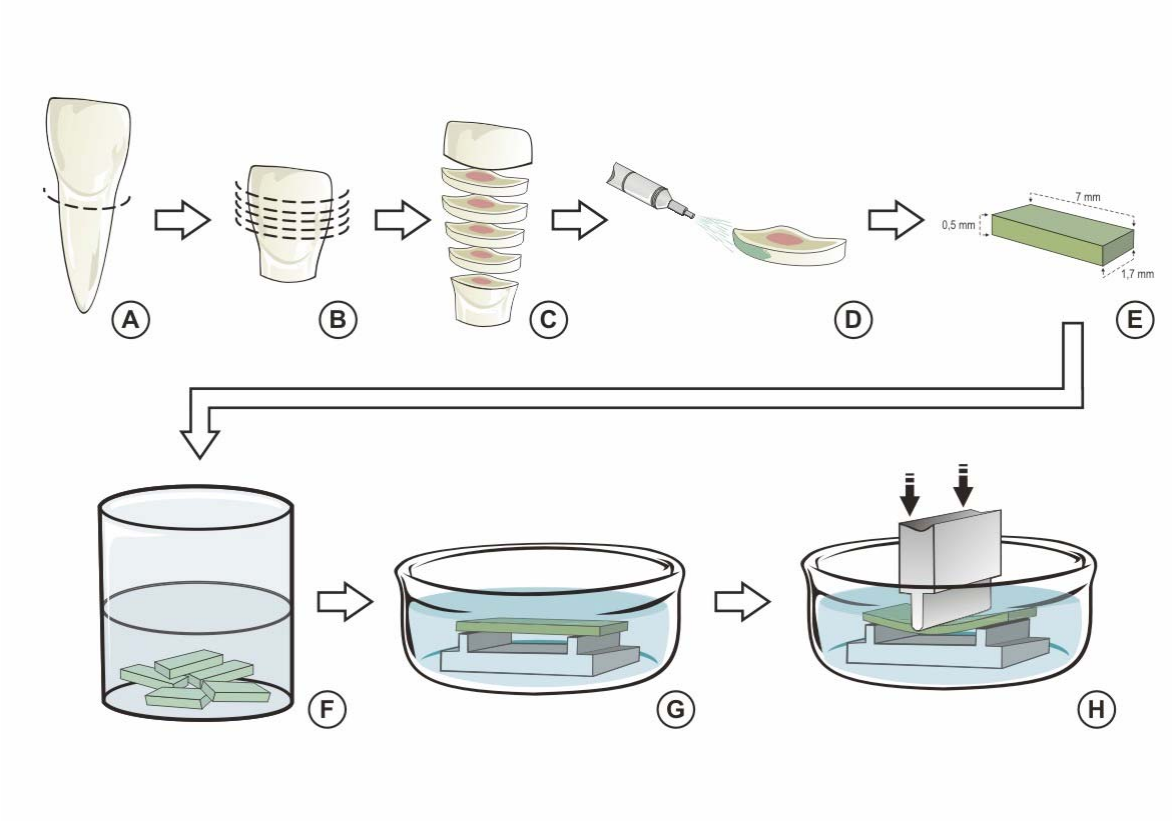

Figure 1 – Specimen preparation (A, B, C), whitening treatments (D), final sample (E), specimens immersion in 10% phosphoric acid solution (F), three point bending testing (G,H).

Capítulo 2

Effect of bleaching on sound enamel and with early artificial caries lesions using confocal laser microscopy

Abstract

Objectives: The purpose of this study was to evaluate effect of experimental and one commercial bleaching agents on the sound enamel (SE) and enamel with early artificial caries lesions (CL) using confocal laser scanning microscopy (CLSM). Methods: Eighty blocks (4 mm thickness x 5 wide x 5 length) of bovine enamel were used and half of them were demineralized using a pH cycling model to induce artificial caries lesions. Eight experimental groups were investigated following the bleaching treatments or not and SE or CL (n=10): SE groups: G1 – unbleached (control); G2 - 4% hydrogen peroxide (4HP); G3 - 4HP containing 0.05% Ca (Ca); G4 - 7.5% hydrogen peroxide (7.5HP) containing amorphous calcium phosphate (ACP). CL groups: G5 – unbleached; G6 – 4HP; G7 - 4HP containing Ca; G8 - 7.5HP containing ACP. The G2, G3, G6, G7 groups were treated with the bleaching agents for 8 hours/day during 14 days, while the samples of G4 and G8 groups were exposed to bleaching agent for 30 minutes twice a day during 14 days. The enamel blocks were stained with 0.1 mM rhodamine B solution and the mineralization was quantified using fluorescence intensity detected by CLSM. Data were analyzed using ANOVA and Fisher's tests ($p<0.05$). Results: For the SE, the bleaching treatments significantly increased the demineralization area when compared to unbleached group, however, in CL no statistically significant difference was observed among the groups ($p>0.05$). Conclusion: The addition of ACP or Ca in the composition of the whitening products did not decrease the enamel demineralization promoted by bleaching treatments.

Keywords: Bovine enamel, Whitening, Confocal laser scanning microscopy

INTRODUCTION

Vital tooth bleaching has gained popularity in recent years as an easy, affordable, and conservative way of treating discolored teeth [20]. Since its introduction [17], bleaching has been accepted as an efficient and simple aesthetic procedure for removing intrinsic and extrinsic stains from teeth [16]. In the last decade, many products (with varying concentrations) and techniques have been indicated for both, in-office use or mouthguard bleaching [19].

As tooth bleaching has been widely used to improve dental appearance, its potentially negative effects are of concern. Some studies found mineral loss, increased susceptibility to erosion or caries, increased surface roughness, reduced enamel tensile strength, reduced fracture stability or a decrease in abrasion resistance of bleached dental hard tissues [5, 6, 8, 27].

In an attempt to decrease tooth sensitivity, to re-establish surface hardness and to support remineralization of initial white spot lesions, some manufacturers have incorporated fluorides into their bleaching gel formulations [1,12]. More recently, amorphous calcium phosphate (ACP) has become available in tooth whitening products; however, up to now the effects of ACP on enamel remineralization have rarely been scrutinized [23]. Calcium ions has also been added in the bleaching agent's composition, however, as ACP little is known about the effectiveness of these components [10].

This study used confocal laser microscopy to evaluate the effect of bleaching agents containing calcium on sound enamel and with early artificial caries lesions. The null hypothesis was that the calcium-containing bleaching agents would not affect the demineralization of sound enamel and not promote remineralization of early simulated caries lesions.

MATERIAL AND METHODS

Specimen preparation

Twenty extracted sound bovine incisors stored in 0.1% thymol solution were used within 1 month of extraction. The roots were separated from the crowns with a water cooled low-speed diamond saw (Buehler-Series 15LC Diamond, Buehler, Lake Bluff, IL, USA). Crowns were sectioned mesio-distally and buccal-lingually to obtain 4 dental blocks (4 mm long X 3 mm wide X 3 mm thick) from each crown. Except for the buccal surfaces, all other surfaces of the dental blocks were sealed with acid-resistant nail polish (Revlon Corp., New York, NY, USA). Eighty dental blocks were randomly assigned to 8 groups (n = 10).

Induction of artificial caries lesions (pH cycling) and bleaching

Forty samples were subjected to a pH cycling model to induce artificial early carious lesions. The specimens were submitted to 8 days pH-cycling regimen simulating. Each day or cycle consisted of the individual immersion in demineration solution (3.12 mL/mm^2 and composition: 1.4 mM Ca, 0.9 mM P, 0.05 M acetate buffer, pH 5.0) and remineration solution (1.56 mL/mm^2 and composition: 1.5 mM Ca, 0.9 mM P, 0.1 M Tris buffer, pH 7.0) for 8 and 16 h, respectively. Between the immersions, and at the end of the cycle, the specimens were rinsed with distilled and deionized water and gently dried with absorbent paper [15, 26]. Immediately after pH cycling the samples were subject to bleaching treatments as described in Table 1. The enamel blocks were daily exposed to bleaching agent with 0.05 mL of artificial saliva and covered with an individual tray.

Post-treatment analysis

Samples were sectioned longitudinally through the treated area with a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) and embedded perpendicular to the demineralized surface in epoxy resin (Buehler, Lake Bluff,

IL, USA). Afterwards, the samples were polished on a water-cooled polishing unit (EcoMet 3000, Buehler, Lake Bluff, IL, USA) with abrasive paper (400-, 600- and 1200-grit) and 0.3 and 0.1 µm diamond mask alumina suspension (Metaldi Supreme, Buehler, Lake Bluff, IL, USA). The polished samples were cleaned ultrasonically in deionized water for 15 minutes to remove the residues from polishing procedure.

Confocal laser scanning microscopy (CLSM)

The samples were immersed in a fresh 0.1 mM rhodamine B solution (Aldrich Chem. Co., Milwaukee, Wisc., USA) for 1 h, without further water-rinsing [13, 29]. Samples were analyzed with CLSM (Zeiss LSM 510, Carl Zeiss, Inc., Germany), using an argon laser with a 529-nm excitation wavelength. Areas were scanned between 10 and 50 µm below the treated surface to reduce the influence of the smear layer created during the cutting and polishing procedure [29]. The quantification of fluorescence demineralization area was analyzed with an image-analysis system (ImageJ 1.41, Wayne Rasband, National of Institutes of Health, USA).

Statistical analysis

The data collected were statistically analyzed using two-way ANOVA (factors: bleaching treatments x enamel conditions) at a 95% confidence interval and Fisher's PLSD test.

RESULTS

The total fluorescence demineralization area (in μm^2) and standard deviations (SD) are shown in Table 2. Two-way ANOVA revealed that there was statistically significant difference for the factor "enamel" ($p<0.0001$) and factor "bleaching treatment" ($p=0.0189$). However, interaction between factors was not statistically significant ($p=0.1510$).

Fisher's PLSD test showed that sound enamel presented increase of demineralization area after bleaching treatments when compared to a control group ($p \leq 0.0004$). The fluorescence area in sound enamel for each bleaching treatment is depicted in the Figure 1. The results of fluorescence area (demineralization) in enamel groups with early artificial caries lesions showed no significant difference among bleaching treatments ($p=0.4795$). The bleached groups were also statistically similar to the control group ($p>0.05$). Figure 2 illustrates the fluorescence areas of the enamel with early lesions groups. The comparison between the enamel condition (sound and enamel with early lesions) showed statistically significant difference ($p<0.0207$). The fluorescence areas of the artificial caries lesions were larger than those of sound bleached enamel ($p<0.0001$).

DISCUSSION

The first use of CLSM in dental research was proposed by Watson in 1989 [30]. The objective of that study was to evaluate the morphology of the tooth-composite interfaces. González-Cabezas *et al*. [13] reported that CLSM could be used with thin enamel sections or half a tooth stained with a 0.1 mM solution of Rhodamine B. This method can replace or supplement microradiography analysis that quantify the demineralization and analyze the remineralization phenomenon. Few studies have used CSLM to evaluate the effects of bleaching agents on tooth structure [14, 22], therefore, this study compared the effect of different formulations of bleaching agents in the enamel demineralization of sound enamel and with early artificial caries lesions by quantifying the fluorescence areas.

The bleaching products tested contained as active ingredient the hydrogen peroxide with two concentrations (4% and 7.5%) and were applied for 8 hours daily or for 30 minutes twice daily, respectively. Experimental bleaching agents contained only the active ingredient or the addition of 0.05% calcium or ACP and were compared to unbleached groups. This current *in vitro* study demonstrated that a 14-days regimen of whitening using different formulations of bleaching agents did not produce different

results on sound enamel and on enamel with early artificial caries lesion. Moreover, the bleached groups showed similar demineralization fluorescence areas to unbleached groups, regardless of enamel conditions. Thus, the null hypothesis of this study was accept, since that the addition of calcium or ACP did not reduce the demineralization area on sound enamel and did not promote remineralization of the enamel with artificial lesions enamel. Some authors have evaluated some commercial bleaching agents containing hydrogen or carbamide peroxides and did not observe alterations in surface morphology after whitening [18, 24, 27]. However, others studies reported that bleaching agents produced mineral loss and formation of porosities on the enamel surface [3, 9, 19, 25]. The surface and subsurface changes correspond to the areas with occurred significant dye penetration (Rhodamine B), as seen in Figures 1 and 2.

Efeoglu *et al.* [11], used computerized tomography to examine human enamel specimens that were treated for 15 days (eight hours daily) with 10% carbamide peroxide. The authors reported that enamel demineralization depth reached 50 μm. Conversely, Bizhang *et al*. [4] evaluated bovine enamel after treatments with 10% carbamide peroxide (8 hours daily for two weeks) or 5.3% hydrogen peroxide (one hour daily for two weeks) and found lesion depths of 4.8 μm and 1.6 μm, respectively. Attin *et al*. [2] showed that the reduction in hardness of enamel was confined to superficial layers, confirming the demineralization in depth. In this current study the demineralization depth caused by pH cycling was 135 μm, which corresponded to the control group (unbleached). The bleached treatments did not increase the demineralization depth for the pH-cycled groups that ranged from 166 (4% Hydrogen Peroxide + 0.05%Ca or 4% Hydrogen Peroxide) to 168 μm (7.5% Hydrogen Peroxide + ACP). The whitening treatment of sound bleached, resulted in a demineralization depth, which ranged from 31 (4% Hydrogen Peroxide) to 52 μm (7.5% Hydrogen Peroxide + ACP).

The pH cycling was chosen since it was possible to reproduce the dynamic process of caries lesion development, alternating deminelization and remineralization periods [15,25]. The role of calcium and phosphate ions in demineralization and

remineralization process is clearly established, but the influence of organic content of saliva should also be considered. This is a limitation of the study, because it was an *in vitro* study and not possible to reproduce all the variables of an *in situ* or *vivo* study.

Increase of dye penetration was observed more for the artificial caries lesions (Figures 2a, 2b. 2c and 2e) than sound bleached enamel due to the mineral loss induced by pH cycling. The Figures 2b and 2d are representatives CSLM images of groups submitted to bleaching agents contained calcium ions and ACP, respectively. Their demineralization fluorescence areas were similar to that observed for 4% hydrogen peroxide (Figure 2c) and control group (Figure 2a). Thus, the induction of artificial caries lesions for pH cycling was the determinant factor for dye penetration and not bleaching treatments. Furthermore, the bleaching composition had no influence on enamel demineralization of sound or with early artificial lesions.

CONCLUSIONS

The bleaching treatments promoted increased sound enamel demineralization, while the addition of calcium ion or ACP did not prevent/reverse the effects caused by the whitening treatment in both conditions of the enamel. Early artificial caries induced by pH cycling model were not affected by the bleaching therapy, regardless of the type of whitening agent.

Acknowledgments

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Table 1 – Material used in the study

Group	Enamel with early artificial caries lesions (CL)	Bleaching Agent	Concentration	Application time	Treatment duration	Manufacturer
1	No	Unbleached	Control Group	_____	_____	_____
2	No	Experimental bleaching	4% Hydrogen Peroxide + 0.05%Ca	8 hours daily	14 days	FGM Prod. Odont., Joinville, SC, Brazil
3	No	Experimental bleaching	4% Hydrogen Peroxide	8 hours daily	14 days	FGM Prod. Odont., Joinville, SC, Brazil
4	No	DayWhite ACP	7.5% Hydrogen Peroxide + ACP	30 min - Twice daily	14 days	Discus Dental, Culver City, CA, USA.
5	Yes	Unbleached	Control Group	_____	_____	_____
6	Yes	Experimental bleaching	4% Hydrogen Peroxide + 0.05%Ca	8 hours daily	14 days	FGM Prod. Odont., Joinville, SC, Brazil
7	Yes	Experimental bleaching	4% Hydrogen Peroxide	8 hours daily	14 days	FGM Prod. Odont., Joinville, SC, Brazil
8	Yes	DayWhite ACP	7.5% Hydrogen Peroxide + ACP	30 min - Twice daily	14 days	Discus Dental, Culver City, CA, USA.

Table 2 – Total area of demineralization (μm^2) of the experimental groups accessed by fluorescent dye using confocal laser microscopy.

Treatments	**Sound enamel (SE)**	**Enamel with early artificial caries lesions (CL)**
Unbleached	4.8 (2.2) ^{B,a}	19.6 (7.6) ^{A,b}
4% Hydrogen Peroxide + 0.05%Ca	10.8 (4.0) ^{A,a}	20.3 (9.0) ^{A,b}
4% Hydrogen Peroxide	11.4 (3.6) ^{A,a}	20.9 (6.6) ^{A,b}
7.5% Hydrogen Peroxide + ACP	10.9 (4.2) ^{A,a}	18.6 (5.0) ^{A,b}

Different upper case letters in columns and lower case letters in rows indicates statistically significant differences between groups (Fisher test, $p<0.05$).

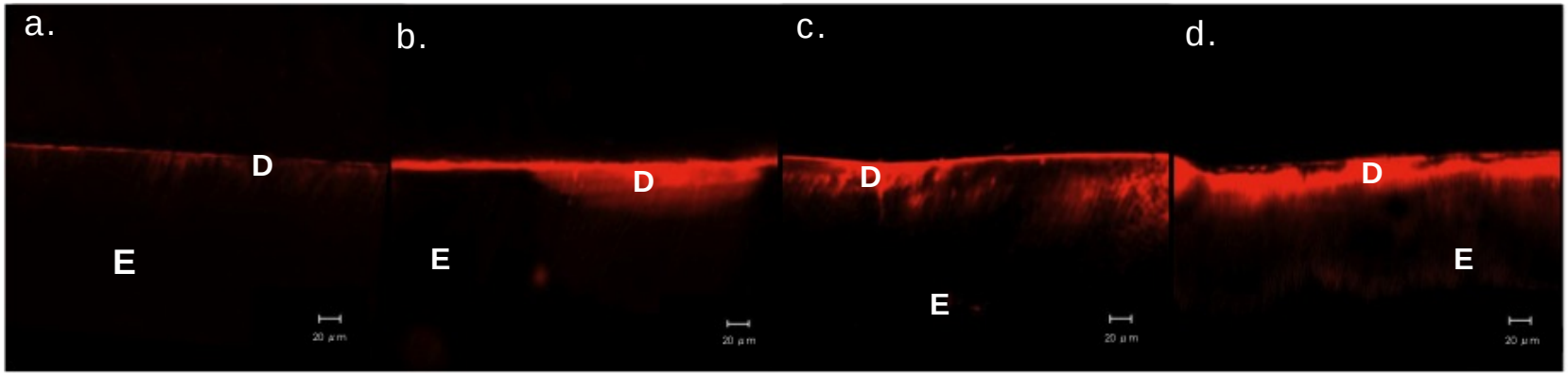

Figure 1. Confocal laser scanning microscopy representative image of sound enamel (D, demineralization; E, sound enamel). (a) Control group; (b) 4% Hydrogen Peroxide + 0.05% Ca; (c) 4% Hydrogen Peroxide; (d) 7.5% Hydrogen Peroxide + ACP.

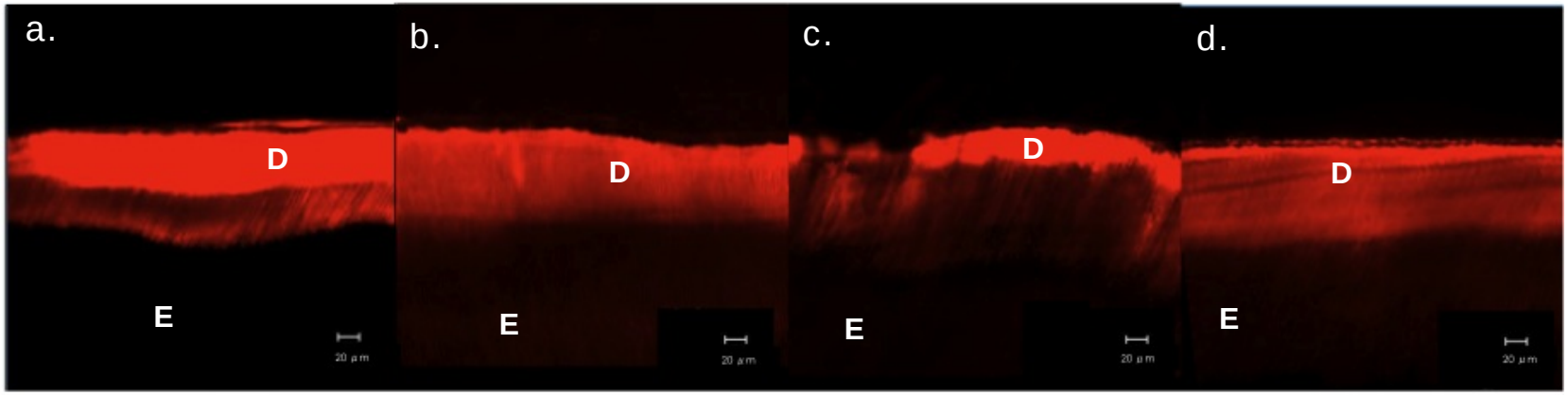

Figure 2. Confocal laser scanning microscopy representative image of artificial caries lesions enamel (D, demineralization; E, sound enamel). (a) control group; (b) 4% Hydrogen Peroxide + 0.05% Ca; (c) 4% Hydrogen Peroxide; (d) 7.5% Hydrogen Peroxide + ACP.

CONSIDERAÇÕES GERAIS

O peróxido de hidrogênio é um composto oxidante instável que apresenta baixo peso molecular e têm a capacidade de produzir radicais livres. Ele se decompõe facilmente quando exposto ao ar em temperatura ambiente (Goldstein *et al*. 1989; Darnell & Moore, 1990; Goldstein & Garber; 1995). O mecanismo de ação dos agentes clareadores é atribuído à oxidação de moléculas que produzem as alterações de coloração ou escurecimento da dentina e do esmalte (Haywood & Heymann, 1989; Albers, 1991; Goldstein & Garber, 1995). Embora pesquisas examinem os efeitos dos agentes clareadores sobre os tecidos duros dentários (Hegedüs *et al*. 1999), o exato mecanismo de ação ainda não está totalmente esclarecido. Entretanto, um agente clareador ideal deveria possuir ação rápida e seletiva, sem causar danos aos tecidos dentários e nem a outros tecidos bucais (Yurdukoru *et al*. 2003).

Devido ao peróxido de hidrogênio possuir baixo peso molecular, os produtos à base deste permitem que ele se difunda através do esmalte e dentina. Quando o carbamida a 10% foi aplicado diretamente na dentina, alguns estudos mostraram que houve redução significativa de microdureza (Lewinstein *et al*. 2004, Basting *et al*. 2005), enquanto outros não relatam esta alteração (Unlu *et al*. 2004). Tam *et al*. (2005), mostraram que a direta aplicação do peróxido de carbamida a 10% na dentina diminuiu a resistência a flexão e módulo de elasticidade deste tecido. Entretanto, quando foi aplicado sobre o esmalte do dente intacto, o peróxido de carbamida 10% não alterou as propriedades mecânicas da dentina subjacente. Os autores relatam que não está claro em qual fase, orgânica ou inorgânica, a dentina é afetada. Porém, Rotstein *et al*. (1996) encontraram redução significativa da razão de Cálcio/Fósforo na dentina após o tratamento com peróxido de carbamida a 10% ou peróxido de hidrogênio a 35%, enquanto que, Kawamoto & Tsujimoto (2004) sugerem que o peróxido de hidrogênio não

tem efeito na hidroxiapatita, mas apenas interfere nos componentes orgânicos da dentina.

Neste estudo foram utilizados géis clareadores que apresentam cálcio e fosfato de cálcio amorfo (ACP) nas formulações. A hipótese testada neste estudo foi que esses componentes agiriam conforme o descrito por Schemehorn & Novak (2007). Esses autores especulam que o cálcio presente no gel clareador promoveria a deposição mineral no esmalte e na dentina, atuaria obliterando os túbulos dentinários, e desta forma reduziria a passagem do peróxido pela estrutura, minimizando os seus efeitos.

No Capítulo 1, foi avaliado 4 agentes clareadores, com diferentes composições e concentrações, no módulo de elasticidade da dentina bovina desmineralizada depois de 24 horas, 7 e 14 dias após o tratamento clareador. Todos os agentes clareadores testados neste estudo reduziram o módulo de elasticidade até 7 dias após o clareamento, quando comparado ao grupo controle não clareado. Entretanto, 14 dias após o clareamento, os grupos tratados não diferiram do controle, exceto quando foi utilizado o peróxido de hidrogênio a 7,5%.

Estudos têm sugerido que géis clareadores são capazes de se difundir no esmalte e dentina (Rotstein, 1991; Hanks *et al*. 1993; Thitnanthapan *et al*. 1999). Este estudo pode confirmar a capacidade do peróxido de se difundir e gerar radicais livres, o qual interage com as estruturas orgânicas, considerando que o esmalte foi mantido intacto durante o tratamento clareador.

Ten Bosch & Coops (1995) relataram que a cor dos dentes é proveniente do conteúdo orgânico do dente, o qual pode ser atacado por componentes dos produtos do clareamento. Os resultados deste estudo mostram que as alterações no módulo de elasticidade da dentina após o clareamento são devidas a efeitos na matriz orgânica, como efeito secundário ou adverso produzido pelos agentes clareadores.

Até o presente momento somente foi relatado na literatura estudos que avaliaram o efeito do tratamento clareador no módulo de elasticidade da dentina mineralizada intacta (Ghanvamnasiri *et al*. 2007, Tam *et al*. 2005a, Tam *et al*. 2005b). Entretanto neste estudo, o conteúdo mineral foi removido e avaliado os efeitos de diferentes agentes clareadores no módulo de elasticidade da matriz orgânica descalcificada. O presente estudo destaca a importância de se avaliar o efeito do tratamento clareador na matriz orgânica do dente, pois o enfraquecimento das estruturas dentárias tem sido considerado como uma das principais causas da redução da resistência de união após claereamento (Cavalli *et al*. 2001; Lai *et al*. 2002).

A recorrência de manchas ou descoloração do dente pode ocorrer ao longo do tempo em função do efeito de reversão da matriz orgânica dentinária promovida pelo agente clareador, bem como o oxigênio residual dos peróxidos pode prejudicar a recuperação de valores de módulo de elasticidade da dentina até 7 dias após o clareamento. Após 14 dias de armazenamento em água, a concentração de oxigênio foi reduzida ou eliminada, o que poderia favorecer a reversão de valores de módulo elástico comprometida da dentina. Este estudo avaliou uma propriedade mecânica de dentina pós-clareamento, no entanto, análises bioquímicas são necessárias para confirmar o efeito reversível observado.

No Capítulo 2, foi avaliado o efeito de agentes clareadores contendo cálcio ou fosfato de cálcio amorfo no esmalte bovino sadio ou com lesão inicial de cárie artificial utilizando microscopia confocal laser de varredura. A hipótese que géis clareadores contendo cálcio ou ACP poderiam reduzir a desmineralização do esmalte sadio e promover uma remineralização nas lesões iniciais de cárie foi rejeitada.

Estudos relatam que a utilização de microscopia confocal laser de varredura pode ser usada para quantificar a demineralização e avaliar a remineralização do esmalte dental (González-Cabezas *et al*. 1998). Géis clareadores contendo cálcio ou fosfato de

cálcio amorfo foram utilizados tanto em esmalte sadio quanto esmalte submetido à ciclagem de pH, para simular lesão inicial de cárie. Nesse último grupo, os grupos tratados apresentaram áreas de fluorescência similares para os grupos não clareados, qualquer que fosse a condição do esmalte.

Efeoglu *et al*. (2005), utilizaram tomografia computadorizada para avaliar os efeitos no esmalte humano após 15 dias (oito horas diárias) de tratamento com peróxido de carbamida a 10%. Os autores relataram que a profundidade de desmineralização do esmalte atingiu 50 μm. Entretanto, Bizhang *et al*. (2006) avaliaram o esmalte bovino após tratamento com peróxido de carbamida a 10% (oito horas por dia, durante duas semanas) ou peróxido de hidrogênio a 5,3% (diariamente por uma hora em duas semanas). As profundidades de desmineralização encontradas foram de 4,8 μm e 1,6 μm, respectivamente. Attin *et al*. (2005) mostraram que a redução na dureza do esmalte foi limitada à camada superficial, confirmando a ausência de desmineralização em profundidade. Neste estudo, a profundidade de desmineralização causada pela ciclagem de pH foi de 135 μm, que correspondeu ao grupo controle. Os tratamentos clareadores não aumentaram significativamente a profundidade de desmineralização dos grupos submetidos à ciclagem de pH, que variaram de 166 (Peróxido de Hidrogênio a 4% + 0,05% Ca ou Peróxido de Hidrogênio a 4%) a 168 μm (Peróxido de Hidrogênio a 7,5% + ACP). Para o esmalte sadio, o tratamento clareador resultou em profundidade de desmineralização, que variou de 31 (peróxido de hidrogênio a 4%) a 52 μm (Peróxido de Hidrogênio a 7,5% + ACP). Desta forma, podemos afirmar que o fator determinante na desmineralização foi a ciclagem de pH, e que a adição de cálcio ou ACP não minimizaram a desmineralização, quando o esmalte sadio foi avaliado.

Este estudo comprova que os géis clareadores são capazes de penetrar no esmalte (Capítulo 2) e dentina (Capítulo 1), e que esta pode ocasionar efeitos deletérios significativos na matriz dentinária (colágeno). A utilização de aditivos nos géis clareadores como cálcio e fosfato de cálcio amorfo não minimizam os efeitos do tratamento clareador

na estrutura dentária, pois os efeitos dos peróxidos talvez sejam mais fortes que estes aditivos. Por outro lado, quando estes foram aplicados sobre lesões iniciais de cárie, não promoveram aumento da desmineralizacão, entretanto, também não promoveram efeito remineralizante.

CONCLUSÃO

1. Os géis clareadores reduzem o módulo de elasticidade da matriz orgânica da dentina bovina, entretanto este pode ser revertido após 14 dias de armazenamento em água. O cálcio e o fosfato de cálcio amorfo não promovem efeitos significativos no módulo de elasticidade da matriz orgânica bovina.

2. A adição de cálcio e fosfato de cálcio amorfo não diminui a desmineralização do esmalte promovida pelo tratamento clareador, tanto para o esmalte sadio, quanto para o esmalte com lesão inicial de cárie artificial.

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