VANESSA CAVALLI

AVALIAÇÕES *'IN VITRO'* DOS EFEITOS PRODUZIDOS POR AGENTES CLAREADORES CONTENDO PERÓXIDO DE CARBAMIDA

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do título de Mestre em Clínica Odontológica, área de Dentística.

Piracicaba

2003

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1. Prof. Dr. MARCELO GIANNINI________________

2. Prof. Dr. CARLOS EDUARDO FRANCCI________________

3. Prof. Dr. MARIO ALEXANDRE COELHO SINHORETI________________

"DE TUDO, FICARAM TRÊS COISAS:
a certeza de que estamos sempre começando...
a certeza de que é preciso continuar...
a certeza de que seremos interrompidos antes de terminar...

PORTANTO DEVEMOS
fazer da interrupção um caminho novo...
da queda um passo de dança...
do medo, uma escada...
do sonho, uma ponte...
da procura... um encontro"

Fernando Sabino

DEDICO ESTE TRABALHO

...aos meus pais, Sônia e Lorival.

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SUMÁRIO

CAPÍTULOS

RESUMO

A valorização e os benefícios proporcionados pelos procedimentos estéticos não invasivos, tornaram o clareamento dental com a utilização do peróxido de carbamida, o tratamento estético comumente realizado em pacientes. O destaque obtido por sua eficiência, simplicidade e segurança, potencializaram a comercialização de diferentes concentrações do agente oxidante, disponíveis tanto para a realização da técnica caseira ou noturna, ou em consultório, sob supervisão direta profissional. Entretanto, dúvidas surgiram a respeito de possíveis efeitos adversos provocados pelo peróxido de carbamida na superfície dental e suas conseqüências. Portanto, essa tese teve como objetivos: 1) analisar a micromorfologia superficial do esmalte após a utilização de peróxido de carbamida em baixas concentrações; 2) avaliar os efeitos de diferentes concentrações de peróxido de carbamida na rugosidade superficial do esmalte humano e examinar as alterações morfológicas da superfície clareada; 3) observar a ação de géis de alta concentração de peróxido de carbamida na rugosidade superficial e susceptibilidade à pigmentação do esmalte humano; 4) investigar os efeitos das concentrações 10%, 15%, 16% e 20% de peróxido de carbamida na resistência à tração do esmalte clareado e suas características morfológicas e 5) quantificar a resistência de união de um compósito resinoso na superfície do esmalte tratado com diferentes concentrações de peróxido de carbamida, 24 horas, 1, 2 e 3 semanas após o clareamento. Baseado nas metodologias experimentais adotadas, concluiu-se que o esmalte humano submetido ao tratamento clareador com peróxido de carbamida apresenta alteração micromorfológica superficial, e a severidade das modificações é proporcional à concentração do gel. Quando a superfície do esmalte foi submetida a maiores concentrações do gel clareador, houve aumento da rugosidade e maior susceptibilidade à pigmentação. A resistência à tração do esmalte clareado foi menor que a do substrato não tratado, e a resistência de união do compósito resinoso foi significantemente diminuída na superfície desse, quando clareado com peróxido de carbamida em concentrações de 10% a 20%. Entretanto, os valores de união foram semelhantes àqueles do esmalte não clareado, quando o procedimento adesivo foi realizado 21 dias após o clareamento.

ABSTRACT

The regards and benefits produced by non-invasive esthetic procedures, brought carbamide peroxide dental bleaching in a commonly esthetic treatment carried out on patients. The appeal based on its efficiency, simplicity and safety, has increased the commercialization of different concentrations of the oxidyzing agent, available either for at-home bleaching techniques, or for in-office bleaching procedures, under diret professional supervision. However, uncertainties concerning possible side effects promoted by carbamide peroxide on enamel surface and their consequences have been raised. Therefore, the aim of this thesis were to: 1) analyze human enamel superficial micromorphology after the application of low concentrations of carbamide peroxide; 2) evaluate the effects of different concentrations of carbamide peroxide on human enamel surface roughness and examine the morphological alterations of the bleached surface; 3) observe the action of high concentrated carbamide peroxide gels on surface roughness and staining susceptibility of human enamel; 4) investigate the effects of 10%, 15%, 16% and 20% carbamide peroxide concentrations on the ultimate tensile strength of bleached enamel and surface morphological characteristics and 5) evaluate the bond strength of composite resin to enamel treated with different carbamide peroxide concentrations, 24 hours, 1, 2 and 3 weeks after bleaching. Based on the adopted experimental methodologies, it was concluded that human enamel submitted to carbamide peroxide treatment presented altered surface morphology, and the severity of the modifications were proportional to gel concentration. Enamel surface presented increased roughness and elevated staining susceptibility when submitted to high-concentrated bleaching solutions. The ultimate tensile strength of bleached enamel was lower than the untreated substrate and the bond strength of composite resin was significantly reduced on the enamel surface when enamel surface was treated with 10% to 20% carbamide peroxide. Nevertheless, bond strengths reached values similar to untreated enamel, when the adhesive procedure was performed 21 days after bleaching.

INTRODUÇÃO

Até o final da década de 80, a técnica de clareamento mais utilizada em dentes vitais com alterações de cor intrínseca ou extrínseca, consistia no condicionamento do esmalte com ácido fosfórico 37% seguido do branqueamento com o peróxido de hidrogênio 30% (H_2O_2) durante 20 a 60 minutos, associado ou não à aplicação de uma fonte de calor por aproximadamente 2 minutos (FEINMAN, GOLDSTEIN & GARBER, 1987, HAYWOOD & HEYMANN, 1989). Entretanto, além da aplicação de calor em dentes vitais gerar grandes preocupações em relação à vitalidade pulpar (ZACH & COHEN, 1965; BOWLES & THOMPSON, 1986; BOWLES & UGWUNERI, 1987; MCEVOY, 1989), a técnica apresentava inconvenientes como a necessidade de várias sessões em consultório, a realização de um cuidadoso isolamento absoluto para os tecidos moles, e o polimento do esmalte após o tratamento, dadas as alterações causadas pelo condicionamento ácido, e devido aos subprodutos do peróxido de hidrogênio serem ácidos (FEINMAN, GOLDSTEIN & GARBER, 1987).

Deste modo, devido aos efeitos adversos e desvantagens fornecidas pela técnica do clareamento externo utilizando-se o peróxido de hidrogênio 35% com condicionamento ácido prévio, e aplicação de uma fonte de calor, diferentes produtos e sistemas de tratamento foram introduzidos. Desde o relato clínico inicial de HAYWOOD & HEYMANN em 1989, o peróxido de carbamida 10% foi o principal agente utilizado para o clareamento de dentes vitais, através da técnica nomeada noturna ou caseira sob supervisão do profissional, na qual o paciente utiliza o produto clareador em uma moldeira individual pelo período de 6 a 8 horas (enquanto dorme), durante estimadamente 2 a 6 semanas (HAYWOOD & HEYMANN, 1989). Atualmente, além dos géis contendo 10% de peróxido de carbamida, concentrações que variam de 15 a 30% estão comercialmente disponíveis (LEONARD, SHARMA & HAYWOOD, 1998; OLTU & GURGAN, 2000, MILLER, 2001), e são também destinadas ao tratamento clareador caseiro ou noturno, constituindo uma variação da técnica.

O clareamento externo noturno com a utilização do peróxido de carbamida 10%, que decompõem-se *in situ* em peróxido de hidrogênio e uréia, tornou-se o procedimento estético mais popular e realizado entre os pacientes (MOKHLIS *et al.*, 2000) devido à sua eficiência clínica (HAYWOOD & HEYMANN, 1989; HOWARD, 1992; GEGAUFF *et al.*, 1993; REINHARDT *et al.*, 1993; CROLL & SASA, 1995; ROSENSTIEL, GEGAUFF & JOHNSTON, 1996; TAM, 1999; JAVAHERI & JANIS, 2000) e segurança (HAYWOOD, 1992; LI, 1996).

Embora ocorra liberação de apenas 3% de peróxido de hidrogênio em 10% de peróxido de carbamida, análises em MEV demonstram que o peróxido de carbamida em baixas concentrações também é capaz de alterar a morfologia do esmalte, provocando dissolução e aumento da porosidade na superfície do substrato (BITTER, 1992; MCGUCKIN, BABIN & MEYER, 1992; BITTER & SANDERS, 1993; SHANNON *et al.*, 1993; JOSEY *et al.*, 1996; ERNST, MARROQUIN & WILLERSHAUSEN-ZONNCHEN, 1996; ZALKIND *et al.*, 1996). Nos capítulos 1 e 2, os trabalhos intitulados *"Vital tooth* *bleaching: microscopic observation of enamel surface following whitening"* e *"Influence of different low concentrations of carbamide peroxide-containing bleaching agents on the human enamel surface roughness and morphology"* foram realizados com o objetivo de constatar-se o efeito do peróxido de carbamida na morfologia do esmalte humano, através de análises em microscopia eletrônica e rugosidade superficial.

Altas concentrações do gel de peróxido de carbamida variando entre 30 a 40% são utilizadas no clareamento de dentes vitais em consultório, efetuado em 3 a 4 sessões, com duração média de uma hora. Apesar dessa técnica ser uma alternativa ao uso de altas concentrações do peróxido de hidrogênio (CLARK & HINTZ, 1998), ou para ser utilizada isolada ou concomitantemente com o tratamento noturno com o objetivo de acelerá-lo (GULTZ *et al.*, 1999; OLTU & GURGAN, 2000), acredita-se que a concentração do peróxido e o tempo de contato do agente na superfície dental, são diretamente proporcionais às alterações provocadas pelo agente no substrato clareado (BITTER & SANDERS, 1993). No capítulo 3, o estudo *"High-concentrated carbamide peroxide bleaching agents effects on enamel surface"* demonstra o efeito de altas concentrações de peróxido de carbamida na superfície do esmalte através de observações em MEV, mensuração da rugosidade superficial e susceptibilidade à pigmentação das superfícies clareadas e não clareadas.

O esmalte humano possui alto conteúdo mineral, correspondendo a 96% de seu peso, formado por fosfato de cálcio cristalino, a apatita, na forma hidroxi, carbonatada ou fluoretada em associação com 4% de água e material orgânico (MECKEL *et al.*, 1965;

STACK, 1954). É um tecido extremamente duro, com alto módulo de elasticidade, que o caracteriza como um material friável (CALDEWELL *et al.*, 1957). Funciona ainda, como uma membrana semi-permeável, permitindo a passagem completa ou parcial de moléculas de baixo peso molecular como o peróxido de hidrogênio que difunde-se através do esmalte e da dentina, liberando radicais livres que reagem com moléculas orgânicas pigmentadas e de alto peso molecular convertendo-as em moléculas menores e mais claras (LYONS & NG, 1998; SUN, 2000).

Alguns estudos *"in vitro"* verificaram que o branqueamento com peróxido de carbamida 10%, é capaz de diminuir a concentração mineral da superfície do esmalte, com conseqüente queda nos seus valores de microdureza (ATTIN *et al.*, 1997; AKAL *et al.*, 2001; RODRIGUES *et al.*, 2001; BASTING, RODRIGUES JR & SERRA, 2001; CIMILLI & PAMEIJER, 2001). Embora, muitos tenham apontado os baixos valores de pH como possível causa da redução do conteúdo mineral (MURCHINSON *et al.*, 1992; LEONARD *et al.*, 1994; BEN-AMAR *et al.*, 1995; ERNST, MARROQUÍN & WILLERSHAUSEN-ZÖNNCHEN, 1996; ZALKIND *et al.*, 1996), atualmente, a maioria dos agentes para uso caseiro ou noturno assumem potencial hidrogeniônico (pH) próximo à neutralidade (PRICE *et al.* 2000). Portanto, a perda de substância mineral na superfície clareada pode estar relacionada à composição do agente e aos seus sub-produtos, pois os radicais livres liberados pelo peróxido de hidrogênio são negativamente carregados, instáveis e inespecíficos e podem reagir tanto com as moléculas orgânicas pigmentadas quanto com a matriz inorgânica do esmalte (SEGHI & DENRY, 1992; ZALKIND *et al.*, 1996; PERDIGÃO *et al.*, 1998).

FEATHERSTONE *et al.*, em 1983, verificaram que mudanças no conteúdo orgânico e inorgânico do esmalte podem alterar as propriedades mecânicas desse substrato. Uma vez que a resistência à propagação de fratura está relacionada com a integridade estrutural da matriz orgânica e mineral, é possível que processos químicos que degradam os componentes estruturais do esmalte, provoquem a diminuição da resistência à fratura do substrato. SEGUI & DENRY (1992) verificaram que o esmalte tratado com peróxido de carbamida a 10%, por 12 horas possui menor resistência à fratura que o esmalte não clareado. Nesse sentido, o estudo apresentado no capítulo 4 *"Effects of different carbamide peroxide concentrations on intrinsic tensile strength of human enamel"* foi conduzido para avaliar se baixas concentrações de peróxido de carbamida, seriam capazes de diminuir a resistência máxima à tração do esmalte, através do ensaio de microtração.

As alterações na micromorfologia do esmalte clareado com peróxido de carbamida, bem como a possibilidade do aprisionamento dos sub-produtos liberados durante seu mecanismo oxidativo podem explicar a redução na resistência de união de compósitos aderidos à superfície do substrato recém clareado (STOKES *et al.*, 1992; GARCIA-GODOY *et al.*, 1993; BEN-AMAR *et al.*, 1995). Essa hipótese também é demonstrada no capítulo 5 com o trabalho *"Bond strength to enamel relative to the elapsed time from bleaching with various concentrations of carbamide peroxide"*. Quando ocorre o tratamento do esmalte com peróxido de carbamida, alguns autores aconselham que o procedimento restaurador adesivo seja adiado por algumas semanas, uma vez que a redução dos valores de união do substrato clareado parece ser transitória e reversível (MCGUCKIN,

THURMOND & OSOVITZ, 1992; TITLEY, TORNECK & RUSE, 1992; MILES *et al.*, 1994, PERDIGÃO *et al.*, 1998). Outros, todavia, sugerem a remoção da camada mais superficialmente clareada, ou a lavagem do substrato tratado com peróxido de carbamida com álcool (BARGHI & GODWIN, 1994), ou ainda, o uso de adesivos contendo solventes orgânicos (SUNG et *al.*, 1999) e até agentes anti-oxidantes (LAI *et al.*, 2002).

Os trabalhos apresentados a seguir, foram delineados com o objetivo de simularmos *"in vitro"*, com o máximo de fidedignidade possível, as condições insubstituíveis do meio oral durante o tratamento clareador. Os estudos focam exclusivamente os efeitos adversos do peróxido de carbamida no esmalte humano e, embora cada capítulo apresente diferentes desvantagens mediante os resultados obtidos, o sistema tampão salivar e sua capacidade de remineralização, além da presença de fluoretos na cavidade oral, podem reverter a ação dos peróxidos na superfície dental. Portanto, o clareamento dental não será contra-indicado. Apenas atentamos para sua segurança quando administrado sobre os tecidos duros, em relação à quantidade, concentração e freqüência de aplicação do agente clareador.

PROPOSIÇÃO

Visto que os aspectos relacionados aos efeitos adversos do peróxido de carbamida permanecem parcialmente elucidados, as propostas desses estudos, divididos em cinco capítulos, foram:

1. Observar em microscopia eletrônica de varredura (MEV), as alterações superficiais produzidas no esmalte após a aplicação de diferentes concentrações de peróxido de carbamida e compará-las àquelas produzidas por refrigerante (Coca-Cola®), pelo condicionamento com ácido fosfórico 37%, e pelo flúor fosfato acidulado.

2. Avaliar os efeitos de diferentes concentrações de peróxido de carbamida, utilizados no clareamento caseiro, na rugosidade superficial do esmalte humano e analisar as alterações morfológicas da superfície tratada através da microscopia eletrônica de varredura (MEV).

3. Verificar em MEV a influência dos géis de alta concentração de peróxido de carbamida e mensurar a rugosidade superficial e susceptibilidade à pigmentação do esmalte humano.

4. Investigar os efeitos das concentrações 10%, 15%, 16%, 20% de peróxido de carbamida na resistência máxima à tração do esmalte clareado.

5. Avaliar a resistência de união de um compósito resinoso na superfície do esmalte tratado com diferentes concentrações de peróxido de carbamida, 24 horas, 1, 2 e 3 semanas após o clareamento.

CAPÍTULO 1

VITAL TOOTH BLEACHING: MICROSCOPIC OBSERVATION OF ENAMEL SURFACE FOLLOWING WHITENING

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TITLE: Vital tooth bleaching: microscopic observation of enamel surface following whitening.

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Abstract

Vital tooth bleaching has been widely used for a long time as a way to improve patients' esthetics and self-esteem. Many papers have already been written on safety, side effects, effectiveness and long-term status of bleaching products. However, there is no consensus on all current literature. This article reviews the use of hydrogen peroxide and carbamide peroxide in vital tooth bleaching, and their effects on bleached enamel surface are presented and compared to other available oral products. Reduction in bond strength, mild sensitivity, gingival irritation and changes in tooth hard tissues seem to be transient, and satisfactory clinical results have been achieved. As a consequence, vital tooth bleaching has gained favor with patients and dentists alike.

Keywords: bleaching, carbamide peroxide, hydrogen peroxide

Introduction

Bleaching methods have been developed and peroxide compounds at different concentrations are used for tooth whitening procedures [1, 9, 20]. Peroxide-containing vital tooth whiteners are classified into three categories, according to application mode and peroxide concentration. Oxidizing agents for professional use only contain high concentrations of carbamide peroxide (35-37%) and hydrogen peroxide (30-35%) solutions, while patient-applied tooth whiteners for at-home bleaching, containing up to 22% carbamide peroxide and 10% hydrogen peroxide, are dispensed and monitored by the dentist. Over-the-counter products are available directly to consumers for home use, however, its safety is questionable [25,30].

Nightguard vital bleaching (NGVB) was first reported in 1989 by Haywood and Heymann [20] as a simple, safe, comparatively inexpensive, dentist-prescribed, home-applied bleaching technique. It offers an effective means of bleaching mildly discolored teeth using a soft nightguard worn by the .patient at night . Generally, NGVB takes 2-6 weeks with the application of 10 to 22% carbamide peroxide gels to obtain maximum lightening. Final results are directly influenced by the exposure time, the degree of discoloration and the concentration of the applied gel.

For patients who are unwilling to take some weeks for desired results, there are shorter treatment protocols, but they must be carried out by using in-office techniques [9]. A 7 to 16 times higher strength was found when using the in-office technique as compared with at-home bleaching products [19,24]. Either a heating element, a light source, or a laser beam can be used to enhance the action of peroxide, but this procedures may also create adverse effects [13,16].

All hydrogen peroxide or carbamide peroxide-based bleaching agents ionize and decompose in order to initiate the redox chemical reaction bleaching process. Hydrogen peroxide (H_2O_2) as an strong oxidizing agent, is the active ingredient responsible for whitening teeth [49]. Carbamide peroxide is water soluble, unstable and contains approximately 35 % H_2O_2. When exposed to tissue, saliva or moisture at body temperature, a 10% carbamide peroxide solution dissociates into 3 % H_2O_2 and 7% urea [24].

$$CO\,(NH_2)_2{\bullet}H_2O_2 \rightarrow H_2O_2 + CO\,(NH_2)_2$$

Urea continues to decompose into CO_2 and ammonia. As a strong base, ammonia offers an elevated pH environment, which is more favorable for bleaching and simultaneously controls the acidity associated with plaque retention [21].

The whitening process is facilitated by the oxidation reaction of peroxide, which transforms the organic colored substances of tooth enamel into lighter chemical intermediates [15]. Free radical ions are unstable and immediately seek an available target to react with. The larger, long-chained, darker colored molecules react easily with the free radicals, altering the optical structure of the molecule and creating a different optical structure. The stain on the tooth surface becomes incolor, as the larger, darker colored molecule becomes virtually dissociated into a smaller, shorter chained, and lighter colored molecule [43].

Daily exposure to carbamide peroxide, based on clinical experiences and researches, is an apparently safe and effective procedure for whitening teeth [22-25, 28]. However, several reports have concerned about possible adverse effects of carbamide peroxide gel, due to the large use of dentist-prescribed bleaching agents.

These effects include changes in ultra-morphological resin-enamel interface [36]; alterations in enamel surface morphology [5, 6, 27, 33, 40], pulpar [10] and gingival irritation [31]; changes in salivary pH [29]; adherence of Streptococcus mutans to bleached enamel [17]; alterations on composite resin [4, 35], and changes on hardness [2] fracture toughness, and abrasion characteristics of human enamel [39].

The aim of this study was to observe surface alterations produced on enamel after the application of different concentrations of carbamide peroxide solutions and compare these alterations with those promoted by a cola beverage, an acid etchant and an acidulated phosphate fluoride gel.

Materials and Methods

Extracted sound erupted third molars stored in a 2% buffered formalin solution were used within one month of extraction.The roots were separated from the crowns, which were sectioned mesiodistally using a water-cooled slow speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, U.S.A). Twenty-eight dental fragments from buccal and lingual enamel surfaces (3.5 x 3.5 x 2.5 mm) were obtained and individually embedded in self-curing polyestyrene resin in a ring mold. Enamel surfaces were flattened with wet 600-, 1000- and 1200-grit aluminum oxide abrasive papers and polished with 6, 3, ½, and ¼ μm–grit diamond pastes on a polishing machine (APL-4, Arotec, Cotia, SP, Brazil).

Specimens were randomly divided into seven groups (n = 4): Group 1- Control, no treatment; Group 2- 10% carbamide peroxide (Opalescence – Ultradent Products); Group 3- 15% carbamide peroxide (Opalescence); Group 4 - 35% carbamide peroxide (Opalescence Quick); Group 5- acid etching with 37% phosphoric acid for 30 seconds; Group 6– samples were immersed into a cola soft drink (Coca-Cola) for 2.5 minutes (approximated amount of time for a subject to drink a 16 oz. soft drink [31]); Group 7 – samples were exposed to acidulated phosphate fluoride (APF) for 1 minute.

Control group was kept in artificial saliva at 37 °C and was not treated. The specimens of low-concentrated carbamide peroxide gels (G2 and G3) were exposed to one daily application of 0.1 mL of carbamide peroxide for 6 hours for 10 consecutive days. After each daily treatment and post bleaching, specimens were stored in artificial saliva solution. For the high-concentrated bleaching group (G4), a 0.1 mL of the carbamide peroxide gel was dispensed on enamel for 30 min. Afterwards, specimens were rinsed with distilled water and stored in artificial saliva at 37°C, until next application. This bleaching procedure was repeated four times with a 72-hour bleaching interval.

Each specimen was sputter-coated with gold (MED 010, Balzers Union, Balzers, Liechtenstein) and observed under a SEM (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, England). Representative areas of the specimens were photographed at 5000X magnification.

In addition, a clinical whitening case was performed. Figures 1 to 4 report a bleaching treatment.

Results

Figures 6 to 11 show alterations on enamel surface produced by a 10% (Opalescence – Ultradent), a 15% (Opalescence) and a 35% carbamide peroxide gels (Opalescence Quick); by acid etching with 37% phosphoric acid for 30 seconds; by exposing the sample to a cola soft drink for 2.5 minutes, and to APF, respectively. These alterations can be compared with Figure 5, which shows an untreated enamel surface (control).

Alterations on enamel surface were evident after exposure to all bleaching products tested (Fig. 6, 7, 8). The surfaces exhibited a muted texture to rounded enamel rods with depressed rod boundaries. When a high concentration of carbamide peroxide is used, a more extensively altered surface can be seen (Fig. 8). Thus, higher peroxide concentrations are likely to produce more changes on enamel surface than low peroxide concentrations.

Surface textures produced by the bleaching agents are much slighter than conventionally etched enamel, which exhibit a high degree of demineralization, exposing core prisms (Fig. 9). The bleached enamel resembled a type 2 acid-etched surface patterns, exhibiting a special loss from the periphery of enamel prisms [41]. As shown in figures 6 and 7, surface alterations produced by low concentrated agents (10% and 15% carbamide peroxide) are not very different from those produced by cola beverage (Fig. 10) or APF (Fig. 11).

Discussion

Morphology and inner structure alterations on enamel surface have been reported by several authors [26, 32, 33, 37, 39, 40]. These enamel alterations can be attributed both to the low molecular weight and consequent penetration on enamel subsurface, and to the low pH of some tooth whitening products. Tooth whitening agents should have a relatively neutral pH to minimize potential damage. However, in a recent study, Price *et al* reported the average tooth whitening products pH as ranging from 3.67 (highly acidic) to 11.13 (highly basic). The most acidic pH means were found for the in-office bleaching products, Opalescence Xtra 35% hydrogen peroxide in-office bleach presenting the lowest pH (3.67) [38]. McCracken and Haywood found no statistical differences on the amount of calcium loss, when the demineralization effects of 10% carbamide peroxide and a popular cola beverage were compared. Although demineralization was statistically different from control group, the authors reported that this loss (about 1 $\mu g/mm^2$) might not be clinically significant [32]. Also, lowered concentrations of calcium and phosphorus, as well as local microstructural and chemical changes in enamel were considered clinically insignificant by Potocnik and others [37].

Cavalli et al [7], reported an increase in enamel surface roughness following bleaching with high-concentrated carbamide peroxide solutions. In this experiment, surface morphologic differences were noted between the unbleached control specimens and those bleached with 35 and 37% carbamide peroxide. High-concentrated carbamide peroxide gels in contact with enamel surface promoted morphologic changes, increasing the surface roughness and etching-like appearance. The bleaching process had little effect in some areas, whereas in other areas, decalcification and increased porosity of the enamel surface was noted.

In vitro studies have indicated that peroxide bleaching agents significantly affect the immediate bond strength of composite to bleached enamel and dentin [3, 5, 11, 14, 34, 42, 44, 46]. Some authors related that the loss in resin adhesiveness to enamel was related to possible presence of residual peroxide, which interfered with resin attachment and inhibited resin polimerization [12, 47, 48], or to changes on the penetration of resin tags induced by hydrogen peroxide treatment [47]. However, Perdigão and others [36] attributed these variations to changes in proteins and in mineral content of the most superficial layers of enamel, rather than to the presence of residual oxygen. To overcome this problem, the authors recommend delays in bonding after bleaching or the use of drying agents or adhesives with high-vapor solvents or elimination of superficial layer of bleached surface.

Recently, it has been suggested [8] that composite-enamel bonds were significantly lower until 2 weeks of time elapsed from cessation of bleaching than those for unbleached enamel. Although Opalescence and Whiteness bleaching agents weakened the resin bond strength, buffering and remineralization potential of artificial saliva probably minimized the bleaching effects without interfering with the resin bonding if it was carried out 3 weeks post bleaching. Thus, the exposure of carbamide peroxide-treated enamel to artificial saliva at 37 °C for 3 weeks was able to eliminate this reduction in bond strength.

The teeth exposed to daily treatment with low-concentrated bleaching gels show surface morphologic alterations similar to that of teeth exposed to cola for 2.5 min (pH 1.5) and APF for 1 min (pH 3.2). Due to its low pH, acidulated phosphate fluoride and cola beverage are able to decalcify enamel. APF raises the ionized calcium concetratation to a point where it exceeds the solubility product constant for calcium fluoride, and precipitates on the surface [18]. As hydroxiapatite looses its mineral content, fluoride reacts with the crystal, originating apatite fluoride.

Figures 1 to 4 depicts a whitening case performed on a 19-year-old-male patient with naturally yellowing teeth. Figure 3 and 4 show that a considerable amount of tooth whitening was achieved after a seven and two-week bleaching treatment. No surface alterations or structural damage were clinically observed on whitened teeth surfaces.

Although enamel surface was microscopically altered after the bleaching regimen, it is consistent with normal daily events and accepted risks, such as drinking a cola beverage [32]. These events are common in the patient population, and it provides a gauge against which the patient can compare the potential demineralizing effects from a daily bleaching regimen. In addition, saliva buffering properties have the potential for remineralization *in vivo*.

Conclusion

Careful diagnosis, case selection, treatment planning, and attention to technique are mandatory for a successful bleaching treatment. According to reviewed literature, bleaching is the simplest, least invasive means available to lighten discolored teeth and diminish or eliminate many stains in both vital and non-vital teeth. Adverse effects have been proved to be transient, and may be reversible and short-lived. In combination with other esthetic procedures such as micro-abrasion, lightening a stained tooth and the adjacent teeth before veneering may also improve the final appearance. The success and acceptance of the NGVB technique has been phenomenal, and, although low concentrated solutions effects on enamel surface have been proved to be much lower (and not different from common substances applied on enamel) than high concentrated peroxide-based bleaching solutions, the in-office bleaching technique continues to be an important option for patients whose lifestyles do not lend themselves to extended treatment times or in association with the NGVB in more severe cases. Even though bleaching with carbamide peroxide and hydrogen peroxide have been proved to be safe and effective, decreased adverse effects, bleaching time and cost, warrant further investigation.

Acknowledgments

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Captions

Figure 1. Preoperative mildly age-stained teeth.

Figure 2. Closer view of the preoperative midly age-stained teeth.

Figure 3. Postoperative after a 7-day bleaching regimen.

Figure 4. Postoperative after a 14-day bleaching regimen.

Figure 5. SEM photograph of an unbleached enamel surface – control group. (Original magnification X5000).

Figure 6. SEM photograph of a 10% carbamide peroxide enamel-bleached surface. (Original magnification X5000).

Figure 7. SEM photograph of a 15% carbamide peroxide enamel-bleached surface. (Original magnification X5000).

Figure 8. SEM photograph of a 35% carbamide peroxide enamel-bleached surface. (Original magnification X5000).

Figure 9. SEM photograph of a 37% acid-etched enamel surface. (Original magnification X5000).

Figure 10. SEM photograph of a cola beverage-produced surface. (Original magnification X5000).

Figure 11. SEM photograph of an acidulate phosphated fluoride-produced surface. (Original magnification X5000).

Fig. 1

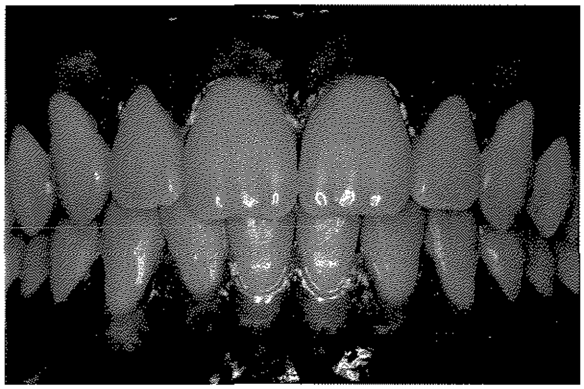


Fig. 2

Fig. 3

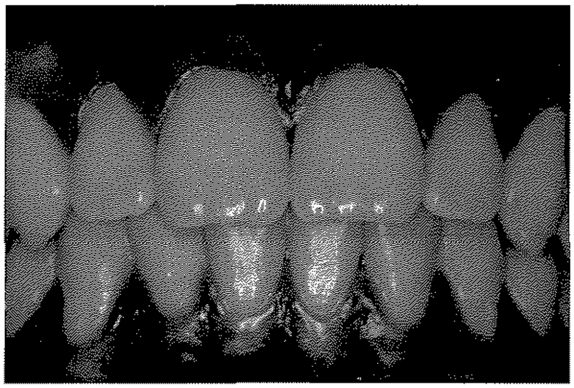


Fig. 4

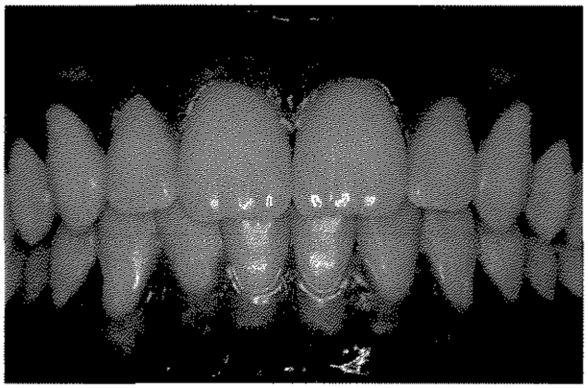


Fig. 5

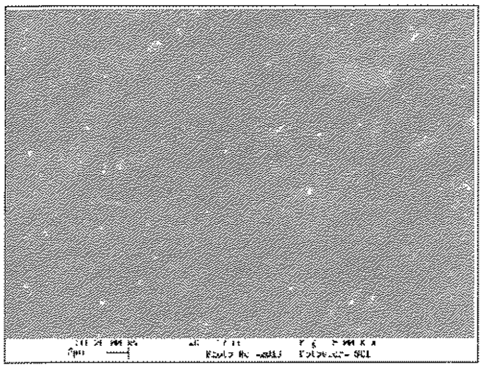


Fig. 6

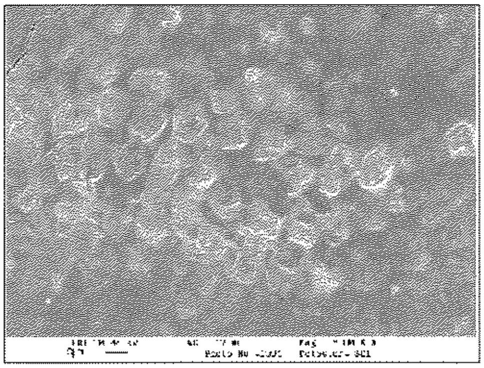


Fig. 7

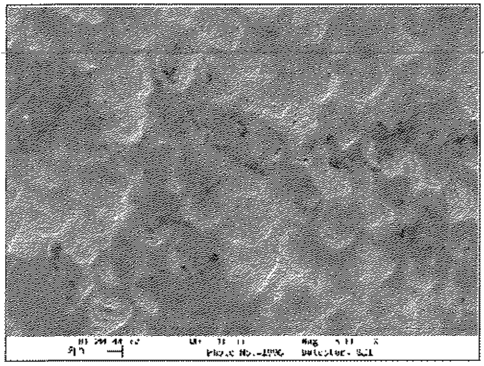


Fig. 8

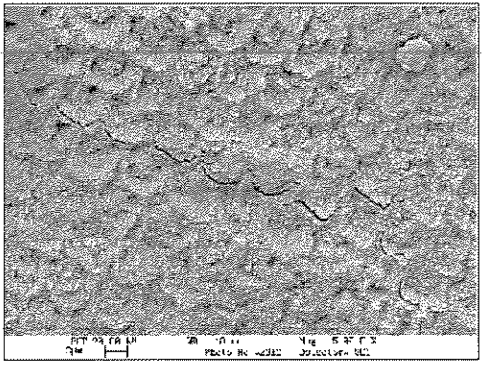


Fig. 9

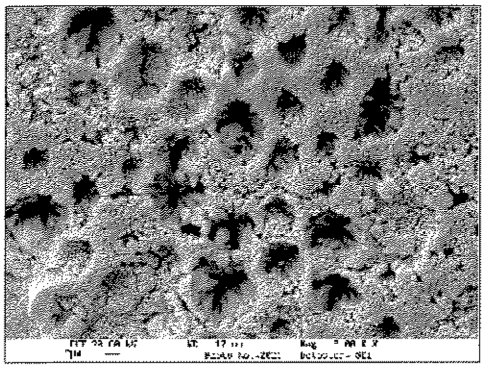


Fig. 10

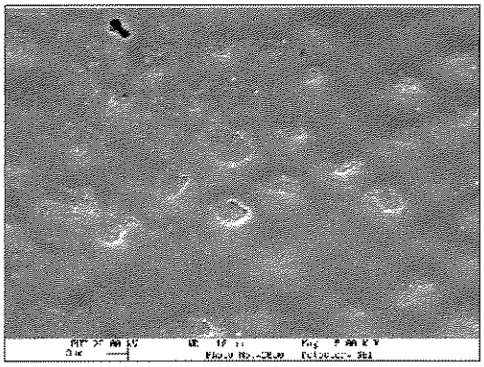
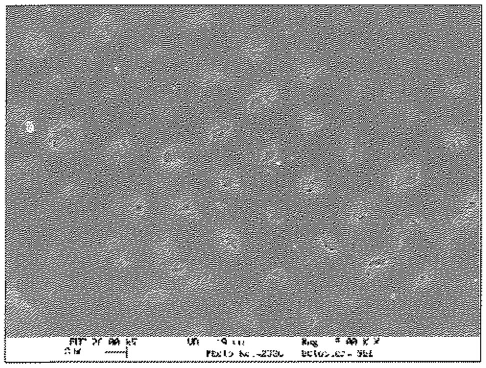


Fig. 11



CAPÍTULO 2

INFLUENCE OF DIFFERENT LOW CONCENTRATIONS OF CARBAMIDE PEROXIDE-CONTAINING BLEACHING AGENTS ON THE HUMAN ENAMEL SURFACE ROUGHNESS AND MORPHOLOGY

Enviado para publicação na revista:
ACTA MICROSCÓPICA
(Anexo 2)

INFLUENCE OF DIFFERENT LOW CONCENTRATIONS OF CARBAMIDE PEROXIDE-CONTAINING BLEACHING AGENTS ON THE HUMAN ENAMEL SURFACE ROUGHNESS AND MORPHOLOGY

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Abstract

This study examined the average surface roughness of enamel before (baseline) and after bleaching; and investigated the influence of different concentrations of carbamide peroxide (CP) on the surface morphology. Sixty flat enamel surfaces obtained from human third molars were randomly assigned into 6 groups (n = 10) and submitted to 5 carbamide peroxide treatments for 14 days: 1- no bleaching treatment (NB-control), 2- 10% CP - Opalescence (O10), 3- 15% CP - Opalescence (O15), 4- 20% CP - Opalescence (O20), 5- 10% CP - Whiteness (W10) and 6- 16% CP - Whiteness (W16). The surface topography was measured by a profilometer and the treated enamel surfaces were examined with a scanning electron microscope (SEM) at × 5,000 and × 20,000 magnifications. Baseline roughness average was statistically similar for all groups ($P > 0.05$). All bleaching agents produced significantly increased surface roughness ($P < 0.05$). Surface morphologic alterations were observed after bleaching treatments. SEM photographs showed that different concentrations of carbamide peroxide caused surface dissolution, exposing a porous surface.

Key Words: dental enamel, tooth bleaching, carbamide peroxide, surface morphology.

Introduction

Carbamide peroxide is the active component in most tooth-whitening agents for patient-applied at-home bleaching (18). This treatment protocol use carbamide peroxide gel contained in a custom fabricated bleaching mounthguard, which is placed over the teeth to facilitate the contact with bleaching agent. Tooth-whitening products are dispensed and monitored by dentists, and daily exposure to the 10% carbamide peroxide for up to 5 weeks (8), based on clinical evaluations and researches, is safe method, conservative and effective procedure for at-home whitening teeth (11,12,15).

Nevertheless, submitting the teeth and oral tissues to a low concentration of carbamide peroxide for an extended period of time may cause side effects (18). Scanning electron microscope evaluations have been conducted on enamel surfaces treated with carbamide peroxide and contradictory evidence of adverse effects has been related. No detectable changes in surface texture and topography were observed between unbleached control group and the bleached groups with 10% carbamide peroxide (7,9,23,28). However, depending on the tooth-whitening product, 10% carbamide peroxide can promote varying degrees of surface porosity and structural change (3,4,13,14,21,25)

In an attempt to improve the whitening effects, bleaching agents with up to 22% carbamide peroxide are also available for at-home bleaching (17,19,22,24). However, the possibility of increased adverse effects with higher concentrations has not been thoroughly studied and still needs to be determined.

The purpose of this study was to evaluate the influence of different concentrations of carbamide peroxide on the surface roughness of human dental enamel. In addition, enamel surface morphology of unbleached control specimens and bleached specimens were investigated using scanning electron microscope (SEM). This study tested the hypothesis that increased carbamide peroxide concentrations may producex proportional adverse effects.

Materials and Methods

Thirty extracted sound erupted third molars stored in 2% buffered formalin solution were used within one month of extraction. The roots were separated from the crowns, which were sectioned mesiodistally using a water-cooled slow speed diamond saw (Isomet - Buehler Ltd.). Sixty dental fragments from buccal and lingual enamel surfaces (3.5 x 3.5 x 2.5 mm) were obtained and individually embedded in self-curing polyestyrene resin in a ring mold. Enamel surfaces were flattened with wet 600-, 1000- and 1200-grit aluminum oxide abrasive papers and polished with 6, 3, ½, and ¼ µm–grit diamond pastes on a polishing machine (APL-4 – Arotec).

Before bleaching treatments, a profilometer (Surf test 211 – Mitutoyo) was used to measure the initial surface roughness (baseline). Three measurements in different directions were recorded and the mean Ra value (μm) was determined for each specimen. Two commercially available bleaching agents, containing different low concentrations of carbamide peroxide (CP) Opalescence (Ultradent Products Inc.) and Whiteness (FGM Produtos Odontológicos) were used in this study.

Specimens were randomly divided into six groups (n = 10): Group 1- Control, no bleaching treatment; Group 2- 10% CP - Opalescence (O10); Group 3- 15% CP Opalescence (O15); Group 4- 20% CP - Opalescence (O20); Group 5- 10% CP – Whiteness (W10) and Group 6- 16% CP – Whiteness (W10). Control group was kept in artificial saliva at 37 °C for 14 days and was not bleached. For bleaching groups (2, 3, 4, 5 and 6), enamel was exposed to one daily application of carbamide peroxide for 8 hours during 14 consecutive days.

In each specimen, approximately 0.1 mL of bleaching agent and 0.05 mL of artificial saliva was applied on the enamel surface and covered with an individual tray. During bleaching, the specimens were placed in 100% relative humidity at 37° C, and after daily bleaching, the specimens were thoroughly rinsed with an air/water spray for 10 seconds and stored in artificial saliva (5) at 37° C. After 14 days, surface roughness was recorded for each specimen again. Profilometric analysis data were statistically analyzed by two-way analysis of variance (ANOVA) and Tukey test at 0.05 level of significance.

Specimens were removed from the acrylic blocks and prepared for the scanning electron microscope (DSM 940A – Zeiss). Then, specimens were sputter coated with gold in a vacuum evaporator (MED 010 – Balzers) and SEM photomicrographs of a representative area of the surfaces were taken at 5,000X and 20,000X.

Results

The average surface roughness of experimental groups are displayed in Table 1. Two-way ANOVA showed significant influence of both factors (treatment and time) on surface roughness. Baseline data were performed in order to verify the similar initial surface smoothness ($P > 0.05$) and to contrast differences between untreated and treated enamel in the same group. Specimens from control group stored in artificial saliva for 14 days present no increase on surface roughness mean values ($P > 0.05$). However, all bleaching agents produced significant increased on the surface roughness ($P < 0.05$). After 14 days of tooth-bleaching, O15, W10 and W16 bleaching agents promoted higher surface roughness ($P < 0.05$) than unbleached control group, which was similar to O10 and O20 ($P > 0.05$).

A representative photomicrograph of polished enamel surface and 14 days stored in artificial saliva control group is shown in Fig. 1. No surface morphologic alterations are noted on unbleached enamel surfaces. The surface morphology of enamel following exposure to 10%, 15%, 16% and 20% carbamide peroxide revealed alterations in surface topography when compared with unbleached control group. Morphological alterations were more evident for O15, O20, W10 and W16 than O10, which presented porosities, irregularities and pits at some sites of the bleached enamel surface and less altered surface area among bleaching agents (Fig. 2). Irregular pattern of enamel etching or erosion is noted in others bleaching agents photomicrographs (Figs 3, 4, 5 and 6).

Discussion

A previous tooth-whitening treatment protocol employed caustic chemical agents (2,6,20), which were capable of causing enamel surface alterations (13,16,28). When hydrogen peroxide-containing bleaching agent was associated with preoperative etching and heat application, extensive structural changes in enamel were observed (21,26). Mouthguard bleaching using 10% carbamide peroxide was developed to substitute the use of high concentrations of hydrogen peroxide in order to minimize their adverse effects (8).

In the present experiment, specimens treated with bleaching agents revealed significant increased on surface roughness, demonstrating that alterations on enamel surface may occur. Storage in artificial saliva did not affect the surface roughness of unbleached control group specimens. SEM observation showed that superficial morphological alterations or pattern of enamel etching were not evident after 14 days of artificial saliva storage for unbleached specimens (Fig. 1).

Differences were noted between unbleached and bleached enamel surfaces under SEM observations. Different concentrations of carbamide peroxide in contact with enamel surface provide morphological changes, causing increased surface roughness and erosion or etching-like appearance. Alterations were not uniform throughout the surfaces, thus, some areas showed little effect of the bleaching process, whereas other areas showed decalcification and increased porosity of the enamel surface.

A lower extensively altered surface was observed for O10 (Fig. 2). Pits and irregularities were noted for O10, which produced surface roughness that was similar to control group after 14 days of bleaching (O10) or artificial saliva storage treatment (control), respectively. O20 was also similar to O10 and control group, however, a more altered surface was noted (Fig. 4). O15, W10 and W16 bleaching agents also promoted morphological alterations, leading to increased surface roughness after bleaching (Figs 3, 5 and 6, respectively).

The oxidative process on enamel surface and pH of tooth-whitening products have been considered as the main adverse effect on mineralized tissues after bleaching treatment. Inquiries have been raised if oxidative process will create porosity in enamel (18). Studies have reported that enamel treated with a low-pH carbamide peroxide agent show slight surface erosion under SEM (4,25) and that the greater peroxide concentration, the more acidic the pH of the bleaching product (27). The dentist-supervised home-bleaching products have a mean pH of 6.48 (24). Regardless of the close to neutral pH level of the bleaching agents, changes on bleached surfaces were presented after bleaching treatments.

Carbamide peroxide (10%) degrades into 3% hydrogen peroxide and 7% urea, which is capable of penetrating into the enamel and affecting the interprismatic regions (1), as observed in bleached specimens of this study (Figs 3, 4, 5 and 6). Thus, urea may contribute to the structural changes and roughness of enamel. However, beneficial side effects can be promoted by alkaline property of urea that decreases the hydrogen ion concentration of the bleaching solution and reduces the whitening adverse effects (10,11).

Conclusions

This investigation showed that *in vitro* use of different low concentrations of carbamide peroxide resulted in significant increased surface roughness and SEM evaluations revealed that tooth-whitening products tested have a potencial demineralizing effect on the enamel surface.

Acknowledgments

The bleaching agents used in this study were generously supplied by Oraltech (São Paulo/SP - Brazil) and FGM Produtos Odontológicos (Joinville/SC -Brazil). The authors are indebted to Dr. E.W. Kitajima (NAP-MEPA/ESALQ-USP) for technical electron microscopy support.

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Table 1. Mean values (±SD) of surface rougheness (μm) of unbleached and bleached enamel surfaces (n=10):

Groups	Control	O10	O15	O20	W10	W16
Baseline	0.12±0.02 Aa	0.11±0.02 Aa	0.11±0.02 Aa	0.12±0.02 Aa	0.12±0.03 Aa	0.12±0.02 Aa
14 days	0.12±0.03 Aa	0.20±0.08 B ab	0.27±0.04 Bb	0.21±0.06 B ab	0.24±0.04 Bb	0.21±0.06 Bb

Means followed by different letters (lower case - horizontal and capital letter - vertical) differ among them by Tukey test ($p<0.05$).

Legends:

Figure 1a and 1b. Enamel surface morphology from unbleached control group. No alterations or pattern of enamel etching are noted.

Figure 2a and 2b. Enamel surface exposed to O10. Pits (arrow), porosities and irregularities are noted.

Figure 3a and 3b. Enamel surface exposed to O15. Irregular patterns of enamel etching and affected interprismatic structures (arrows) can be seen (C - enamel prism core).

Figure 4a and 4b. Enamel surface exposed to O20. Irregular patterns of enamel etching and affected interprismatic structures (arrows) can be seen (C - enamel prism core).

Figure 5a and 5b. Enamel surface exposed to W10. Erosion or mild demineralized areas showing irregular patterns of enamel etching (arrows).

Figure 6a and 6b. Enamel surface exposed to W16. Erosion or mild demineralized areas showing irregular patterns of enamel etching (arrows).

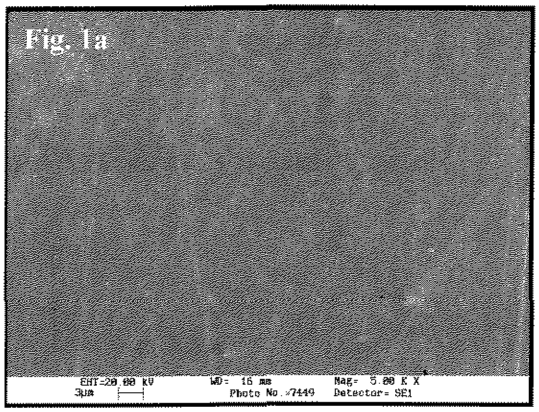

Fig. 1a

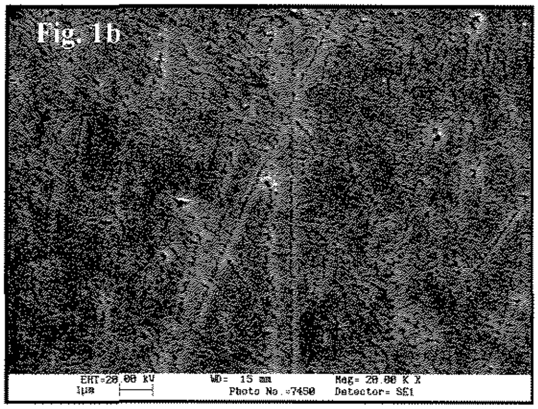

Fig. 1b

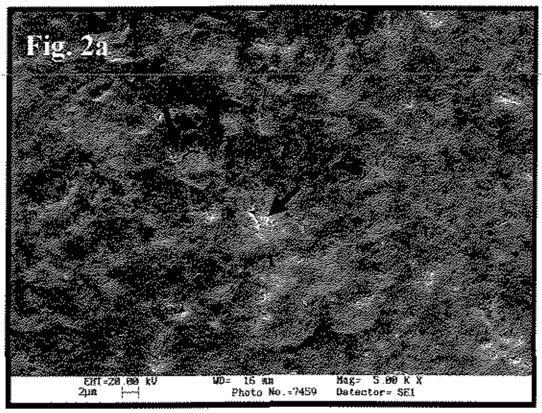

Fig. 2a

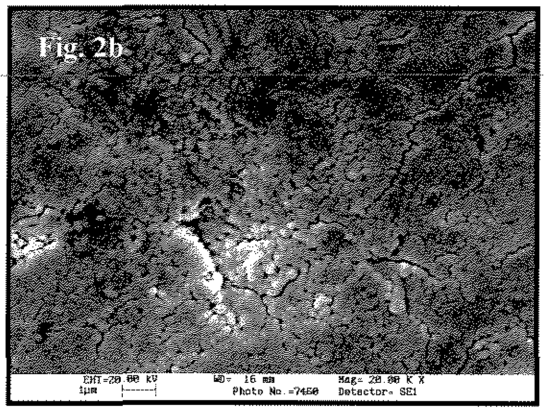

Fig. 2b

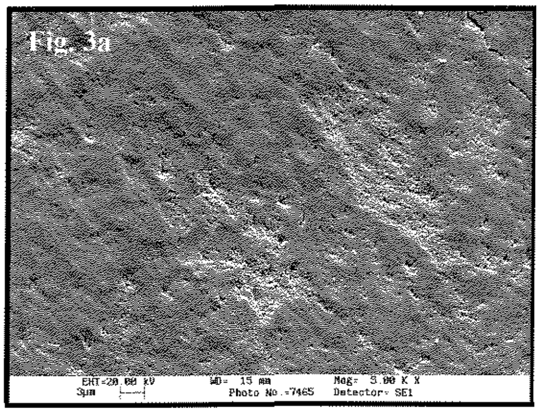

Fig. 3a

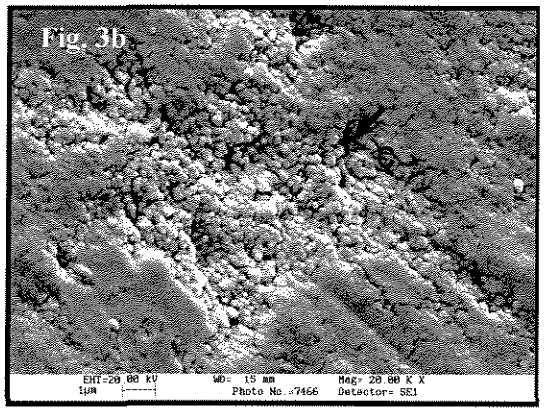

Fig. 3b

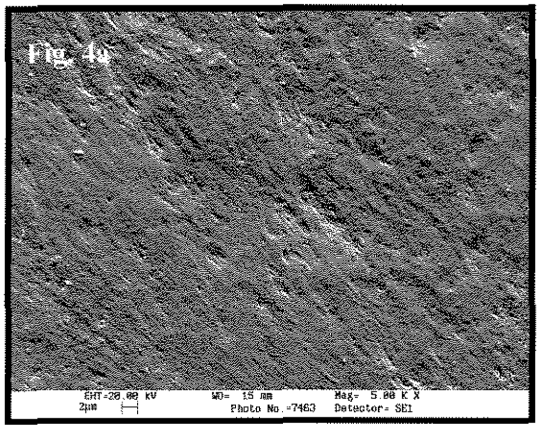
Fig. 4a

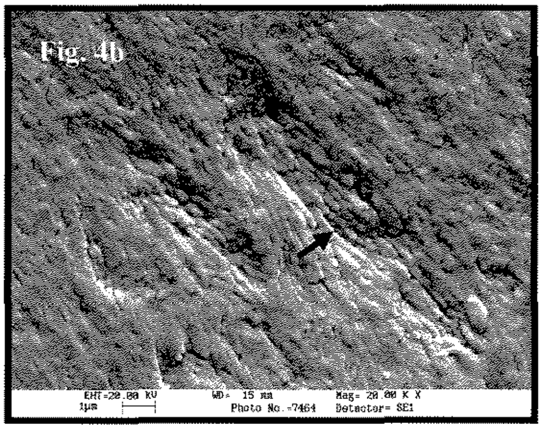
Fig. 4b

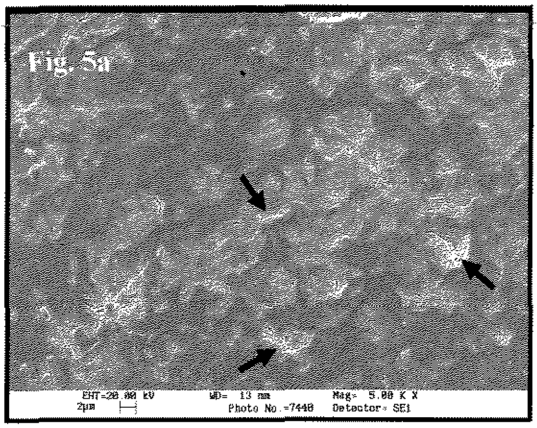
Fig. 5a

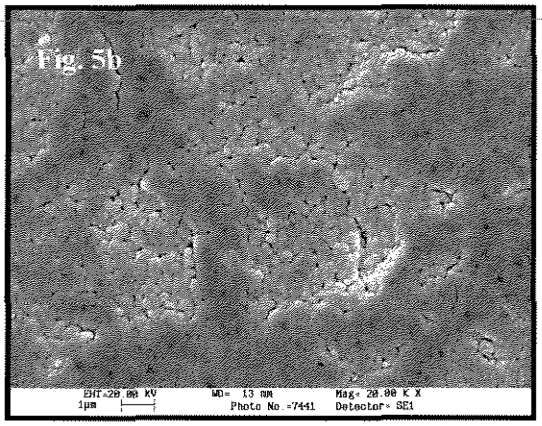
Fig. 5b

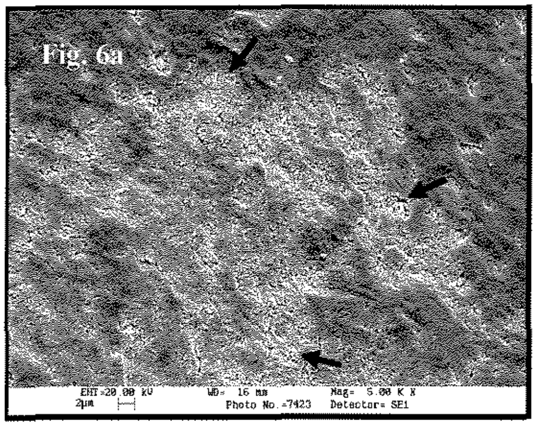
Fig. 6a

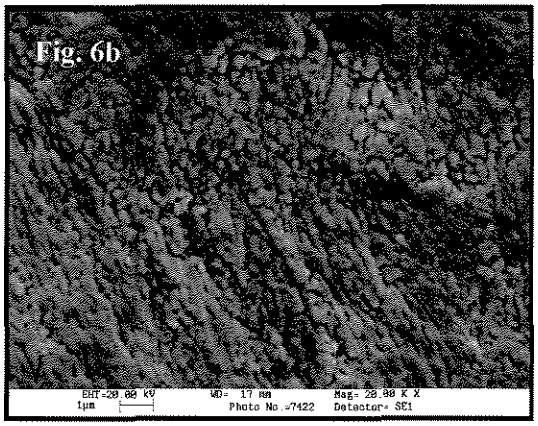
Fig. 6b

CAPÍTULO 3

HIGH-CONCENTRATED CARBAMIDE PEROXIDE BLEACHING AGENTS EFFECTS ON ENAMEL SURFACE

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(Anexo 3)

HIGH-CONCENTRATED CARBAMIDE PEROXIDE BLEACHING AGENTS EFFECTS ON ENAMEL SURFACE

ALTERATIONS IN ENAMEL SURFACE FOLLOWING BLEACHING (running title)

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KEYWORDS: bleaching, enamel, carbamide peroxide, roughness, staining, surface morphology

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HIGH-CONCENTRATED CARBAMIDE PEROXIDE BLEACHING AGENTS EFFECTS ON ENAMEL SURFACE

SUMMARY Concern has been expressed regarding the adverse effects of peroxide-containing tooth bleaching agents on enamel surface. This study examined enamel average surface roughness before (baseline) and after an in-office bleaching protocol and investigated the influence of high concentrations of carbamide peroxide gels on its surface staining and morphology. Flat enamel surfaces were submitted to 35% and 37% carbamide peroxide or to no bleaching treatment (n = 10) and evaluated with a profilometer. Eigth specimens from each group were randomly selected and immersed in a 2% methylene blue solution. Afterwards, specimens were ground into powder and prepared for the spectrophotometric analysis. Two remained specimens of each group were examined using a scanning electron microscope. Data were subjected to analysis of variance and Tukey test ($P > 0.05$). Baseline roughness average was statistically similar for all groups, however, 35% carbamide peroxide produced the roughest enamel surfaces. Different concentrations of carbamide peroxide produced similar staining means and enamel surface morphologic alterations.

Introduction

Bleaching methods have been developed and peroxide compounds at different concentrations are used for tooth whitening procedures (Arens, Rich & Healey, 1972; Haywood & Heymann, 1989; Clark & Hintz, 1998). Peroxide-containing vital tooth whiteners are classified into three categories, according to application mode and peroxide concentration. Oxidizing agents for professional use only contain high concentrations of carbamide peroxide (35-37%) and hydrogen peroxide (30-35%) solutions, while patient-applied tooth whiteners for at-home bleaching, containing up to 20% carbamide peroxide and 10% hydrogen peroxide, are dispensed and monitored by dentist. Over-the-counter products are available directly to consumers for home use, however, its safety is questionable (Haywood, 1992; Li, 1996).

Daily exposure to 10% carbamide peroxide for up to 5 weeks (Haywood & Heymann, 1989), based on clinical evaluations and researches, is a safe, conservative and effective procedure for at-home tooth whitening (Haywood, 1992; Haywood *et al.*, 1994; Haywood & Robinson, 1997; Kelleher & Roe, 1999). Nevertheless, concerns have been raised regarding changes in enamel surface morphology due to oxidative process produced by the bleaching gel. Low concentrations of carbamide peroxide can promote varying degrees of surface porosity and structural change, depending on the bleaching agent (McGuckin, Babin & Meyer, 1992; Bitter, 1993; Shannon *et al.*, 1993; Bem-Amar *et al.*, 1995; Josey *et al.*, 1996; Hegedüs *et al.*, 1999).

Techniques and materials have been introduced for in-office tooth whitening procedures. The use of concentrations of carbamide peroxide of up to 40% are an alternative to nightguard vital bleaching and to high concentrations of hydrogen peroxide in-office bleaching. Moreover, high concentrations of carbamide peroxide can also be used as a pre-treatment and associated with at-home bleaching (Clark & Hintz, 1998; Gultz *et al.*, 1999). Treatment times vary from a few minutes to a couple of hours and consist of gel placed on teeth, alone or it contained in a mouthguard, with the possibilty of repetitive aplications. Alterations on enamel surface can be produced by the high concentrations of carbamide peroxide used in in-office technique (Gultz *et al.*, 1999; Oltu & Gurgan, 2000), however, their adverse effects have not been thoroughly studied.

The purpose of this study was to evaluate the influence of high concentrations of carbamide peroxide gels on the surface roughness and staining susceptibility of human dental enamel. In addition, enamel surface morphology of untreated control and bleached specimens were investigated by scanning electron microscopy (SEM) observations.

Materials and methods

Fifteen extracted sound erupted third molars stored in a 2% buffered formalin solution were used within one month of extraction. The teeth were obtained according to protocols that were analyzed and approved by the appropriate institutional review board of the Piracicaba School of Dentistry – University of Campinas and with the informed consent of the donors.

The roots were separated from the crowns, which were sectioned mesiodistally using a water-cooled slow speed diamond saw*. Thirty dental fragments from buccal and lingual enamel surfaces (3.5 x 3.5 x 2.5 mm) were obtained and individually embedded in self-curing polyestyrene resin in a ring mold. Enamel surfaces were

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* Isomet, Buehler Ltd., Lake Bluff, IL, U.S.A.

† APL-4, Arotec, Cotia, SP, Brazil.

‡ Ultradent Products Inc., Salt Lake City, UT, U.S.A.

flattened with wet 600-, 1000- and 1200-grit aluminum oxide abrasive papers and polished with 6, 3, ½, and ¼ μm–grit diamond pastes on a polishing machine[†].

Specimens were randomly divided into three groups (n = 10): Group 1- Control, no bleaching treatment; Group 2- 35% carbamide peroxide Opalescence Quick®[‡] and Group 3- 37% carbamide peroxide / Whiteness Super®[*]. Control group was kept in artificial saliva at 37 °C and was not bleached. For bleaching groups, a 0.1 mL of the carbamide peroxide gel was dispensed on enamel for 30 min. Afterwards, specimens were rinsed with distilled water and stored in artificial saliva (Cavalli *et al.*, 2001) at 37°C, until next application. The bleaching procedure was repeated four times with a 72-hour bleaching interval.

A profilometer[†] was used to measure the surface roughness, before (baseline) and after bleaching procedures. Three measurements in different directions were recorded and the average surface roughness (Ra) was determined for each specimen. Eight specimens from each group were randomly assigned to the staining test. Specimens were immersed in a 2% methylene blue solution at 37 °C for 24 hours. Specimens were rinsed with distilled water for 30 seconds, air-dried, removed from the acrylic blocks and ground into powder in a mill for hard tissues[#]. The resulting powder was placed separately into test tubes, which were filled with 5 ml of absolute alcohol. After 24 hours, the solutions were centrifuged at 3000 rpm for 3 minutes[‡] and the

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[*] FGM Produtos Odontológicos, Joinville, SC, Brazil.

[†] Surf test – 211, Mitutoyo, Tokyo, Japan.

[‡] IC-15AN, Tomy Seiko Co. LTD. Tokio, Japan.

[§] DU 65, Beckman Instruments Inc, Fullerton, CA, U.S.A.

[#] Marconi – Siemens, Piracicaba, SP, Brazil supernatant used to determine the absorbance in a spectrophotometer[§]. Standard solutions of methylene blue in 5 ml of absolute alcohol were prepared, containing from 0 to 4 μg of dye/ml. The absorbance of the standard solutions were determined at wave lengths ranging from 500-700 ηm, and the best results were obtained at 596 ηm. Prior to determining the absorbance of experimental solutions at 596 ηm, the correlation coefficient (r) between dye concentration and absorbance of the standard solutions was calculated, an r value of 0.997 was obtained. To estimate the dye concentration on the experimental specimens, a linear regression was obtained. The regression equation expressed as:

$$y = 0.024x - 0.00071,$$

where y is the absorbance and x is the dye concentration. The dye uptake of each specimen was expressed in μg dye/ml, lower values indicating lower staining susceptibility. Data from profilometric examination and spectrophotometric analysis were statistically analyzed by one-way analysis of variance and Tukey test at 0.05 level of significance.

Two specimens of each group that were not submitted to the staining test were prepared for the scanning electron microscope[*]. Specimens were sputter coated with gold in a vacuum evaporator[†] and photomicrographs of a representative area of the surfaces were taken at 5,000X.

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[*] DSM 940A, Zeiss, Oberkochen, Germany.

[†] MED 010, Balzers, Balzer, Liechtenstein.

Results

The average surface roughness and dye uptake for experimental groups are displayed in Table 1. Baseline data were performed in order to verify the similar initial surface smoothness ($P > 0.05$) and to contrast differences between untreated and treated enamel in the same group. The use of 35% carbamide peroxide increased surface roughness significantly ($P < 0.05$), while control group and 37% carbamide peroxide promoted no alterations on surface roughness ($P > 0.05$).

Regarding the dye uptake, no statistical significant differences were observed when enamel surfaces were treated with 35% or 37% carbamide peroxide ($P > 0.05$). However, unbleached specimens presented more stain resistant surfaces than specimens bleached with 37% carbamide peroxide ($P < 0.05$) and similar to 35% carbamide peroxide bleached ones ($P > 0.05$).

A representative photomicrograph of a polished and unbleached enamel surface (control) that was stored in artificial saliva for 12 days is shown in Fig. 1. The surface morphology of enamel following exposure to 35% and 37% carbamide peroxide gels revealed alterations in the surface topography when compared to unbleached control group. Irregular pattern of enamel erosion was evident in bleached specimens, which resemble a type II acid-etching pattern (Figs 2 and 3).

Discussion

Previous in-office vital tooth bleaching techiniques have used high concentrations of hydrogen peroxide (Cohen & Parkins, 1970; Arens *et al.*, 1972;

McEvoy, 1989), which promoted enamel surface alterations (Ledoux *et al.*, 1985; Zalkind *et al.*, 1996; Hegedüs *et al.*, 1999). When a hydrogen peroxide-containing bleaching agent was associated with preoperative etching and heat application, extensive structural changes in enamel were observed (Titley, Torneck & Smith, 1988; McGuckin *et al.*, 1992). Nightguard vital bleaching using a 10% carbamide peroxide gel was developed as a conservative and safe method to minimize the adverse effects, often associated with in-office bleaching (Haywood & Heymann, 1989). The effects of increased carbamide peroxide concentrations have been studied in a attempt to promote an improved whitening effect and reduced whitening protocol time (Gultz *et al.*, 1999; Oltu & Gurgan, 2000). High concentrations of carbamide peroxide have been indicated for in-office bleaching procedures, in order to substitute the use of hydrogen peroxide solutions (Clark & Hintz, 1998).

In the present experiment, surface morphologic differences were noted between the unbleached control specimens and those bleached with 35 and 37% carbamide peroxide. The bleaching process comprises peroxide penetration into enamel, oxidizing tooth stains and discolorations (Haywood, 1992). Enamel surface in contact with high-concentrated carbamide peroxide gels provide morphologic changes, causing increasing the surface roughness and etching-like appearance. The bleaching process had little effect in some areas, whereas in other areas, decalcification and increased porosity of the enamel surface was notice. Therefore, alterations were not uniform throughout the surfaces.

Regardless of the close to neutral pH level of the bleaching agents, enamel changes on bleached surfaces were observed under SEM. Carbamide peroxide degrades into hydrogen peroxide, which breaks down to water and oxygen, and urea, which breaks down to carbon dioxide and ammonia. Arends *et al.* (1984) described that urea was capable of penetrating into enamel, affecting the interprismatic regions, as observed in bleached specimens of this study (Figs 2 and 3). Thus, urea may contribute to the structural changes and roughness of enamel. However, it can promote beneficial side-effects due to urea alkaline property that raises the hydrogen ion concentration (pH) of the bleaching solution and reduces the adverse effects (Haywood, 1992; Haywood & Heymann, 1991).

Although enamel exposed to 35% or 37% carbamide peroxide revealed similar alterations on surface morphology, Opalescence Quick that has a pH of 6.5, promoted significant increase on enamel surface roughness. No alterations in surface morphology or enamel etching pattern were noted in the unbleached specimens (Fig. 1). Whiteness Super produced similar surface roughness to control group and its pH ranges from 6.5 to 7.2. Peroxide-containing vital tooth bleaching agents, which are a mixture of different ingredients, have been shown to produce a specific effect on enamel in spite of their almost neutral pH (McGukin *et al.*, 1992; Li, 1996). In contrast, other reports have shown that bleaching agents neutrality is a important property to avoid roughening of enamel surface (Bitter, 1992; Shannon *et al.*, 1993).

Scanning electron microscope evaluations have been conducted on enamel surfaces treated with high concentration of carbamide peroxide and contradictory evidence of adverse effects have been related. No differences in enamel surface morphology and enamel mineral profile were observed between unbleached control group and bleached groups with 35% carbamide peroxide (Gultz *et al.*, 1999; Ngo *et al.*, 1999). However, Oltu & Gurgan (2000) reported that lower concentrations of carbamide peroxide are recomended over higher concentrations to avoid changes on enamel surface.

Unbleached specimens were more stain resistant to methylene blue than those bleached with 37% carbamide peroxide, although no significant differences were observed between bleaching agents tested. An absorbance spectrophotometer was used in order to quantify the dye uptake by enamel surfaces submitted or not to bleaching. The staining susceptibility of enamel cannot be related to the surface roughness alone, but to enamel composition, water absorption rate due to permeability alterations, and irregularities left on bleached enamel surfaces, which could favor the accumulation of dye (Arends et al., 1984; Titley *et al.*, 1988; Rotstein *et al.*, 1996; Hegedüs *et al.*, 1999; Ngo *et al.*, 1999).

In summary, the results suggested that high concentrations of carbamide peroxide can promote alterations on enamel surface. The surface roughness and staining susceptibility can also be affected depending on the bleaching agent.

Acknowledgments

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Table 1. Mean values (± SD) of surface roughness (μm) and dye uptake (μg dye/ml) of unbleached and bleached enamel surfaces.

Group	Surface Roughness			Dye Uptake
	Baseline		12 days	
Control	0,120 (± 0,024) Aa	NS	0,129 (± 0,032) A a	0,046 (± 0,006) a
Carbamide Peroxide 35%	0,104 (± 0,031) A a	S	0,158 (± 0,055) Bb	0,071 (± 0,026) ab
Carbamide Peroxide 37%	0,095 (± 0,015) Aa	NS	0,118 (± 0,057) Aa	0,224 (± 0,167) b

Means followed by same vertical letter were not significantly different by Tukey test ($P > 0.05$).

Legends:

Figure 1. Enamel surface morphology of an unbleached specimen (control group). No alterations or pattern of enamel etching are noted.

Figure 2. Enamel surface exposed to 35% carbamide peroxide. Affected interprismatic structures (arrows) and irregular patterns of enamel etching can be seen (C - enamel prism core).

Figure 3. Enamel surface exposed to 37% carbamide peroxide. Affected interprismatic structures (arrows) and increased porosity can be seen (C - enamel prism core).

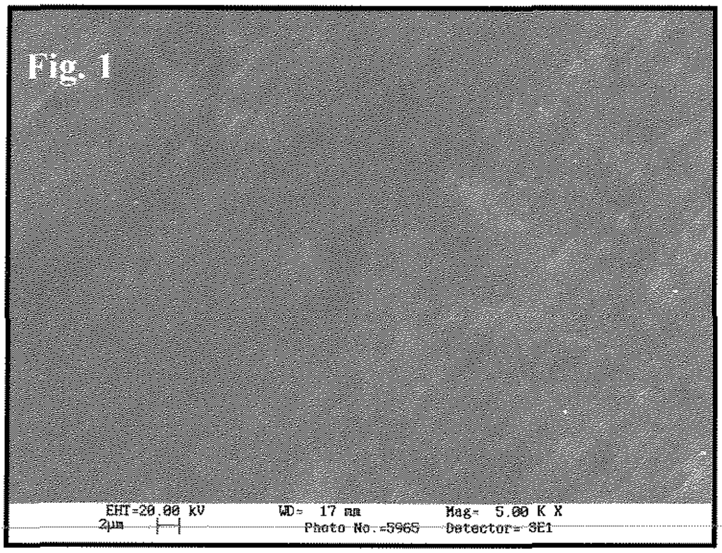
Fig. 1

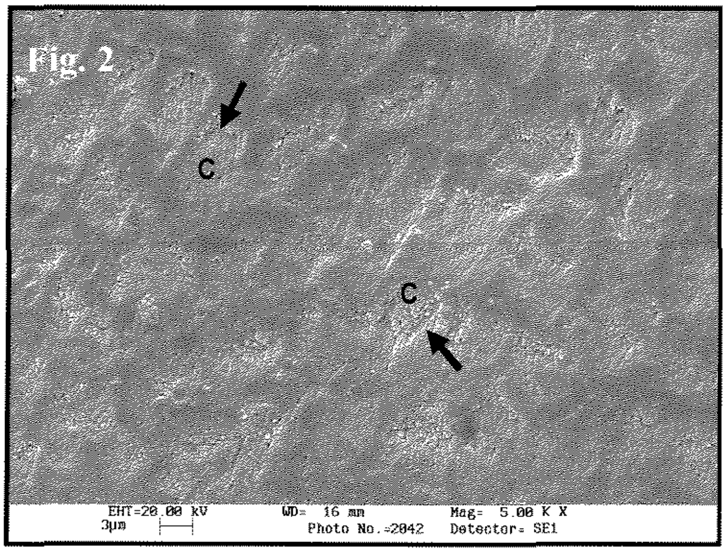
Fig. 2

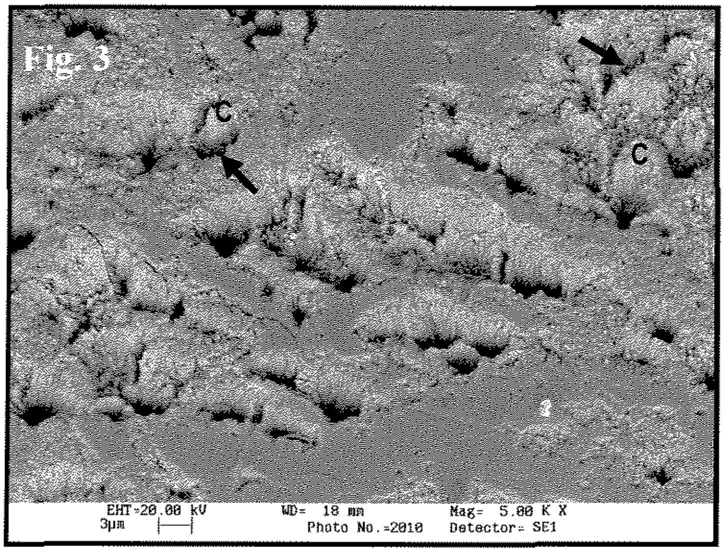
Fig. 3

CAPÍTULO 4

EFFECT OF CARBAMIDE PEROXIDE BLEACHING AGENTS ON TENSILE STRENGTH OF HUMAN ENAMEL

Enviado para publicação na revista:
DENTAL MATERIALS

Effect of carbamide peroxide bleaching agents on tensile strength of human enamel

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Short Title: Effect of bleaching on enamel strength

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Keywords: carbamide peroxide, bleaching agent, enamel, microtensile test.

Abstract

Objectives: The aim of this study was to evaluate the effects of low concentrations of carbamide peroxide bleaching agents (CP) on the ultimate tensile strength of enamel (UTS).

Methods: A "cube-like" resin composite structure (TPH Spectrum) was built-up on the bonded occlusal surface of twelve sound third molars to facilitate posterior preparation for the microtensile test. The bonded teeth were serially sectioned in a buccal-lingual direction in slices of approximately 0.7 mm thick. Each slice was trimmed with a fine diamond bur to reduce the buccal, internal slope enamel of the cusps to a dumb-bell shape with a cross-sectional area at the "neck" of less than 1 mm^2. The sample was randomly divided into 6 groups of 10 specimens each: Control (C- untreated); Opalescence 10% (O10), Opalescence 15% (O15), Opalescence 20% (O20), Whiteness 10% (W10) and Whiteness 16% (W16). Specimens in each group received the application of the bleaching agents for 8 hours/day, at 37° C, during 14 days and were stored in artificial saliva in between each application. After bleaching regimen, specimens were rinsed in water and tested with the microtensile method at 0,5 mm/min. Data were analyzed by ANOVA and Tukey test.

Results: Mean tensile strengths were (MPa): C= 47.5 ± 6.2^a; O10= $37,6 \pm 5.8^b$; O15= 33.2 ± 6.0^b; O20= 31.2 ± 3.5^b; W10= 32.5 ± 6.1^b; W16= 30.6 ± 7.7^b. Specimens subjected to the bleaching regimen presented significantly lower tensile strength than control group ($p<0.05$). No differences were found among the type and concentration of the bleaching agents ($p>0.05$).

Significance: Results suggested that bleaching enamel with carbamide peroxide agents could significantly reduce its tensile strength within a 14 days treatment.

Introduction

General concerns regarding the vital bleaching technique comprise potential adverse or side effects during and after treatment. Despite the absence of clinical reports on fractures or cracks of dental structures after carbamide peroxide application, a general concern is expressed regarding possible weakening of the tooth structure [1]. It has been shown that the fracture toughness of enamel was reduced about 30% after 12 hours exposure to 10% carbamide peroxide [2]. Changes in the mechanical properties indicate that structural alterations are likely to occur in enamel subjected to such bleaching agents.

Carbamide peroxide dissociates into hydrogen peroxide and urea when in contact with soft-tissues or saliva at oral temperature. Peroxide solutions can flow freely through enamel and dentin due to its low molecular weight. While hydrogen peroxide further degrades into oxygen and water, urea degrades into ammonia and carbon dioxide [3]. The action mechanism of the bleaching agents comprises the ability of hydrogen peroxide to form oxygen free radicals that interact with adsorbed colored organic molecules and break these stain macromolecules and pigments, producing dental discoloration into smaller and lighter molecules. The shift of the visible absorption spectrum from a longer to a shorter wavelength results in a colorless or less dark compounds, producing color changes and whitening action [2,3].

Studies have analyzed the effects of carbamide peroxide on enamel with different results. Although some scanning electron microscopy investigations of bleached enamel have shown little or no topographic alterations [4, 5, 6, 7, 8, 9], enamel surface changes after carbamide peroxide bleaching have been reported. These include increased porosity, pitting, erosion and demineralization of enamel prisms periphery [10, 11, 12, 13, 14, 15, 16,17, 18]. Additionally, hardness analysis of enamel has also demonstrated conflicting evidences. Depending on the bleaching agent or application time, Knoop hardness of enamel can decrease [11, 19, 20] or remain unaltered after whitening [2, 12, 21].

Nowadays, there is a widespread use of bleaching agents to whiten teeth for esthetic reasons. While there are little questions about their efficacy, various concerns have been expressed regarding the safety of carbamide peroxide-containing tooth whiteners on dental hard tissues, specially enamel structural integrity. Thus, the objective of this study was to test the null hypothesis that the ultimate tensile strength of enamel is not altered when exposed to carbamide peroxide-containing bleaching agents.

Materials and Methods

Twelve sound, extracted human third molars, stored in 0.1% thymol solution for no longer than two weeks after extraction were used in this study. The teeth were obtained after informed consent by the patients and under the protocol (42/2001) that was analyzed and approved by the Ethical Research Committee of Piracicaba School of Dentistry / UNICAMP, Brazil. The teeth were cleaned of gross debris and placed in deionized water for twenty-four hours before beginning the experiment.

Intact enamel occlusal surface was etched with 35% phosphoric acid for 30 s, air dried and bonded with Single Bond adhesive system (3M ESPE, St. Paul, MN, USA). Bonded surfaces received three layers of TPH Spectrum resin composite (Dentsply Caulk, Milford, DE, USA) to build up a "cube-like" crown of approximately 6.0 mm in height (Fig. 1a). Each resin layer was light cured for 40 s with a XL 3000 light-curing unit (3M ESPE, St. Paul, MN, USA) and the bonded teeth were stored in water at 37°C. After 24 h, the roots were removed and the crowns were vertically, serially sectioned into 0.7 mm thick slabs with a diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA), under water lubrication (Fig. 1b). Each slab (Fig. 1c) was trimmed from both sides with a fine diamond bur (1040, KG Sorensen Ind. e Com. Ltda, Barueri, SP, Brazil) (Fig. 1d) under water lubrication to conform the specimen to an "hour-glass" shape and reduce the cross-sectional area of the "neck" to approximately 0.5 mm^2 (Fig. 1e). Specimens were prepared from the enamel located at the internal slope of the buccal cusps of the teeth [22].

Bleaching procedures

Sixty slabs were obtained from the 12 teeth (5 each). They were then randomly assigned to 6 groups (n = 10), according to bleaching agents and concentrations. The bleaching agents used in this study are listed in the Table 1.

Treated groups were as follow:

Group 1- Control, no bleaching treatment.

Group 2- Bleaching with 10% carbamide peroxide - Opalescence.

Group 3- Bleaching with 15% carbamide peroxide - Opalescence.

Group 4- Bleaching with 20% carbamide peroxide - Opalescence.

Group 5- Bleaching with 10% carbamide peroxide - Whiteness.

Group 6- Bleaching with 16% carbamide peroxide - Whiteness.

Control group was kept in artificial saliva [23] (Serra & Cury-1992) at 37 °C for the same time period of the treated groups. For treated groups (2, 3, 4, 5 and 6), the trimmed enamel area was daily exposed to a mixture of 0.1 mL of the respective bleaching agent with 0.05 mL of artificial saliva for 8 hours, during 14 consecutive days (Fig. 1f). During bleaching period, the specimens were placed in 100% relative humidity at 37° C. After daily bleaching, the specimens were thoroughly rinsed with deionized for 10 seconds and stored in 0.5 mL of artificial saliva at 37° C. After the end of the bleaching regimen (14th day), specimens were rinsed and stored in deionized water for 24 h at 37° C before being tested.

Testing

Each specimen was fixed to the "grips" of a microtensile test device with cyanoacrylate glue (Zapit, DVA, Corona, CA, USA) and tested in tension in a universal testing machine (4411, Instron Co., Canton, MA, USA) at 0.5 mm/min until failure (Fig. g). After testing, specimens were carefully removed from the fixtures with a scalpel blade and the cross-sectional area at the site of fracture measured to the nearest 0.01 mm with a digital caliper (727-6/150, Starret, SP, Brazil) to calculate ultimate tensile strength that was expressed in MPa. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey test at $\alpha = 0.05$.

Microscopy

Tested specimens were allowed to dry in an oven overnight and the fractured edges were sputter-coated with gold (MED 010, Balzers, Balzer, Leichtenstein) and observed under a scanning electron microscope (DSM 940A, Zeiss, Oberkochen, Germany).

Results

There was a statistically significant difference among the groups ($p=0.00002$). All bleaching agents produced significant decrease in the ultimate tensile strength (UTS) of enamel as compared to the control, untreated substrate ($p<0.05$), however, no significant differences were found among the treated groups ($p>0.05$). The ultimate tensile strength of untreated enamel was 47.5 ± 6.1 MPa, while the UTS of bleached enamel ranged from 30.6 ± 7.7 MPa to 37.6 ± 5.8 MPa (Table 2). Regardless of brand and concentration, bleaching resulted in approximately 20% to 35% reduction of the UTS of enamel.

Analysis of fractured specimens showed that fracture mode of unbleached and bleached were different. Unbleached fractured enamel showed a typical, transversal fracture pattern as the load was applied perpendicular to the orientation of the prisms. A compact structure with no clear porosity was observed (Fig. 2). Bleached fractured enamel showed evidences of the attack of the bleaching agents. No major differences were observed among the different agents. There was an apparent preference for dissolution of the boundaries between the prism and the interprismatic substance. Upon fracture, prisms dislodged in a cone-like shape and their fractured ends seemed porous (Fig. 3).

Discussion

Early, in-office vital tooth bleaching techniques used preoperative acid etching, strong chemical oxidizing agents and heat to promote tooth whitening [24,25,26]. Oxidizing agents comprised high concentrations of hydrogen peroxide solutions (30-35%) and were indicated for professional use only. As result of these bleaching techniques, enamel has been shown to present superficial alterations [17, 27] and reduction in the calcium/phosphorus (Ca/P) ratio [28]. However, no internal structure changes or decreased intrinsic strength of enamel has been reported as a consequence of such in-office bleaching techniques.

Low concentrations of carbamide peroxide (10-22%) are dispensed and monitored by dentists while used by patients at home during 5 to 8 hours daily for 2 to 5 weeks. When used under the supervision of the dentist, peroxide bleaching is considered safe regarding general toxicity, risk of cell mutation or carcinogenesis [29,30,31]. Conversely, contradicting evidences have been reported about possible alterations in the organic and mineral content of enamel after bleaching. Elemental surface analysis of enamel bleached with high concentrations of hydrogen peroxide for 60 minutes showed increased nitrogen content, but no other significant chemical changes were detected [32]. Another report showed no significant changes or volume loss produced by hydrogen peroxide bleaching on enamel [33]. However, a significant reduction in the Ca/P ratio was found following treatment with hydrogen peroxide for 7 days [28].

For carbamide peroxide bleaching agents, while studies demonstrated no significant reduction in Ca/P ratio [28], others showed that some carbamide peroxide formulas caused calcium dissolution from enamel [19]. Urea is a sub product of such bleaching agents and has been shown to be able to remove enamel proteins and related mineral elements [34], attacking the core or intra-prismatic area and producing porosities at the prism surfaces [35]. A significant decrease in Ca and P concentration after carbamide peroxide bleaching can also produce morphological alterations in the most superficial enamel crystallites [18]. Although the amount of calcium loss has been described as not being clinically relevant [36], the summation of adverse effects could result in mechanical changes of enamel.

The 10% to 15% carbamide peroxide solutions dissociate into 3% to 5% hydrogen peroxide and approximately 7% to 10% urea. Despite the low hydrogen peroxide concentration released, at-home tooth-whitening materials are used over prolonged periods and extensive application time raises concerns of possible teeth damage. The daily application of carbamide peroxide for 8 hours and 14 consecutive days used in this study simulated the home-applied vital bleaching technique [37]. Results showed that under these conditions, bleached enamel presented lower tensile strength than specimens that were not subjected to carbamide peroxide treatment. No significant effects were observed regarding the concentration of the bleaching agents. Although structural changes of the enamel may be limited to its surface, it is possible that due to the small dimensions of the trimmed specimens such damages ultimately affected the tensile property of the entire specimen. Porosities created by the bleaching agent along the exposed area of enamel may have acted as stress raisers during tension and resulted in premature failures. Although the tensile forces used in this study are not readily extrapolated to clinical situations, the weakening effect of carbamide peroxide bleaching agents on enamel is evident and may explain the clinical observation of post-bleaching incisal fractures of teeth. The results may also offer an additional explanation as to why bond strengths of resins to bleached enamel are usually lower than to unbleached enamel [37, 39]. Lower "bond strengths" may be due to premature fracture of the weakened underlying enamel rather than due to the oxidizing effect of hydrogen peroxide [38]. It is therefore advisable that studies attempting to investigate the effects of bleaching agents on the bond strength of resins to enamel, to properly evaluate the mode of fracture of their specimens before reaching any conclusion.

The main detrimental effects of bleaching enamel are related to surface morphological changes [10, 11, 12, 13, 14, 16, 17, 18], decreased enamel microhardness [19,20,11] and reduction of fracture toughness [2]. The oxidative effect, pH of the solution and the composition of the bleaching agent have been claimed to produce surface dissolution, rendering the surface porous. The acidity of bleaching agents has always been associated with enamel demineralization. However, this may have not been the case in this study since the pH of the bleaching agents used are close to neutral, according to the manufacturers. Urea may provide beneficial effects due to the pH raise of the bleaching solution [3, 5] however, due to its protein denaturing ability, it could also lead to enamel intraprismatic degradation [24].

The overall reduction in the UTS of enamel reported here was close to that reported by [2]. These authors attributed their findings to possible alterations in enamel organic matrix promoted by non specific and potentially reaction free radicals, which resulted in fracture toughness reduction. As enamel presents 1-2% of organic material [40], the influence of bleaching on enamel matrix must be further investigated, as it could lead to changes in the mechanical properties.

The effects of bleaching agents on the mechanical properties of enamel have not been extensively studied. Although it is quite difficult to clinically associate enamel cracking or fractures with previous bleaching treatments, there are increasing evidences that enamel structural changes may occur due to exposure to such substances, and that may ultimately compromise its strength. This study showed that the UTS of enamel was significantly reduced followed a routinely used bleaching regimen. The clinical implications of that must be further investigated.

Conclusion

Significant reductions in the ultimate tensile strength of enamel were observed after exposure to carbamide peroxide agents under a clinically simulated bleaching regimen. This may have clinical implications on the post-treatment outcomes.

Acknowledgements

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Table 1. Bleaching agents composition, pH, and batch numbers.

Bleaching agent	Composition	%	pH	Batch numbers
Opalescence	Carbamide peroxide, glycerin, carbopol.	10	6.0 – 6.5	44QT
		15	6.0 – 6.5	44QT
		20	6.0 – 6.5	44QT
Whiteness	Carbamide peroxide, glycol, carbopol, potassium ions, deionized water.	10	6.5 – 7.2	20031998
		16	6.5 – 7.2	20031998

Table 2. Ultimate tensile strength mean values (MPa) for experimental groups.

Group	Mean	SD	Tukey test
Control	47.5	6.1	a
Opalescence 10%	37.6	5.8	b
Opalescence 15%	33.1	6.0	b
Opalescence 20%	31.2	3.5	b
Whiteness 10%	32.5	6.1	b
Whiteness 16%	30.6	7.7	b

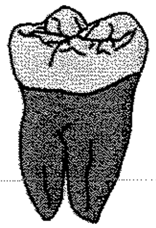
Means followed by different letters are significantly different at $p < 0.05$.

Legends:

Figure 1. Schematic representation of specimen preparation.

Figure 2a and 2b. Fractured enamel surface of unbleached specimens. A typical fracture pattern that occurs transversally to prismatic orientation is seen (2a). Cone-like, protruding prisms indicate the uneven fracture plane of the compact enamel (2b)

Figure 3a, 3b, 4a and 4b. Fractured enamel surface of bleached specimens. The general appearance was that enamel seemed more porous than the unbleached specimens. A similar, uneven fracture plane could be observed regardless of the bleaching agent used (3a, 3b, 4a, 4b). The bleaching agents seemed to preferentially attack the boundaries between the prisms and the interprismatic substance (3a, 4a-arrows). Porosities are seem on the surface of the transversally fractured prisms (3b, arrowheads).



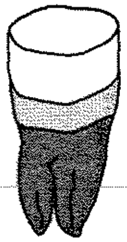


Fig. 1a

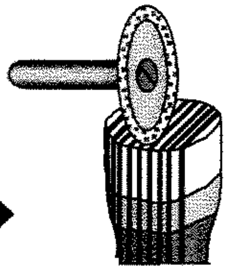


Fig. 1b

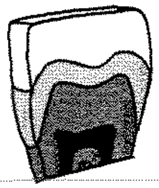


Fig. 1c

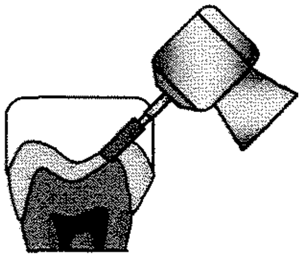


Fig. 1d

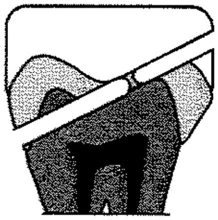


Fig. 1e

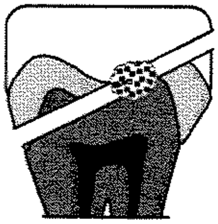


Fig. 1f

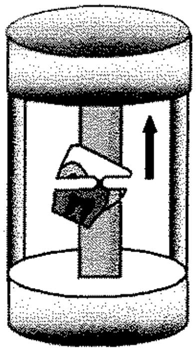


Fig. 1g

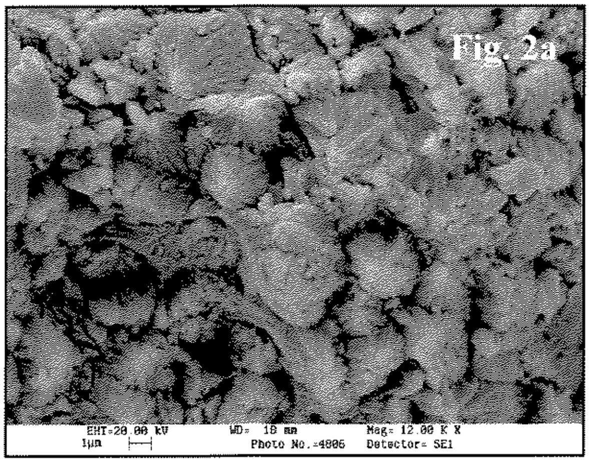


Fig. 2a
EHT=20.00 kV
1µm
WD= 18 mm
Photo No.=4806
Mag= 12.00 K X
Detector= SE1

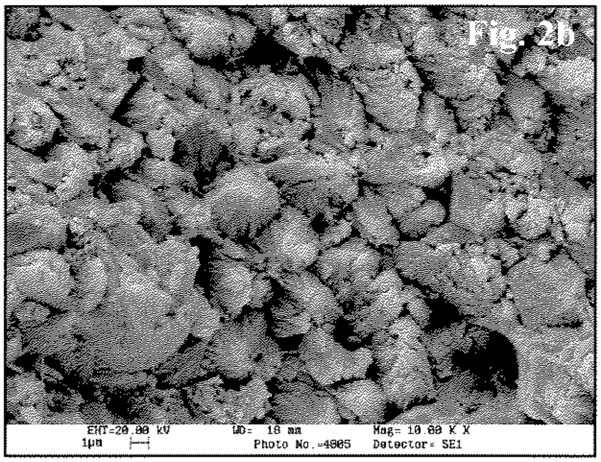


Fig. 2b
EHT=20.00 kV
1µm
WD= 18 mm
Photo No.=4805
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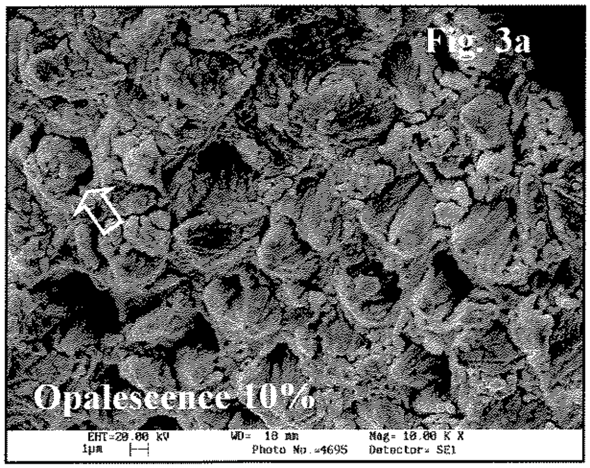
Fig. 3a
Opalescence 10%
EHT=20.00 kV
1µm
WD= 18 mm
Photo No.=4695
Mag= 10.00 K X
Detector= SE1

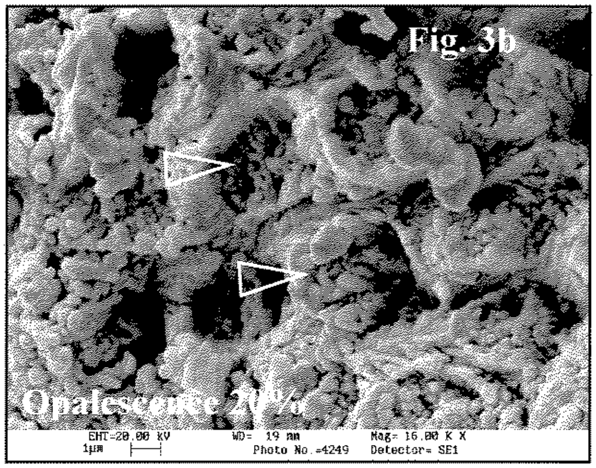
Fig. 3b
Opalescence 20%
EHT=20.00 kV
1µm
WD= 19 mm
Photo No.=4249
Mag= 16.00 K X
Detector= SE1

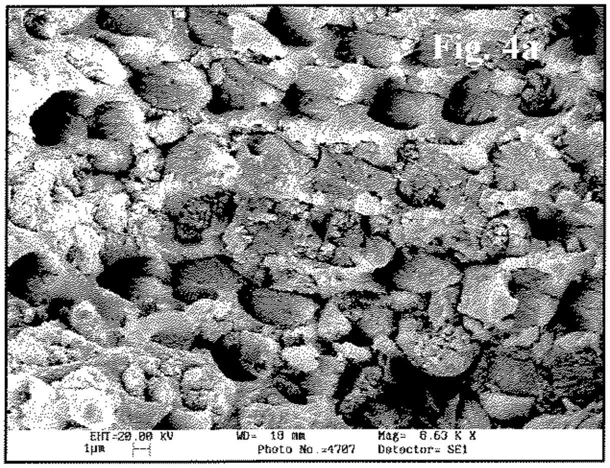
Fig. 4a
EHT=20.00 kV
1µm
WD= 18 mm
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Mag= 8.63 K X
Detector= SE1

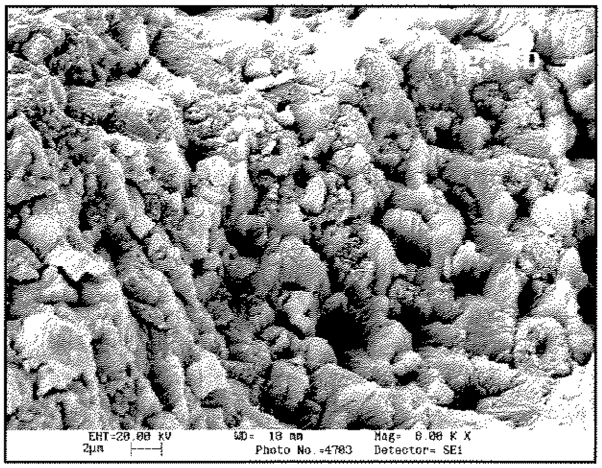
Fig. 4b
EHT=20.00 kV
2µm
WD= 18 mm
Photo No.=4703
Mag= 8.00 K X
Detector= SE1

CAPÍTULO 5

THE EFFECT OF ELAPSED TIME FOLLOWING BLEACHING ON ENAMEL BOND STRENGTH OF RESIN COMPOSITE

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(Anexo 4)

THE EFFECT OF ELAPSED TIME FOLLOWING BLEACHING ON ENAMEL BOND STRENGTH OF RESIN COMPOSITE

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Clinical Relevance

Bond strength of resin composite to enamel can be significantly reduced after bleaching. However, the effects of carbamide peroxide on bond strength may be reversible and short-lived.

SUMMARY

Recent studies have concluded that carbamide peroxide bleaching agents significantly affect the bond strength of composite to bleached enamel. The objective of this study was to evaluate the effects of bleaching regimen with different carbamide peroxide concentrations and post treatment times on composite bond strength to enamel. Two hundred and four flat buccal and lingual enamel surfaces obtained from erupted sound third molars were randomly divided into 17 groups (n = 12). Sixteen experimental groups comprised the evaluation of four carbamide peroxide home bleaching agents (Opalescence 10% - 20 % and Whiteness 10% - 16%) and four time intervals after bleaching (1 day, 1, 2 and 3 weeks). Specimens of control group were not submitted to bleaching and were stored in artificial saliva at 37° C for 10 days. The specimens of experimental groups were exposed to one daily application of carbamide peroxide for 6 hours for 10 consecutive days. After each daily treatment and post bleaching, the specimens were stored in artificial saliva solution. Bonds were formed with Scothbond MP and Z-100 composite resin, and shear bond test was carried out 24 hours after adhesive-composite application. Two-way ANOVA showed that the bond strengths were significantly different ($P < 0.05$). For the first two weeks post bleaching, the bond strengths of resin to enamel was low. After a lapse of three weeks, the bond strength returned to that of the untreated control group. Increased concentration did not prolong the time needed prior to bonding.

INTRODUCTION

Nightguard vital bleaching as an esthetic dentistry treatment was introduced by Haywood & Heymann (1989). A great number of dentists have indicated to treat intrinsically discolored teeth with bleaching agents due to current interest in aesthetic dentistry (Chong, 1993; Lyons & Ng, 1998; Cibirka & others, 1999; Leonard & others, 1999). This original technique involved the application of a carbamide peroxide gel to the teeth within the confines of a soft mouthguard for some hours during 2-5 weeks. Daily exposure to carbamide peroxide, based on clinical experiences and researches, is an apparently safe and effective procedure for whitening teeth (Haywood, 1992; Haywood & others, 1994; Haywood & Robinson, 1997; Kelleher & Roe, 1999).

Several reports have concerned about possible adverse effects of carbamide peroxide gel, due to the large use of dentist-prescribed bleaching agents. These effects include changes in ultra-morphological resin-enamel interface (Perdigão & others, 1998); alterations in enamel surface morphology (McGuckin, Babin & Meyer, 1992a; Bitter, 1993; Shannon & others, 1993; Ben-Amar & others, 1995; Josey & others, 1996); pulpar (Cooper, Bokmeyer & Bowles, 1992) and gingival irritation (Marshall, Cancro & Fischman, 1995); changes in salivary pH (Leonard Jr, Bentley & Haywood, 1994); adherence of Streptococcus mutans to bleached enamel (Gurgan, Bolay & Alaçam, 1997); alterations on composite resin (Bailey & Swift, 1992; Monaghan, Lim & Lautenschlager, 1992) and changes on fracture toughness, hardness and abrasion characteristics of human enamel (Seghi & Denry, 1992).

Some adverse effects of carbamide peroxide are clinically relevant when it is necessary to bond composite and porcelain restorations or orthodontic brackets to bleached enamel surfaces. For up to one week following bleaching a decrease in bond strength was noted (Cvitko & others, 1991; Stokes & others, 1992; Garcia-Godoy & others, 1993; Ben-Amar & others, 1995). The use of the acid-etch technique on bleached enamel with normal bond strength values has been a challenge for esthetic dentistry (McGuckin & others, 1992b; Titley, Torneck & Ruse, 1992; Barghi & Godwin, 1994; Sung & others, 1999)

This *in vitro* study evaluated the effect of different carbamide peroxide gel concentrations, using the mouthguard technique, on the shear bond strength of resin composite to bleached enamel surface after various post-bleaching intervals (one day and, one, two or three weeks).

MATERIALS AND METHODS

One hundred and two freshly extracted erupted sound human third molars were stored in 10% formalin. The roots were separated from their crowns using a diamond disc (KG Sorensen, Barueri, SP 06454-920, Brazil). The crowns were sectioned mesiodistally to obtain two similar enamel halves, lingual and buccal. Each half-crown was embedded in self-curing polyestyrene resin cylinders (2.0 cm diameter by 2.0 cm high), and lingual or buccal enamel surfaces were polished with wet 600-, 1000- and 1200-grit aluminum oxide abrasive paper on a polishing machine (APL-4; Arotec, Cotia, SP 06700-000, Brazil) to create a flat enamel surface. Specimens were randomly divided into 17 groups of 12 specimens each; one control group and 16 experimental groups. The control group was not bleached and after 10-day storage in artificial saliva at 37° C it was tested in shear.

The artificial saliva, with an electrolyte composition similar to that of human saliva, was prepared from 1g sodium carboxymethylcellulose, 4.3 g xylitol, 0.1 g potassium chloride, 0.1 g sodium chloride, 0.02 mg sodium fluoride, 5 mg magnesium chloride, 5 mg calcium chloride, 40 mg potassium phosphate, 1 mg potassium thiocyanate and 100 g distilled deionized water.

A heat and vacuum tray forming machine (Plastivac P7, Bioart, São Carlos, SP 13568-000, Brazil) was used to fabricate 192 individual trays from 0.02-inch soft plastic. There was a reservoir in each tray to keep bleaching gel and artificial saliva in contact with the teeth. Two commercially available bleaching agents, Opalescence (Ultradent Products, Inc, Salt Lake City, UT 84124, USA) and Whiteness (FGM Produtos Odontológicos, Joinville, SC 89219-310, Brazil), with two different concentrations of carbamide peroxide for each bleaching agent were used in this study: Opalescence 10%, Opalescence 20%, Whiteness 10% and Whiteness16% (Table 1).

Experimental groups were exposed to one daily application of carbamide peroxide for 6 hours during 10 consecutive days. In each specimen, approximately 0.1 mL of bleaching agent and 0.05 mL of artificial saliva was applied on the enamel surface and covered with an individual tray. During bleaching, the specimens were placed in 100% relative humidity at 37° C, and after daily bleaching, the specimens were thoroughly rinsed with an air/water spray for 10 seconds and stored in artificial saliva at 37° C. The effect of carbamide peroxide on shear bond strength to enamel was tested on the first day, one week, two and three weeks post bleaching treatments and storage in artificial saliva at 37° C.

Bleached and unbleached (control group) flat enamel surfaces were washed in tap water and dried with oil free compressed air, for the bond strength test. A circular hole, 2 mm in diameter, was punched in adhesive tape which was positioned on the enamel surfaces. The demarcated area was etched with 35% phosphoric acid for 15 seconds, rinsed by using an air/water syringe for 15 seconds and dried with compressed air for 5 seconds. Scotchbond multipurpose adhesive (3M Dental Products, St. Paul, MN 55144,USA) was applied on the demarcated enamel bonding area in a thin layer and light cured for 10 seconds. The embedded half-crowns were mounted in an assembly apparatus as described by Kamel and others (1990). A split Teflon mold with a circular hole, 2 mm in diameter and 4 mm high, was locked in the device. Two increments of composite resin (Z-100; 3M Dental Products, St. Paul, MN 55144,USA) were placed into the opening of the split mold, and each one was light cured for 40 seconds. After curing, the split mold was removed, and specimens were stored in 100% relative humidity at 37°C for 24 hours prior to testing.

Each specimen was locked in a special device which was seated on the compression load cell of a universal testing machine (DL 500; Emic, São José dos Pinhais, PR 83020-250, Brazil). A shear load was applied to the base of the composite cylinder with a knife-edge rod with 0.5 mm width at a crosshead speed of 0.5 $mm.min^{-1}$. The shear bond strengths were calculated and expressed in MPa. Results were statistically analyzed by two-way analysis of variance (split plot - ANOVA) and additional treatment (control group), Dunnett's test and Tukey test at the 5% level of significance. Means of bond strength were correlated with the respective post bleaching time and analyzed by linear regression at $\alpha = 0.05$.

RESULTS

Table 2 displays the mean shear bond strengths and standard deviations for bleached groups and the control group. ANOVA revealed a statistically significant difference among groups ($P < 0.05$), therefore, data were further analyzed by Dunnett's and Tukey test. Dunnett's test indicated that the shear bond strength of all bleached groups, except for the groups tested 3 weeks post bleaching, were lower than control group ($P < 0.05$). Analysis of the data by Tukey test showed no significant differences in shear bond strength among treatments for the same post bleaching time ($P > 0.05$). Linear regression showed a direct relationship between bond strength and post bleaching time for the four treatments ($P < 0.05$) (Figures 1, 2, 3 and 4). Increased concentration did not prolong the time needed prior to bonding.

DISCUSSION

Previous studies have shown changes in enamel surfaces (Titley, Torneck & Smith, 1988a) and reduction on bond strength of composite resin to enamel exposed to high-concentration of hydrogen peroxide (Torneck & others, 1991; Stokes & others, 1992). Alterations in bleached enamel surface morphology with the use of 35% hydrogen peroxide could have resulted from exposure to acid solutions and peroxide, which also has a low pH, or from both agents (Titley & others, 1988b; McGuckin & others, 1992a). The loss in resin adhesiveness to enamel was related to possible presence of residual peroxide, which interfered with resin attachment and inhibited resin polimerization (Torneck & others, 1990; Titley & others, 1993; Dishman, Covey & Baughan, 1994), or to changes on the penetration and structure of resin tags induced by hydrogen peroxide treatment (Titley & others, 1991). The use of 25-35% hydrogen peroxide in office as a vital bleaching procedure was indicated before nightguard home bleaching.

Products currently available on dental market for the nighguard vital bleaching technique use 10% and higher concentrations of carbamide peroxide with pH close to neutral. This solution is unstable and disassociate into 3% hydrogen peroxide and 7% urea on contact with tissue or saliva. The hydrogen peroxide further degrades into oxygen and water, while the urea degrades into ammonia and carbon dioxide, which elevate the pH. The oxidizers remove some unattached organic matter from the tooth without dissolving the enamel matrix, and these pigments are removed by diffusion, leading to bleaching. In this technique, after carbamide peroxide breakdown, the concentration of peroxide is lower than previous in-office bleaching technique. However, home bleaching comprises daily applications of five-eight hours for two-five weeks (Haywood, 1992, Haywood & Robinson, 1997).

Hegedüs & others (1998) found that 30% hydrogen peroxide solution caused more severe changes in the enamel surfaces than carbamide peroxide gel. It was presumed that the difference in groove depth after treatment was caused by difference in hydrogen peroxide concentration. Morphologic alterations of the external enamel structure after carbamide peroxide bleaching have been reported (McGuckin & others, 1992a; Bitter, 1993; Shannon & others, 1993; Ben-Amar & others, 1995; Josey & others, 1996), however, other studies demonstrated minimal topographic alteration in bleached enamel (Haywood & others, 1990; Haywood, Houck & Heymann, 1991; Wandera & others, 1994; Ernst, Marroquin & Willershausen-Zönnchen, 1996; Zalkind & others, 1996).

It has been hypothesized that the carbamide peroxide-containing bleaching agents affect the mineral content and organic phase of the superficial layer and the inner structure of enamel (Hegedüs & others, 1998; Perdigão & others, 1998). Carbamide peroxide is a denaturing agent for proteins (Yip, Beeley & Stevenson, 1995). Urea is capable of attacking protein structures and penetrating into the enamel affecting the surface and interprismatic regions (Arends & others, 1984; Goldberg & others, 1983). The mineral loss can be noted by decreased enamel micro-hardness after bleaching (Seghi & Denry, 1992). Thus, these changes in mechanical properties may be responsible for the alterations in the superficial bleached enamel crystallites and on ultra-structure of resin-enamel interfaces (Perdigão & others, 1998).

Reduction in bond strength due to carbamide peroxide has been evaluated *in vitro* (Stokes & others, 1992; Garcia-Godoy & others, 1993; Ben-Amar & others, 1995). Authors have advised delays in bonding one week after bleaching, because the reduction of composite bond strength to freshly bleached enamel has been shown to be transient. (McGuckin & others, 1992b; Titley, Torneck & Ruse, 1992; Miles & others, 1994). Similarly, removal of superficial layer (Cvitko & others, 1991), pretreatment of bleached enamel with alcohol (Barghi & Godwin, 1994) and use of adhesives containing organic solvents (Sung & others, 1999) can result in complete reversal of the reduced enamel bonds.

Statistical evaluation of the data obtained by shear testing indicated a reduction in bond strength until 2 weeks post bleaching, and that the bleaching agent concentration did not seem to affect the bond strength for the same post treatment time. It took three weeks for the enamel to return to conditions that lead to normal bond strength. Over an extensive period of time, exposure of bleached enamel to artificial saliva may leach out peroxide absorbed by enamel during bleaching and reestablish the superficial morphology.

For bleached enamel surfaces, linear regression showed a tendency towards a reduction of the shear bond strength of the composite resin to enamel, which varied according to the post bleach storage period. Although Opalescence and Whiteness bleaching agents weakened the resin bond strength, buffering and remineralization potential of artificial saliva probably minimized the bleaching effects without interfering with the resin bonding if it was carried out three weeks post-bleaching.

CONCLUSION

The results of this study suggested that bonding of composite to enamel bleached with 10%, 16% and 20% carbamide peroxide gels result in a significant decrease on bond strength. Bond strength returns to values close to those of non-bleached enamel within three weeks following the procedure.

Acknowledgments

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Table 1. *Home Bleaching Agents Evaluated.*

Product	Bleaching Agent	Concentration	Other Components	pH
Opalescence	Carbamide peroxide	10 % and 20%	Carbopol, glycerin	6.5
Whiteness	Carbamide peroxide	10 % and 16%	Carbopol, glycol, potassium ions, humectant, deionized water	6.5 – 7.2

Table 2. *Means and standard deviations of shear bond strengths (MPa).*

Treatment	Post Treatment Time			
	1 day	1 week	2 weeks	3 weeks
Opalescence 10%	5.13 ± 1.76 *	8.37 ± 2.53 *	8.36 ± 2.54 *	13.84 ± 4.13
Opalescence 20%	5.54 ± 2.08 *	8.16 ± 1.48 *	6.20 ± 1.77 *	14.72 ± 4.03
Whiteness 10%	6.95 ± 2.15 *	6.86 ± 1.80 *	7.89 ± 2.26 *	13.37 ± 4.71
Whiteness 16%	7.47 ± 2.66 *	7.28 ± 2.26 *	7.17 ± 2.06 *	14.94 ± 3.81

Control Group: 16,62 ± 3.29

* Significant differences from the control group (unbleached enamel) by Dunnett's test ($P < 0.05$).

Means joined by vertical line were not significantly different by Tukey test ($P > 0.05$).

Legends

Figure 1. Enamel shear bond strengths after bleaching with Opalescence 10%. The solid circles represent the mean bond strength at each time interval after the bleaching treatment.

Figure 2. Enamel shear bond strengths after bleaching with Opalescence 20%. The solid circles represent the mean bond strength at each time interval after the bleaching treatment.

Figure 3. Enamel shear bond strengths after bleaching with Whiteness 10%. The solid circles represent the mean bond strength at each time interval after the bleaching treatment.

Figure 4. Enamel shear bond strengths after bleaching with Whiteness 16%. The solid circles represent the mean bond strength at each time interval after the bleaching treatment.

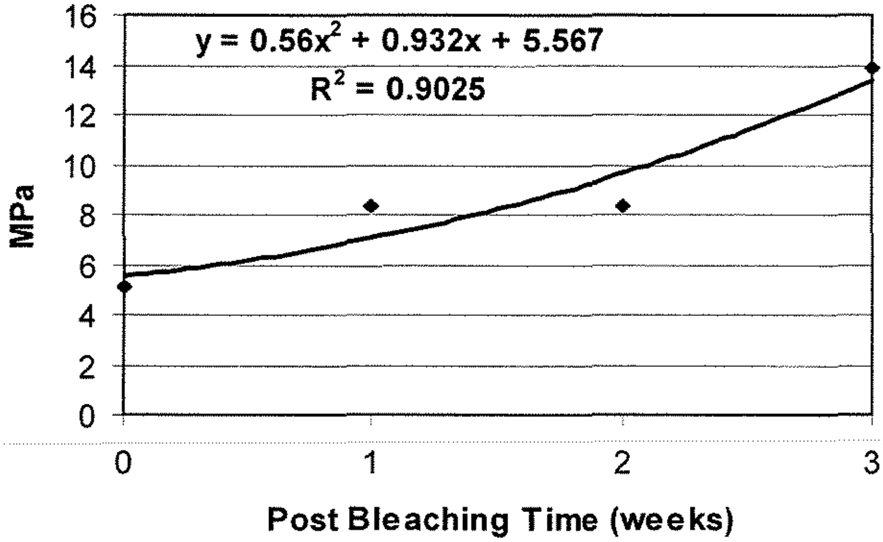

FIGURE 1

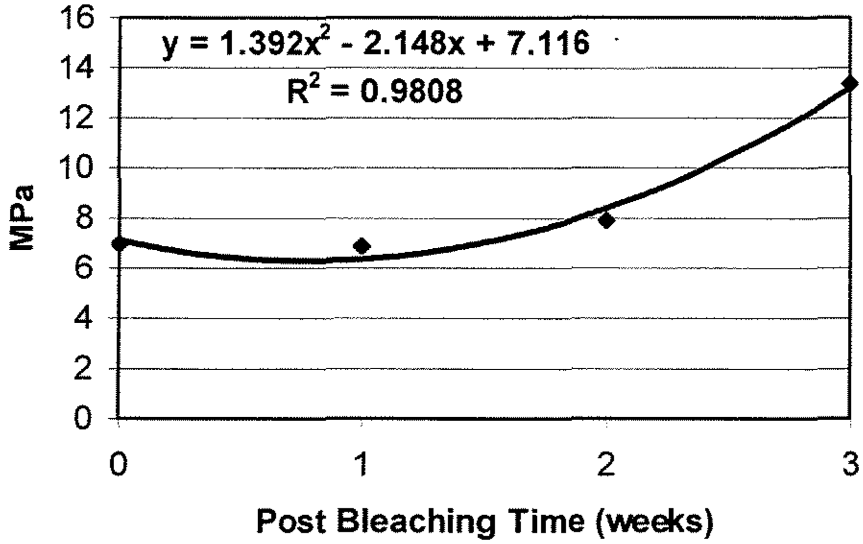

FIGURE 2

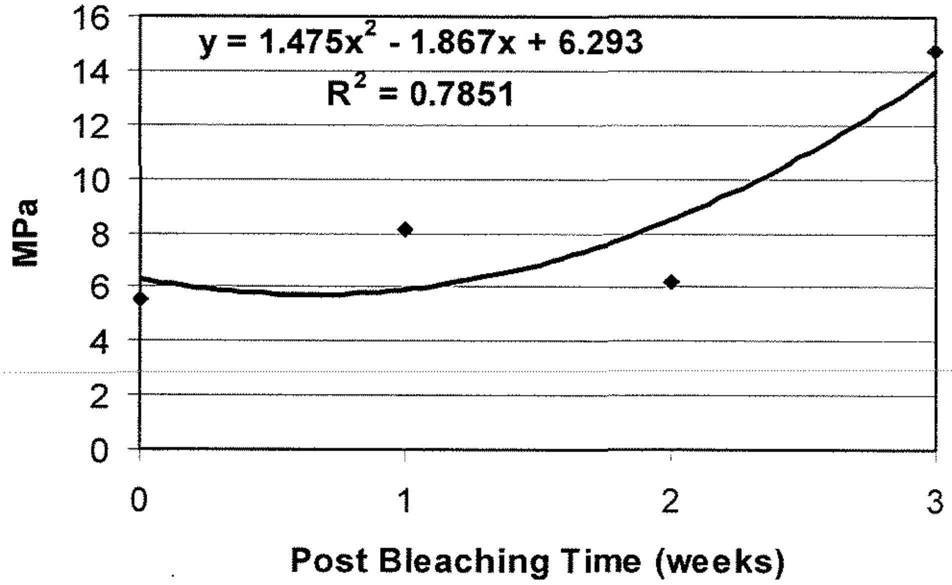

FIGURE 3

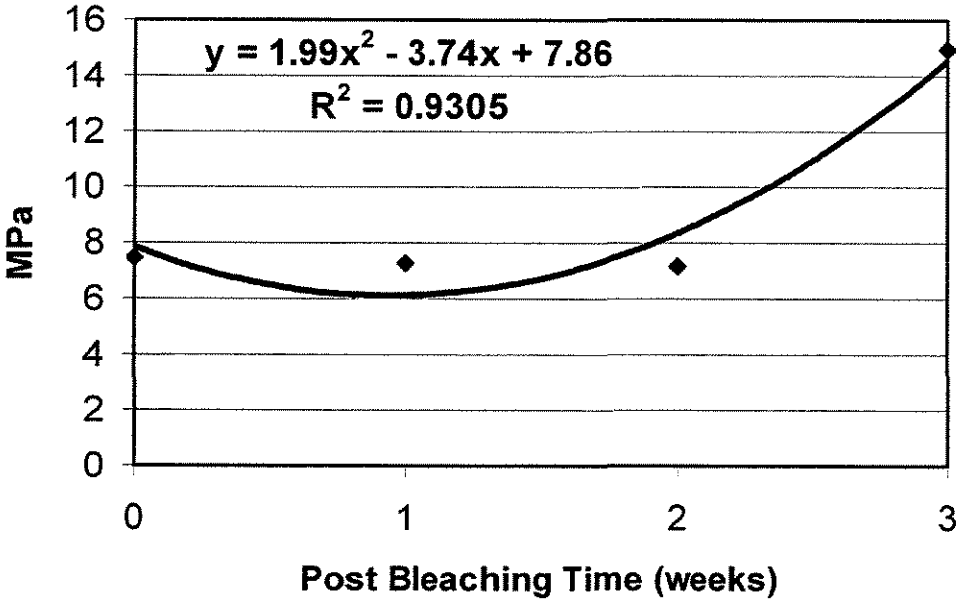

FIGURE 4

CONCLUSÃO GERAL

De acordo com as condições experimentais adotadas nos cinco capítulos apresentados, pode-se concluir que:

1. Diferentes concentrações de peróxido de carbamida – 10%, 16%, 15% e 20% - produzem alterações micromorfológicas no esmalte clareado, sendo que as mudanças na morfologia superficial foram diretamente proporcionais à concentração do agente clareador utilizado. O condicionamento com ácido fosfórico a 37% provocou maior efeito desmineralizante no esmalte, e baixas concentrações de peróxido de carbamida produziram efeitos similares aos produzidos pelo refrigerante de cola e fluoreto de fosfato acidulado.

2. Baixas concentrações de peróxido de carbamida, utilizadas de acordo com o protocolo clínico, podem aumentar a rugosidade superficial do esmalte, dependendo do agente clareador.

3. O gel de peróxido de carbamida em concentrações variando de 35 a 37%, normalmente empregado no tratamento em consultório, também provoca alteração na morfologia superficial do esmalte clareado, aumento da rugosidade da superfície e maior susceptibilidade à pigmentação.

4. O esmalte tratado com peróxido de carbamida a 10%, 15%, 16%, 20% apresenta menor resistência à tração que o esmalte não clareado.

5. Ocorre diminuição da resistência de união do compósito resinoso na superfície do esmalte tratado com baixas concentrações de peróxido de carbamida: 24 horas, 1 e 2 semanas após o clareamento Entretanto, após 3 semanas do término do clareamento, os valores de união do esmalte clareado são estatisticamente iguais ao do esmalte não tratado.

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ANEXOS

ANEXO 1

URBAN & FISCHER

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Journal Ästhetische Zahnmedizin
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Dr. V. Cavalli, DDS, MS
University of Campinas, Piracicaba School of
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Avenida Limeira 901
Piracicaba, SP, 13414-018
Brazil

Tel.: 089/5383-403
p.muenzel@urbanfischer.de
München, 14. November 2001

Dear Dr. Cavalli,

The „Journal Ästhetische Zahnmedizin, Parodontologie und Implantologie" informs German dentists about the latest influences, techniques and methods in the State of Art in Dentistry. It does not only attract with excellent articles written by international and national experts, but catches the readers attention with its appealing, fresh layout. It appears with five issues a year in the most honourable publishing house Urban&Fischer - a house formed of two of Germany`s oldest scientific publishing houses.

Dear Dr. Cavalli, we would be very honored to publish your results as read in:

„The Effect of Elapsed Time Following Bleaching on Enamel Bond Strength of Resin Composite"
Operative Dentistry 26 (2001), 597-602

since I found your knowledge outstanding and therefore do not want to withold it from our German readers. In order not to conflict with copyrights we would appreciate if you could support the article with some new pictures. We would care about the translation in German.

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I am very much looking forward to hearing from you and remain
Sincerely yours

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ANEXO 1

Marcelo Giannini

De: Münzel, Petra <P.Muenzel@urbanfischer.de>
Para: 'Giannini, Marcelo' <giannini@fop.unicamp.br>
Enviada em: quarta-feira, 24 de julho de 2002 05:08
Assunto: Your manuscript for the Journal Ästhetische Zahnmedizin

Dear Dr. Giannini,

Your article arrived today - thank you very much. Probably it will appear in issue 1/2003. I will tell you, as soon as the date is fixed.

Best regards

Petra Münzel-Kaiser
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ANEXO 2

CONGRESSO DA SOCIEDADE BRASILEIRA
DE MICROSCOPIA E MICROANÁLISE

São Carlos, 24 de setembro de 2001.

Prezado(a) Senhor (a) Vanessa Cavalli

Vimos informar que o Comitê Científico do XVIII Congresso da Sociedade Brasileira de Microscopia e Microanálise – CSBMM2001 analisou o resumo estendido abaixo identificado, submetido por V.Sa. para publicação em suplemento da revista *Acta Microscópica*, tendo considerado que o mesmo pode ser aceito para publicação **após realizadas as alterações ou correções indicadas pelo assessor.** O trabalho será apresentado como pôster no Congresso.
Informamos também, que seu trabalho poderá ser aceito para publicação como um *Trabalho Completo na referida revista* (com até seis páginas), caso seja de seu interesse.
Neste caso, o trabalho completo deverá ser submetido até o próximo dia 08/janeiro/2002 e somente será publicado após nova revisão pelo Comitê Científico. As normas para publicação na revista serão colocadas na homepage do evento: http://www.nit.ufscar.br/csbmm
A forma de encaminhamento será a mesma descrita a seguir para o resumo estendido revisado.
Encaminhamos em anexo seu resumo estendido juntamente com o parecer do assessor, aguardando o envio da versão final do mesmo. A versão final do trabalho(*) deverá ser enviada em uma via original impressa com qualidade laser de impressão e com fotos originais e também em disquete com o texto em Word até o término do Congresso. Favor ajustar brilho e contraste das imagens (JPG) no arquivo (.doc). Colocar no disquete o código do trabalho. Lembramos que esse material final poderá ser entregue diretamente na Secretaria do Congresso ou encaminhado, antecipadamente, para:

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Acompanhe pela homepage o horário de sua apresentação.

Cordiais saudações,

Prof. Dr. Alberto Moreira Jorge Júnior
Coordenador do XVIII CSBMM

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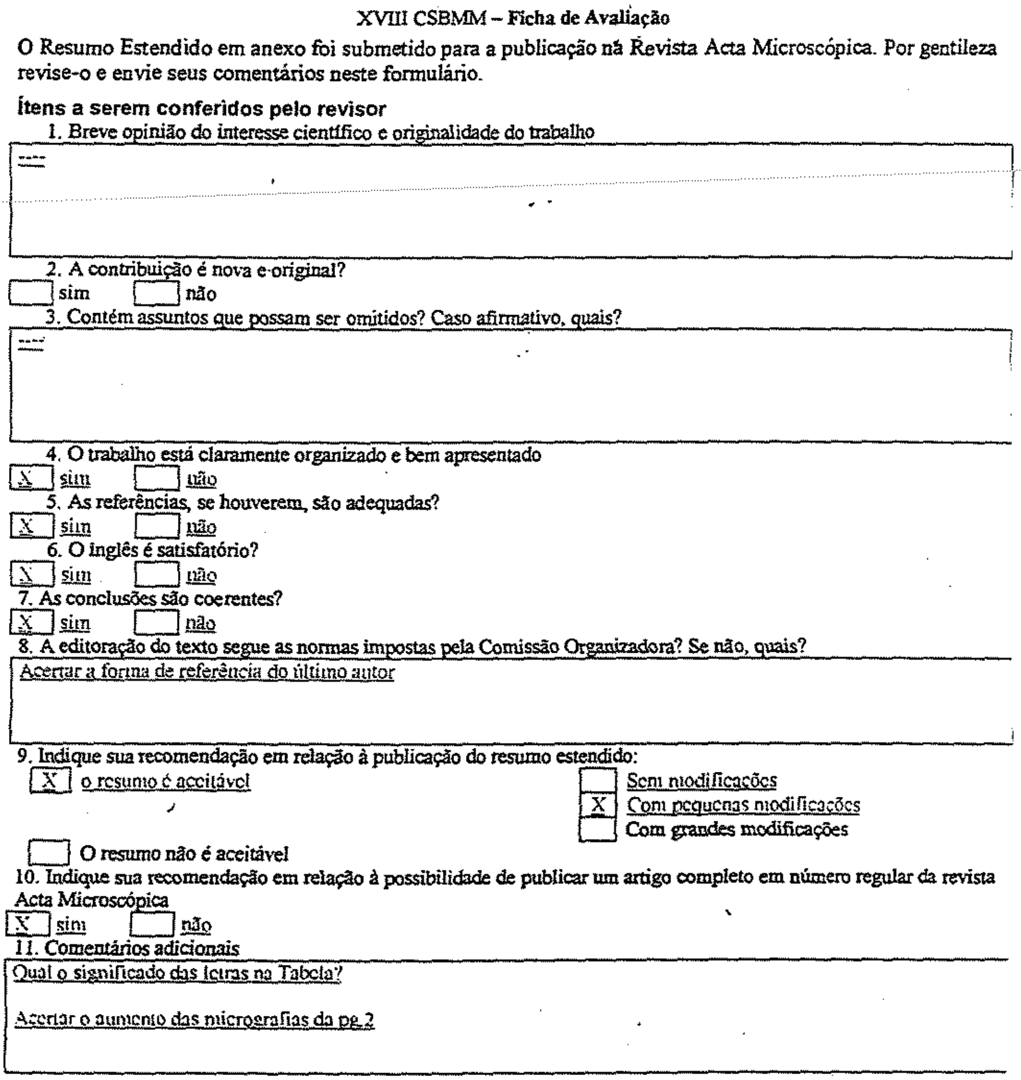
Título: SCANNING ELECTRON MICROSCOPY OBSERVATION OF HUMAN BLEACHED ENAMEL SURFACE
Autores: *V. CAVALLI (1) ; C.A.G. ARRAIS (1); M. GIANNINI (1)

(*) Veja mais informações na homepage: http://www.nit.ufscar.br/csbmm

ANEXO 2



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2. A contribuição é nova e original?

☐ sim ☐ não

3. Contém assuntos que possam ser omitidos? Caso afirmativo, quais?

4. O trabalho está claramente organizado e bem apresentado

☒ sim ☐ não

5. As referências, se houverem, são adequadas?

☒ sim ☐ não

6. O inglês é satisfatório?

☒ sim ☐ não

7. As conclusões são coerentes?

☒ sim ☐ não

8. A editoração do texto segue as normas impostas pela Comissão Organizadora? Se não, quais?

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ANEXO 3

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14th February 2002

Dear Professor Giannini

Re : " High concentrated carbamide peroxide bleaching agents effects on enamel surface "

Thank you for sending the above manuscript to the Journal. I can now report that the paper is acceptable for publication in the Journal. There are some minor changes required with regard to style :-

a) Title page - please add " running title " and also " key words (6) ".
b) Page 15 (references) - 2nd down - Haywood, FONDA & Robinson
- reposition Ledoux et al from page 16 to above Li.

Please let me have a hard copy of the revised script together with a new floppy disc which holds the complete revised manuscript. Also return the enclosed File Description Form. A Copyright Assignment Form also needs to be signed and this can be printed off the Journal Web Site.

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With best wishes,

Yours sincerely,

©*Operative Dentistry, 2001,* **26**, 597-602

The Effect of Elapsed Time Following Bleaching on Enamel Bond Strength of Resin Composite

V Cavalli • AF Reis
M Giannini • GMB Ambrosano

Clinical Relevance

Bond strength of resin composite to enamel can be significantly reduced after bleaching. However, the effects of carbamide peroxide on bond strength may be reversible and short-lived.

SUMMARY

Recent studies have concluded that carbamide peroxide bleaching agents significantly affect the bond strength of composite to bleached enamel. This study evaluated the effects of bleaching regimen with different carbamide peroxide concentrations and post-treatment times on composite bond strength to enamel. Two hundred and four flat buccal and lingual enamel surfaces obtained from erupted sound third molars were randomly divided into 17 groups (n=12). Sixteen experimental groups comprised the evaluation of four carbamide peroxide home bleaching agents (Opalescence 10%—20 % and Whiteness 10%—16%) and four time intervals after bleaching (one day, one, two and three weeks). Specimens of control group were not submitted to bleaching and were stored in artificial saliva at 37° C for 10 days. The specimens of experimental groups were exposed to one daily application of carbamide peroxide for six hours for 10 consecutive days. After each daily treatment and post-bleaching, the specimens were stored in artificial saliva solution. Bonds were formed with Scothbond MP and Z-100 composite resin, and shear bond test was carried out 24 hours after adhesive-composite application. Two-way ANOVA showed that the bond strengths were significantly different ($p<0.05$). For the first two weeks post-bleaching, the bond strengths of resin to enamel were low. After a lapse of three weeks, the bond strength returned to that of the untreated control group. Increased concentration did not prolong the time needed prior to bonding.

INTRODUCTION

Nightguard vital bleaching as an esthetic dentistry treatment was introduced by Haywood & Heymann (1989). Many dentists have indicated treating intrinsically discolored teeth with bleaching agents due to current interest in aesthetic dentistry (Chong, 1993; Lyons

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