ROBERTA TARKANY BASTING

Estudos 'in vitro' e 'in situ' do efeito de agentes clareadores contendo peróxido de carbamida sobre a microdureza de tecidos dentais hígidos e desmineralizados

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para a obtenção do título de Doutor em Clínica Odontológica, área de concentração em Dentística

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Orientadora: Profª Drª Mônica Campos Serra

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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 17 de Outubro de 2001, considerou a candidata ROBERTA TARKANY BASTING aprovada.

1. Profa. Dra. MONICA CAMPOS SERRA

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3. Prof. Dr. JESUS DJALMA PÉCORA

4. Prof. Dr. LUIZ ANDRE FREIRE PIMENTA

5. Prof. Dr. ANTONIO CARLOS PEREIRA

Dedicatória

"...porque não importa a distância, no coração sempre estarão perto;

não importam as diferenças, no coração sempre terão um ponto de acordo;

não importam as brigas, no coração sempre haverá um lugar para o perdão;

não importam as circunstâncias, sempre haverá um ombro para recostar, mãos par ajudar, olhos para enxergar e chorar de alegria e dor, bocas para expressar verdades e sorrir...".

(Autor desconhecido)

Ao meu marido, RICARDO, pela manifestação
plena do amor verdadeiro, forte, paciente e
compreensivo e com quem aprendi o
significado de amar e ser amada

Aos meus pais, ROBERTO e ELISABETA, pela
alegria de sua presença, compreensão, estímulo
e confiança no meu trabalho.

Aos meus irmãos, ROBERTO E ROSANNA,
pela felicidade que trazem à minha vida.

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A Deus, que tem

proporcionado uma vida

tão maravilhosa

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Sumário

Resumo

A utilização do peróxido de carbamida nas técnicas de clareamento dental caseiro vem se tornando cada vez mais freqüente nos consultórios odontológicos por ser um método simples e eficaz para minimizar ou remover manchas intrínsecas ou extrínsecas dos dentes. Devido ao íntimo contato do agente clareador com os tecidos dentais por um prolongado período de tempo e às características inerentes aos componentes produto, alterações da microdureza superficial do esmalte e dentina podem ser esperados. Desse modo, os objetivos deste trabalho, composto por cinco artigos científicos, foram: a) avaliar *in vitro* a microdureza do esmalte dental humano hígido submetido ao tratamento com dois agentes clareadores de peróxido de carbamida a 10% em diferentes tempos; b) avaliar *in vitro* a microdureza do esmalte dental humano hígido submetido ao tratamento com dois agentes clareadores de peróxido de carbamida a 10% associado à utilização de dois dentifrícios dessensibilizantes em diferentes tempos e em um período pós-clareamento; c) avaliar *in vitro* a microdureza da dentina humana hígida submetida ao tratamento com dois agentes clareadores de peróxido de carbamida a 10% em diferentes tempos e em um período pós-clareamento; d) avaliar *in vitro* a microdureza do esmalte dental humano hígido submetido ao tratamento com diferentes concentrações de agentes clareadores de peróxido de carbamida em diferentes tempos e em um período pós-clareamento; e) avaliar *in situ* a microdureza do esmalte e da dentina hígidos e desmineralizados submetidos ao tratamento com um agente clareador de peróxido de carbamida a 10%, pelo período de três semanas. De acordo com a metodologia utilizada, concluiu-se que diferentes agentes

clareadores com a mesma concentração apresentam diferentes efeitos sobre a microdureza superficial do esmalte dental humano hígido. A utilização de dentifrícios dessensibilizantes pode manter ou aumentar a microdureza do esmalte dental humano quando submetido ao tratamento com agentes de peróxido de carbamida a 10%. O peróxido de carbamida a 10% pode diminuir a microdureza superficial da dentina humana hígida em função do tempo; contudo, os valores de microdureza no período pós-clareamento são semelhantes aos valores iniciais devido ao efeito remineralizante da saliva artificial pelo tempo de 14 dias. Diferentes concentrações de peróxido de carbamida podem diminuir a microdureza do esmalte dental humano hígido em função do tempo, embora a saliva artificial apresente um efeito no aumento da microdureza no período pós-clareamento. No estudo *in situ*, os resultados sugerem que há alterações da microdureza do esmalte dental humano hígido e desmineralizado após o tratamento com o peróxido de carbamida a 10% pelo período de três semanas, embora tais alterações não ocorram para a dentina humana hígida e desmineralizada.

Abstract

Nightguard vital bleaching with carbamide peroxide agent has become the most frequent used treatment modality for improving esthetic appearance of teeth, because of its effectiveness and easy application for removing intrinsic and extrinsic stains from the teeth. As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extensive period of time and due to the characteristics of the components of the bleaching product, changes in enamel and dentin superficial microhardness can occur. This study, composed by five scientific articles, had the following objectives: a) to *in vitro* evaluate the microhardness of sound human enamel treated with two 10% carbamide peroxide bleaching materials at different time intervals; b) to *in vitro* evaluate the microhardness of sound human enamel treated with a 10% carbamide peroxide agent associated with two desensitizing dentifrices at different bleaching times; c) to *in vitro* evaluate the microhardness of sound human dentin treated with two 10% carbamide peroxide agents at different bleaching times; d) to *in vitro* evaluate the microhardness of sound human enamel exposed to different concentrations of carbamide peroxide agents at different bleaching times; e) to *in situ* evaluate the microhardness of sound and demineralized enamel and dentin submitted to treatment with a 10% carbamide peroxide for three weeks. According to the methodology employed in these studies, it was concluded that different carbamide peroxide bleaching materials with the

same concentration have different effects on the sound human enamel superficial microhardness. The use of desensitizing dentifrices associated with the treatment of a 10% carbamide peroxide bleaching agent could maintain or enhance the human enamel microhardness. Ten percent carbamide peroxide bleaching agents decrease the dentin microhardness over time, but 14 days after the completion of the treatment, the baseline microhardness values are recovered due to the remineralizing effect of artificial saliva. Different concentrations of carbamide peroxide bleaching agents decrease the enamel microhardness over time, but the artificial saliva presented a remineralizing effect after 14 days of the completion of the treatment, increasing the enamel microhardness. In the *in situ* study, the treatment with 10% carbamide peroxide agent can alter the microhardness of sound and demineralized enamel, although it does not seem to affect the microhardness of sound and demineralized dentin.

I) Introdução

Desde a sua introdução por Haywood & Heymann, em 1989, o clareamento de dentes vitais com peróxido de carbamida a 10% tem sido bastante utilizado por ser um procedimento simples e efetivo para a remoção de manchas intrínsicas e extrínsicas (Haywood, 1992; Haywood, 1994). O protocolo clínico emprega a utilização do produto clareador em uma moldeira individual pelo período de 8 horas diárias – enquanto o paciente dorme – num intervalo de tempo estimado de clareamento satisfatório entre 2 a 6 semanas (Haywood & Heymann, 1989; Goldstein & Garber, 1995).

Diferentes produtos e sistemas de clareamento dental foram introduzidos desde 1989, tais como a utilização em consultório do peróxido de hidrogênio a 35%, peróxido de carbamida a 37% e dos produtos chamados *over the counter*[1] (Haywood, 1992; Haywood & Robinson, 1997). Entretanto, o peróxido de carbamida parece apresentar maior efetividade (Christensen, 1998; Russell & others, 1996) e segurança (Berry, 1990; Curtis & others, 1996[a]; Curtis & others, 1996[b]), além da aprovação da *American Dental Association (ADA)* para alguns produtos na concentração a 10% (Haywood, 1993; Haywood & Robinson, 1997). Variações da técnica também foram apresentadas, incluindo a utilização do peróxido de carbamida nas concentrações entre 15 a 22% (Leonard, Sharma & Haywood, 1998; Oltu & Gurgan, 2000). O carbopol e a glicerina aumentam a aderência do agente clareador sobre

[1] *over-the-counter* é um termo em inglês que se refere aos produtos para clareamento dental que estão disponíveis aos pacientes sem a necessidade de controle supervisionado pelo cirurgião dentista. São também chamados de *produtos sobre a prateleira*, segundo Baratieri (comunicação pessoal).

a estrutura dental e permitem um maior tempo de liberação do componente ativo (Haywood, 1994; McCracken & Haywood, 1995).

Uma vez que o procedimento de clareamento vital envolve o contato direto entre a superfície das estruturas dentais com o agente clareador por um prolongado período de tempo, muitos estudos têm avaliado os efeitos do peróxido de carbamida a 10% sobre a micromorfologia superficial. Nas análises de microscopia eletrônica de varredura, foram observadas alterações em esmalte (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst, Marroquin & Willershausen-Zonnchen, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McGuckin, Babin & Meyer, 1992; Shannon & others, 1993; Smidt & others, 1998; Zalkind & others, 1996) e em dentina (Zalkind & others, 1996), com a presença de poros com diâmetros aumentados, erosões e rugosidade superficial.

Entretanto, não somente a micromorfologia dos tecidos dentais pode ser afetada pelos agentes clareadores. Alterações do conteúdo mineral do esmalte e da dentina devem ser avaliadas devido às propriedades ácidas desses materiais (Ben-Amar & others, 1995; Ernst & others, 1996; Leonard, Bentley & Haywood, 1994; Murchinson, Charlton & Moore, 1992; Smidt & others, 1998; Zalkind & others, 1996). Perda do conteúdo mineral ou desmineralização altera a microdureza do esmalte e da dentina (Featherstone & others, 1983; Rotstein & others, 1996), apesar da presença da saliva, de fluoretos ou de outras soluções remineralizantes serem capazes de manter o equilíbrio entre os processos de desmineralização e remineralização.

Alguns estudos *in vitro* mostram alterações da microdureza e do conteúdo mineral do esmalte após a exposição aos agentes clareadores de peróxido de carbamida (McCracken

& Haywood, 1995; McCracken & Haywood, 1996; Rotstein & others, 1996; Smidt & others, 1998). Attin & others (1997) observaram que, mesmo com o uso concomitante de uma solução para bochecho fluoretada ou de verniz fluoretado, uma significativa diminuição dos valores de microdureza foi encontrada. Nos capítulos I e IV, os trabalhos *"Effects of 10 % carbamide peroxide bleaching materials on enamel microhardness"* e *"Effects of seven carbamide peroxide bleaching agents on enamel microhardness at different time intervals"* foram realizados com o objetivo de se avaliar o efeito do peróxido de carbamida sobre a microdureza do esmalte dental hígido. O efeito da associação de agentes dessensibilizantes durante e após a aplicação do agente clareador foi verificado no capítulo II, com a condução da pesquisa *"Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals"*.

Avaliações da microdureza da dentina também foram realizadas por Nathoo, Chmielewski & Kirkup (1994), mostrando não haver alterações da microdureza ao se utilizar o peróxido de carbamida a 10%. Entretanto, Pécora & others (1994) e Rotstein & others (1996) observaram significativas alterações da microdureza e do conteúdo mineral da dentina ao se utilizar tais agentes clareadores. Nesse sentido, o trabalho *"Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals"* foi conduzido para averiguar o efeito do peróxido de carbamida a 10% sobre a microdureza da dentina hígida, apresentado no capítulo III.

O efeito desses materiais clareadores sobre lesões iniciais de cárie também é desconhecido, uma vez que o diagnóstico da lesão precoce de cárie é difícil ou não

interpretável pela maioria dos cirurgiões dentistas. Além disso, o efeito remineralizante da saliva, a presença de fluoretos na cavidade bucal e o controle de placa realizado pelo paciente podem interferir na degradação e nos efeitos do peróxido de carbamida. Desse modo, um estudo *in situ* intitulado *" The effect of 10 % carbamide peroxide on the microhardness of sound and demineralized enamel and dentin 'In situ'"* foi realizado para se avaliar o efeito de um agente clareador sobre a microdureza do esmalte e da dentina hígidos e desmineralizados quando inseridos na cavidade bucal, sendo apresentado na capítulo V.

Apesar de estudos laboratoriais não substituírem as condições clínicas do meio bucal, estudos *in vitro* e *in situ* devem ser conduzidos por permitirem a verificação de algumas propriedades dos tecidos dentais mineralizados de uma maneira mais exeqüível e menos onerosa.

II) Proposição

Este trabalho, composto por cinco artigos científicos, apresentou como objetivo geral avaliar a microdureza de tecidos dentais hígidos e desmineralizados submetidos ao tratamento com agentes clareadores de peróxido de carbamida através de modelos de estudo *in vitro* e *in situ*. Os objetivos específicos foram avaliar a microdureza:

A) do esmalte dental humano hígido submetido ao tratamento *in vitro* com dois agentes clareadores de peróxido de carbamida a 10% em diferentes tempos;

B) do esmalte dental humano hígido submetido ao tratamento *in vitro* com dois agentes clareadores de peróxido de carbamida a 10% associado à utilização de dois dentifrícios dessensibilizantes em diferentes tempos e em um período pós-clareamento;

C) da dentina humana hígida submetida ao tratamento *in vitro* com dois agentes clareadores de peróxido de carbamida a 10% em diferentes tempos e em um período pós-clareamento;

D) do esmalte dental humano hígido submetido ao tratamento *in vitro* com diferentes concentrações de agentes clareadores de peróxido de carbamida em diferentes tempos e em um período pós-clareamento;

E) do esmalte e da dentina hígidos e desmineralizados submetidos ao tratamento *in situ* com um agente clareador de peróxido de carbamida a 10%, pelo período de três semanas.

Capítulo I

Effects of two 10 % percent carbamide peroxide bleaching materials on enamel microhardness at different time intervals

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JOSÉ AUGUSTO RODRIGUES; ROBERTA TARKANY BASTING; MÔNICA CAMPOS SERRA; ANTONIO LUIZ RODRIGUES JR

Effects of two 10 percent carbamide peroxide bleaching materials on enamel microhardness at different time intervals

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Abstract

Purpose: The objective of this *in vitro* study was to evaluate the enamel microhardness treated with two different 10% carbamide peroxide bleaching materials at different time intervals. *Materials and Methods*: Two bleaching agents were analyzed: Opalescence / Ultradent (OPA) and Rembrandt / Den-Mat Corporation (REM). The control group (CON) consisted of dental fragments maintained in artificial saliva. Bleaching agents were accomplished for eight hours per day and stored during the remaining diary time in an individual recipient with artificial saliva. Microhardness testing was performed before the initial exposure to the treatments and after 1, 7, 14, 21, 28, 35 and 42 days in the enamel tissue. *Results*: The Analysis of Variance, followed by the Bartlet and Tukey tests, showed significant differences for treatments ($\rho < 0.00001$) from the seventh to the forty-second day. From the seventh to the fourteenth day, OPA presented an increase of enamel microhardness over time while REM presented a decrease of microhardness. Statistical differences were not found between REM and the control group (OPA > CON = REM). From the twenty-first to the thirty-fifth day, enamel fragments bleached with OPA and REM presented a decrease of microhardness. Statistical differences of microhardness were verified among all the treatments (OPA > CON > REM). On the forty-second day, statistical differences were not found between OPA and the control group, but they were found between REM and the control group (OPA = CON > REM). The polynomial regression showed an increase of microhardness for OPA until the twenty-first day, followed by a decrease of microhardness up to the forty-second day. A decrease of microhardness for REM bleaching agent was verified. *Conclusions:* There were alterations in enamel microhardness as a function of bleaching time when using the two different 10% carbamide peroxide whiteners. Over a 42-day treatment time, bleaching with REM agent caused a decrease in enamel microhardness. The OPA agent initially increased the microhardness, then returned to the control level. Different bleaching materials with the same concentration of carbamide peroxide have different effects on the enamel.

Clinical significance: The potential effects caused by bleaching with 10% carbamide peroxide agents on the tooth structure must be known by dentists. The effects of bleaching agents on the enamel microhardness over time are important parameters to evaluate if a demineralization process is occurring.

Introduction

In the past decades, the demand for conservative aesthetic dentistry has dramatically grown and so has the rapid development of new nonrestorative treatments for discolored teeth[1]. Frequently, vital teeth present changes in color that substantially compromise the smile. As nightguard vital bleaching[1] has gained popularity with patients and dentists as a conservative technique to lighten natural teeth, laboratories have rapidly introduced bleaching products into the market[2]. Many of the newer systems contain 10% carbamide peroxide[3,4] with carboxypolymethylene polymer as a thickening agent to improve tissue adherence and allow for a time or sustained release of the whitening agent[5]. The original technique involves the application of the bleaching agent in a custom-fitted vinyl nightguard for 6 to 8 hours a night from 2 to 6 weeks[6].

The exact mechanism of bleaching is unclear. It is an oxidation reaction whereby the 10% carbamide peroxide in the presence of saliva releases 3% of hydrogen peroxide (which penetrates enamel and dentin to lighten the tooth) and 7% urea. The hypothesis of bleaching is that as the oxidizing agent diffuses through the interprismatic substance of the enamel, the highly pigmented carbon ring compounds are opened and converted into chains, which are lighter in color. Ideally, this is the point (the saturation point) at which whitening should be terminated[7,8]. As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extensive period of time, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. Ben-Mar *et al.*[9], Bitter (1992)[10], Bitter (1998)[11], Bitter & Sanders[12], Covington *et al.* (1990)[13], Covington *et al.* (1991)[14], Flaitz & Hicks[7], Josey *et al.*[15], McGuckin *et*

al.[16], Shannon *et al.*[17] and Zalkind *et al.*[18] reported changes in enamel surface morphology using SEM analysis with varying degrees of surface porosity and alteration. On the other hand, Ernst *et al.*[19], Haywood *et al.* (1990)[20] and Haywood *et al.* (1991)[21] showed no significant changes in surface morphology of human enamel using SEM evaluations.

Enamel microhardness evaluations after exposure to 10% carbamide peroxide under a variety of *in vitro* conditions have been reported[22,23,24]. Furthermore, a combined *in vitro-in vivo* study[17] demonstrated an increase in enamel microhardness due to a possible remineralization phenomenon of saliva. McCracken & Haywood (1995)[25], McCracken & Haywood (1996)[26] and Rotstein *et al.*[27] showed that teeth exposed to 10% carbamide peroxide lost calcium. Loss of mineral content from the outer tooth structure or demineralization alters enamel microhardness[28,29], even though saliva, fluorides or other remineralizing solutions can maintain the equilibrium between the phenomena of demineralization and remineralization.

The lack of conclusive evidence of the effects of bleaching agents on enamel microhardness suggests the need for additional research. Little is known about the long term consequences of bleaching agents on the enamel surface and the influence of saliva as a function of bleaching time on human enamel treated with different 10% carbamide peroxide materials.

Therefore, the purpose of this *in vitro* study was to evaluate the enamel microhardness treated with two different 10% carbamide peroxide materials at different bleaching times.

Materials and methods

1) Experimental design

The factors under study were:

A) "Treatment" at three levels: Opalescence / Ultradent, Rembrandt / Den-Mat Corporation (experimental group) and artificial saliva (control group);

B) "Time" at eight levels: 0, 1, 7, 14, 21, 28 , 35 and 42 days.

The experimental units consisted of 63 dental fragments, randomly and evenly assigned to the three different treatments. The experimental group was treated with the bleaching agents 8 h per day while the control group remained in artificial saliva. Repeated measurements of microhardness were taken from each specimen at specific times.

The response variable was microhardness evaluated quantitatively. With "time" an independent variable, it was possible to obtain a response-surface curve by means of linear models[30]. The study diagram is illustrated in Figure 1.

2) Preparation of the dental fragments

Twelve unerupted third molars freshly extracted were used. After extraction, the teeth were kept in 2% formaldehyde (pH 7.0). The teeth were submitted to a soft-tissue debridement with periodontal curettes and cleaned with a slurry of pumice stone in a webbed rubber cup in a slow-speed handpiece. The roots were removed approximately 2 to 3 mm apical to the cementoenamel junction. The crowns were sectioned longitudinally to obtain dental slabs (4mm x 4mm x 3mm) using a double-faced diamond disc, producing 63 dental fragments.

The dental fragments were individually embedded in polystyrene resin in a ring mold, allowing only one side of the dental fragment left unsealed by the polystyrene resin. The specimens were serially polished by means of 400, 600 and 1000 grade sandpaper. These procedures were conducted to form parallel planar surfaces for the microhardness tests.

An uniform $7mm^2$ area of exposed enamel was created on the specimens by covering the remaining dental fragment with a nail varnish. The specimens were randomly allocated to the "treatment" groups (n = 21).

3) Specification of the bleaching materials

Two commercial bleaching agents were investigated: Opalescence (batch number: 2HQB/ Ultradent Co, South Jordan, UT, USA) and Rembrandt (batch number: 001059200/ Den-Mat Corporation, Santa Maria, CA, USA). These materials are ADA approved, syringe delivered 10% carbamide peroxide base glycerin gel and do not contain fluoride.

4) Exposure of the dental fragments to the bleaching materials

The specimens in the experimental group were treated with the bleaching agents 8 h per day for a total 42 days, by covering the dental fragments with 0.02 ml of each bleaching material. After the bleaching period, the carbamide peroxide gel was removed under running deionized and distillated water. During the remaining diary time, the fragments were individually kept in 20 ml of artificial saliva.

The specimens in the control group were kept in 20 ml of artificial saliva 24 h per day. The artificial saliva was daily changed after washing the dental fragments under running deionized and distillated water. During these cycles, the experimental and control specimens were kept in humid atmosphere at 37^0 C. The artificial saliva used was proposed by Featherstone *et al.*[31], as described by Serra & Cury[32].

5) Microhardness tests

Microhardness measurements were performed before the initial exposure to the treatments and after 1, 7, 14, 21, 28, 35 and 42 days. The tests were conducted immediately following 8 hours of bleaching for the experimental groups. For the control groups, the dental fragments were removed from the storage and tested just before changing the artificial saliva.

Knoop microhardness was measured keeping the long axis of the diamond perpendicular to the outer enamel surface using a microhardness tester (Future Tech Corporation- FM-1e, Tokyo, Japan). Three indentations at each specimen were made with 50g load applied for 20 seconds at each time. The microhardness measurements were taken on the subsurface enamel from the cut section of each dental fragment with each indentation randomly located 100, 200 and 300 μm from the outer enamel surface to the enamel-dentin junction. Those since there were multiple tests times (eight test times of three indentations in each time), the distance between each indentation were 200μm from the occlusal to the cervical surface. One single indentation was measured only one time. The average of the three indentations was used as the value for each time period.

6) Statistical analysis

The statistical analysis considered the average of those three replicates in micrometers taken from each specimen, in each time. The average of the three indentations in each dental fragment was used to obtain the Knoop Hardness Number (KHN) by the following calculation:

$$KHN = \frac{14.23 \times 10^3 \times F}{d^2}$$

where F is the value of the applied load (in g) and d is the diagonal indentation (in μm).

Statistical analysis using the Analysis of Variance (ANOVA) for completely randomly design was employed, considering "treatment" in each "time". The method of decomposition of sum of square followed by Tukey test was applied for get pairwise comparisons among "treatment" in each "time". The data were analyzed by software STATA(STATA Software, Computing Resource Center, Santa Mônica, CA, USA)[33] .

Results

The ANOVA showed no significant differences for "treatment" up to the first day, but showed significant differences ($\rho = 0.000$) from the seventh to the forty-second day. Table 1 shows mean values for microhardness, number of specimens, standard deviation and the Analysis of Variance (ANOVA) for the control and experimental groups. Tukey test is also shown in table 1.

On the seventh and fourteenth days, there were not statistical differences in microhardness among the enamel fragments submitted to the bleaching with REM and the

control group. Enamel fragments bleached with OPA presented the highest values of microhardness within this period.

Statistical differences of microhardness were verified among all the treatments from the twenty-first to the thirty-fifth day. Enamel specimens bleached with OPA presented the highest values of microhardness, followed by the control group and the fragments bleached with REM.

On the forty-second day, there were no statistical differences in microhardness among the enamel fragments submitted to the bleaching with OPA and the control group due to a decrease of microhardness of the specimens treated with OPA. Specimens bleached with REM differed from the others, presenting the lowest values of microhardness.

Linear regression was used to verify "time" factor (log-scale) in each "treatment". The mathematical model and the comparisons among the mean Knoop hardness for each bleaching material or artificial saliva in each time are presented in figure 2. The control group did not present significant differences in microhardness as a function of time, being represented as a constant function ($Y = 224.77$).

The linear regression illustrated the mathematical models that express the microhardness of the dental fragments of the control and experimental groups as a function of time. An increase in microhardness was noticed in the enamel fragments bleached with OPA up to the twenty-first day, followed by a decrease of microhardness up to forty-second day. For the control group specimens kept in artificial saliva, there were no significant differences of microhardness as a function of time (represented by a constant function). The

enamel fragments bleached with REM presented a decrease of microhardness as a function of time.

Discussion

Despite the beneficial effects on home-applied whitening agents in reducing or eliminating stains, the findings in the current study pose certain concerns with both carbamide peroxide gels evaluated. Many researchers have reported that changes in enamel surface are evident[7,9,10,11,12,13,14,15,16,17,18]. Although surface morphology was not a factor studied in this experiment, both positive and negative alterations in enamel microhardness were found with both products evaluated as a function of bleaching time.

In the original protocol prescribed by Haywood & Heymann[1], the average time recommended for optimal change color is 6 weeks, although slight effects may be noted as early as 2 weeks. Some manufacturers, however, instructed dentists to allow patients to use their own discretion to determine the duration of treatment, stating that "the product should be used until the desired result is achieved"[12].

By means of microhardness tests, the mineral profile of the enamel resulting from a demineralization and remineralization process could be obtained[28].

Demineralization of enamel structure occurs at a critical pH of 5.5[28,31,34]. In fact, the bleaching agent Rembrandt with a low pH between 4.9 to 5.2[17] induced a decrease of the microhardness values as compared to the control group from the twenty-first day to the forty-second day due to demineralization. The results of this investigation show that REM can cause a demineralization effect on enamel surface. Significant alterations of enamel

surface micromorfology were also observed by Shannon *et al.*[17] when using REM bleaching agent for four weeks. Haywood *et al.*[20], however, affirmed that other 10% carbamide peroxide solutions *in vitro* did not cause any significant changes in enamel surface morphology, regardless of pH levels. The present study employed storage in artificial saliva which contains calcium and phosphate ions that increase the remineralization potential and may approximate this condition to that found in oral environment[31,32,35,36]. It is possible that the remineralization effect was not observed because the intact enamel is less receptive to remineralization than demineralized enamel[34]. Furthermore, it was pointed out that a moderate low-pH bleaching solution *in vivo* reduces the pH of saliva in the mouth during the first 5 minutes and that after 15 minutes of treatment, the pH increases above baseline, probably attributed to the chemical reactions of neutralization of acidic carbamide peroxide by saliva[5]. A crucial shortcoming of this study is that we do not have exactly a measurement of the pH products. Also crucial to the clinical application of this data is that the pH in the mouth may rise[5] as cited above, but we neither know what the pH in this study does during 8 hours application, nor if the pH of the bleaching products behaves similarly.

As this study did not evaluate the microhardness in a post-bleaching period, with a potential for remineralization, it cannot demonstrate that the reported decrease in microhardness is a significantly clinical relevant problem that persists following the active bleaching phase. A recovery toward pretreatment microhardness values might be expected. Important factors such as salivary flow, buffering capacity of saliva, oral hygiene [11] and

the use of topical fluorides [7,37] may increase remineralization of bleached enamel. So, a remineralization effect could well be expected *in vivo*.

On the other hand, enamel fragments treated with OPA, with a pH reported between 5.5 to 6.5[9,18,19,23], presented an increase of microhardness as a function of bleaching time until the twenty-first day. After this period, there was a decrease of microhardness but not inferior to the microhardness found in the control group. It was also observed that, on the forty-second day, there were no statistical differences of microhardness between the specimens bleached with OPA and the control group. Murchison, Charlton and Moore[22] also showed no statistical differences in pre- or postbleaching microhardness values for OPA using artificial saliva as a storage agent. A significant decrease of hardness was found by Smidt *et al.*[23] for OPA product after 16 days. The present study used a longer period of bleaching time corresponding clinically with the prolonged time used by the patients. We can also point out that remineralization and demineralization processes are occurring during the entire evaluation time. Clinically speaking, the use of this bleaching material for 6 weeks does not cause an increase in tooth demineralization. The small decrease in enamel microhardness observed from 28 days through 42 days in this investigation could be the result achieved by the saturation point in bleaching. In this study, the decrease in microhardness did not seem to be enough to injure the enamel matrix because this decrease was not significantly different from the control group at the end of the experiment. It is unknown if more time had been spent to bleach the teeth with OPA – e.g. when patients want an "over" whitening – if a certain degree of damage could be achieved by affecting the enamel microhardness and surface morphology. This emphasizes that at-home

whitening agents require professional supervision to ensure correct selection of the bleaching agent, proper application, recommended amount of gel/paste, length of treatment and steps to prevent adverse reactions[7].

Conclusions

Even thought products have the same 10 % concentration of carbamide peroxide, other compositional factors of materials may alter their effects on tooth structure. In this study, one product caused an increase in microhardness while another caused a decrease as compared a control group.

Acknowledgements

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Table 1: Mean values for microhardness, standard deviation (SD), number of specimens and the Analysis of Variance (ANOVA) for control and experimental groups. Tukey test (represented by the letters) compared the factor "treatments" in each "time" (the subsets are specific for separate days).

Time	Measures	Control Group	Experimental Group		p
		Artificial Saliva	OPA	REM	
0	Mean	[a] 217,6	[a] 224,1	[a] 222,6	0,8959
	SD	39,2	50,4	50,9	
	n	21	20	20	
1d	Mean	[a] 233,7	[a] 239,8	[a] 218,8	0,4586
	SD	62,3	42,7	53,8	
	n	21	19	20	
7d	Mean	[a] 219,5	[b] 303,6	[a] 177,3	0,0000
	SD	48,1	49,5	38,0	
	n	21	20	20	
14d	Mean	[a] 204,1	[b] 287,4	[a] 166,0	0,0000
	SD	42,6	52,2	41,9	
	n	21	20	21	
21d	Mean	[b] 222,7	[c] 303,2	[a] 162,1	0,0000
	SD	41,2	47,42	32,4	
	n	20	20	20	
28d	Mean	[b] 216,9	[c] 279,5	[a] 134,3	0,0000
	SD	36,4	33,68	35,1	
	n	21	20	20	
35d	Mean	[b] 208,7	[c] 272,6	[a] 122,3	0,0000
	SD	39,2	37,01	47,5	
	n	21	20	21	
42d	Mean	[b] 215,9	[b] 255,4	[a] 130,8	0,0000
	SD	44,0	35,28	52,4	
	n	21	20	21	

Equal superscript letters horizontally indicate mean values that are not significantly different.

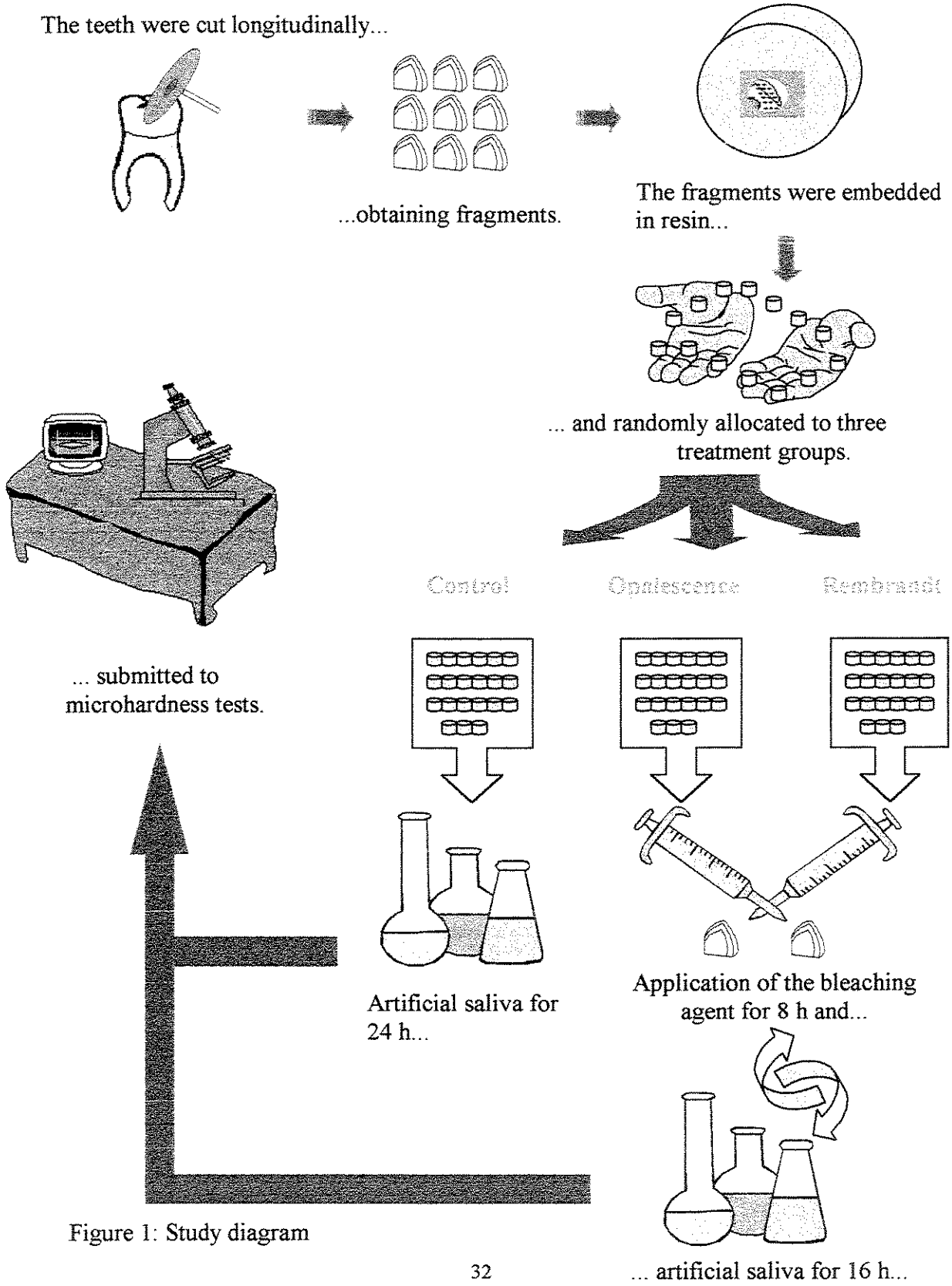

Figure 1: Study diagram

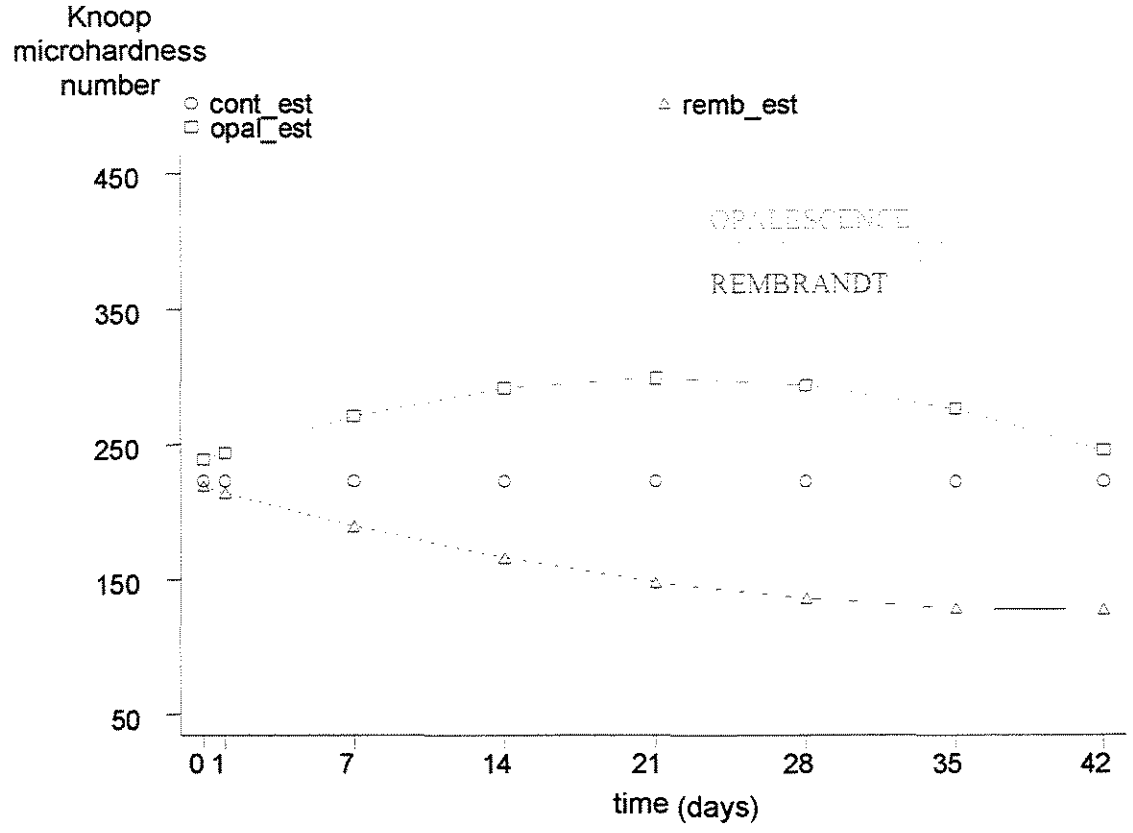

Figure 2: Mean Knoop microhardness for enamel fragments bleached with Opalescence, Rembrandt and for the control group as a function of time and the mathematical model.

Capítulo II

Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals

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Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals

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Abstract

Purpose: The aim of this *in vitro* study was to evaluate the microhardness of enamel submitted to the treatment of a 10% carbamide peroxide agent associated with two desensitizing dentifrices at different bleaching times. *Materials and methods*: A 10% carbamide peroxide bleaching agent was evaluated - Rembrandt 10%/ Den-Mat Corporation (REM) - and a placebo agent was used as a control group (PLA). The bleaching and the placebo agents were applied to the human enamel dental fragments for eight hours per day, followed by immersion for 5 minutes in a slurry solution of desensitizing dentifrices: Sensodyne/Stafford-Miller (S) or Sensodyne Fluor/Stafford-Miller (SF). During the remaining time, the enamel fragments were individually stored in 13.5 ml of artificial saliva. Knoop microhardness measurements were performed at baseline, 8 hours, 7, 14, 21, 28, 35 and 42 days of treatment and at 7 and 14 days of a post-treatment period. *Results:* The Analysis of Variance and Tukey test showed no differences in enamel microhardness for REM + SF (p-value=0.0688) and PLA + SF (p-value=0.9265) within each time interval. The dental fragments treated with REM + S and PLA + S showed an increase in microhardness values within each time interval (p-value<0.0001). There were significant differences among the treatment agents from the twenty-eighth day to fifty-sixth day. The use of 10% carbamide peroxide bleaching agent associated with a desensitizing dentifrice significantly increased the enamel microhardness values during the bleaching treatment and after 14 days after the completion of the treatment. After the post-treatment period, the enamel fragments treated with a placebo agent and with a 10% carbamide peroxide agent associated with a desensitizing fluoride dentifrice maintained the baseline values.

Clinical Significance: The use of desensitizing dentifrices associated with the treatment of a 10% carbamide peroxide bleaching agent could maintain or enhance human enamel microhardness.

Introduction

Nightguard vital bleaching has become the most frequently used treatment modality for improving the esthetic appearance of teeth, primarily because of its relative ease of application, the safety of 10% carbamide peroxide bleaching materials, reduced time with the patient in-chair, the lower cost and the high percentage of successful treatments[1].

The mechanism of bleaching is not clear, but it consists of an oxidation reaction of 10% carbamide peroxide that in contact with saliva and the oral fluids is dissociated into 7% urea and 3% hydrogen peroxide. The hydrogen peroxide diffuses through enamel and dentin and releases free radicals that can break the pigmented carbon rings of high molecular weight into smaller molecules which are lighter in color[2].

As the bleaching involves the direct contact of the whitening agent on the surface of the teeth for an extensive period of time and due to the acidic properties of these products[3-8], many studies have evaluated the potential adverse effects of these carbamide peroxide agents on the physical and chemical structure of the enamel. *In vitro* evaluations have reported alterations in microhardness[7, 9-12] and in the micromorphology of the enamel after exposure to 10% carbamide peroxide[3, 4, 7, 8, 13-18].

Microhardness changes are related to a loss or gain of mineral (demineralization or remineralization) of the dental structure[19]. The first defense mechanism capable of neutralizing the acids brought about by the low pH of the bleaching agents is the saliva buffering capacity through bicarbonates that provides a dilution and neutralization of the acids[20].

However, the side effects of the use of bleaching agents cannot be related only to the physical and chemical structure of the teeth, but also to the development of tooth sensitivity[1, 21]. Occasional tooth sensitivity associated with nightguard vital bleaching is attributed to the passage of the hydrogen peroxide through the enamel and dentin to the pulp, resulting in mild irritation[1, 21, 22]. This ceases on termination of the treatment and can be modulated by the use of anti-inflammatory or pain medications, fluorides or desensitizing materials in the bleaching tray and by desensitizing dentifrices[1].

Desensitizing dentifrices, as well as fluoride dentifrices, seem to be a practical and efficient method for reducing tooth sensitivity[23-26, 27, 28]. Due to the components of the dentifrices, an obliteration of the dentin tubules can be obtained[26, 28-30]. The presence of fluorides in desensitizing dentifrices also act as a therapeutic agent[27, 28] that can maintain the equilibrium between demineralization and remineralization processeses[31]. The use of desensitizing dentifrices associated with the bleaching treatment with 10% carbamide peroxide agents seems to be important in avoiding tooth sensitivity and the demineralization of the enamel during the bleaching period.

The purpose of this *in vitro* study was to evaluate the microhardness of the enamel submitted to the treatment of a 10% carbamide peroxide agent associated with two desensitizing dentifrices during the bleaching treatment at different times and at a post-treatment period.

Materials and methods

1. Experimental design

The factors under study were:

1) Treatment agents (four levels): Rembrandt 10% + Sensodyne (REM + S), Rembrandt 10% + Sensodyne Fluor (REM + SF), placebo agent + Sensodyne (PLA + S), placebo agent + Sensodyne Fluor (PLA + SF).

2) Time (ten levels): baseline, 8 hours, 7, 14, 21, 28, 35, 42 days of treatment and at 7 and 14 days of a post-treatment period (corresponding to 49 and 56 days after the beginning of the treatment).

The experimental units consisted of 80 sound human enamel dental fragments, randomly and evenly assigned to the four different treatment agents (20 dental fragments per group). The Knoop microhardness response was evaluated by quantitative methods. Repeated measurements of Knoop microhardness were taken on the surface of each specimen at each time interval for a Split-Plot design.

2. Preparation of the dental fragments

Eighty freshly extracted non-erupted third molars were used. After extraction, the teeth were kept in 10% formaldehyde (pH 7.0). The teeth were submitted to a soft-tissue debridement with periodontal curettes and cleaned with a slurry of pumice stone in a webbed rubber cup at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil, 89221-040). The roots were removed approximately 2 to 3 mm apical to the cement-enamel junction. The crowns were longitudinally sectioned to obtain the dental fragments using

double-faced diamond discs (K.G.Sorensen, Barueri, SP, Brazil) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil). The fragments that presented stains or cracks after the observation on a stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan) were discarded. The size of the fragments was required to be larger than 4mm x 4 mm x 3 mm.

Eighty dental fragments were obtained and embedded individually in a self-curing polyester resin in a PVC ring mold of 2.0 cm in diameter, with the external surface of the enamel exposed. The resin was left to polymerize for 24 hours, the molds were removed and all the external surfaces of the dental fragments were leveled by a water-cooling mechanical grinder (Maxgrind/ Solotest, São Paulo, SP, Brazil). Aluminum oxide discs were used in a sequential granulation of 400, 600 and 1000 grit (Carborundum/ 3M do Brasil Ltda., Sumaré, SP, Brazil) refrigerated with water. The polishing was performed with polishing cloths (Arotec Ind. e Com. Ltda., Cotia, SP, Brazil) and diamond pastes of 6, 3, 1 and ¼ μm (Arotec Ind. e Com. Ltda., Cotia, SP, Brazil) refrigerated with mineral oil (Arotec Ind. e Com. Ltda., Cotia, SP, Brazil). The polishing was considered satisfactory when there were no scratches on the dental surface after observation on a stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan). These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests.

A uniform area of $9mm^2$ (3mm x 3 mm) of exposed enamel was created on the specimens by covering the remaining dental fragment with 2 coatings of nail varnish (Colorama/ CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil). Afterwards, the eighty

specimens were randomly distributed to the four treatment agents, with 20 dental fragments for each group.

3. Specification of the materials

A 10% carbamide peroxide bleaching agent recognized by the American Dental Association (ADA)[32] was evaluated: Rembrandt 10%/ Den-Mat Corporation. The pH level of the bleaching agent was measured by using a pH meter (Procyon, SA 720, São Paulo, SP, Brazil, 04530-970).

The control group consisted of a placebo agent prepared with carbopol and glycerin. The color and consistency of the placebo agent was similar to the bleaching agent, but it was pH neutral and had no carbamide peroxide. Table 1 presents the composition, batch number, pH level and the manufacturer of the bleaching and placebo agents.

Sensodyne/ Stafford Miller and Sensodyne Fluor/ Stafford Miller were used as desensitizing dentifrices. Table 2 presents the composition, batch numbers and the manufacturer of the desensitizing dentifrices.

4. Exposure of the enamel fragments to the treatment agents

Previous to the treatment period, an individual tray was manufactured for each specimen using a 0.4 mm thick flexible ethyl vinyl acetate (EVA) polymer (Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000) in a vacuum forming machine (P7/ Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000).

The enamel fragments were submitted to the treatment with the bleaching or the placebo agents for 8 hours per day for a period of 42 days. During the treatment period, 0.02 ml of each agent were applied to each specimen using a syringe. The specimens were individually covered with the tray and soaked in individual closed containers with 13.5 ml of artificial saliva (pH=7.0), at 37° C. The artificial saliva consisted of a remineralization solution that was proposed by Featherstone *et al.*[20] and modified by Serra and Cury[33]. After 8 hours, the specimens were taken away from the storage media and the trays were removed. The treatment agents were washed from the surface of the enamel fragments under running distillated and deionized water for 5 seconds.

A 1:3 (w/w) dentifrice slurry of each desensitizing dentifrice in distillated and deionized water was made fresh within 30 minutes of each use. The specimens were individually immersed in 13.5 ml of slurry for 5 minutes and washed under running distillated and deionized water for 5 seconds.

During the remaining time (16 hours per day), the fragments were maintained in individual closed containers with 13.5 ml of artificial saliva (pH=7.0), at 37° C that was changed daily.

5. Post-treatment phase

After the 42 day treatment period, the specimens were maintained in their individual containers with 13.5 ml of artificial saliva (pH=7.0) at 37°C which was changed daily. The bleaching and the placebo agents were not applied, but the treatment with the desensitizing dentifrices continued for 14 days.

6. Microhardness tests

Microhardness measurements were performed before the initial exposure to the treatments (baseline values) and after 8 hours, 7, 14, 21, 28, 35 and 42 days and also at 7 and 14 days after the completion of the treatment period (corresponding to 49 and 56 days after the initial application of the treatment agents). A Knoop indentor was used, keeping the long axis of the diamond parallel to the outer enamel surface in a microhardness testing machine (Future Tech - FM-1e, Tokyo, Japan, 140). Three indentations on each specimen were made with a load of 25g applied for 5 seconds.

7. Statistical analysis

For the statistical analysis, the average of the three Knoop Hardness Numbers was taken. Statistical analysis was made by a parametric method using the Analysis of Variance, in a "Split-Plot" design (repeated measurements at the same experimental unit). The Tukey test (α=0.005) was used to compare the differences among ***treatment agents*** in each ***time*** interval at a 5% level of significance.

Results

Table 3 shows the mean Knoop microhardness values, standard deviation and the clusters to identify significant differences among the experimental treatments at each time interval. The Analysis of Variance (ANOVA) and Tukey test showed significant differences for REM + S and PLA + S (p-value<0.0001), but no differences in

microhardness for REM + SF (p-value=0.0688) and PLA + SF (p-value=0.9265) within each time interval. The use of a desensitizing dentifrice associated with a 10% carbamide peroxide bleaching agent or with a placebo agent increases the microhardness values of the enamel fragments overtime. Tukey test showed significant differences among the treatment agents from the twenty-eighth day to fifty-sixth day. Figure 1 shows the mean Knoop microhardness values of each treatment agent at different time intervals.

Discussion

Sensitivity caused by the bleaching treatment may be related to the easy passage of the hydrogen peroxide to the pulp, resulting in a reversible pulpitis[1, 21, 22]. Increased concentrations of carbamide peroxide, more frequent applications and the reduced water content of the bleaching agent may increase the side effect of tooth sensitivity, which is the major deterrent to bleaching success[1, 21]. The occurrence of severe sensitivity may result in termination of treatment by the patient prior to successful outcome[1].

A more important result of these observations is the option for the treatment with 10% carbamide peroxide, which can reduce the chances of developing tooth sensitivity by reducing the concentration of the bleaching material. When teeth are to be whitened, it would be prudent to use a product and a technique that are as efficacious as possible but cause minimal side effects. Lower concentrations of carbamide peroxide agents would result in fewer side effects than the gels with higher concentrations while providing a similar shade change[34].

Carbamide peroxide bleaching agents with a lower pH also have been implicated in the development of side effects by possibly removing minerals from the tooth[35]. Practitioners would not want to enhance the esthetic appearance of their patient's smile at the risk of developing tooth sensitivity and demineralizing enamel – which occurs at a critical pH of 5.5[20].

In this study, Rembrandt was used as a 10% carbamide peroxide bleaching agent. It contains carbopol and glycerin and the pH was 6.19. Others studies also reported that Rembrandt has a pH level ranging from 4.9 to 6.8[13, 36] showing that this product presents acidic properties. The acidic properties of 10% carbamide peroxide bleaching agents and the presence of glycerin and carbopol can affect the physical and chemical structure of the enamel[3, 4, 7-18] and these components are related to tooth sensitivity[1, 22, 35].

Among the methods of decreasing sensitivity during the whitening process[1], desensitizing dentifrices present certain advantages due to their frequency of application, cost and availability to the patients[23, 25, 27-29]. Although the decrease of tooth sensitivity during the bleaching treatment can only be assessed by *in vivo* studies, *in vitro* observations can be conducted to evaluate the effects of desensitizing dentifrices on dental structure submitted to the treatment with bleaching agents. It is possible that desensitizing dentifrices can help to inhibit the loss of mineral caused by the pH of 10% carbamide peroxide agent, the presence of carbopol and glycerin and the prolonged contact time between the agent and the enamel since they contain some components - strontium chloride, potassium nitrate or others – that could increase the enamel microhardness values.

A decrease in surface enamel microhardness and loss of mineral after the treatment with 10% carbamide peroxide bleaching agents has been demonstrated by McCracken and Haywood[9], Rotstein *et al.*[10] and Smidt *et al.*[7]. However, the effects of saliva or a remineralization solution and the use of dentifrices were not evaluated in these studies.

The use of artificial saliva during the bleaching treatment with Rembrandt 10% was evaluated in a previous study[12]. An initial decrease in enamel microhardness and a final increase of its values after 42 days was observed, showing that REM causes demineralization on enamel surface. However, the microhardness in a post-bleaching period, with a potential for remineralization, was not evaluated. It could not be demonstrated if the reported decrease in microhardness persists following the bleaching period. A recovery toward pretreatment microhardness values might be expected because important factors - such as saliva and the use of dentifrices - may increase remineralization of bleached enamel. Although Murchinson *et al.*[6] also used artificial saliva in their study, no statistical differences in pre- or postbleaching Knoop microhardness values for enamel treated with three bleaching agents and the control group (no treatment) were reported.

In this study, the microhardness of the enamel surface in a post-bleaching period and the concomitant use of desensitizing dentifrices during and after the bleaching treatment were evaluated when submitting human enamel fragments to 10% carbamide peroxide agent (REM). A desensitizing fluoride dentifrice was used as a control group to provide comparisons with the effects of a desensitizing dentifrice without fluoride. Immediately after the application of the treatment agents for 8 hours, there was a slight decrease in microhardness values that could be related to some mineral loss. The

remineralization effect can be verified during and after the treatment period. There was a significant increase in the microhardness values for the enamel treated with Sensodyne after 28 days and no significant differences in microhardness for the enamel treated with Sensodyne Fluor.

The desensitizing dentifrice without fluoride (Sensodyne) caused a significant increase in microhardness for REM and PLA. Sensodyne has 10% strontium chloride as an active ingredient that unites with the dental structure and occludes the lumen of dentin tubules[37]. It is also probable that strontium chloride or other components of Sensodyne could increase the enamel microhardness values or react with the sub-products of the carbamide peroxide reaction, enhancing the microhardness over time.

In this *in vitro* experiment, the most remarkable finding was that the Sensodyne Fluor used daily for 6 weeks in association with 10% carbamide peroxide bleaching agent or with a placebo agent did not increase the enamel microhardness as Sensodyne dentifrice did. Attin *et al.*[11] reported a decrease of the enamel microhardness values during bleaching treatment, even with the concomitant use of a fluoride mouthrinse solution and a fluoride varnish that are concentrated, high in fluoride materials. The use of fluoride dentifrices favors the formation of a calcium fluoride-like layer that is later dissolved, allowing fluoride to diffuse into the underlying enamel or saliva covering the tooth, supporting the remineralization of enamel[31, 38, 39]. However, as Sensodyne Fluor presents sodium fluoride as the active ingredient, it is also possible that this unstable component[40] reacted with the products of the degradation of the carbamide peroxide, inhibiting the remineralization potential of the fluoride and the increase of the enamel microhardness. The effects of

toothbrushing were not evaluated in this study and it is also possible that it could enhance the uptake of particulate material onto the dental surface as shown by Absi *et al.*[30], increasing the enamel microhardness.

Although there were differences in microhardness values when using Sensodyne dentifrice associated with a bleaching agent, these differences represent an increase in microhardness values over time that do not damage the enamel structure. When associating Sensodyne Fluor dentifrice, the bleaching treatment with 10% carbamide peroxide agent does not decrease the enamel microhardness.

Conclusions

The use of 10% carbamide peroxide bleaching agent associated with a desensitizing dentifrice significantly increased the enamel microhardness values during the bleaching treatment and after 14 days after the completion of the treatment. After the post-treatment period, the enamel fragments treated with a placebo agent and with a 10% carbamide peroxide agent associated with a desensitizing fluoride dentifrice maintained the baseline values.

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Table 1: Composition, batch number, pH level and the manufacturer of the bleaching and placebo agents.

Agents	*Composition**	*pH level*	*Manufacturer*
Rembrandt 10% ***batch number*** ***567708***	10% carbamide peroxide; glycerin; sodium citrate; carbopol; flavor; triethanolamine	6.19	Den-Mat Corporation, Santa Maria, USA
Placebo	5% glycerin; 1.2% carbopol 940	7.0	Mixed formula, Proderma - Pharmacy, Piracicaba, Brazil

* According to the manufacturer.

Table 2: Composition, batch numbers and the manufacturer of the desensitizing dentifrices.

Dentifrices	*Batch numbers*	*Composition*	*Manufacturer*
Sensodyne	003004; 003005; 909001	10% strontium chloride; distilled water; glycerin; Mirj; flavoring; paraben; sodium saccharine; titanium dioxide; silicon dioxide; sorbitol; calcium carbonate; cellosize; igpon; DC 28 red coloring.	Stafford-Miller Ind. Ltd., Rio de Janeiro, RJ, Brazil
Sensodyne Fluor	912027; 002038	5% potassium nitrate; sodium fluoride (1450 ppm); distilled water; sodium bicarbonate; glycerin; hydrated silica; silicon dioxide; sodium lauril sulfate; flavoring; cellosize; titanium dioxide; sodium saccharine.	Stafford-Miller Ind. Ltd., Rio de Janeiro, RJ, Brazil

Table 3: Mean Knoop hardness numbers (KHN), standard deviation and the results of the Tukey test at a 5% level of significance of each treatment agent at each time interval.

Time	Measures	Placebo		Rembrandt	
		S (n=20)	SF (n=20)	S (n=20)	SF (n=20)
baseline	**mean**	*A 101.79 a*	*A 90.28 a*	*A 94.25 ab*	*A 78.82 a*
	SD	*38.86*	*73.50*	*73.97*	*44.74*
8 hours	**mean**	*A 80.76 a*	*A 81.34 a*	*A 53.35 a*	*A 67.88 a*
	SD	*45.85*	*67.25*	*24.42*	*40.50*
7 days	**mean**	*A 82.19 a*	*A 89.00 a*	*A 68.46 a*	*A 71.47 a*
	SD	*34.55*	*76.83*	*43.92*	*51.68*
14 days	**mean**	*A 98.07 a*	*A 72.28 a*	*A 87.45 a*	*A 65.87 a*
	SD	*46.57*	*48.12*	*57.03*	*24.80*
21 days	**mean**	*A 100.12 a*	*A 69.18 a*	*A 105.29 ab*	*A 80.05 a*
	SD	*40.35*	*41.89*	*77.60*	*46.18*
28 days	**mean**	*AB 102.35 a*	*A 70.26 a*	*B 120.46 bc*	*AB 85.05 a*
	SD	*44.33*	*36.59*	*64.44*	*40.95*
35 days	**mean**	*AB 105.54 a*	*A 73.60 a*	*C 153.91 c*	*A 98.87 a*
	SD	*63.27*	*39.07*	*86.01*	*32.88*
42 days	**mean**	*A 110.45 a*	*A 72.93 a*	*B 163.03 c*	*A 104.23 a*
	SD	*54.33*	*37.22*	*119.03*	*36.68*
49 days	**mean**	*A 118.76 a*	*A 80.50 a*	*B 181.95 c*	*A 103.54 a*
	SD	*53.67*	*42.76*	*76.91*	*41.77*
56 days	**mean**	*B 129.10 a*	*A 76.00 a*	*C 208.34 d*	*AB 103.97 a*
	SD	*63.43*	*38.22*	*79.43*	*33.87*

- Equal letters indicate mean values that are not significantly different.
- Capital letters (on the left) represent the experimental treatment comparisons in each time (line); lower cases (on the right) represent the time comparisons in the same experimental treatment (column).

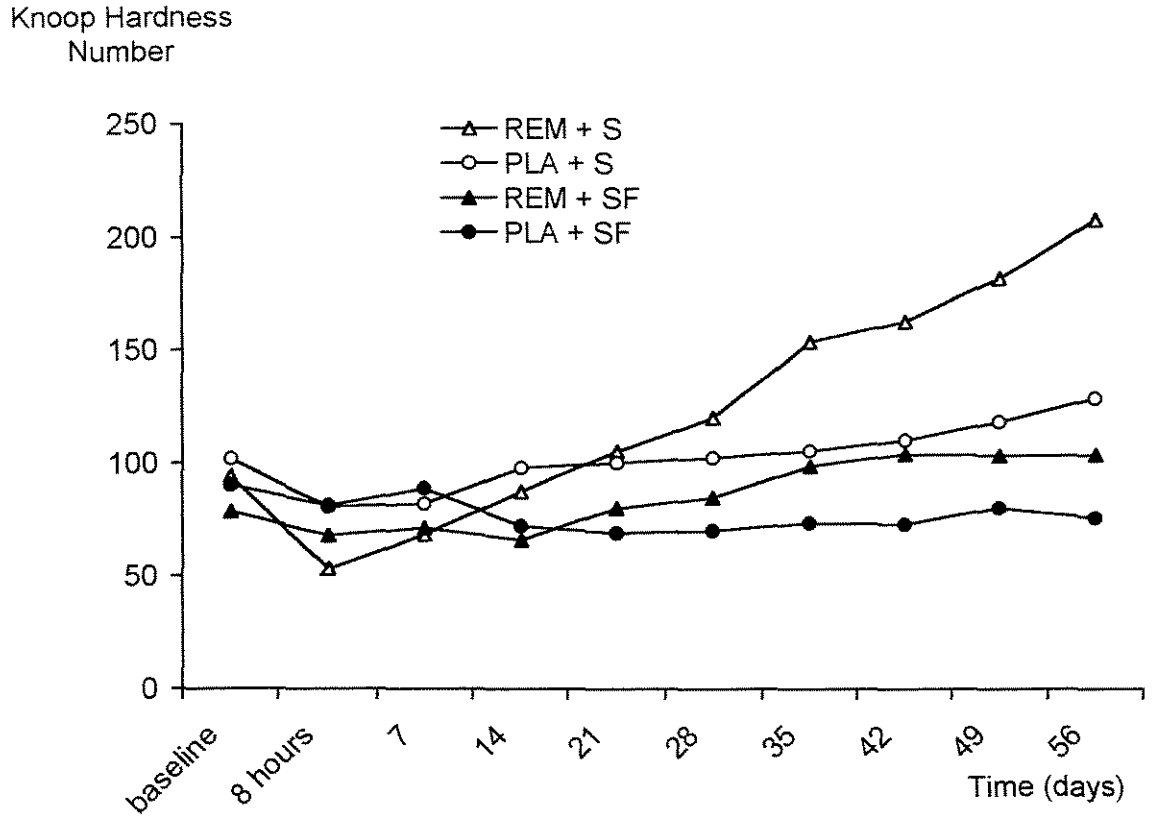

Figure 1: Mean Knoop microhardness values of each treatment agent at different time intervals.

Capítulo III

Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals

ENVIADO PARA PUBLICAÇÃO PARA *QUINTESSENCE INTERNATIONAL*

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Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals

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Abstract

Objectives: The purpose of this *in vitro* study was to evaluate the microhardness of the human dentin exposed to two 10% carbamide peroxide agents at different bleaching times. *Method and Materials:* Opalescence 10%/ Ultradent (OPA) and Rembrandt 10%/ Den-Mat Corporation (REM) were analyzed. A placebo agent was used as a control group (PLA). The bleaching and the placebo agents were applied on the surface of human dentin dental fragments for 8 hours and then stored in individual recipients with artificial saliva for the remaining time each day. Microhardness testing was performed at baseline, 8 hours, 7, 14, 21, 28, 35 and 42 days of the treatment and at 7 and 14 days of a post-treatment period. *Results:* The Analysis of Variance (ANOVA) and Tukey test showed differences in microhardness values for dentin treated with OPA, REM and PLA within each time interval ($p<0.0001$). There was a decrease in the microhardness values for both bleaching agents after 8 hours of treatment. Fourteen days after the completion of the treatment, the microhardness values for dentin surface treated with OPA and REM reached the baseline values, although the dental surface treated with PLA showed an increase in microhardness values at a post-treatment period. *Conclusions:* Ten percent carbamide peroxide bleaching agents decrease the dentin microhardness over time, but 14 days after the completion of the treatment in artificial saliva storage, the baseline microhardness values are recovered.

Key words: carbamide peroxide, bleaching agents, dentin, microhardness, hydrogen peroxide.

Clinical relevance: The 10% carbamide peroxide bleaching agents alter the dentin microhardness, but 14 days after the completion of the treatment, the artificial saliva presented a remineralizing effect.

Introduction

Nightguard vital bleaching has been suggested as an efficient and simple procedure in removing intrinsic and extrinsic stains from the teeth .[1, 2, 3]

Different products and systems have appeared on the market for in-office use such as 35% hydrogen peroxide or as over-the-counter products.[2] However, 10% carbamide peroxide bleaching agents are still the most utilized at-home bleaching technique, supported by several reports of their safety and effectiveness and by ADA approval.[4, 5]

As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extensive period of time[6] and reaches the dentin in areas of enamel defects or abrasions, exposed root surfaces, and the marginal areas between dentin and restorations, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. When using SEM evaluations, changes in enamel[7, 8, 9, 10, 11, 12, 13, 14 15, 16] and in dentin surface morphology [16] were reported.

Due to the acidic property of the bleaching agents, [7, 10, 15, 16, 17, 18] changes in the mineral content of the teeth can be expected. Loss of mineral content or demineralization alters enamel and dentin microhardness,[19, 20] even though saliva, fluorides or other remineralizing solutions can maintain the balance between the phenomena of demineralization and remineralization.

Some *in vitro* studies reported that there were no significant changes in enamel microhardness when using 10% carbamide peroxide agents, [18, 21, 22, 23] although others observed a decrease in microhardness values or mineral loss when using these bleaching agents.[15, 20, 24, 25, 26, 27] In a combined *in vitro-in vivo* study, an increase in enamel microhardness was demonstrated due to a possible remineralization by saliva.[14]

In dentin, Nathoo *et al.*[22] showed that there are no microhardness changes, although Pécora *et al.*[28] and Rotstein *et al.*[20] showed significant alterations in the microhardness and mineral content when using 10% carbamide peroxide agents. Since the observations in dentin are still controversial, there is a need for additional research of the effects of 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals.

The aim of this paper was to evaluate *in vitro* the dentin microhardness when exposed to two 10% carbamide peroxide bleaching materials and a placebo agent for 42 days of treatment and at 7 and 14 days of a post-treatment period.

Method and materials

1. Experimental design

The factors under study were:

1) Treatment agents: in three levels - Opalescence 10%/ Ultradent (OPA), Rembrandt 10%/ Den-Mat Corporation (REM), and a placebo agent (PLA) as a control;

2) Time: in ten levels - baseline, 8 hours, 7, 14, 21, 28, 35, 42 days of treatment and at 7 and 14 days at a post-treatment period (corresponding to 49 and 56 days after the beginning of the treatment).

The experimental units consisted of 60 sound human dentin dental fragments, randomly and evenly assigned to the three different treatment agents (20 dental fragments per group). Knoop microhardness response was evaluated by quantitative methods. Repeated measurements of Knoop microhardness were taken on the surface of each specimen at each time intervals considering a Split-Plot design.

2. Preparation of the dental fragments

Thirty freshly extracted non-erupted third molars were used. Immediately after extraction, the teeth were kept in 10% formaldehyde (pH 7.0). The crows were removed approximately to the cementoenamel junction and the roots were longitudinally sectioned to obtain 60 dental fragments using double-faced diamond discs (K.G.Sorensen, Barueri, SP, Brazil, 06454-920) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil, 89221-040). The apical third was discarded and only the cervical region was used. Care was taken not to leave the dental fragments dehydrated for a long period of time and then they were soaked in distilled and deionized water at 37° C.

The dental fragments size was required to be larger than 4mm x 4 mm x 3 mm and those fragments that presented stains or cracks after the observation on stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan, 356) were not used. The 60 dentin fragments were embedded individually in a self-curing polyester resin in a PVC ring mold

of 2.0 cm in diameter, with the external surface of the dentin exposed and the resin was left to polymerize for 24 hours. The molds were removed and the external surfaces of the dental fragments were leveled by a water-cooling mechanical grinder (Maxgrind/ Solotest, São Paulo, SP, Brazil, 01328-000). Aluminum oxide discs were used in a sequential granulation of 600, 1000, and 1200 grit (Carborundum/ 3M do Brasil Ltda., Sumaré, SP, Brazil, 13001-970) refrigerated with water. These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests.

A standardized area of $9mm^2$ (3mm x 3 mm) of exposed dentin was created on the specimens by covering the remaining dental fragment with two coatings of nail varnish (Colorama/ CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil, 05113-900).

3. Specification of the materials

In this study, two 10% carbamide peroxide bleaching agents were evaluated: Opalescence 10%/ Ultradent and Rembrandt 10%/ Den-Mat Corporation. The control group consisted of a placebo agent prepared with carboximethilcelulose and glycerin. The color and consistency of the placebo agent was similar to one of the bleaching agents (Opalescence 10%/ Ultradent), but the placebo was pH neutral and had no carbamide peroxide.

Table 1 presents the basic composition, lot number, pH level and the manufacturer of each treatment agent.

4. Exposure of the dental fragments to the bleaching materials

The dentin fragments were submitted to the treatment agents (experimental and control) for 8 hours per day for a period of 42 days.

An individual tray was manufactured for each specimen using a 0.4 mm thick flexible ethyl vinyl acetate (EVA) polymer (Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000) in a vacuum forming machine (P7/ Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000).

For the application of the treatment agents, 0.02 ml of each agent were applied to each specimen using a syringe. The specimens were individually covered with the tray and soaked in individual closed containers with 13.5 ml of artificial saliva (pH=7.0), at 37° C.

After 8 hours, the specimens were taken away from the storage media and the trays were removed. The treatment agents were washed from the surface of the dentin fragments under running distillated and deionized water for 5 seconds.

During the remaining daily time (16 hours per day), the fragments were maintained in individual recipients with 13.5 ml of artificial saliva (pH=7.0), at 37° C. The artificial saliva was daily changed. The artificial saliva used a remineralization solution proposed by Featherstone *et al.*[29] and modified by Serra and Cury.[30]

5. Post-treatment phase

After the 42 day treatment period, the specimens were maintained in their individual containers with 13.5 ml of artificial saliva (pH=7.0), at 37°C which was daily changed.

Microhardness tests were performed at 7 and 14 days after finishing the completion of the treatment period (49 and 56 days after the beginning of the treatment).

6. Microhardness tests

Microhardness measurements were performed before the initial exposure to the treatments (baseline values) and after 8 hours, 7, 14, 21, 28, 35 and 42 days and also at 7 and 14 days at a post-treatment phase (corresponding to 49 and 56 days after the initial application of the treatment agents). A Knoop indentor was used, keeping the long axis of the diamond parallel to the dentin surface in a microhardness testing machine (Future Tech - FM-1e, Tokyo, Japan, 140). Three indentations on each specimen were made with a load of 10g applied for 5 seconds at each time.

7. Statistical analysis

For the statistical analysis, the average of the three Knoop Hardness Numbers was taken. Statistical analysis was made by a parametric method using the Analysis of Variance, in a Split-Plot design (repeated measurements at the same experimental unit). The Tukey test (α=0.005) was used to compare the differences among treatment agents in each time interval at 5% level of significance.

Results

Graph 1 and Table 2 shows the mean Knoop microhardness values of each treatment agent at different time intervals and the results of the Tukey test at a 5% level of significance.

The Analysis of Variance (ANOVA) and Tukey test showed significant differences for each treatment agent within each time interval (p-value<0.0001). Since the Tukey test verified significant differences in microhardness values among the treatment agents at baseline, comparisons between PLA and OPA, OPA and REM and PLA and REM within each time interval were not carried out.

There was a decrease in the microhardness values of dentin treated with OPA and REM during the treatment period, although the baseline values were reached after 14 days of the completion of the treatment. For PLA, the microhardness values for dentin remained the same during the treatment period and there was an increase above baseline values in a post-treatment period.

Discussion

The chemistry of the carbamide peroxide bleaching agents is based upon its ability to generate free radicals, which are highly reactive. These free radicals – the hydrogen peroxide – are non-specific, extremely unstable, and can potentially react not only with the pigmented carbon rings,[6] but with other organic molecules to achieve stability. Other radicals can be generated and breakdown of the organic matrix can occur.[21]

For enamel, several studies were conducted to evaluate the potential effects of 10% carbamide bleaching agents.[14, 15, 18, 20, 21, 22, 23, 24, 26, 27]

The findings of this *in vitro* investigation shows that human dentin exposed to two 10% carbamide peroxide bleaching agents presents a decrease in the microhardness values over time. Pécora *et al.*[28] also showed a decrease in microhardness values when applying a 10% carbamide peroxide agent on dentin for 72 hours and Rotstein *et al.*[20] observed a significant reduction in the mineral content after immersing the dentin in the three 10% carbamide peroxide agents and incubating for 7 days. Immediately after the application of OPA and REM for 8 hours, there was a significant decrease in microhardness values that could be related to some mineral loss. Although the dental fragments were immersed in artificial saliva during the application of the bleaching agents, it was not enough to allow a remineralization effect during the first 8 hours of the treatment period. However, the artificial saliva should be enough to provide the dissociation of the bleaching agents due to the results obtained in this experiment. The remineralization effect was verified in the post-treatment period, when there was a significant increase in the microhardness values that reached the baseline values.

Comparisons between each treatment agent within each time interval could not be carried out probably due to the methodology employed in this experiment. Differences in the diameter of the dentinal ducts along the extension of the root and the procedures of leveling and polishing the dentin surface could be responsible for significant differences in microhardness values at baseline.

The pH of 10% carbamide peroxide bleaching agents is reported to range from 4.6 to 7.4.[7, 10, 13, 15, 16, 17, 18] In our study, the pH of the bleaching agents was 6.68 for OPA and 6.19 for REM, showing that the products evaluated present acidic properties that could affect the physical and chemical structure of the dentin. The major concern of this property is the dentin demineralization, which occurs at a pH lower than 6.5, while for enamel it is 5.5.[19, 29]

These acidic properties of the bleaching agents could be responsible for decreasing the dentin microhardness during the treatment period. Leonard *et al.*[17] pointed out that a moderately low-pH bleaching solution *in vivo* reduces the pH of saliva in the mouth during the first 5 minutes. After 15 minutes of treatment, the pH increases above baseline, probably attributed to the chemical reactions of neutralization of acidic carbamide peroxide by saliva. Although OPA and REM did not present a very low pH level, there was a significant decrease in dentin microhardness values. However, when using a placebo agent during the same period, there were no differences in dentin microhardness until the 42nd day of treatment. The placebo agent consisted of a neutral pH glycerin and carboxypolymethylene polymer solution and was considered a better choice to adequate the control group, providing equal hydration of the samples. Glycerin and carboximethilcelulose are inactive ingredients and the manufacturers do not make any claims about the action of those products. However, in a enamel microhardness evaluation comparing two 10% carbamide peroxide bleaching agents with and without carbopol, McCracken and Haywood[24] showed a significant decrease in microhardness in the outer 25 μm of the enamel surface after the treatment with the product containing carbopol. This

Continuing education

Choose the correct item for each question:

1) Ten percent carbamide peroxide bleaching agents:

a) decrease the enamel and dentin microhardness during the treatment period;

b) alter the pH of the dental structure;

c) increase dentin microhardness over time;

d) increase dentin microhardness over time depending on the amount of carbopol and glycerin of the bleaching agent used.

Mark true (T) or false (F) for each question:

2) Changes in dentin microhardness when using 10% carbamide peroxide agents are related to:

a) (T) pH of the bleaching agent;

b) (T) the presence of carbopol and glycerin;

c) (T) the presence of remineralizing solutions or saliva;

d) (F) the presence of fluoride incorporated into the structure of dentin.

3) Ten percent carbamide peroxide agents associated with the use of remineralizing solutions, as artificial saliva, can affect dentin by:

a) (T) decreasing the microhardness during the treatment period;

b) (F) increasing the microhardness after exposing dentin in artificial saliva for 8 hours;

c) (F) increasing the microhardness during the treatment period;

d) (T) increasing the microhardness in a post-treatment period.

4) A recovery of the initial microhardness values of dentin after the treatment with 10% carbamide peroxide bleaching agents is related to:

a) (T) the use of a remineralizing solution;

b) (F) the use of an individual tray that could dissociate the bleaching agent;

c) (F) the diameter of the dentinal ducts;

d) (F) the presence of glycerin and carbopol in the bleaching agents.

Acknowledgments

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Tables

Table 1: Basic composition, lot number, pH level and the manufacturer of each treatment agent.

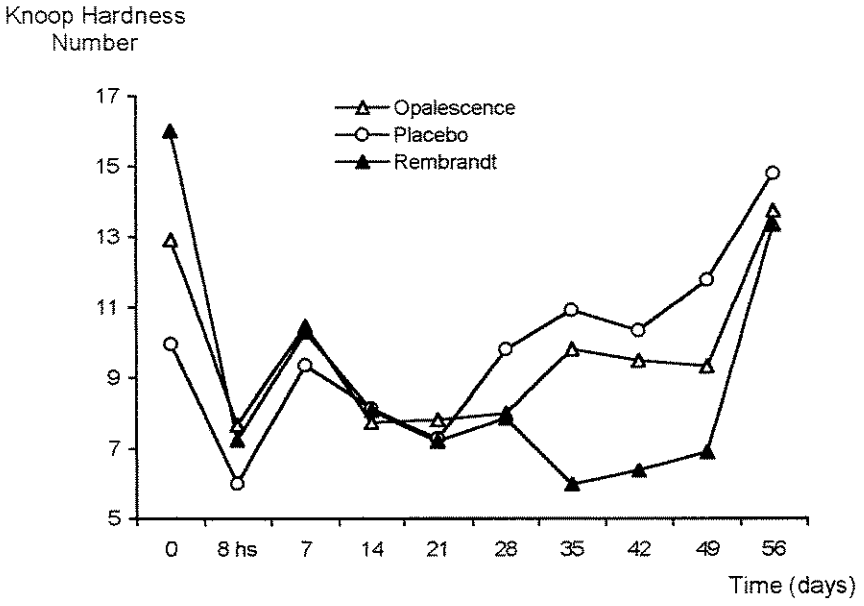
TREATMENT AGENTS	*COMPOSITION*	*pH*	*MANUFACTURER*
OPALESCENCE 10% (OPA) ***(regular)*** **(lot nº 393N)**	10% carbamide peroxide; carbopol; glycerin; flavoring	6.68	*Ultradent* Products Ind., Utah, USA
REMBRANDT 10% (REM) *(regular)* **(lot nº 567708)**	10% carbamide peroxide; glycerin; sodium citrate; carbopol; flavor; triethanolamine	6.19	Den-Mat Corporation, Santa Maria, USA
PLACEBO (PLA)	5% glycerin; 1.2% carbopol 940	7.0	Mixed formula, Proderma - Pharmacy, Piracicaba, Brazil

Table 2: Mean Knoop microhardness values, standard deviation (SD) and number of dentin fragments for each treatment agent at different time intervals. The Tukey test compared the differences among each time interval at 5% level of significance.

Time	Measures	Placebo		Opalescence		Rembrandt	
baseline	**mean**	*9.97*	*ab*	*12.91*	*bc*	*16.03*	*c*
	SD (n)	*4.06 (20)*		*5.94 (20)*		*8.30 (20)*	
8 hours	**mean**	*6.00*	*a*	*7.68*	*a*	*7.24*	*a*
	SD (n)	*1.47 (20)*		*2.99 (20)*		*2.58 (20)*	
7 days	**mean**	*9.36*	*ab*	*10.48*	*abc*	*10.31*	*ab*
	SD (n)	*3.74 (20)*		*3.20 (20)*		*2.62 (20)*	
14 days	**mean**	*8.16*	*ab*	*7.75*	*a*	*8.10*	*a*
	SD (n)	*3.66 (20)*		*3.13 (20)*		*2.31 (20)*	
21 days	**mean**	*7.29*	*ab*	*7.82*	*a*	*7.22*	*a*
	SD (n)	*2.61 (19)*		*2.45 (20)*		*1.97 (20)*	
28 days	**mean**	*9.82*	*ab*	*8.00*	*ab*	*7.87*	*a*
	SD (n)	*2.39 (20)*		*2.55 (20)*		*2.31 (20)*	
35 days	**mean**	*10.93*	*abc*	*9.82*	*abc*	*5.98*	*a*
	SD (n)	*3.63 (20)*		*4.57 (20)*		*2.26 (20)*	
42 days	**mean**	*10.34*	*abc*	*9.50*	*abc*	*6.39*	*a*
	SD (n)	*3.46 (20)*		*3.18 (19)*		*2.13 (18)*	
49 days	**mean**	*11.77*	*bc*	*9.32*	*abc*	*6.91*	*a*
	SD (n)	*4.81 (20)*		*4.10 (19)*		*2.82 (20)*	
56 days	**mean**	*14.80*	*c*	*13.74*	*c*	*13.34*	*bc*
	SD (n)	*4.60 (20)*		*4.91 (20)*		*3.90 (20)*	

- Equal letters indicate mean values that are not significantly different.
- Lower cases represent the time comparisons for the same treatment agent.

Graphs



Graph 1: Mean Knoop microhardness of the dentin fragments treated with the different agents at different time intervals.

Capítulo IV

Effects of seven carbamide peroxide bleaching agents on enamel microhardness at different time intervals

ENVIADO PARA PUBLICAÇÃO PARA *OPERATIVE DENTISTRY*

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"Effects of seven carbamide peroxide bleaching agents on enamel microhardness at different time intervals"

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Summary

The purpose of this *in vitro* study was to evaluate the microhardness of human enamel exposed different concentrations of carbamide peroxide agents at different bleaching times. Seven bleaching agents were analyzed: Nite White 10% Excel/ Discus Dental (NW10); Nite White 16% Excel/ Discus Dental (NW16); Nite White 22% Excel/ Discus Dental (NW22); Opalescence 10%/ Ultradent Products (OPA10); Opalescence PF 20%/ Ultradent Products (OPA20); Rembrandt 15%/ Den-Mat Corporation (REM15) and Nupro Gold/ Dentsply (NG10). A placebo agent was used in a control group (PLA). The bleaching and the placebo agents were applied on the surface of human dental fragments for eight hours per day and between bleaching treatment, the fragments were stored in individual vials with artificial saliva. Microhardness testing was performed at baseline, 8 hours, 7, 14, 21, 28, 35 and 42 days of treatment and at 7 and 14 days of a post-treatment period. The Analysis of Variance showed statistical differences for treatment agents, time and treatment agents-time interaction (p-value < 0.0001). Linear regression showed that human enamel treated with different concentrations of carbamide peroxide or a placebo agent presents a decrease in the microhardness values over time in a similar profile, except the enamel fragments exposed to OPA20. Until the 49^{th} day, the enamel surface exposed to OPA20 presented the lowest microhardness differences from baseline values than the enamel treated with other treatment agents, but did not differ from the surface exposed to REM15 on the 56^{th} day. After 14 days of a post-treatment period, enamel treated with PLA, NW10, NW16 and NW22 showed the highest microhardness differences from baseline values than the enamel exposed to other agents. There was an increase in enamel microhardness of the fragments stored in artificial saliva at the post-treatment period, although baseline values were not reached. For OPA20, the enamel microhardness values surpassed the baseline values at a post-treatment period.

Clinical Relevance: All carbamide peroxide bleaching agents evaluated decrease the enamel microhardness, but 14 days after the completion of the treatment, fragments stored in artificial saliva, while not reaching baseline values, showed an increase in enamel microhardness. Enamel exposed to a 20 % carbamide peroxide bleaching agent with 3% potassium nitrate and 0,11% ion fluoride showed microhardness values that surpassed baseline values at a post-treatment period.

Introduction

Since its introduction by Haywood & Heymann (1989), nightguard vital bleaching has been suggested as an efficient and simple procedure in removing intrinsic and extrinsic stains from the teeth (Haywood, 1992; Haywood, 1994).

Different products and systems have appeared on the market for in-office use such as 35% hydrogen peroxide or as over-the-counter products. However, the application of carbamide peroxide bleaching agents is the most commonly used at-home bleaching technique, supported by several reports (Haywood, 1992; Haywood, 1994; Leonard, Bentley & Haywood, 1994) about its safety and effectiveness and the ADA approval of several 10% carbamide peroxide bleaching products (Haywood & Robinson, 1997).

Variations of the technique have been introduced, including the use of higher concentrations of carbamide peroxide agents (from 10% to 22%) with carboxypolymethylene polymer as a thickening agent to improve tissue adherence and a timed or sustained release of the whitening agent.

Ten percent carbamide peroxide in the presence of saliva releases 3% of hydrogen peroxide and 7% urea. It is supposed that peroxide-containing bleaching agents remove tooth discolorations through oxidation. Although dental hard tissues are highly mineralized,

their organic content can also play an important role in the bleaching process. In the presence of decomposition catalysts, enzymes and saliva, the hydrogen peroxide ionization occurs and the free radicals diffuses through the interprismatic substance of the enamel, opening and converting the highly pigmented carbon rings into chains (Goldstein & Garber, 1995), which are lighter in color. Higher concentrations of carbamide peroxide agents contain higher amounts of hydrogen peroxide; for this reason, whitening results are obtained more quickly than with the use of 10% carbamide peroxide (Kihn & others, 2000; Leonard, Sharma & Haywood, 1998).

As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extensive period of time, many *in vitro* studies have evaluated the potential adverse effects of 10% carbamide peroxide agents on enamel micromorphology (Ernst, Marroquin & Willershausen-Zönnchen, 1996; Flaitz & Hicks, 1996; Hegedus & others, 1999; Potocnik, Kosec & Gasperic, 2000; Smidt & others, 1998; Zalkind & others, 1996). The acidic property of the bleaching agents (Ernst & others, 1996; Leonard & others, 1994; Price, Sedarous & Hiltz, 2000; Smidt & others, 1998; Zalkind & others, 1996) also can cause changes in the mineral content of the enamel (Goldstein & Garber, 1995).

Studies have reported loss of calcium and some alterations in enamel microhardness after exposure to 10% carbamide peroxide (McCracken & Haywood, 1996; Potocnik & others, 2000; Rotstein & others, 1996; Smidt & others, 1998). In a combined *in vitro-in vivo* study, an increase in enamel microhardness was demonstrated due to remineralization by saliva (Shannon & others, 1993). Attin & others (1997) showed a decrease in the enamel

microhardness values with the use of highly concentrated fluoride solutions. Rodrigues & others (2001) reported an initial decrease in enamel microhardness values at different time intervals when using a 10% carbamide peroxide agent, and a final increase in its values after 42 days, although an inverse situation was reported for another product with the same concentration.

For higher concentrations of carbamide peroxide, Pinheiro Junior & others (1996) showed that a 16% carbamide peroxide bleaching agent decreased the enamel microhardness values when compared with four different products of 10% carbamide peroxide agents. When using infrared spectroscopy and x-ray diffraction analysis, Oltu & Gürgan (2000) observed that 35% carbamide peroxide agent affect the structure of enamel, although 10 and 16% did not change the enamel after the treatment for 8 hours per day for 6 weeks. However, the effects of long term regimens of different concentrations of carbamide peroxide bleaching agents on enamel microhardness and at a post-bleaching period are still unknown. Different concentrations of this agent could induce higher or faster decreases in microhardness values due to the content of the hydrogen peroxide released, low pH levels or changes in the inorganic/organic ratio in enamel caused by the breakdown of the organic matrix. Furthermore, since the clinical procedure of bleaching treatment with carbamide peroxide agents requires repeated and prolonged exposure times, it is also important to evaluate the effects of higher concentrations of these agents at different bleaching time intervals. The aim of this work was to evaluate the microhardness of the enamel exposed to different concentrations of carbamide peroxide agents at different bleaching times.

Methods and materials

1. Experimental design

The factors under study were:

1) Treatment agents: in eight levels - Nite White 10% Excel/ Discus Dental (NW10); Nite White 16% Excel/ Discus Dental (NW16); Nite White 22% Excel/ Discus Dental (NW22); Opalescence 10%/ Ultradent Products (OPA10); Opalescence PF 20%/ Ultradent Products (OPA20); Rembrandt 15%/ Den-Mat Corporation (REM15); Nupro Gold/ Dentsply (NG10) and a placebo agent (PLA) as a control;

2) Time: in ten levels - baseline, 8 hours, 7, 14, 21, 28, 35, 42 days of treatment and at 7 and 14 days of a post-treatment period (corresponding to 49 and 56 days after the beginning of the treatment).

The experimental units consisted of 120 sound human enamel dental fragments, randomly and evenly assigned to the eight different treatment agents (15 dental fragments per group).

The Knoop microhardness response variable was evaluated by quantitative methods. Three measurements of Knoop microhardness were taken on the surface of each specimen at each time interval for a nested design.

2. Preparation of the dental fragments

Fifty non-erupted freshly extracted third molars were used. Immediately after extraction, the teeth were kept in 10% formaldehyde (pH 7.0). The roots were removed approximately 2 to 3 mm apical to the cementoenamel junction and the crowns were

longitudinally sectioned to obtain 120 dental fragments using double-faced diamond discs (K.G.Sorensen, Barueri, SP, Brazil, 06454-920) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil, 89221-040). Care was taken not to leave the dental fragments in air for a long period of time to prevent dehydration. They were immersed in distilled and deionized water at 37° C.

The dental fragments were required to be larger than 4 mm x 4 mm x 3 mm and those fragments that presented stains or cracks after the observation on stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan, 356) were not used. The fragments were embedded individually in a self-curing polyester resin in a PVC ring mold of 2.0 cm in diameter, with the external surface of the enamel to be exposed and the resin was left to polymerize for 24 hours. The molds were removed and the external surfaces of the dental fragments were leveled by a water-cooling mechanical grinder (Maxgrind/ Solotest, São Paulo, SP, Brazil, 01328-000). Aluminum oxide discs of 400, 600 and 1000 grit (Carborundum/ 3M do Brasil Ltda., Sumaré, SP, Brazil, 13001-970) were used sequentially. The polishing was performed with polishing cloths (Top, Gold and Ram, Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150) and diamond pastes of 6, 3, 1 and ¼ μm (Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150) with mineral oil (Lubrificante azul modelo LA, Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150). These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests. A standardized area of $9mm^2$ (3mm x 3 mm) of exposed enamel (size of the window for testing) was created on the specimens by covering the remaining dental fragment with two coatings of enamel nail varnish (Colorama/ CEIL Com. Exp. Ind. Ltda.,

São Paulo, SP, Brazil, 05113-900). Afterwards, the 120 specimens were randomly distributed to the treatment agents, with 15 dental fragments for each group and stored in distilled and deionized water at 37°C until their exposure to the treatment agents.

3. Specification of the materials

In this study, seven carbamide peroxide bleaching agents were evaluated: Nite White 10% Excel/ Discus Dental; Nite White 16% Excel/ Discus Dental; Nite White 22% Excel/ Discus Dental; Opalescence 10%/ Ultradent Products; Opalescence PF 20%/ Ultradent Products; Rembrandt 15%/ Den-Mat Corporation; Nupro Gold/ Dentsply. The control group consisted of a placebo agent prepared with carbopol and glycerin. The color and consistency of the placebo agent was similar to one of the bleaching agents (Opalescence 10%/ Ultradent Products, South Jordan, UT, NT 84095). The pH levels of the bleaching agents were measured by using a pH meter (Procyon, SA 720, São Paulo, SP, Brazil, 04530-970). Table 1 presents the basic composition, lot number, pH level and the manufacturer of each treatment agent.

4. Exposure of the dental fragments to the treatment agents

The enamel fragments were exposed to the *treatment agents* (experimental and control) for 8 hours per day for a period of 42 days.

Previous to the treatment period, an individual tray was manufactured for each specimen using a 0.4 mm thick flexible ethyl vinyl acetate (EVA) polymer (Bio-Art Equip.

Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000) in a vacuum forming machine (P7/ Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000).

For the application of the treatment agents, 0.02 ml of each agent was applied to each specimen using a syringe. The specimens were individually covered with the tray and immersed in individual closed vials with 13.5 ml of artificial saliva (pH=7.0), at 37° C.

After 8 hours, the specimens were taken from the storage media and the trays were removed. The treatment agents were washed from the surface of the enamel fragments under running distillated and deionized water for 5 seconds.

During the remaining time (16 hours per day), the fragments were immersed in individual closed containers with 13.5 ml of artificial saliva (pH=7.0), at 37° C. The artificial saliva of the containers was changed daily. The artificial saliva consisted of a remineralization solution that was proposed by Featherstone & others (1986) and modified by Serra & Cury (1992). After the 42 day treatment period, the specimens were maintained in their individual vials and immersed in 13.5 ml of artificial saliva (pH=7.0) at 37°C that was changed daily.

Microhardness measurements were performed before the initial exposure to the treatments (baseline values) and after 8 hours, 7, 14, 21, 28, 35 and 42 days and also at 7 and 14 days of the post-treatment phase (corresponding to 49 and 56 days after the initial application of the treatment agents). A Knoop indentator was used, keeping the long axis of the diamond parallel to the outer enamel surface in a microhardness testing machine (Future Tech - FM-1e, Tokyo, Japan, 140). Knoop microhardness test was used because both hardened and softened materials can be obtained (Anusavice, 1996). Three

indentations at each specimen were made with a load of 25g applied for 5 seconds at each time.

The data was evaluated by a classical linear model – Analysis of Variance (ANOVA) – in a nested design into a hierarchical structure of fixed effects. The time trend of microhardness was adjusted by linear regression and it was used to verify the factor *time* in each *treatment agent*. The Tukey test was used to compare the differences among *treatment agents* at each *time* at a 5% level of significance. (Montgomery, 1996).

Results

Table 2 shows the mean Knoop microhardness numbers at baseline, the differences from baseline values, the standard error and the Tukey test clusters for each treatment agent at each time. Chart 1 shows estimated Knoop microhardness differences at each time that were obtained from linear regression for each treatment agent. The Analysis of Variance showed statistical differences for *treatment agents*, *time* and *treatment agents-time* interaction (p-value < 0.0001).

There were significant differences in the mean microhardness values among *treatment agents* at each *time* (p-value < 0.0001). The Tukey test verified that, after 8 hours, the enamel microhardness exposed to PLA did not differ from the enamel microhardness exposed to other bleaching agents, except from NW10 and OPA20. From the 7^{th} to the 35^{th} days, microhardness values were practically similar over time for all materials, but showing differences among them in each time. On the 42^{nd} day, there were no differences in the microhardness values among NW22, NG and REM15. Until the 49^{th}

day, the enamel surface exposed to OPA20 presented the lowest microhardness differences from baseline values than the enamel treated with other treatment agents, but did not differ from the surface exposed to REM15 on the 56th day. After 14 days of a post-treatment period, enamel treated with PLA, NW10, NW16 and NW22 showed the highest microhardness differences from baseline values than the enamel exposed to other agents.

Linear regression showed that the enamel microhardness of fragments exposed to all the treatment agents, except OPA20, were similar during the bleaching period. The human enamel treated with different concentrations of carbamide peroxide or a placebo agent presents a decrease in the microhardness values. During the treatment period and after 7 or 14 days of a post-treatment period, there was a decrease in the enamel microhardness differences from baseline values for all treatment agents. For OPA20, the enamel microhardness values surpassed the baseline values at a post-treatment period.

Discussion

Changes in the chemical or morphological structure of enamel must be of concern when using bleaching techniques as a treatment for whitening teeth. Although some studies reported that there were no significant changes in enamel microhardness when using short term regimens of carbamide peroxide (Nathoo, Chmielewski & Kirkup, 1994; Potocnik & others, 2000; Seghi & Denry, 1992; Shannon & others, 1993), others observed a decrease in enamel microhardness when using these bleaching agents for 2 weeks or more (McCracken & Haywood, 1995; Rodrigues & others, 2001; Smidt & others, 1998), even with the additional use of concentrated fluoride solutions (Attin & others, 1997). When using higher

concentrations of carbamide peroxide bleaching agents, only Pinheiro Junior & others (1996) showed that a 16% carbamide peroxide agent significantly decreased the enamel microhardness values when compared with three 10% carbamide peroxide agents, suggesting that a higher concentration of carbamide peroxide could lead to deleterious effects on the dental structure. No other reports about the effects of carbamide peroxide bleaching agents in the concentrations of 15, 16, 20 and 22% on enamel microhardness have been available until now.

The findings of this *in vitro* investigation shows that human enamel exposed to the application of carbamide peroxide with different concentrations or a placebo agent presents a decrease in the microhardness values. Immediately after the application of the treatment agents for 8 hours, there was a significant decrease in microhardness values. Although the dental fragments were immersed in artificial saliva during the application of the bleaching agents, this was not enough to allow a recovery of the microhardness values during the first 8 hours of the treatment period. One of the possible reasons for the rapid decrease in microhardness values could be the pH of 10% carbamide peroxide bleaching agents. Some studies reported that the pH of these agents ranges from 4.6 to 7.4 (Ernst & others, 1996; Leonard & others, 1994; Price & others, 2000; Smidt & others, 1998; Zalkind & others, 1996), and that can also affect the physical and chemical structure of the enamel (Goldstein & Garber, 1995). In our study, the pH of the bleaching agents ranged from 6.22 to 7.84, showing that some of the products present acidic properties. However, none were lower than pH 5.5 and, therefore, would not contribute to the enamel demineralization (Featherstone & others, 1983; Featherstone & others, 1986). In addition, Leonard & others

(1994) pointed out that a moderate low-pH bleaching solution *in vivo* reduces the pH of saliva in the mouth during the first 5 minutes, but an increase above baseline is expected after 15 minutes of treatment, probably due to the chemical reactions of the neutralization of acidic carbamide peroxide by saliva. One might also expect that the tray used during the application of the treatment agents did not allow for this remineralization during the 8 hours of application of the agents since it acted as a barrier to the contact of the artificial saliva with the dental fragments. The clinical application of our data is the expectation that the pH of saliva, plaque and bleaching agent in the mouth may rise due to the factors found in the oral environment and that the presence of peroxidases, enzymes e saliva may neutralize the hazard effects of hydrogen peroxide.

An intriguing result of this experiment was that there were no differences in enamel microhardness between PLA and some of the bleaching agents evaluated at different times of treatment. The placebo agent consisted of a neutral pH glycerin and a carbopol gel and was selected as an adequate choice for the control group since it provided equal hydration of the samples. Manufactures have made no claims about the action of glycerin and carbopol, thus these agents have generally been considered to be inactive ingredients. However, when comparing two 10% carbamide peroxide bleaching agents with and without carbopol, McCracken & Haywood (1995) showed a significant decrease in microhardness in the outer 25 μm of the enamel surface after the treatment with the product containing carbopol. This result was related not only to the pH level of the products, but also to the presence of carbopol. This can also suggest that glycerin or the carbopol could act as a demineralizing agent (McCracken & Haywood, 1995) or as an impermeable barrier,

inhibiting the penetration of artificial saliva solution through the enamel surface and promote the recovery of baseline values. Probably, some bleaching agents evaluated can exhibit higher amounts of carbopol and glycerin in the compositions, showing a microhardness profile over time similar to the placebo agent.

Despite the decrease in enamel microhardness values, a remineralization effect could be verified during the treatment period and at the post-treatment period, when there was a continuos decrease in the microhardness differences from baseline values. The use of remineralization solutions or fluorides could inhibit the decrease in microhardness caused by the bleaching agents. Remineralization potential exists in saliva substitutes that contain calcium and phosphate ions (Featherstone & others, 1983; Featherstone & others, 1986), such as the artificial saliva used in this study. When using 10% carbamide peroxide agents, Rodrigues *et al.* (2001) showed the remineralization potential of this artificial saliva. Even though the baseline microhardness values were higher than the post-bleaching period values for all the agents – except for OPA20 - , the microhardness differences values obtained on the 7^{th} and 14^{th} days of a post-bleaching period were lower than the microhardness differences values obtained during the treatment period. It is possible that the storage of the dental fragments in the artificial saliva for a greater period of time would have allowed a recovery of baseline microhardness values. This recovery toward baseline microhardness values also might be expected in *in vivo* conditions due to some important factors such as salivary flow, the buffering capacity of saliva, oral hygiene and the use of topical fluorides that may increase the remineralization of bleached enamel.

OPA20 showed the best results in microhardness profile over time. Due to some ingredients - as potassium nitrate and fluoride – the microhardness differences from baseline values during the treatment period (8 hs to the 42nd day) were not so significant as compared to the other agents evaluated. At the post-treatment period, there was an increase in enamel microhardness that surpassed baseline values. The presence of fluoride in OPA20 could act as a remineralizing agent by forming a calcium fluoride layer on enamel that inhibits the demineralization or a decrease in microhardness values (Crawford & others, 1994; Featherstone & others, 1982; Miller & others, 1994; White & Featherstone, 1987). Potassium nitrate also could be responsible for an increase in enamel microhardness, although its benefits are related to reduce dentin hypersensitivity by occluding dentin tubules (Absi, Addy & Adams, 1995; Collins & Perkens, 1984; Martens & Surmont, 1991; Miller & others, 1994; Topbasi, Turkmen & Gunday, 1998) or blocking nerve conduction (Peacock & Orchadson, 1999). It is also probable that potassium nitrate could react with the sub-products of the carbamide peroxide reaction, enhancing the microhardness over time.

On the 56th day, the enamel exposed to REM15 also showed an increase in microhardness values that were not different from the results showed by enamel treated with OPA20. Even though the microhardness of the enamel exposed to REM15 presented a profile that were similar to the other agents evaluated, there was a rapid increase in microhardness on the 56th day. REM15 contain sodium citrate that is effective in controlling tooth hypersensitivity (Clark, Al-Joburi & Chan, 1987; Ong & Strahan, 1989; Zinner, Duany & Lutz, 1977). Probably, sodium citrate can also increase enamel microhardness over time by reacting with the sub-products of the carbamide peroxide.

As the enamel microhardness of fragments exposed to all the bleaching agents were similar during the bleaching period (except OPA20), one might suppose that the use of higher concentrations of carbamide peroxide are not more deleterious than the use of 10% carbamide peroxide agents; therefore, when one wants to achieve faster whitening of teeth, higher concentrations from properly selected materials might be chosen. With 10, 15, 16 or 22% carbamide peroxide concentrations, the same aesthetic results can be obtained in a shorter period of time when compared with the results with lower concentrations of carbamide peroxide agents (Leonard & others, 1998), even though an increase in tooth sensitivity can occur (Haywood, 1994; Leonard & others, 1998). When combining other components in the same product – as OPA20 and REM15 – a reduction in tooth sensitivity can be observed due to the presence of fluoride, potassium nitrate and/or sodium citrate and an increase in microhardness values over time can be demonstrated.

The decrease in microhardness values during the treatment with carbamide peroxide bleaching agents seems to be the result of damage to the enamel structure regardless of concentration. A post-bleaching period allowed for a recovery - for OPA20 - or an increase in the microhardness values – for PLA, NW10, NW116, NW22, OPA10, REM15 and NG -, although the baseline values were not reached for these products. This emphasizes that at-home whitening agents require professional supervision to ensure the proper application of the bleaching agents, recommended amount of gel/paste, length of treatment and steps to prevent adverse reactions.

Conclusion

All concentrations of carbamide peroxide bleaching agents decrease enamel microhardness during treatment period. After fourteen days of storage in artificial saliva following the completion of the bleaching treatment, there is a small increase in the enamel microhardness values that did not reach baseline values. When using a 20 % carbamide peroxide bleaching agent with 3% potassium nitrate and 0,11% ion fluoride, enamel microhardness values surpassed baseline values at a post-treatment period.

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Tables

Table 1: Composition, lot number, pH level and the manufacturer of each treatment agent.

TREATMENT AGENT	COMPOSITION	pH	MANUFACTURER
NITE WHITE 10% EXCEL* (NW10) (peppermint)* (lot nº 9FK)	10% carbamide peroxide; polyethylene glycol; propylene glycol; hydroxypolicellulose; carbopol, flavor, sodium hydroxide	7.49	Discus Dental, Inc. Los Angeles, California, USA
NITE WHITE 16% EXCEL (NW16) (peppermint) (lot nº 8HJ)	16% carbamide peroxide; polyethylene glycol; propylene glycol; hydroxypolicellulose; carbopol, flavor, sodium hydroxide	7.46	Discus Dental, Inc. Los Angeles, California, USA
NITE WHITE 22% EXCEL (NW22) (peppermint) (lot nº 9KD)	22% carbamide peroxide; polyethylene glycol; propylene glycol; hydroxypolicellulose; carbopol, flavor, sodium hydroxide	7.84	Discus Dental, Inc. Los Angeles, California, USA
OPALESCENCE 10%* (OPA10) (regular) (lot nº 3MPL)	10% carbamide peroxide; carbopol; glycerin; flavoring	6.68	Ultradent Products Ind., South Jordan, Utah, USA
OPALESCENCE PF 20% (OPA20) (regular) (lot nº 3MTJ)	20% carbamide peroxide; carbopol; glycerin; flavoring; 3% potassium nitrate; 0.11% ion fluoride	6.70	Ultradent Products Ind., South Jordan, Utah, USA
REMBRANDT 15% (REM15) (regular) (lot nº 030371545)	15% carbamide peroxide; glycerin; sodium citrate; carbopol; flavor; triethanolamine	6.22	Den-Mat Corporation, Santa Maria, California, USA
NUPRO GOLD (NG10) (regular) (lot nº 9911021)	10% carbamide peroxide; glycerin; carbopol; flavor	6.24	Dentsply Preventive Care, New York, Pennsylvania, USA
PLACEBO (PLA)	5% glycerin; 1.2% carbopol	7.0	Mixed formula, Proderma - Pharmacy, Piracicaba, Brazil

* - Products that are ADA approved

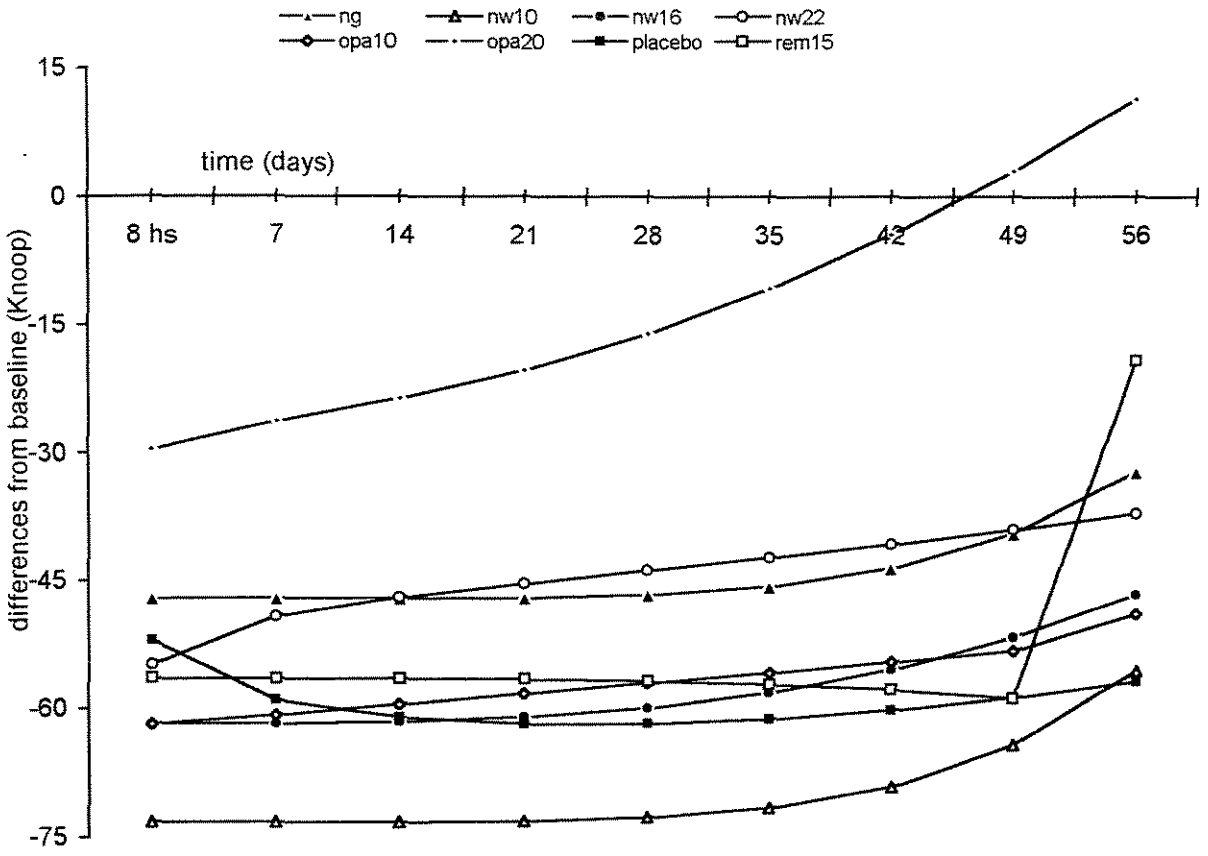
Table 2: Mean Knoop microhardness numbers at baseline, the differences from baseline values, the standard error (SE) and the Tukey test clusters (5% level of significance) for each treatment agent at each time (n=15).

Time	Estimates	PLA	NW10	NW16	NW22	OPA10	OPA20	REM15	NG
Baseline	Mean	106.7	120.3	105.6	90.8	110.4	77.1	83.7	102.7
	SE	15.6	16.4	15.9	8.5	15.9	11.1	9.6	12.1
8 hs	Mean	-50.7 b	-69.5 c	-59.6 bc	-56.1 bc	-63.9 b	-32.7 a	-58.0 bc	-50.0 b
	SE	9.4	16.6	13.6	8.8	16.2	12.3	14.7	11.0
7	Mean	-63.4 cde	-74.5 e	-64.9 de	-47.1 b	-62.2 cde	-19.9 a	-56.2 bcd	-49.3 bc
	SE	11.1	18.3	14.1	* 11.4	17.4	7.8	16.3	10.8
14	Mean	-59.9 cd	-75.8 e	-61.8 de	-45.6 bc	-55.9 bcd	-18.0 a	-58.3 cd	-43.9 b
	SE	10.5	18.1	17.7	11.3	15.9	14.3	16.5	11.9
21	Mean	-61.0 cd	-72.4 d	-61.1 cd	-44.3 b	-54.5 bc	-24.3 a	-57.0 bc	-45.5 b
	SE	* 12.0	17.8	15.3	11.5	16.1	11.4	16.9	12.0
28	Mean	-60.7 cd	-71.2 d	-56.9 bc	-45.7 b	-57.9 bcd	-20.1 a	-55.8 bc	-46.6 bc
	SE	11.0	19.3	14.9	10.9	16.2	13.9	19.2	* 13.1
35	Mean	-57.9 cd	-69.4 d	-56.8 bcd	-44.9 bc	-57.9 cd	-15.9 a	-52.6 bc	-43.4 b
	SE	10.9	18.4	15.2	9.4	16.6	14.7	14.3	12.8
42	Mean	-61.5 de	-74.0 e	-59.8 de	-42.6 b	-58.0 cd	-7.5 a	-53.3 bcd	-44.9 bc
	SE	10.8	16.9	13.3	11.4	17.2	16.7	15.2	12.7
49	Mean	-62.4 ef	-65.5 f	-50.9 cd	-36.7 b	-50.7 cde	14.0 a	-33.4 b	-40.9 bcd
	SE	11.2	18.0	13.7	11.6	17.5	24.1	13.8	12.5
56	Mean	-55.0 e	-54.1 e	-46.0 de	-36.7 cd	-49.0 de	7.8 a	-19.2 ab	-31.4 bc
	SE	10.3	20.0	13.1	11.1	17.8	18.4	12.4	14.0

Equal letters indicate mean values that are not significantly different (lines)

* n = 14 (1 missing value)

Graphs



Graph 1: Estimated Knoop microhardness differences at each time obtained from linear regression for each treatment agent.

Capítulo V

The effect of 10 % carbamide peroxide on the microhardness of sound and demineralized enamel and dentin 'In situ'

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The effect of 10 % carbamide peroxide on the microhardness of sound and demineralized enamel and dentin 'In situ'

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Clinical Relevance

In clinical situations, the treatment with 10% carbamide peroxide agent can alter the microhardness of sound and demineralized enamel, although it does not seem to affect the microhardness of sound and demineralized dentin.

Summary

The purpose of this *in situ* study was to evaluate the microhardness of sound and demineralized enamel and dentin submitted to treatment with 10% carbamide peroxide for three weeks. A 10% carbamide peroxide bleaching agent - Opalescence / Ultradent (OPA) – was evaluated against a placebo agent (PLA). Two hundred and forty dental fragments - 60 sound enamel fragments (SE), 60 demineralized enamel fragments (DE), 60 sound dentin fragments (SD) and 60 demineralized dentin fragments (DD) – were randomly fixed on the vestibular surface of the first superior molars and second superior premolars of 30 volunteers. The volunteers were divided into two groups that received the bleaching or the placebo agent at different sequences and periods at a double blind 2 x 2 cross-over study with a wash-out period of two weeks. Microhardness tests were performed on the enamel and dentin surface. The SE and DE submitted to the treatment with OPA showed lower microhardness values than the SE and DE submitted to the treatment with PLA. There were no statistical differences in microhardness values for SD and DD submitted to the treatment with OPA and PLA. The results suggest that treatment with 10% carbamide peroxide bleaching material for 3 weeks alters the enamel microhardness, although it does not seem to alter the dentin microhardness.

Introduction

The demand for conservative aesthetic dentistry has dramatically grown and so has the rapid development of new nonrestorative treatments for discolored teeth. Frequently, vital teeth present changes in color that substantially compromise the smile. As nightguard vital bleaching has gained popularity with patients and dentists as a conservative technique to lighten natural teeth (Haywood & Heymann, 1989), modifications, improvements and variations of the technique were introduced, including the use of a soft custom-fitted tray (from 0.2 to 0.4 mm thick), different concentrations of carbamide peroxide agents (10 to 22%) with carbopol and the use of these agents for one or more intervals during the day. Different products and systems have appeared on the market for in-office use such as 35% hydrogen peroxide or as over-the-counter products (Haywood, 1994). However, 10% carbamide peroxide is still the most used at-home bleaching technique with several reports in literature about its safety and effectiveness. The decomposition products of carbamide peroxide - water, oxygen, ammonia and carbon dioxide - are easily found in the normal processes of the human body. Therefore, the Food and Drug Administration (Haywood, 1993) classifies carbamide peroxide as being safe and effective for human use as an oral antiseptic in the concentration of 10%, and has ADA approval (Haywood & Robinson, 1997).

Ten percent carbamide peroxide dissociates into 3% hydrogen peroxide and 7% urea. These products are obtained after the dissociation of the bleaching product with the contact of saliva and oral fluids. Urea is degraded into ammonia and carbon dioxide. The hydrogen peroxide, because of its instability and ease of decomposition into water and

oxygen, penetrates through the pores of enamel and dentin to provide the lightening of the teeth. Due to the low amount of hydrogen peroxide in the home bleaching products (only 3%), there is a need for a prolonged contact of the agent with the dental structure to initiate the oxidation process (Goldstein & Kiremidjian-Schumacher, 1993; Goldstein & Garber, 1995). Bleaching agents affect the lightening of tooth structure through decomposition of peroxides into free radicals. The free radicals break down large pigmented molecules in enamel and dentin into smaller and less pigmented molecules. This is the point (the saturation point) at which whitening should be terminated (Goldstein & Garber, 1995). If a prolonged period of time is spent to bleach the teeth – e.g. when patients want their teeth "over" whitened – protein matrix breaks can occur in enamel and dentin (Goldstein & Garber, 1995). Therefore, one of the possible side-effects of bleaching products is that the enamel and dentin may be weakened by oxidation of the organic or inorganic elements.

As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extensive period of time, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. When using SEM evaluations, changes in enamel (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst, Marroquin & Willershausen-Zonnchen, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McGuckin, Babib & Meyer, 1992; Nam, Kugel & Habib, 1999; Shannon & others, 1993; Smidt & others, 1998; Zalkind & others, 1996) and dentin surface morphology (Zalkind & others, 1996) were reported.

However, not only the micromorphology of the dental tissues can be affected by bleaching agents, but changes in the mineral content of the enamel and dentin should be

evaluated as well, due to the acidic property of the bleaching agents (Ben-Amar & others, 1995; Ernst & others, 1996; Leonard & others, 1994; Murchinson, Charlton & Moore, 1992; Smidt & others, 1998; Zalkind & others, 1996) that could affect the tooth structure. Loss of mineral content or demineralization alters enamel and dentin microhardness (Featherstone & others, 1983; Rotstein & others, 1996), even though saliva, fluorides or other remineralizing solutions can maintain the balance between the phenomena of demineralization and remineralization.

In vitro studies have reported some alterations in enamel microhardness and loss of calcium after exposure to 10% carbamide peroxide (McCracken & Haywood, 1996; Rotstein & others, 1996; Attin & others, 1997; Smidt & others, 1998; Rodrigues & others, 2001). No changes in enamel microhardness were reported by Murchinson & others (1992), Seghi & Denry (1992), Nathoo, Chmielewski & Kirkup (1994) and McCracken & Haywood (1995). In dentin, Nathoo, Chmielewski & Kirkup (1994) showed that there are no changes in dentin microhardness, although Pécora & others (1994) and Rotstein & others (1996) showed significant alterations in the mineral content when using 10% carbamide peroxide agents.

In a combined *in vitro-in vivo* study – the bleaching treatment was performed in *in vitro* conditions and the remineralization period was performed inside the mouth – Shannon & others (1993) showed a slight increase in enamel microhardness due to a possible remineralization phenomenon of saliva. However, the interactions of the bleaching agent with the oral environment were not evaluated. Therefore, *in situ* studies should be

conducted to evaluate the direct interaction among product, saliva, soft tissue and sound or demineralized teeth.

It is also possible that bleaching agents have been applied on active caries lesions in enamel and dentin because there is a frequent absence of procedures to arrest incipient lesions before the aesthetic/restorative procedures. Since the bleaching agent penetrates through sound or demineralized dental tissues, there is a need for additional research on the effects of the nightguard vital bleaching agent on these tissues in clinical situations.

The aim of this paper was to evaluate *in situ* the microhardness of sound and demineralized enamel and dentin when submitted to treatment with 10% carbamide peroxide bleaching material and a placebo agent for 3 weeks.

Materials and methods

A) Experimental design

Thirty volunteers took part in this double-blind experiment, performed in two periods, with a wash-out period of 2 weeks. The volunteers were randomly divided into two equal groups of 15 volunteers. Each group received the bleaching or the placebo agent for 3 weeks in different sequences, in two distinct periods (bleaching agent – placebo agent; placebo agent – bleaching agent) in a cross-over 2 x 2 study (Montgomery, 1991).

The factors under study were:

Treatment agents: (two levels) experimental – Opalescence/ Ultradent; control – placebo agent;

Quality of dental tissue fragments: (two levels) sound and demineralized.

The experimental units consisted of 240 dental fragments: 60 sound enamel fragments; 60 demineralized enamel fragments; 60 sound dentin fragments; and 60 demineralized dentin fragments. One fragment of each dental tissue was randomly distributed in complete blocks among 30 volunteers. Each volunteer was considered as a block. All of the volunteers were submitted to the treatment with the bleaching agent for 3 weeks and with the placebo agent for another period of 3 weeks.

B) Selection of Volunteers

The volunteers were 30 adults (23 females and 7 males) from 19 to 25 years of age. Each volunteer was informed about the objectives of the research, benefits and possible risks involved in this experiment and participated only after providing written formal consent. This study had the approval of the FOP/UNICAMP Ethical Committee Guidelines, in agreement with the National Health Council (Brazil, 1996).

The volunteers were under-graduate students from the Dental School of Piracicaba, São Paulo, Brazil who were candidates for bleaching treatment. The need for a bleaching treatment was evaluated for each volunteer by the assessment of the color of the teeth using a Vita shade guide (Vita Zahnbabrik, H. Rauter GmbH & Co. KG, Bad Säckingen, Germany, D-79704). The exclusion criteria for participation in this study were: volunteers that wore fixed or removable dentures or orthodontic appliances, pregnant or nursing women, smokers, volunteers with dentin sensitivity.

C) Tray (nightguard) preparation

Superior and inferior dental arch impressions were taken with alginate (Jeltrate/ Dentsply, Mildford, DE, USA, 19963) and stone cast molds were made (Vigodent, Rio de Janeiro, RJ, Brazil, 21041-150). The maxillary casts were horseshoe shaped and without a palate to avoid interference with the efficiency of the vacuum pull on the hot thermoplastic sheet.

In the molds, vestibular reservoirs with 3 coatings of nail varnish (Colorama/ CEIL Com. Exp. Ind. Ltda., São Paulo, SP, Brazil, 05113-900) were prepared on all teeth except for the last teeth of the arches.

On the superior first molars and superior second premolars (or, when the latter were missing, the superior first premolars), larger reservoirs of 5mm x 5 mm x 4 mm were prepared with composite resin (Charisma/ Heraus Kulzer, Wehrheim, TS, D-61273) corresponding to the dental fragments that would be fixed in the volunteers.

Two scalloped trays were manufactured for each volunteer in a vacuum forming machine (P7/ Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000) using a flexible ethyl vinyl acetate (EVA) polymer (Bio-Art Equip. Odontológicos Ltda., São Carlos, SP, Brazil, 13568-000) that was 0.4 mm thick.

D) Specification of the materials

A 10% carbamide peroxide bleaching agent (Opalescence/ Ultradent, Utah, USA, 84095) – recognized by the American Dental Association (ADA) – was evaluated. The pH level of the bleaching agent was measured by using a pH meter (Procyon, SA 720, São

Paulo, SP, Brazil, 04530-970). The control group consisted of a placebo agent prepared with carboximethilcelulose and glycerin. The color, taste, flavor, consistency and packaging of the placebo agent was similar to the bleaching agent, but the placebo was pH neutral and had no active component (carbamide peroxide). Table 1 presents the basic composition, pH level, packaging and the manufacturer of each treatment agent.

E) Preparation of the dental fragments

Forty non-erupted third molars were used in this study. Immediately after extraction, the teeth were kept in 10% formaldehyde at pH 7.0. The teeth were sectioned with double-faced diamond discs (K.G.Sorensen, Barueri, SP, Brazil, 06454-920) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil, 89221-040), dividing the root from the coronary portion. In the root portion, the apical third was discarded and only the cervical region was used.

Two hundred and forty dental fragments (a hundred and twenty enamel fragments and a hundred and twenty dentin fragments) were obtained. The fragments that presented stains or cracks after the observation on stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan, 356) were not used. The size of the fragments was required to be 4mm x 4 mm x 2 mm.

The dental fragments were embedded individually in a self-curing polyester resin with the external surface of the enamel or dentin exposed. The external surfaces of the dental fragments were leveled by a water-cooling mechanical grinder (Maxgrind/ Solotest, São Paulo, SP, Brazil, 01328-000). For the enamel fragments, aluminum oxide discs were

used in a sequential granulation of 400, 600 and 1000 grit (Carborundum/ 3M do Brasil Ltda., Sumaré, SP, Brazil, 13001-970) refrigerated with water. The polishing was performed with polishing cloths (Top, Gold and Ram, Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150) and diamond pastes of 6, 3, 1 and ¼ μm (Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150) refrigerated with mineral oil (Lubrificante azul modelo LA, Arotec Ind. e Com. Ltda., Cotia, SP, Brazil, 06709-150). For the dentin fragments, only aluminum oxide discs were used in a sequential granulation of 600, 1000 and 1200 grit (Carborundum/ 3M do Brasil Ltda., Sumaré, SP, Brazil, 13001-970) refrigerated with water.

The fragments were removed from the polystyrene resin with a probe. All dental fragments were immersed in containers with distillated and deionized water and steam sterilized (Tuttnauer 2340MK, Ronkonkoma, NY, USA, 11779) for 20 minutes at 121° C. The steam sterilization is the most effective method to avoid the bacterial contamination (Pantera & Schuster, 1990; Amaechi, Highan & Edgar, 1998; Dewald, 1997) and does not seem to change the mineral content of the teeth (Amaechi & others, 1998, Oliveira, Sperandio & Souza, 1999).

F) Induction of artificial caries lesion

To obtain 60 demineralized enamel fragments and 60 demineralized dentin fragments, caries-like lesions were generated by a dynamic model of demineralization and remineralization cycles, similar to the model proposed by Featherstone & others (1986) and modified by Serra & Cury (1992).

The enamel fragments were submitted to 7 cycles of de-remineralization (Serra & Cury, 1992), while the dentin fragments were submitted to 3 cycles of de-remineralization (Hara & others, 2000). The 60 dental fragments of enamel and 60 of dentin, which made up the sound group of each dental tissue, were not submitted to the de-remineralization cycles but kept immersed in distillated and deionized water.

G) Preparation of the volunteers for the experimental phase

The initial color of the teeth was determined by Vita scale and photographs were taken to compare the initial to the final color after the experimental phase.

Two weeks before the initiation of the experiment, toothbrushes (Oral B 35/ Gillette do Brasil Ltda., Manaus, AM, Brazil, 69075-900) and fluoride toothpastes (Colgate MFP/ Kolynos do Brasil Ltda., Osasco, SP, Brazil, 06020-170) were given to the volunteers. Instructions were given on how to perform the Bass dental hygiene technique to standardize the toothbrushing method and the fluoride levels in the mouth. This phase was called the *run-in* period and it lasted for 2 weeks.

The thirty volunteers were randomly divided into two equal groups of 15 volunteers. Group I received the bleaching treatment while group II received the placebo treatment. In a second phase, group I received the placebo treatment while group II received the bleaching treatment.

H) Experimental phases

Four dental fragments, one of sound enamel, one of demineralized enamel, one of sound dentin, and one of demineralized dentin, were randomly fixed to the vestibular surfaces of the superior first molars and superior second premolars (or, when the latter were missing, the superior first premolars) of each volunteer (Figure 1). The fragments were fixed using an adhesive system (Scotchbond Multi-purpose/ 3M, St. Paul, MN, USA, 55144-1000) and a composite resin (Charisma/ Heraus Kulzer, Wehrheim, TS, D-61273). The bleaching or placebo treatment was applied in the superior dental arch of each volunteer where the experimental units were attached (vestibular surfaces of the teeth).

Fifteen volunteers were instructed to apply the bleaching agent (Group I) while fifteen volunteers were instructed to apply the placebo agent (Group II) in the tray and to wear it during the night for about 8 hours. They were instructed to clean the tray after its removal from the mouth and keep it in a container provided.

After 3 weeks of the treatment with the bleaching or placebo agent (experimental phase I), the fragments were removed with an appropriate pliers. The composite resin that adhered to the volunteer's tooth was removed with resin polishing carbide burs (K.G.Sorensen, Barueri, SP, Brazil, 06454-920) and aluminum oxide discs (Sof-Lex/ 3M, St. Paul, MN, USA, 55144-1000).

The volunteers were submitted to a wash-out period of 2 weeks to eliminate the residual effects of the treatment previously applied. New toothbrushes and toothpastes were given to the volunteers and the toothbrushing technique was reinforced. New trays were

used to eliminate the possibility that residues left by the agent previously applied would interfere in the effects of the other agent to be used.

Other four dental fragments – sound and demineralized enamel and sound and demineralized dentin – were fixed in the same way as described for experimental phase I. This time the volunteers used the treatment agent (placebo or bleaching agent) that they had not received at the experimental phase I (experimental phase II) for another period of 3 weeks.

The fragments were removed again with an appropriate pliers. During the experimental phases I and II, a syringe of bleaching or placebo agent was given to the volunteers weekly. The experimental phases and periods of the study can be observed in Table 2.

I) Microhardness tests

An acrylic device allowed the fragments to be held keeping the long axis of the indentator perpendicular to the dental surface. Three microhardness indentations were performed on the leveled surface of each enamel and dentin fragment with a microhardness tester (Future Tech - FM-1e, Tokyo, Japan, 140) and a Knoop indentator. A load of 25 gr. was used for the enamel fragments and a load of 10 gr. was used for the dentin for 5 seconds.

J) Statistical analysis

For the statistical analysis, the average of the three Knoop Hardness Numbers was taken. Before the Analysis of Variance, the *carry-over* effect was determined by the *t-Student* test, in each volunteer. The Analysis of Variance for Greco-Latin Squares 2x2 design was employed to compare the treatment agents, using a three-dimensional block composed of "different sequences", "periods" and "quality of the dental fragment" (Montgomery, 1991). The statistical analysis was made by Statgraphics plus software (Manugistics, Inc., Rockville, Maryland, USA, 20852).

Results

The t-Student test did not show the presence of the *carry-over* effect for enamel (p-value=0.0269) or for dentin (p-value=0.0356). These results permitted a comparison between treatment agents and quality of the dental fragments without the *carry-over* effect at the 5% level of significance.

The mean of the Knoop microhardness values for enamel and dentin according to the quality of the dental fragments, treatment agents and periods are shown in Table 3. Mean Knoop microhardness values for each group, period and quality of the dental fragments are illustrated in Graphs 1 and 2.

The Analysis of Variance of the experimental design considering the Greco-Latin Squares 2 X 2 was employed. For enamel, there was significant differences between bleaching and placebo agents (p-value=0.0045) and between sound and demineralized dental fragments (p-value<0.0001). The sound and demineralized enamel submitted to 10%

carbamide peroxide bleaching agent for 3 weeks showed significant lower values of microhardness than those submitted to a placebo agent. The Knoop microhardness values for sound enamel fragments were significantly higher than the Knoop microhardness values for demineralized enamel fragments in both treatments.

For dentin, there was significant differences between sound and demineralized dental fragments (p-value<0.0001). There was not significant differences between sound and demineralized dentin treated with bleaching or placebo agents, but the sound and demineralized dentin submitted to 10% carbamide peroxide bleaching agent for 3 weeks showed slightly higher values of microhardness than those submitted to a placebo agent. The Knoop microhardness values for sound dentin fragments were significantly higher than the Knoop microhardness values for demineralized dentin fragments for bleaching and placebo agents.

Discussion

Since its introduction by Haywood & Heymann (Haywood & Heymann, 1989), nightguard vital bleaching is a procedure that has dramatically grown in the dental offices due to its efficiency and simplicity in removing intrinsic or extrinsic stains from the teeth (Haywood, 1992; Haywood, 1994). Many of the bleaching products contain 10% carbamide peroxide (Goldstein & Kiremidjan-Schumacher, 1993; Haywood, 1992) with carboxypolymethylene polymer as a thickening agent to improve tissue adherence and allow for a time or sustained release of the whitening agent (Haywood, 1994).

Although the bleaching agent is applied on the enamel surface, the oxidation processes of the carbamide peroxide take place within the teeth by an interaction with their structural components. The effects and the mechanism of the bleaching agents should be evaluated to understand possible damages in detriment to the benefits of a more aesthetic smile offered by this technique.

In vitro studies using SEM analysis have demonstrated that the application of 10% carbamide peroxide on enamel surface causes morphological changes with an increase in porosity and erosions (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst & others, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McGuckin & others, 1992; Nam & others, 1999; Shannon & others 1993; Smidt & others, 1998; Zalkind & others, 1996). In dentin, an increase of the superficial roughness was verified (Zalkind & others, 1996). Although only one paper reported no alterations in enamel (Haywood & others, 1990), the acidic properties of the bleaching agents, the prolonged contact time between the lightening product and the dental surface, and the presence of greater amounts of carbopol have been claimed as the possible factors that can cause these superficial changes. Clinically, the increased porosity allows the bleaching agent to easily penetrate through enamel and dentin and could explain the transitory dental sensitivity during its use.

Regarding the inorganic and organic components in the dental structure, studies should take into account the structure's mechanical properties. Changes in the inorganic and organic components ratio could be deleterious to the teeth (Featherstone & others, 1983; Featherstone & others, 1986). The free radicals of the decomposition of carbamide peroxide may react with the organic components of the dental structure and the low pH of

the bleaching systems may cause demineralization. Loss of mineral can be related to the acidic properties of the bleaching agents, even though Leonard & others (1994) observed that there is an increase of the pH levels of 10% carbamide peroxide after its dissociation in the mouth. A loss of calcium after the exposure of enamel to 10% carbamide peroxide was observed in some *in vitro* studies (McCracken & Haywood, 1996; Rotstein & others, 1996), though this reduction of mineral content was not clinically significant.

Differences in organic and inorganic content can also be verified by microhardness tests (Featherstone & others, 1983). Although some *in vitro* studies have shown that there were no microhardness changes on sound enamel and dentin submitted to the treatment with 10% carbamide peroxide (Murchinson & others, 1992; Seghi & Denry, 1992; Nathoo & others, 1994; McCracken & Haywood, 1995), significant differences were found in other experiments (Pécora & others, 1994; Attin & others, 1997; Rodrigues & others, 2001; Shannon & others, 1993; Smidt & others, 1998).

A dynamic model of inducing artificial caries lesions through pH cycles of demineralization and remineralization solutions (Featherstone & others, 1986; Serra & Cury, 1992; Hara & others, 2000) was used in this study. This model presents a correlation with the initiation and progression of carious lesions in patients at high-risk for caries (Featherstone & others, 1986), leading to changes in the mineral and organic content of the teeth. The applicability of the model for inducing caries can be verified by the significant differences between the quality of the enamel and dentin fragments (p-value<0.0001; p-value<0.0001). It shows that the demineralization-remineralization model was effective in producing artificial caries lesions. The Knoop microhardness values for sound enamel

fragments were significantly higher than those for the demineralized enamel fragments in both treatments. The same occurred for the sound and demineralized dentin fragments.

For the enamel fragments, it was verified that the sound and demineralized fragments submitted to the effects of the bleaching agent presented lower microhardness values than the sound and demineralized fragments submitted to the placebo agent. These results were obtained by fixing the dental fragments in a vestibular location – close to the parotid salivary gland duct exit where there is a low-risk for caries. Because the fragments were maintained in the mouth during the whole period of the experimental phases and at the same location (with the constant flow of saliva, temperature and pH changes, fluorides, toothbrush abrasiveness and effects of liquids and foods in the oral environment), we consider that the results are reliable. The bleaching agent caused a mineral loss in human dental fragments, even though saliva, plaque control and fluorides were present in the oral environment. These factors could be responsible for maintaining the balance between the demineralization and remineralization phenomena. This mineral loss can be related not only to the pH level of the bleaching agent. Leonard & others (1994) showed that a 10% carbamide peroxide solution with a moderately low-pH presented an increase of its pH level after 5 minutes of degradation, reaching a neutral pH. However, the pH level of OPA used in this experiment was not so acidic, but an increase of OPA pH level can also be expected. So, the prolonged contact between the product and the dental structure can be the responsible for the decrease in the microhardness values Due to a high level of mineral content of enamel, the bleaching agent seems to cause a demineralization effect in the enamel structure, even though a slight decrease in the organic content in the enamel could

take place (the percentage of organic content and water in enamel is around 4% (Ten Cate, 1988).

For the dentin fragments, there was no significant difference between the bleaching and the placebo agent. The percentage of mineral content in dentin (70%) is lower than in enamel (Ten Cate, 1988). Therefore, our results also suggest that, although some alterations occur in the dentin, these effects do not damage the inorganic/organic content in dentin structure, but significantly affect the mineral content of the enamel fragments.

This experiment can also elucidate the importance of not applying bleaching agents on early carious lesions due to their damaging effects. On sound dental structures, the bleaching agents can be used as an aesthetic treatment, but one should be aware of the lower microhardness values obtained in this study. There is a possibility that the human enamel could be damaged, although the effects of higher concentrations of fluorides and a post-bleaching time were not evaluated. Perhaps a prolonged time contact between the dental structure with saliva and fluorides could be helpful to reverse the ratio between organic and inorganic mineral content and to return to the initial conditions which could increase the enamel microhardness values. This emphasizes that at-home bleaching agents require the need for future researches, even though professional supervision can ensure correct selection of the proper case, correct application, and steps to prevent adverse reactions.

Conclusions

The results suggest that :

a) the treatment with 10% carbamide peroxide bleaching material for 3 weeks alters the microhardness of sound and demineralized enamel;

b) the treatment with 10% carbamide peroxide bleaching material for 3 weeks does not seem to alter the microhardness of sound and demineralized dentin.

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TABLES

Table 1: Composition, pH, presentation form and manufacturer of each treatment agent

TREATMENT AGENTS	*COMPOSITION*	*pH*	*PACKAGING*	*MANUFACTURER*
OPALESCENCE **(batch nº 393N)**	10% carbamide peroxide; carbopol; glycerin; flavoring*	6.68	Dispensable syringe	*Ultradent* Products Inc., Utah, 84095, USA
PLACEBO	5% glycerin; 1.2% carbopol 940	7.0	Dispensable syringe with package identical to Opalescence	Mixed formula, Proderma - Pharmacy, Piracicaba, 13414-000, Brasil

* The manufacturer does not indicate the percentage of each component.

Table 2: Experimental phases and periods of the study according to the volunteer groups.

PHASE	PERIOD	GROUP I (15 volunteers)	GROUP II (15 volunteers)
RUN-IN	2 weeks	Standardized brushing with toothbrushes and toothpastes provided; tray manufacturing	Standardized brushing with toothbrushes and toothpastes provided; tray manufacturing
EXPERIMENTAL PHASE I	3 weeks	Bleaching agent	Placebo agent
WASH-OUT	2 weeks	Standardized brushing with new toothbrushes and toothpastes provided	Standardized brushing with new toothbrushes and toothpastes provided
EXPERIMENTAL PHASE II	3 weeks	Placebo agent	Bleaching agent
POST-EXPERIMENTAL PHASE	Time required for the volunteer	Evaluation; continuation of the bleaching treatment on both arches; follow-up	Evaluation; continuation of the bleaching treatment on both arches; follow-up

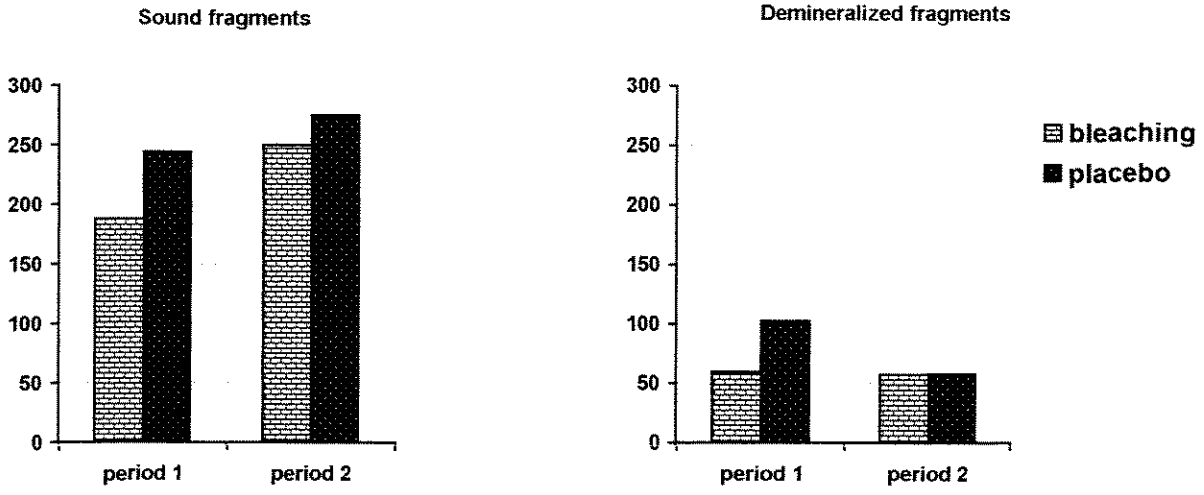
Table 3 : Exploratory estimates for enamel and dentin responses on Knoop microhardness according to the quality of dental fragments, treatment agents and periods.*

Quality of the dental fragments	Treatment Agents	Enamel		Dentin	
		Period 1	Period 2	Period 1	Period 2
Demineralized	**OPA**	59.6 (7.0)	57.4 (14.4)	12.7 (1.5)	16.7 (1.0)
	PLA	102.6 (14.7)	57.7 (13.9)	11.8 (1.2)	15.3 (0.9)
Sound	**OPA**	187.4 (21.6)	250.2 (13.8)	30.8 (4.2)	39.8 (2.7)
	PLA	244.2 (14.9)	275.5 (13.4)	29.8 (2.5)	38.8 (3.2)

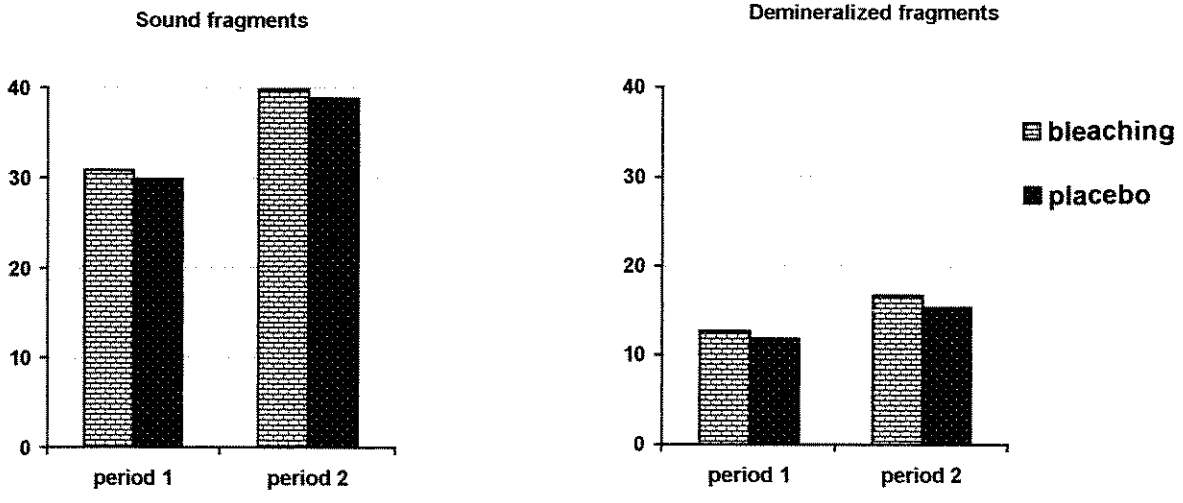
* Mean (standard error) - m (se) - confidence interval at 95% is taken by m ± 1.96.se

Graphs

Graph 1: Bar diagram of the mean Knoop microhardness illustrating the effects of the treatment agents, quality of the dental fragments and periods for the enamel fragments.



Graph 2: Bars diagram of the mean Knoop microhardness illustrating the effects of the treatment agents, quality of the dental fragments and periods for the dentin fragments.



Figures

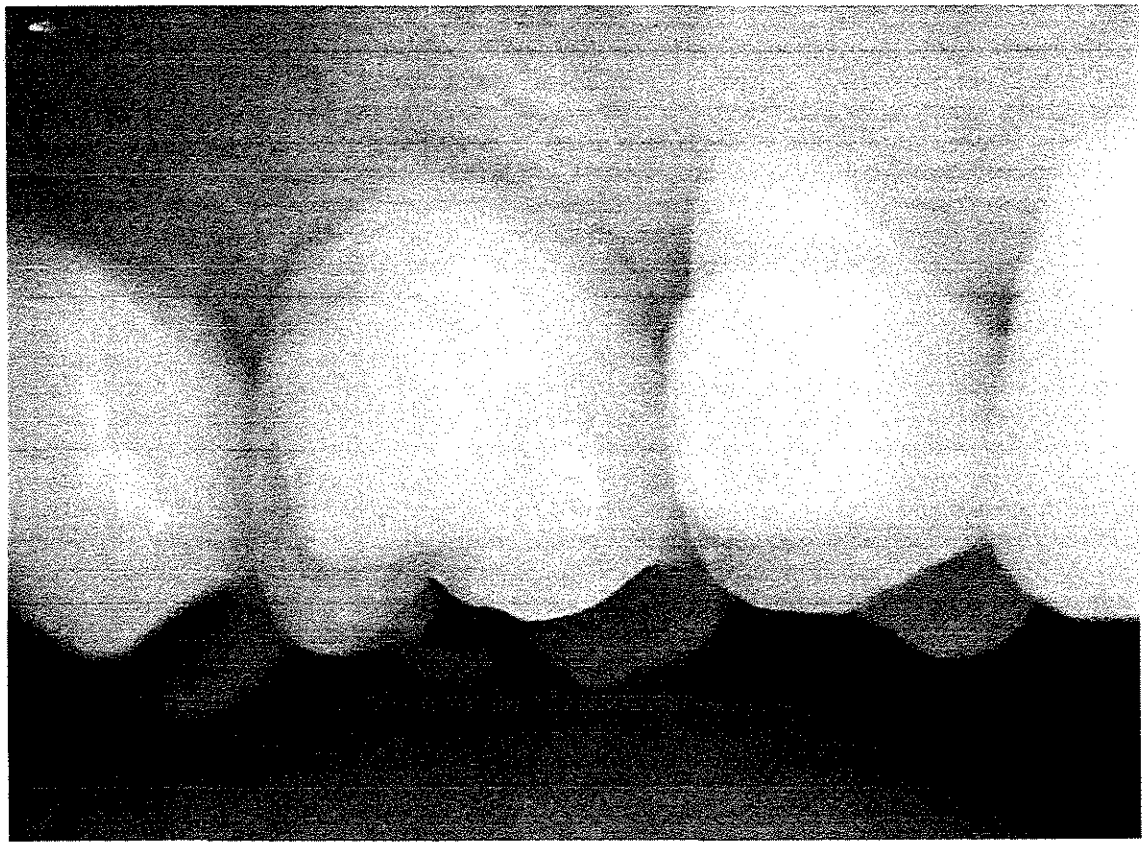


Figure 1: Dental fragments fixed to the vestibular surfaces of the superior first molar and second premolars.

IV) Conclusão

Nas condições em que estes cinco estudos foram conduzidos e de acordo com a análise dos resultados obtida, conclui-se que os agentes clareadores de peróxido de carbamida podem alterar a microdureza dos tecidos dentais e que a saliva, no período pós-clareamento, apresenta um efeito remineralizante. Especificamente, conclui-se que:

A) Diferentes agentes clareadores com a mesma concentração apresentam diferentes efeitos sobre a microdureza superficial do esmalte dental humano hígido;

B) A utilização de dentifrícios dessensibilizantes pode manter ou aumentar a microdureza do esmalte dental humano quando submetido ao tratamento com agentes de peróxido de carbamida a 10%;

C) O peróxido de carbamida a 10% pode diminuir a microdureza superficial da dentina humana hígida em função do tempo; contudo, os valores de microdureza no período pós-clareamento são semelhantes aos valores iniciais devido ao efeito remineralizante da saliva artificial pelo tempo de 14 dias;

D) Agentes clareadores contendo peróxido de carbamida nas concentrações de 10, 15, 16 e 22% diminuem a microdureza do esmalte dental humano hígido em função do tempo, embora a saliva artificial apresente um efeito no aumento da microdureza no período pós-clareamento;

E) No estudo *in situ*, os resultados sugerem que há alterações da microdureza do esmalte dental humano hígido e desmineralizado após o tratamento com o

peróxido de carbamida a 10% pelo período de três semanas, embora tais alterações não ocorram para a dentina humana hígida e desmineralizada.

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Anexo I: Comprovante de publicação do artigo *"Effects of two 10 percent carbamide peroxide bleaching materials on enamel microhardness at different time intervals"*.

C.E. Article #7-201 (1 credit/AGD code 780) Research Article

Effects of 10% carbamide peroxide bleaching materials on enamel microhardness

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ABSTRACT: *Purpose*: To evaluate the microhardness of enamel treated with two different 10% carbamide peroxide bleaching materials at different time intervals. *Materials and Methods*: Two bleaching agents were analyzed: Opalescence (OPA) and Rembrandt (REM). The control group (CON) consisted of dental fragments maintained in artificial saliva. Bleaching was accomplished for 8 hrs per day and stored during the remaining time in an individual recipient with artificial saliva. Enamel microhardness testing was performed before the initial exposure to the treatments and after 1, 7, 14, 21, 28, 35 and 42 days. *Results*: The ANOVA, followed by the Bartlet and Tukey tests, showed significant differences for treatments ($P < 0.00001$) from day 7-day 42. From the 7th to the 14th day, OPA presented an increase of enamel microhardness over time while REM presented a decrease of microhardness. Statistical differences were not found between REM and the control group (OPA > CON = REM). From the 21st–35th day, enamel fragments bleached with OPA and REM presented a decrease of microhardness. Statistical differences of microhardness were verified among all the treatments (OPA > CON > REM). On the day 42, statistical differences were not found between OPA and the control group, but they were found between REM and the control group (OPA = CON > REM). The polynomial regression showed an increase of microhardness for OPA until the 21st day, followed by a decrease of microhardness up to the 42nd day. A decrease of microhardness for REM was verified. There were alterations in enamel microhardness as a function of bleaching time when using the two different 10% carbamide peroxide whiteners. Over a 42-day treatment time, bleaching with REM agent caused a decrease in enamel microhardness. The OPA agent initially increased the microhardness, then returned to the control level. Different bleaching materials with the same concentration of carbamide peroxide have different effects on the enamel. (*Am J Dent* 2001;14:67-71).

CLINICAL SIGNIFICANCE: The potential effects caused by bleaching with 10% carbamide peroxide agents on enamel must be known by dentists. The effects of bleaching agents on the enamel microhardness over time are important parameters to evaluate if a demineralization process is occurring.

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Introduction

In the past few decades, the demand for conservative esthetic dentistry has dramatically grown and so has the rapid development of new nonrestorative treatments for discolored teeth.[1] Frequently, vital teeth present changes in color that substantially compromise esthetics. As nightguard vital bleaching[1] has gained popularity with patients and dentists as a conservative technique to lighten natural teeth, laboratories have rapidly introduced bleaching products into the market.[2] Many of the newer systems contain 10% carbamide peroxide[3,4] with carboxypolymethylene polymer as a thickening agent to improve tissue adherence and allow for a time or sustained release of the whitening agent.[5] The original technique involves the application of the bleaching agent in a custom-fitted vinyl nightguard for 6-8 hrs a night from 2-6 wks.[6]

The exact mechanism of bleaching is unclear. It is an oxidation reaction whereby the 10% carbamide peroxide in the presence of saliva releases 3% of hydrogen peroxide (which penetrates enamel and dentin to lighten the tooth) and 7% urea. The hypothesis of bleaching is that as the oxidizing agent diffuses through the interprismatic substance of the enamel, the highly pigmented carbon ring compounds are opened and converted into chains, which are lighter in color. Ideally, this is the point (the saturation point) at which whitening should be terminated.[7,8] As the bleaching of vital teeth involves the direct contact of the whitening agent on the outer enamel surface for an extended period of time, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. Using SEM analysis, several studies,[7,9-18] reported changes in enamel surface morphology with varying degrees of porosity and alteration. On the other hand, other studies[19-21] showed no changes in surface morphology of human enamel using SEM evaluations.

Some alterations in enamel microhardness, though without clinical significance, after exposure to 10% carba-mide peroxide under a variety of *in vitro* conditions, have been reported.[22-24] Furthermore, a combined *in vitro/in vivo* study[17] demonstrated a decrease of the initial microhardness of enamel, followed by an increase in enamel microhardness due to a possible remineralization phenomenon of saliva. Teeth exposed to 10% carbamide peroxide lost calcium, although this reduction of mineral content was not significant.[25-27] Loss of mineral content from the outer tooth structure or demineralization alters enamel microhardness,[28,29] even though saliva, fluorides or other remineralizing solutions can maintain the equilibrium between the phenomena of demineralization and remineralization.

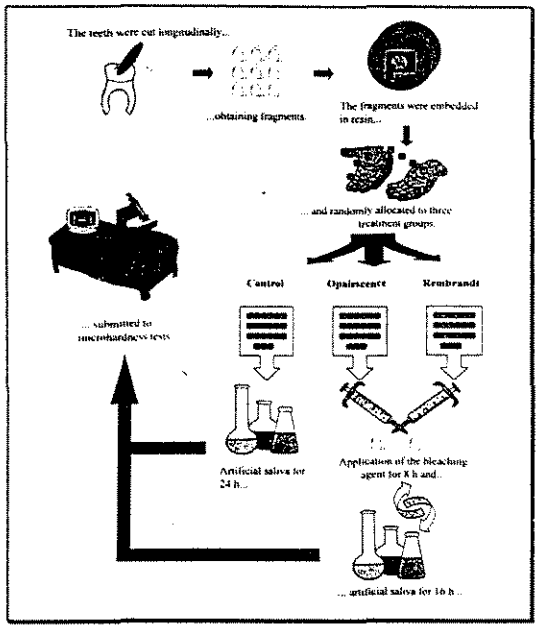

Fig. 1. Study diagram.

The lack of conclusive evidence of the effects of bleaching agents on enamel microhardness suggests the need for additional research. Little is known about the long term consequences of bleaching agents on the enamel surface and the influence of saliva as a function of bleaching time on human enamel treated with different 10% carbamide peroxide materials.

This *in vitro* study evaluated the microhardness of enamel treated with two different 10% carbamide peroxide materials at different bleaching times.

Materials and Methods

Experimental design

The factors under study were three treatments: Opalescence,[a] Rembrandt,[b] (experimental groups) and artificial saliva (control group); and 8 time levels: 0, 1, 7, 14, 21, 28, 35 and 42 days.

The experimental units consisted of 63 dental fragments, randomly and evenly assigned to the 3 different treatments. The experimental group was treated with the bleaching agents 8 hrs per day while the control group remained in artificial saliva. Repeated measurements of microhardness were taken from each specimen at specific times.

The response variable was microhardness evaluated quantitatively. With "time" an independent variable, it was possible to obtain a response-surface curve by means of linear models.[30] The study diagram is illustrated in Fig. 1.

Preparation of the dental fragments

Twelve unerupted freshly extracted third molars were used. After extraction, the teeth were kept in 2% formaldehyde (pH 7.0). The teeth were submitted to a soft-tissue debridement with periodontal curettes and cleaned with a slurry of pumice in a webbed rubber cup in a slow-speed handpiece. The roots were removed approximately 2-3 mm apical to the cementoenamel junction. The crowns were sectioned longitudinally to obtain dental slabs (4 mm x 4 mm x 3 mm) using a double-faced diamond disc, producing 63 dental fragments.

The dental fragments were individually embedded in polystyrene resin in a ring mold, allowing only one side of the dental fragment left unsealed by the polystyrene resin. The specimens were serially polished by means of 400, 600 and 1000 grade sandpaper. These procedures were conducted to form parallel planar surfaces for the microhardness tests.

A uniform 7 mm^2 area of exposed enamel was created on the specimens by covering the remaining dental fragment with a nail varnish, which was impermeable to 10% carbamide peroxide and water. The specimens were randomly allocated to the "treatment" groups (n = 21).

Specification of the bleaching materials

Two commercial bleaching agents were investigated: Opalescence and Rembrandt. These materials are ADA approved, syringe delivered 10% carbamide peroxide base glycerin gel and do not contain fluoride.

Exposure of the dental fragments to the bleaching materials

The specimens in the experimental group were treated with the bleaching agents 8 hrs per day for a total of 42 days, by covering the dental fragments with 0.02 ml of each bleaching material. After the bleaching period, the carbamide peroxide gel was removed under running deionized and distilled water. During the remaining time, the fragments were individually kept in 20 ml of artificial saliva.

The specimens in the control group were kept in 20 ml of artificial saliva 24 hrs per day. The artificial saliva was changed daily after washing the dental fragments under running deionized and distilled water. During these cycles, the experimental and control specimens were kept in a humid atmosphere at 37°C. The artificial saliva used was proposed by Featherstone *et al*,[31] as described by Serra & Cury.[32]

Microhardness tests

Microhardness measurements were performed before the initial exposure to the treatments and after 1, 7, 14, 21, 28, 35 and 42 days. The tests were conducted immediately following 8 hrs of bleaching for the experimental groups. For the control groups, the dental fragments were removed from storage and tested just before changing the artificial saliva.

Knoop microhardness was measured keeping the long axis of the diamond perpendicular to the outer enamel surface using a microhardness tester (Model FM-1e[c]). Three indentations were made on each specimen with 50g load applied for 20 s each time. The microhardness measurements were taken on the subsurface enamel from the cut section of each dental fragment with each indentation randomly located 100, 200 and 300 µm from the outer enamel surface to the dentin-enamel junction. Since there were multiple test times (eight test times of three indentations each), the distance be-

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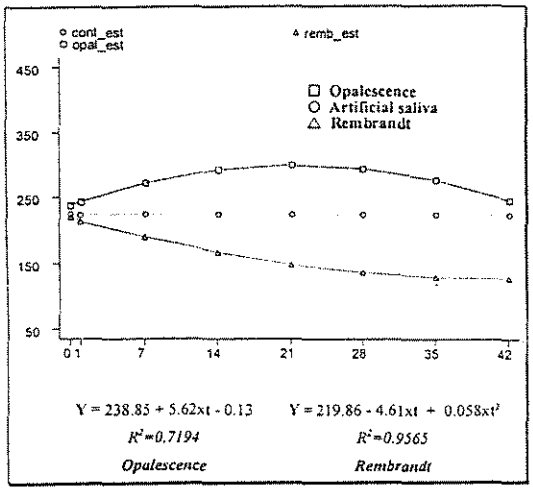

Fig. 2. Mean Knoop microhardness for enamel fragments bleached with Opalescence, Rembrandt and for the control group as a function of time and the mathematical model.

Linear regression was used to verify the "time" factor (log-scale) in each "treatment". The mathematical model and the comparisons among the mean Knoop hardness for each bleaching material or artificial saliva in each time are presented in Fig. 2. The control group did not present significant differences in microhardness as a function of time, being represented as a constant function (Y = 224,77).

The linear regression illustrated the mathematical models that express the microhardness of the dental fragments of the control and experimental groups as a function of time. An increase in microhardness was noticed in the enamel fragments bleached with OPA up to the 21st day, followed by a decrease of microhardness up to the 42nd day. For the control group specimens kept in artificial saliva, there were no significant differences of microhardness as a function of time (represented by a constant function). The enamel fragments bleached with REM presented a decrease of microhardness as a function of time.

Discussion

Despite the beneficial effects on home-applied whitening agents in reducing or eliminating stains, the findings of the current study pose certain concerns with both carbamide peroxide gels evaluated. Many studies have reported that changes in enamel surface are evident.[7,9-18] Although surface morphology was not a factor studied in this experiment, both positive and negative alterations in enamel microhardness were found with both products evaluated as a function of bleaching time.

In the original protocol prescribed by Haywood & Heymann,[1] the average time recommended for optimal change color is 6 wks, although slight effects may be noted as early as 2 wks. Some manufacturers, however, instructed dentists to allow patients to use their own discretion to determine the duration of treatment, stating that "the product should be used until the desired result is achieved".[12]

By means of microhardness tests, the mineral profile of the enamel resulting from a demineralization and remineralization process could be obtained.[28] Demineralization of enamel structure occurs at a critical pH of 5.5.[28,31,34] In fact, the bleaching agent Rembrandt with a low pH between 4.9-5.2[17] induced a decrease of the microhardness values as compared to the control group from the 21st day to the 42nd day due to demineralization. The results of this investigation showed that REM can cause a demineralization effect on enamel surface. Significant alterations of enamel surface microhardness values were also observed by Shannon *et al*[17] when using REM bleaching agent for 4 wks. Haywood *et al*,[20] however, affirmed that other 10% carbamide peroxide solutions *in vitro* did not cause any significant changes in the enamel surface morphology, regardless of pH levels. The present study employed storage in artificial saliva which contains calcium and phosphate ions that increase the remineralization potential and may approximate this condition to that found in oral environ-ment.[31,32,35,36] It is possible that the remineralization effect was not observed because the intact enamel is less receptive to remineralization than demineralized enamel.[34]

Furthermore, it was pointed out that a moderate low-pH bleaching solution *in vivo* reduces the pH of saliva in the mouth during the first 5 mins and that after 15 mins of treatment, the pH increases above baseline, probably attributed to the chemical reactions of neutralization of acidic carbamide peroxide by saliva.[5] A crucial shortcoming of this study is that we do not have the exact pH value of the products. Also crucial to the clinical application of this data is that the pH in the mouth may rise[5] as cited above, but we do not know what the pH in this study did during the 8-hr application, nor if the pH of the bleaching product behaves similarly.

As this study did not evaluate the microhardness in a post-bleaching period, with the potential for remineralization, it cannot demonstrate that the reported decrease in microhardness is a significant clinical problem that persists following the active bleaching phase. A recovery toward pretreatment microhardness values might be expected. Important factors such as salivary flow, buffering capacity of saliva, oral hygiene[11] and the use of topical fluorides[7,37] may increase remineralization of bleached enamel. So, a remineralization effect could well be expected *in vivo*.

On the other hand, enamel fragments treated with OPA, with a pH between 5.5-6.5,[9,18,19,23] presented an increase of microhardness as a function of bleaching time until the 21st day. After this period, there was a decrease of microhardness but not less than the microhardness found in the control group. It was also observed that, on the 42nd day, there was no statistical difference of microhardness between the specimens bleached with OPA and the control group. Murchison *et al*[22] also showed no statistical differences in pre- or postbleaching microhardness values for OPA using artificial saliva as a storage agent. A slight decrease of hardness was found by Smidt *et al*[23] for OPA product, but without statistically significant differences after 16 days. The present study used a longer period of bleaching time corresponding clinically with the prolonged time used by the patients. We can also point out that

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Table. Mean values for microhardness, standard deviation (SD), number of specimens and the Analysis of Variance (ANOVA) for control and experimental groups. Tukey test (represented by the letters) compared the factor "treatments" in each "time" (the subsets are specific for separate days).

Time	Measures	Control Group Artificial saliva	Experimental Group OPA	Experimental Group REM	P
0	Mean	^{a}217.6	^{a}224.1	^{a}222.6	
	SD	39.2	50.4	50.9	0.8959
	n	21	20	20	
1d	Mean	^{a}233.7	^{a}239.8	^{a}218.8	
	SD	62.3	42.7	53.8	0.4586
	n	21	19	20	
7d	Mean	^{a}219.5	^{b}303.6	^{a}177.3	
	SD	48.1	49.5	38.0	0.0000
	n	21	20	20	
14d	Mean	^{a}204.1	^{b}287.4	^{a}166.0	
	SD	42.6	52.2	41.9	0.0000
	n	21	20	21	
21d	Mean	^{b}222.7	^{c}303.2	^{a}162.1	
	SD	41.2	47.42	32.4	0.0000
	n	20	20	20	
28d	Mean	^{b}216.9	^{c}279.5	^{a}134.3	
	SD	36.4	33.68	35.1	0.0000
	n	21	20	20	
35d	Mean	^{b}208.7	^{c}272.6	^{a}122.3	
	SD	39.2	37.01	47.5	0.0000
	n	21	20	21	
Mean 42d		^{b}215.9	^{b}255.4	^{a}130.8	
	SD	44.0	35.28	52.4	0.0000
	n	21	20	21	

Equal superscript letters horizontally indicate mean values that are not significantly different.

tween each indentation was 200 µm from the occlusal to the cervical surface. Each indentation was measured only once. The average of the three indentations was used as the value for each time period.

Statistical analysis

The statistical analysis considered the average of the three measurements taken from each specimen. The average of the three indentations in each dental fragment was used to obtain the Knoop hardness number (KHN) by the following calculation:

$$KHN = \frac{14.23 \times 10^3 \times F}{d^2}$$

where F was the value of the applied load (in g) and d was the diagonal indentation (in μm).

Statistical analysis using the ANOVA for a completely random design was employed, considering "treatment" in each "time". The method of decomposition of sum of square followed by Tukey test was applied for pairwise comparisons among "treatment" in each "time". The data were analyzed by software STATA.[d,33]

Results

The ANOVA showed no significant differences for "treatment" at day 1, but showed significant differences (P= 0.000)[f] from day 7 to day 42. The Table shows the mean values for microhardness, number of specimens, standard deviation and the ANOVA for the control and experimental groups. Tukey test is also shown in the Table.

On the 7th and 14th days, there were no statistical differences in microhardness among the enamel fragments submitted to bleaching with REM and the control group. Enamel fragments bleached with OPA presented the highest values of microhardness within this period.

Statistical differences of microhardness were verified among all the treatments from the 21st to the 35th day. Enamel specimens bleached with OPA presented the highest values of microhardness, followed by the control group and the fragments bleached with REM.

On the 42nd day, there were no statistical differences in microhardness among the enamel fragments submitted to bleaching with OPA and the control group due to a decrease of microhardness of the specimens treated with OPA. Specimens bleached with REM differed from the others, presenting the lowest values of microhardness.

remineralization and demineralization processes are occurring during the entire evaluation time.

Clinically, the use of this bleaching material for 6 wks may not cause an increase in tooth demineralization. The small decrease in enamel microhardness observed from 28 days through 42 days in this investigation could be the result achieved by the saturation point in bleaching. In this study, the decrease in microhardness did not seem to be enough to injure the enamel matrix because this decrease was not significantly different from the control group at the end of the experiment. It is unknown if more time had been spent to bleach the teeth with OPA, *e.g.* when patients want "over" whitening, if a certain degree of damage could be achieved by affecting the enamel microhardness and surface morphology. This emphasizes that at-home whitening agents require professional supervision to ensure correct selection of the bleaching agent, proper application, recommended amount of gel/paste, length of treatment and steps to prevent adverse reactions.[7] Even though products have the same 10% concentration of carbamide peroxide, other compositional factors of materials may alter their effects on tooth structure. In this study, one product caused an increase in microhardness while another caused a decrease as compared a control group.

a. Ultradent Co., South Jordan, UT, USA.
b. Den-Mat Corporation, Santa Maria, CA, USA.
c. Future Tech Corporation, Tokyo, Japan.
d. Computing Resource Center, Santa Monica, CA, USA.

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Anexo II: Comprovante de recebimento do artigo *"Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals"* para avaliação pelos assessores.

Date: Tue, 15 May 2001 14:43:36 -0400
From: Franklin Garcia-Godoy <f.garcia-godoy@tufts.edu>
Subject: Manuscript received
To: mcserra@forp.usp.br
Organization: Tufts University

Dear Dr. Serra:

I received your manuscript "Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals", submitted for consideration for publication in the American Journal of Dentistry.

I will send the paper to two referess for their comments and will contact you immediately after I hear from them.

Sincerely,

Prof. Dr. Franklin Garcia-Godoy

Anexo III: Comprovante de recebimento do artigo *"Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals"* para avaliação pelos assessores.

Quintessence International

Editor-in-Chief: Thomas G. Wilson, Jr, DDS

May 24, 2001

Dr. Mônica Campos Serra
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Dept. de Odontologia Restauradora-Dentistica
Avenida do Café, s/n°
CEP: 14040-904 Ribeirao Preto-SP
Brazil

Dear Dr. Serra:

Thank you for submitting the manuscript "Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals." Your submission included:

- manuscript (3 copies)
- 1 mandatory submission form
- 1 disk
- 2 tables (+ 2 duplicate sets)
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Marti Tiedeman
Assistant Editor

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Anexo IV: Comprovante de recebimento do artigo "*Effects of seven carbamide peroxide bleaching agents on enamel microhardness at different time intervals*" para avaliação pelos assessores.

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To: "'rbasting@yahoo.com'" <rbasting@yahoo.com>

Subject: RE: Information about our article

Date: Mon, 9 Apr 2001 12:01:38 -0500

Dear Dr. Basting,

Your manuscript was received by our office on March 13, 2001. On March 15, 2001, copies were sent to reviewers and a letter was mailed to you acknowledging receipt of your paper. I'm sorry if the mailing did not reach you, but your paper is currently under review and we will notify you when the process is complete. Thank you for your inquiry.

Dr. Michael Cochran, Editor

Anexo V: Comprovante de publicação do artigo *"The effect of 10 % carbamide peroxide on the microhardness of sound and demineralized enamel and dentin 'In situ' "*.

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Clinical Research

The Effect of 10% Carbamide Peroxide Bleaching Material on Microhardness of Sound and Demineralized Enamel and Dentin *In Situ*

RT Basting • AL Rodrigues Jr • MC Serra

Clinical Relevance

In clinical situations, treatment with 10% carbamide peroxide agent can alter the microhardness of sound and demineralized enamel although it does not affect the microhardness of sound and demineralized dentin.

SUMMARY

This *in situ* study evaluated the microhardness of sound and demineralized enamel and dentin submitted to treatment with 10% carbamide peroxide for three weeks. A 10% carbamide peroxide bleaching agent—Opalescence/Ultradent (OPA)—was evaluated against a placebo agent (PLA). Two hundred and forty dental fragments—60 sound enamel fragments (SE), 60 demineralized enamel fragments (DE), 60 sound dentin fragments (SD) and 60 demineralized dentin fragments (DD)—were randomly fixed on the vestibular surface of the first superior molars and second superior premolars of 30 volunteers. The volunteers were divided into two groups that received bleaching or the placebo agent at different sequences and periods at a double blind 2 x 2 crossover study with a wash-out period of two weeks. Microhardness tests were performed on the enamel and dentin surface. The SE and DE submitted to treatment with OPA showed lower microhardness values than the SE and DE submitted to treatment with PLA. There were no statistical differences in microhardness values for SD and DD submitted to the treatment with OPA and PLA. The results suggest that treatment with 10% carbamide peroxide bleaching material for three weeks alters the enamel microhardness, although it does not seem to alter the dentin microhardness.

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Mônica Campos Serra, professor

INTRODUCTION

The demand for conservative aesthetic dentistry has dramatically grown. So has the rapid development of new non-restorative treatments for discolored teeth.

Frequently, vital teeth present changes in color that substantially compromise the smile. As nightguard vital bleaching has gained popularity with patients and dentists as a conservative technique to lighten natural teeth (Haywood & Heymann, 1989), modifications, improvements and variations of the technique were introduced, including using a soft custom-fitted tray (from 0.2 to 0.4 mm thick), different concentrations of carbamide peroxide agents (10 to 22%) with carbopol and using these agents for one or more intervals during the day. Different products and systems have appeared on the market for in-office use, such as 35% hydrogen peroxide or as over-the-counter products (Haywood, 1994). However, 10% carbamide peroxide is still the most used at-home bleaching technique, with several reports in literature about its safety and effectiveness. The decomposition products of carbamide peroxide—water, oxygen, ammonia and carbon dioxide—are easily found in the normal processes of the human body. Therefore, the Food and Drug Administration (Haywood, 1993) classifies carbamide peroxide as safe and effective for human use as an oral antiseptic in 10% concentration and has ADA approval (Haywood & Robinson, 1997).

Ten percent carbamide peroxide dissociates into 3% hydrogen peroxide and 7% urea. These products are obtained after the dissociation of the bleaching product with the contact of saliva and oral fluids. Urea is degraded into ammonia and carbon dioxide. The hydrogen peroxide, because of its instability and ease of decomposition into water and oxygen, penetrates through the pores of enamel and dentin to provide the lightening of the teeth. Due to the low amount of hydrogen peroxide in the home bleaching products (only 3%), there is a need for a prolonged contact of the agent with the dental structure to initiate the oxidation process (Goldstein & Kiremidjian-Schumacher, 1993; Goldstein & Garber, 1995). Bleaching agents affect the lightening of tooth structure through decomposition of peroxides into free radicals. The free radicals break down large pigmented molecules in enamel and dentin into smaller, less pigmented molecules. This is the point (the saturation point) at which whitening should be terminated (Goldstein & Garber, 1995). If a prolonged period is spent—that is, when patients want their teeth "over" whitened—protein matrix breaks can occur in enamel and dentin (Goldstein & Garber, 1995). Therefore, one possible side-effect of bleaching products is that the enamel and dentin may be weakened by oxidation of the organic or inorganic elements.

As bleaching of vital teeth involves direct contact of the whitening agent on the outer enamel surface for an extensive period of time, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. When using SEM evaluations, changes in enamel (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst, Marroquin & Willershausen-Zonnchen, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McGuckin, Babib & Meyer, 1992; Nam, Kugel & Habib, 1999; Shannon & others, 1993; Smidt & others, 1998; Zalkind & others, 1996) and dentin surface morphology (Zalkind & others, 1996) were reported.

However, not only the micromorphology of the dental tissues can be affected by bleaching agents, but changes in the mineral content of the enamel and dentin should be evaluated as well, due to the acidic property of the bleaching agents (Ben-Amar & others, 1995; Ernst & others, 1996; Leonard & others, 1994; Murchinson, Charlton & Moore, 1992; Smidt & others, 1998; Zalkind & others, 1996) that could affect tooth structure. Loss of mineral content or demineralization alters enamel and dentin microhardness (Featherstone & others, 1983; Rotstein & others, 1996), even though saliva, fluorides or other remineralizing solutions can maintain the balance between the phenomena of demineralization and remineralization.

In vitro studies have reported some alterations in enamel microhardness and loss of calcium after exposure to 10% carbamide peroxide (McCracken & Haywood, 1996; Rotstein & others, 1996; Attin & others, 1997; Smidt & others, 1998; Rodrigues & others, 2001). No changes in enamel microhardness were reported by Murchison & others (1992), Seghi & Denry (1992), Nathoo, Chmielewski & Kirkup (1994) and McCracken & Haywood (1995). In dentin, Nathoo, Chmielewski & Kirkup (1994) showed no changes in dentin microhardness, although Pécora & others (1994) and Rotstein & others (1996) showed significant alterations in the mineral content when using 10% carbamide peroxide agents.

In a combined *in vitro-in vivo study*—the bleaching treatment was performed in *in vitro* conditions and the remineralization period was performed inside the mouth—Shannon & others (1993) showed a decrease in the initial microhardness of enamel due to the bleaching agent, followed by an increase in enamel microhardness resulting from a possible remineralization phenomenon of saliva. However, interactions of the bleaching agent with the oral environment were not evaluated. Therefore, *in situ* studies should be conducted to evaluate the direct interaction among product, saliva, soft tissue and sound or demineralized teeth.

It is also possible that bleaching agents have been applied on active carious lesions in enamel and dentin because there is a frequent absence of procedures to arrest incipient lesions before the aesthetic/restorative procedures. Since the bleaching agent penetrates through demineralized dental tissues, there is a need for additional research on the effects of the nightguard vital bleaching agent on these tissues in clinical situations.

Spelled Denhy in references.

ANEXO V

This paper evaluated *in situ* the microhardness of sound and demineralized enamel and dentin when submitted to treatment with 10% carbamide peroxide bleaching material and a placebo agent for three weeks.

METHODS AND MATERIALS

A) Experimental Design

Thirty volunteers took part in this double-blind experiment performed in two periods with a two-week washout period. The volunteers were randomly divided into two groups of 15. Each group received the bleaching or the placebo agent for three weeks in different sequences, in two distinct periods (bleaching agent—placebo agent; placebo agent—bleaching agent) in a crossover 2 x 2 study (Montgomery, 1991).

The factors under study were:

Treatment Agents: (two levels) experimental—Opalescence/ Ultradent; control –placebo agent;

Quality of Dental Tissue Fragments: (two levels) sound and demineralized.

The experimental units consisted of 240 dental fragments: 60 sound enamel fragments; 60 demineralized enamel fragments; 60 sound dentin fragments and 60 demineralized dentin fragments. One fragment of each dental tissue was randomly distributed in complete blocks among 30 volunteers. Each volunteer was considered a block. All the volunteers underwent treatment with the bleaching agent for three weeks, then for another three weeks with the placebo agent.

B) Selection of Volunteers

The volunteers were 30 adults (23 females and 7 males) from 19 to 25 years of age. Each volunteer was informed of the objectives, benefits and possible risks involved in this experiment and participated only after providing written formal consent. This study had the approval of the FOP/UNICAMP Ethical Committee Guidelines in agreement with the National Health Council (Brazil, 1996).

The volunteer candidates for bleaching treatment were undergraduate students from the Dental School of Piracicaba, São Paulo, Brazil. Each volunteer's need for a bleaching treatment was evaluated by assessing tooth color using a Vita shade guide (Vita Zahnbabrik & H Rauter GmbH & Co KG, Bad Säckingen, Germany, D-79704). Exclusion criteria for participating in this study was whether volunteers wore fixed or removable dentures or orthodontic appliances, were pregnant or nursing women, smokers or had dentin sensitivity.

C) Tray (Nightguard) Preparation

Superior and inferior dental arch impressions were taken with alginate (Jeltrate/Dentsply, Mildford, DE 19963, USA) and stone cast molds were made (Vigodent, Rio de Janeiro, RJ, Brazil, 21041-150). The maxillary casts were horseshoe-shaped without a palate to avoid interference with the efficiency of the vacuum pull on the hot thermoplastic sheet.

In the molds, vestibular reservoirs with three coatings of nail varnish (Colorama/CEIL Com Exp Ind Ltda, São Paulo, SP, Brazil, 05113-900) were prepared on all teeth except for the last teeth of the arches.

On the superior first molars and superior second premolars (or, when the latter were missing, the superior first premolars), larger reservoirs of 5 mm x 5 mm x 4 mm were prepared with composite resin (Charisma/Heraus Kulzer, Wehrheim, TS, D-61273) corresponding to the dental fragments that would be fixed in the volunteers.

Two scalloped trays were manufactured for each volunteer in a vacuum-forming machine (P7/Bio-Art Equip Odontológicos Ltda, São Carlos, SP, Brazil, 13568-000) using a flexible ethyl vinyl acetate (EVA) polymer (Bio-Art Equip Odontológicos Ltda, São Carlos, SP, Brazil, 13568-000) that was 0.4 mm thick.

D) Specification of the Materials

A 10% carbamide peroxide bleaching agent (Opalescence/Ultradent, South Jordan, Utah 84095, USA)—recognized by the American Dental Association (ADA)—was evaluated. The pH level of the bleaching agent was measured using a pH meter (Procyon, SA 720, São Paulo, SP, Brazil, 04530-970). The control group consisted of a placebo agent prepared with carboximethilcelulose and glycerin. The color, taste, flavor, consistency and packaging of the placebo agent was similar to the bleaching agent, but the placebo was pH neutral and had no active component (car-

Table 1: *Composition, pH, Presentation Form and Manufacturer of Each Treatment Agent*

Treatment Agents	Composition	pH	Packaging	Manufacturer
Opalescence	10% carbamide peroxide, carbopol; glycerin; flavoring*	6.68	Dispensable syringe	Ultradent Products Inc, South Jordan, Utah 84095 USA
Placebo	5%glycerin; 1.2% carbopol 940	7.0	Dispensable syringe with package identical to Opalescence	Mixed formula, Proderma–Pharmacy, Piracicaba, 13414-000, Brasil

* The manufacturer does not indicate the percentage of each component

bamide peroxide). Table 1 presents the basic composition, pH level, packaging and the manufacturer of each treatment agent.

E) Preparation of the Dental Fragments

Forty non-erupted third molars were used in this study. Immediately after extraction, the teeth were kept in 10% formaldehyde at pH 7.0. They were sectioned with double-faced diamond discs (KG Sorensen, Barueri, SP, Brazil, 06454-920) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil, 89221-040), dividing the root from the coronary portion. In the root portion, the apical third was discarded and only the cervical region was used.

Two hundred and forty dental fragments 4 mm x 4 mm x 2 mm (120 enamel fragments and 120 dentin fragments) were obtained. The fragments presenting stains or cracks after observation on stereomicroscope at 30x (Meiji Techno EMZ Series, Saitama, Japan, 356) were not used.

The dental fragments were embedded individually in a self-curing polyester resin with the external surface of the enamel or dentin exposed. The external surfaces of the dental fragments were leveled by a water-cooling mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil, 01328-000). For the enamel fragments, aluminum oxide discs of 400, 600 and 1000 grit were used sequentially (Carborundum/3M do Brasil Ltda, Sumaré, SP, Brazil, 13001-970) with water coolant. Polishing was performed with polishing cloths (Top, Gold and Ram, Arotec Ind e Com Ltda, Cotia, SP, Brazil, 06709-150) and diamond pastes of 6, 3, 1 and 0.25 μm (Arotec Ind e Com Ltda) with mineral oil coolant (Lubrificante azul modelo LA, Arotec Ind. e Com Ltda). For the dentin fragments, only aluminum oxide discs were used in a sequential granulation of 600, 1000 and 1200 grit (Carborundum/3M do Brasil Ltda) with water coolant.

The fragments were removed from the polystyrene resin with a probe. All dental fragments were immersed in containers with distillated and deionized water and steam sterilized (Tuttnauer 2340MK, Ronkonkoma, NY 11779, USA) for 20 minutes at 121°C. Steam sterilization is the most effective method to avoid bacterial contamination (Pantera & Schuster, 1990; Amaechi, Highan & Edgar, 1998; Dewald, 1997) and does not change the mineral content of the teeth (Amaechi & others, 1998, Oliveira, Sperandio & Souza, 1999).

F) Induction of Artificial Caries Lesion

To obtain 60 demineralized enamel fragments and 60 demineralized dentin fragments, caries-like lesions were generated by a dynamic model of demineralization and remineralization cycles similar to the model proposed by Featherstone & others (1986) and modified by Serra & Cury (1992).

The enamel fragments were submitted to seven cycles of de-remineralization (Serra & Cury, 1992), while the dentin fragments were submitted to three cycles of de-remineralization (Hara & others, 2000). The 60 enamel dental fragments and 60 dentin fragments that made up the sound group of each dental tissue were not submitted to de-remineralization cycles but kept immersed in distillated and deionized water.

G) Preparing the Volunteers for the Experimental Phase

The initial color of the teeth was determined by Vita scale and photographs were taken to compare the initial to the final color after the experimental phase.

Two weeks before initiation of the experiment, volunteers received toothbrushes (Oral B 35/Gillette do Brasil Ltda, Manaus, AM, Brazil, 69075-900) and fluoride toothpastes (Colgate MFP/ Kolynos do Brasil Ltda, Osasco, SP, Brazil, 06020-170). They received instructions on how to perform the Bass dental hygiene technique to standardize the toothbrushing method and the fluoride levels in the mouth. This phase was called the run-in period and lasted for two weeks.

The 30 volunteers were randomly divided into two groups of 15. Group 1 received the bleaching treatment while Group 2 received the placebo. In a second phase, Group 1 received the placebo treatment while Group 2 received the bleaching treatment.

H) Experimental Phases

Four dental fragments, one of sound enamel, one of demineralized enamel, one of sound dentin and one of demineralized dentin, were randomly fixed to the vestibular surfaces of the superior first molars and superior second premolars (or, when the latter were missing, the superior first premolars) of each volunteer

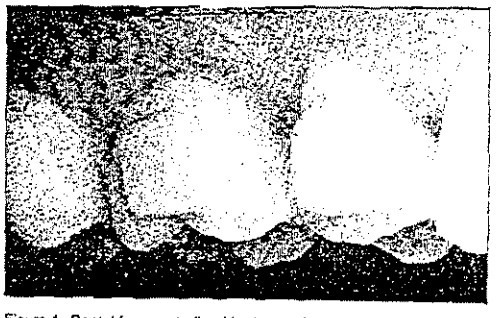

Figure 1. *Dental fragments fixed to the vestibular surfaces of the superior first molar and second premolars.*

Table 2: : Experimental Phases and Periods of the Study According to the Volunteer Groups

Phase	Period	Group 1 (15 volunteers)	Group 2 (15 volunteers)
Run-In	2 weeks	Standardized brushing with toothbrushes and toothpastes provided; tray manufacturing	Standardized brushing with toothbrushes and toothpastes provided; tray manufacturing
Experimental Phase I	3 weeks	Bleaching agent	Placebo agent
Wash-Out	2 weeks	Standardized brushing with new toothbrushes and toothpastes provided	Standardized brushing with new toothbrushes and toothpaste provided
Experimental Phase II	3 weeks	Placebo agent	Bleaching agent
Post-Experimental Phase	Time required for the volunteer	Evaluation; continuation of the bleaching treatment on both arches; follow-up	Evaluation; continuation of the of the bleaching treatment on both arches; follow-up

(Figure 1). The fragments were fixed using an adhesive system (Scotchbond, Multi-purpose/3M Dental Products, St Paul, MN 55144-1000 USA) and a composite resin (Charisma/Heraus Kulzer). The bleaching or placebo treatment was applied in the superior dental arch of each volunteer, where the experimental units were attached (vestibular surfaces of the teeth).

Fifteen volunteers applied the bleaching agent (Group 1), while 15 volunteers applied the placebo agent (Group 2) in the tray and wore it during the night for about eight hours. They were instructed to clean the tray after removing it from the mouth and keep it in a container provided.

After three weeks of the treatment with the bleaching or placebo agent (experimental Phase 1), the fragments were removed with appropriate pliers. The composite resin that adhered to the volunteer's tooth was removed with resin polishing carbide burs (KG Sorensen) and aluminum oxide discs (Sof-Lex/3M).

Volunteers were then submitted to a washout period of two weeks to eliminate the residual effects of the treatment previously applied. Volunteers received new toothbrushes and toothpastes and the toothbrushing technique was reinforced. New trays were used to eliminate any possible residues left by the previously applied agent, eliminating the possibility of it interfering with the effects of the second agent used.

Four other dental fragments—sound and demineralized enamel and sound and demineralized dentin—were fixed in the same way as that used for experimental Phase 1. This time the volunteers used the treatment agent (placebo or bleaching agent) not received at experimental Phase 1 (experimental Phase 2) for another three weeks.

The fragments were again removed with appropriate pliers. During experimental Phases 1 and 2, volunteers received a weekly syringe of bleaching or placebo agent. Table 2 shows the experimental phases and periods of the study.

I) Microhardness Tests

An acrylic device allowed the fragments to be held, keeping the long axis of the indentator perpendicular to the dental surface. Three microhardness indentations were performed on the leveled surface of each enamel and dentin fragment with a microhardness tester (Future Tech-FM-1e, Tokyo, Japan, 140) and a Knoop indentator. A load of 25 gr was used for the enamel fragments and a load of 10 gr was used for the dentin for five seconds.

J) Statistical Analysis

For statistical analysis, the average of the three Knoop Hardness Numbers was taken. Before the Analysis of Variance, the *carry-over* effect was determined by the *t*-Student test, in each volunteer. The Analysis of Variance for Greco-Latin Squares 2x2 design was employed to compare the treatment agents, using a three-dimensional block composed of "different sequences," "periods" and "quality of the dental fragment" (Montgomery, 1991). The statistical analysis was made by Statgraphics plus software (Manugistics, Inc. Rockville, Maryland 20852, USA).

RESULTS

The *t*-Student test showed no presence of the carry-over effect for enamel (p-value=0.0269) or for dentin (p-value=0.0356). These results permitted a comparison between treatment agents and quality of the dental fragments without the *carry-over* effect at the 5% level of significance.

Table 3 shows the mean of the Knoop microhardness values for enamel and dentin according to the quality of the dental fragments, treatment agents and periods. Mean Knoop microhardness values for each group, period and quality of the dental fragments are illustrated in Figures 2 and 3.

The Analysis of Variance of the experimental design considering the Greco-Latin Squares 2x2 was employed. For enamel, there were significant differences between bleaching and placebo agents (p-value=0.0045) and between sound and demineralized dental fragments (p-value<0.0001). The sound and demineralized enamel submitted to 10% carbamide peroxide bleaching agent for three weeks showed sig-

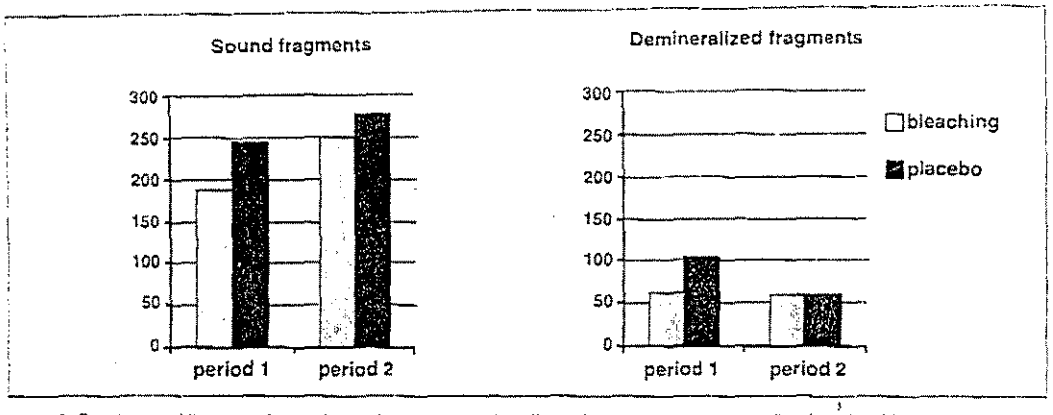

Figure 2. *Bar diagram of the mean Knoop microhardness illustrating the effects of the treatment agents, quality of the dental fragments and periods for the enamel fragments.*

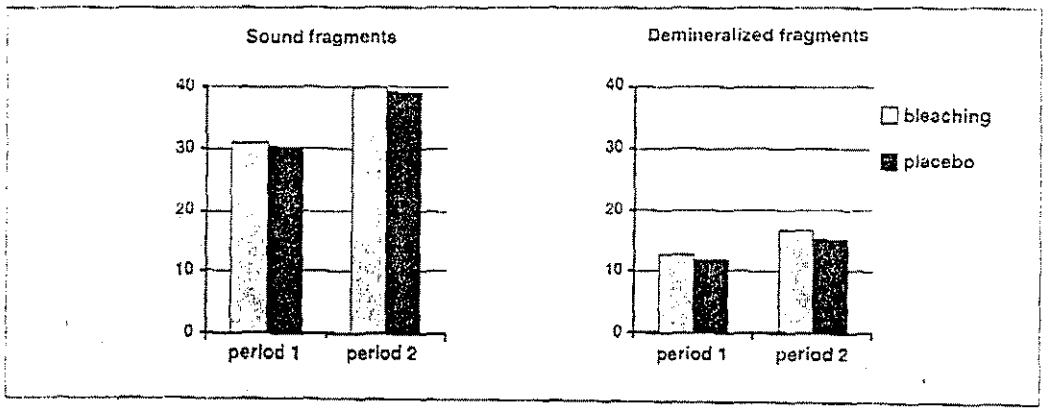

Figure 3. *Bars diagram of the mean Knoop microhardness illustrating the effects of the treatment agents, quality of the dental fragments and periods for the dentin fragments.*

nificant lower values of microhardness than those submitted to a placebo agent. The Knoop microhardness values for sound enamel fragments were significantly higher than the Knoop microhardness values for demineralized enamel fragments in both treatments.

For dentin, there were significant differences between sound and demineralized dental fragments (p-value<0.0001). There were no significant differences between sound and demineralized dentin treated with bleaching or placebo agents, but the sound and demineralized dentin submitted to 10% carbamide peroxide bleaching agent for three weeks showed slightly higher values of microhardness than those submitted to a placebo agent. The Knoop microhardness values for sound dentin fragments were significantly higher than the Knoop microhardness values for demineralized dentin fragments for bleaching and placebo agents.

DISCUSSION

Since its introduction by Haywood & Heymann (1989), nightguard vital bleaching is a procedure that has dramatically grown in dental offices due to its efficiency and simplicity in removing intrinsic or extrinsic stains from teeth (Haywood, 1992; Haywood, 1994). Many bleaching products contain 10% carbamide peroxide (Goldstein & Kiremidjian-Schumacher, 1993; Haywood, 1992) with carboxypolymethylene polymer as a thickening agent to improve tissue adherence and allow for a time or sustained release of the whitening agent (Haywood, 1994).

Table 3: *Exploratory Estimates* for Enamel and Dentin Responses on Knoop Microhardness According to the Quality of Dental Fragments, Treatment Agents and Periods*

Quality of the Dental Fragments	Treatment Agents	Enamel		Dentin	
		Period 1	Period 2	Period 1	Period 2
Demineralized	OPA	59.6 (7.0)	57.4 (14.4)	12.7 (1.5)	10.7 (1.0)
	PLA	102.6 (14.7)	57.7 (13.9)	11.8 (1.2)	15.3 (0.9)
Sound	OPA	187.4 (21.6)	250.2 (13.8)	30.8 (4.2)	39.8 (2.7)
	PLA	244.2 (14.9)	275.5 (13.4)	29.8 (2.5)	38.8 (3.2)

* Mean (standard error) - m (se) - confidence interval at 95% is taken by m ± 1.96 se

Although the bleaching agent is applied on the enamel surface, the oxidation processes of carbamide peroxide take place within the teeth by an interaction with their structural components. The effects and the mechanism of the bleaching agents should be evaluated to understand possible damages in detriment to the benefits of a more aesthetic smile offered by this technique.

In vitro studies using SEM analysis have demonstrated that applying 10% carbamide peroxide on enamel surface causes morphological changes with an increase in porosity and erosions (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst & others, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McGuckin & others, 1992; Nam & others, 1999; Shannon & others 1993; Smidt & others, 1998; Zalkind & others, 1996). In dentin, an increase in the superficial roughness was verified (Zalkind & others, 1996). Although only one paper reported no alterations in enamel (Haywood & others, 1990), the acidic properties of bleaching agents, the prolonged contact time between the lightening product and dental surface, and the presence of greater amounts of carbopol have been claimed as possible factors that can cause these superficial changes. Clinically, increased porosity allows the bleaching agent to easily penetrate through enamel and dentin and could explain the transitory dental sensitivity during its use.

Regarding inorganic and organic components in dental structure, studies should take into account the structure's mechanical properties. Changes in the inorganic and organic components ratio could be deleterious to teeth (Featherstone & others, 1983; Featherstone & others, 1986). Free radicals of the decomposition of carbamide peroxide may react with organic components of the dental structure and the low pH of bleaching systems may cause demineralization. Loss of mineral can be related to the acidic properties of bleaching agents, even though Leonard & others (1994) observed an increase in the pH levels of 10% carbamide peroxide after its dissociation in the mouth. A loss of calcium after the exposure of enamel to 10% carbamide peroxide was observed in some *in vitro* studies (McCracken & Haywood, 1996; Rotstein & others, 1996), though this reduction of mineral content was not clinically significant.

Differences in organic and inorganic content can also be verified by microhardness tests (Featherstone & others, 1983). Although some *in vitro* studies have shown no microhardness changes on sound enamel and dentin submitted to the treatment with 10% carbamide peroxide (Murchison & others, 1992; Seghi & Denry, 1992; Nathoo & others, 1994; McCracken & Haywood, 1995), significant differences were found in other experiments (Pécora & others, 1994; Attin & others, 1997; Rodrigues & others, 2001; Shannon & others, 1993; Smidt & others, 1998).

A dynamic model of inducing artificial caries lesions through pH cycles of demineralization and remineralization solutions (Featherstone & others, 1986; Serra & Cury, 1992; Hara & others, 2000) was used in this study. This model presents a correlation with the initiation and progression of carious lesions in patients at high-risk for caries (Featherstone & others, 1986), leading to changes in the mineral and organic content of teeth. The applicability of the model for inducing caries can be verified by the significant differences between the quality of enamel and dentin fragments (p-value<0.0001; p-value<0.0001). It shows that the demineralization-remineralization model was effective in producing artificial caries lesions. The Knoop microhardness values for sound enamel fragments were significantly higher than those for demineralized enamel fragments in both treatments. The same occurred for sound and demineralized dentin fragments.

For enamel fragments, it was verified that sound and demineralized fragments submitted to effects of the bleaching agent presented lower microhardness values than sound and demineralized fragments submitted to the placebo agent. These results were obtained by fixing the dental fragments in a vestibular location—close to the parotid salivary gland duct exit where there is a low-risk for caries. Because the fragments were maintained in the mouth during all experimental phases and at the same location (with constant flow of saliva, temperature and pH changes, fluorides, toothbrush abrasiveness and effects of liquids and foods in the oral environment), the authors consider the results reliable. The bleaching agent caused a mineral loss in human dental fragments, even though saliva, plaque control and fluorides were present in the oral environment. These factors could be responsible for maintaining the balance between the demineralization and

remineralization phenomena. This mineral loss can not only be related to the pH level of the bleaching agent. Leonard & others (1994) showed that a 10% carbamide peroxide solution with a moderately low-pH presented an increase of its pH level after five minutes of degradation, reaching a neutral pH. However, the pH level of OPA used in this experiment was not as acidic, but an increase of OPA pH level can also be expected. Thus, prolonged contact between the product and dental structure can be responsible for the decrease in microhardness values. Due to a high level of mineral content of enamel, the bleaching agent seems to cause a demineralization effect in the enamel structure, even though a slight decrease in organic content in the enamel could take place (the percentage of organic content and water in enamel is around 4% (ten Cate, 1988).

For dentin fragments, there was no significant difference between the bleaching and placebo agent. The percentage of mineral content in dentin (70%) is lower than in enamel (ten Cate, 1988). Therefore, the authors' results also suggest that, although some alterations occur in the dentin, these effects do not damage the inorganic/organic content in dentin structure, but significantly affect the mineral content of the enamel fragments.

This experiment can also elucidate the importance of not applying bleaching agents on early carious lesions due to their damaging effects. On sound dental structures, bleaching agents can be used as an aesthetic treatment, but one should be aware of the lower microhardness values obtained in this study. There is a possibility that human enamel could be damaged, although the effects of higher concentrations of fluorides and a post-bleaching time were not evaluated. Perhaps a prolonged time contact between the dental structure with saliva and fluorides could help reverse the ratio between organic and inorganic mineral content and to return to the initial conditions which could increase the enamel microhardness values. This emphasizes that at-home bleaching agents require future research, even though professional supervision can ensure correct selection of the proper case, correct application and steps to prevent adverse reactions.

CONCLUSIONS

The results suggest that:

a) treatment with 10% carbamide peroxide bleaching material for three weeks alters the microhardness of sound and demineralized enamel;

b) treatment with 10% carbamide peroxide bleaching material for three weeks does not alter the microhardness of sound and demineralized dentin.

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ANEXO VI

Anexo VI: Certificado do Comitê de Ética em Pesquisa para o desenvolvimento do projeto *"Effects of ten percent carbamide peroxide bleaching agent associated with desensitizing dentifrices on enamel microhardness at different time intervals"*.

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COMITÊ DE ÉTICA EM PESQUISA

Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba
CEP-FOP-UNICAMP

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito da utilização do peróxido de carbamida a 10% associado à aplicação de dentifrício fluoretado sobre a microdureza do esmalte em diferentes tempos", sob o protocolo nº **99/99**, do Pesquisador(a) ***Rogério de Oliveira,*** sob a responsabilidade do Prof(a). Dr(a). **Mônica Campos Serra**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 24 de fevereiro de 2000

We certify that the research project with title "Effect of a carbamide peroxide 10% agent associated with the aplication of a fluoride dentifrice on enamel microhardness at different time intervals ", protocol nº **99/99**, by Researcher ***Rogério de Oliveira,*** responsibility by Prof. Dr. **Mônica Campos Serra,** is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, Febrwary 02 2000

Prof. Dr. Pedro Luiz Rosalen
Secretário - CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador - CEP/FOP/UNICAMP

ANEXO VII

Anexo VII: Certificado do Comitê de Ética em Pesquisa para o desenvolvimento do projeto *"Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals"*.

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Faculdade de Odontologia de Piracicaba
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CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito do peróxido de carbamida a 10% sobre a microdureza da dentina em diferentes tempos", sob o protocolo nº **98/99**, do Pesquisador(a) ***Patricia Moreira de Freitas*** sob a responsabilidade do Prof(a). Dr(a). **Mônica Campos Serra**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 22 de fevereiro de 2000

We certify that the research project with title "Effect of 10% carbamide peroxide agents on dentin microhardness at different time intervals ", protocol nº **98/99**, by Researcher ***Patricia Moreira de Freitas***, responsibility by Prof. Dr. **Mônica Campos Serra**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, Febrwary 22 2000

Prof. Dr. Pedro Luiz Rosalen
Secretário - CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador - CEP/FOP/UNICAMP

Anexo VIII: Certificado do Comitê de Ética em Pesquisa para o desenvolvimento do projeto "*Effects of seven carbamide peroxide bleaching agents on enamel microhardness at different time intervals*".

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Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba
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CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito de agentes clareadores de peróxido de carbamida sobre a microdureza do esmalte em diferentes tempos", sob o protocolo nº **91/99**, do Pesquisador(a) ***Roberta Tarkany Basting*** sob a responsabilidade do Prof(a). Dr(a). **Mônica Campos Serra**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 15 de março de 2000

We certify that the research project with title "Effect of 10 percent peroxide carbamide agents on enamel microhardness at different bleaching times", protocol nº **91/99**, by Researcher ***Roberta Tarkany Basting***, responsibility by Prof. Dr. **Mônica Campos Serra**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, March 15 2000

Prof. Dr. Pedro Luiz Rosalen
Secretário - CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador - CEP/FOP/UNICAMP

Anexo IX: Certificado do Comitê de Ética em Pesquisa para o desenvolvimento do projeto *"The effect of 10 % carbamide peroxide on the microhardness of sound and demineralized enamel and dentin 'In situ' "*.

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PARECER DO CEP — FOP/UNICAMP

Comunicamos que o Protocolo de Pesquisa referente ao Projeto No. 84/98:

Título do Projeto de Pesquisa: Microdureza de tecidos dentais e desmineralizados submetidos à ação "in situ"do gel clareador de peróxido de carbamida à 10%

Pesquisador Orientador/Orientado:
Profª Monica Campos Serra / Roberta Tarkany Basting

apresentado a este Comitê para análise ética, segundo a Resolução CNS 196/96, do Conselho Nacional de Saúde, de 10/10/96, e de acordo com cópia do projeto arquivada em nossa secretaria, foi considerado:

[X] Aprovado, em reunião realizada em _02/12/98_.
[ ] Aprovado com pendência, devendo o Pesquisador encaminhar as modificações sugeridas em anexo para complementação da análise do Projeto.
[ ] Com pendência.
[ ] Reprovado.

Análise e parecer do relator (com resumo do projeto):
O projeto visa avaliar a microdureza de tecidos dentais hígidos e desmineralizados quando submetido à ação do gel clareador de peróxido da carbamida a 10% pelo período de 03 semanas. Para o experimento será utilizado 240 fragmentos dentais sendo 60 de esmalte hígido, 60 desmineralizadas e 60 de dentina. Estes fragmentos serão fixados nas faces vestibulares dos 2°s. molares e 1°s. m,olares superiores de 30 voluntários, (alunos de graduação e pós-graduação). Sobre os fragmentos será aplicado o agente clareador comercial e placebo. A avaliação dos tecidos dentais hígidos e desmineralizados. serão realizados através de ensaios de microdureza. Considerando ser um trabalho de valor clínico apreciável, uma vez que está sendo realizado aplicações caseiras, aleatórias, com procedimentos inadequados podendo ser considerado prejudicial ao paciente, sugerimos que o projeto seja APROVADO.

CEP-FOP/UNICAMP
Prof. Dr. Antonio Benito Alves de Moraes
Coordenador
CEP-FOP/UNICAMP