PATRICIA MOREIRA DE FREITAS

CIRURGIÃ-DENTISTA

EFEITO DE AGENTES CLAREADORES CASEIROS SOBRE O CONTEÚDO MINERAL DOS TECIDOS DENTAIS

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

PIRACICABA

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A Comissão Julgadora dos trabalhos de Defesa de Tese de MESTRADO, em sessão pública realizada em 27 de Fevereiro de 2003, considerou a candidata PATRICIA MOREIRA DE FREITAS aprovada.

1. Profa. Dra. MONICA CAMPOS SERRA _____

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3. Prof. Dr. JAIME APARECIDO CURY _____

DEDICATORIA

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"É melhor tentar e falhar,

que preocupar-se e ver a vida passar;

é melhor tentar, ainda que em vão,

que sentar-se fazendo nada até o final.

Eu prefiro na chuva caminhar,

que em dias tristes em casa me esconder.

Prefiro ser feliz, embora louco,

que em conformidade viver ..."

Martin Luther King

SUMÁRIO

RESUMO

O clareamento dental caseiro vem sendo cada vez mais empregado no tratamento estético de dentes com alteração de cor, por ser considerado uma alternativa conservativa e, na maioria das vezes, efetiva. Contudo, ainda existem dúvidas quanto aos efeitos dos agentes clareadores sobre a estrutura dental. Desta forma, o presente trabalho teve como objetivos: (1) avaliar a microdureza da dentina humana hígida e desmineralizada submetida ao tratamento clareador com agentes contendo peróxido de carbamida, pelo período de 42 dias e 7 e 14 dias após o término do clareamento e (2) apresentar e discutir os possíveis fatores relacionados às alterações no conteúdo mineral da estrutura dental ocorridos durante e após o tratamento clareador. Nos estudos *in vitro* foram utilizadas amostras de dentina radicular humana para analisar o efeito de seis agentes clareadores (Nite White Excel 2Z 10% e 22%, Opalescence 10% e 20%, Rembrandt 10% e 22%) sobre a microdureza da dentina ao longo do clareamento e no período pós-tratamento. Concluiu-se que, na dependência do agente clareador utilizado, a dentina hígida ou desmineralizada pode apresentar um aumento ou redução transitória nos valores de microdureza. Foi elaborado um artigo de revisão com o intuito de discutir o efeito da saliva, do flúor e da composição e subprodutos dos géis clareadores nas alterações do conteúdo mineral do esmalte e dentina ocorridos durante e após o clareamento caseiro.

PALAVRAS-CHAVE: clareamento dental caseiro, conteúdo mineral, dentina, esmalte, microdureza, peróxido de carbamida.

ABSTRACT

Nightguard vital bleaching has become the most indicated treatment for improving the esthetic appearance of discolored teeth, as it is considered conservative and effective. However, there is still some concern about the effects of these bleaching agents on dental structure. Therefore, the present study aimed to: (1) evaluate the microhardness of sound and demineralized dentin throughout the 42 days of whitening treatment and 7 and 14 days after concluding the bleaching period and (2) elucidate the possible factors related to alterations of tooth mineral content occurring during and after bleaching procedures. The *in vitro* studies were conducted using samples of human root dentin to analyze the effect of six whitening agents (Nite White Excel 2Z 10% and 22%, Opalescence 10% and 20%, Rembrandt 10% and 22%) on dentin microhardness during the bleaching treatment and at a post-treatment period. It was concluded that, depending on the bleaching agent applied, the sound or demineralized dentin might show an increase or transitory decrease in microhardness values. A review article was written describing the possible factors related to alterations of tooth mineral content occurring during and after bleaching procedures.

KEY WORDS: carbamide peroxide, dentin, enamel, home tooth bleaching, microhardness, mineral content.

1. INTRODUÇÃO GERAL

A crescente procura por uma melhor aparência estética associada à filosofia de preservação da estrutura dental, têm contribuído de forma significativa para o aumento da indicação do clareamento caseiro para o tratamento de dentes escurecidos, em detrimento de procedimentos mais invasivos e onerosos.

A técnica de clareamento dental caseiro foi primeiramente descrita por HAYWOOD & HEYMANN, em 1989, e empregava, originalmente, o peróxido de carbamida a 10% em uma moldeira individual por 8 horas diárias, pelo período de 2 a 6 semanas. Apesar de sua natureza pouco previsível, o tratamento clareador apresenta uma série de vantagens, como o baixo custo e facilidade técnica, e tem sido descrito como uma alternativa segura e efetiva na maioria dos casos (HAYWOOD, 1992; HAYWOOD, 2000).

O peróxido de carbamida, quando em contato com a água ou fluidos salivares, decompõe-se em peróxido de hidrogênio e uréia (HAYWOOD & HEYMANN, 1989; GOLDSTEIN & GARBER, 1996). De acordo com GOLDSTEIN & GARBER (1996), o agente ativo, peróxido de hidrogênio, libera água e radicais livres de oxigênio e, simultaneamente, hidrogênio e peridroxil em proporções dependentes do pH do meio de degradação. Em meio básico, uma maior quantidade de radicais livres de hidrogênio e peridroxil é produzida, promovendo a oxidação das macromoléculas carbonadas pigmentadas, em moléculas menores e descoradas (GOLDSTEIN & GARBER, 1996). Considerando-se o mecanismo de ação do agente clareador e a sua capacidade em difundir livremente pela estrutura dental (HAYWOOD, 1990; HANKS *et al.*, 1993; GOLDSTEIN & GARBER, 1996), alterações morfológicas e químicas no esmalte e dentina podem ser esperadas.

Análises quantitativas da composição (ROTSTEIN *et al.*, 1996; CIMILLI & PAMEIJER, 2001) ou avaliação dos valores de microdureza (ATTIN *et al.*, 1997; SMIDT *et al.*, 1998; BASTING *et al.*, 2001; RODRIGUES *et al.*, 2001) revelaram diminuição do conteúdo mineral do esmalte durante ou após o clareamento; outros trabalhos, porém, afirmam não encontrar mudanças significativas (MURCHINSON *et al.*, 1992; SEGHI & DENRY, 1992; SHANNON *et al.*, 1993; NATHOO *et al.*, 1994; MCCRACKEN & HAYWOOD, 1995; POTOCNIK *et al.*, 2000; LEONARD *et al.*, 2001; LOPES *et al.*, 2002).

Em busca de resultados estéticos mais rápidos, agentes clareadores caseiros a base de peróxido de carbamida em concentrações mais elevadas (15-22%) foram introduzidos no mercado (LEONARD *et al.*, 1998). Alterações mais severas foram encontradas quando aplicados agentes clareadores em maiores concentrações (FLAITZ & HICKS, 1996; OLTU & GURGAN, 2000); porém, existem resultados controversos demonstrando que os efeitos sobre o esmalte e a dentina independem da concentração dos materiais utilizados (ROTSTEIN *et al.*, 1996; ZALKIND *et al.*, 1996; CIMILLI & PAMEIJER, 2001).

Acredita-se que o pH (SHANNON *et al.*, 1993; FREITAS *et al.*, 2002) e componentes (ZALKIND *et al.*, 1996; ROTSTEIN *et al.*, 1996; RODRIGUES *et al.*, 2001) de alguns agentes clareadores podem estar intimamente relacionados com o ganho ou perda mineral durante o tratamento clareador. A capacidade tampão e a presença de minerais, enzimas e flúor na saliva parecem contribuir de forma imperativa na manutenção das propriedades dos tecidos dentais (MARSHALL *et al.*, 2001; LOPES *et al.*, 2002). Além disso, subprodutos do gel clareador também foram referidos como substâncias capazes de elevar o pH durante a decomposição do peróxido de

carbamida (LEONARD *et al.*, 1994a, 1994b) e, possivelmente, evitar alterações no conteúdo mineral (FLAITZ & HICKS, 1996; OLIVEIRA *et al.*, 2002).

Muitos questionamentos ainda persistem quanto ao efeito do clareamento caseiro sobre a dentina humana (NATHOO *et al.*, 1994; ROTSTEIN *et al.*, 1996; ZALKIND *et al.*, 1996; BASTING *et al.*, 2001; FREITAS *et al.*, 2002). Pouco se sabe a respeito das conseqüências da exposição da dentina a elevadas concentrações desses produtos oxidantes e de que forma um prolongado período de exposição a diferentes marcas comerciais desses agentes pode induzir alterações na microdureza do substrato dentinário. Os efeitos do clareamento seriam mais acentuados sobre a dentina, em função da sua maior composição orgânica e do seu pH crítico de desmineralização (6,2-6,7) (HOPPENBROUWERS *et al.*, 1987; WEFEL, 1994). Com o intuito de avaliar a microdureza da dentina humana antes e durante o período de 6 semanas de clareamento caseiro com diferentes agentes a base de peróxido de carbamida e após 2 semanas de armazenamento em saliva, o estudo "Dentine microhardness throughout and after whitening treatments" (capítulo 1) foi delineado e realizado.

A possibilidade do agente clareador entrar em contato com uma superfície de dentina desmineralizada pode ser considerada, uma vez que o estágio inicial de lesões de cárie pode não ser detectado clinicamente (NYVAD *et al.*, 1997). Desta forma, BASTING *et al.* (2001) avaliaram o efeito do peróxido de carbamida a 10% sobre a microdureza da dentina hígida e desmineralizada. Contudo, altas concentrações dos agentes clareadores caseiros, de diferentes marcas comerciais, bem como um prolongado período de tratamento não foi até o presente momento avaliado. Desta forma, o trabalho "Monitoring of demineralized dentin microhardness throughout and after bleaching" (capítulo 2) foi desenvolvido.

As dúvidas quanto ao mecanismo de ação dos peróxidos e de que forma eles podem estar relacionados às alterações do conteúdo mineral levou à elaboração do capítulo 3, entitulado "Dental mineral equilibrium during and after home-bleaching: a review ". O artigo objetivou elucidar os possíveis fatores relacionados a perda e ganho de minerais do esmalte e da dentina que podem ocorrer durante e após o tratamento clareador caseiro.

2. PROPOSIÇÃO

O presente trabalho, composto por 3 artigos científicos, teve como objetivos:

1. Avaliar *in vitro* o efeito de diferentes agentes clareadores sobre a microdureza da dentina hígida e desmineralizada ao longo do tratamento clareador pelo período de 42 dias e 7 e 14 dias após o término do tratamento;

2. Discutir os possíveis fatores relacionados a perda e ganho mineral do esmalte e dentina ocorridos durante e após o clareamento dental.

CAPÍTULO 1

Dentine microhardness throughout and after whitening treatments

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"Dentine microhardness throughout and after whitening treatments"

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CLINICAL RELEVANCE

Different bleaching agent brands may change the dentine mineral content during whitening treatment, but saliva is capable of maintaining or recovering microhardness values.

SUMMARY

This *in vitro* study evaluated the effect of bleaching agents on dentine microhardness during and after bleaching. Specimens were randomly assigned into 7 groups (n=15): NiteWhite Excel2Z [NW] 10% and 22%, Rembrandt [REM] 10% and 22%, Opalescence [OPA] 10% and 20%, and a placebo agent. The 42 days whitening treatment consisted of the agents daily application on dentine surfaces for 8 h, followed by immersion in artificial saliva for 16 h. After the bleaching treatment, specimens were kept immersed in artificial saliva for 14 days. Microhardness was measured at *baseline*, 8 h, 7, 14, 21, 28, 35 and 42 days of bleaching and in the post-treatment period (7 and 14 days). The ANOVA for *Split-plot* showed a significant effect on the interaction between bleaching agent and time (α=0.05). Tukey's test and regression analyses revealed that, during the bleaching period, the agents NW 10%, NW 22% and OPA 20%, which did not differ from each other, did not alter dentine microhardness, showing constant microhardness values. There were no differences among REM 10%, REM 22% and OPA 10%, which showed a significant reduction in microhardness after day 14 compared to other agents. After bleaching procedures there was an increase in dentine microhardness for all groups.

INTRODUCTION

The demand for conservative aesthetic dentistry has grown dramatically and there has been rapid development of new non-restorative treatment for discoloured teeth. Within the conservative techniques, nightguard vital bleaching has become very popular due to its safety and effectiveness (Haywood, 1992; Goldstein & Kiremidjian-Schumacher, 1993; Goldstein & Garber, 1996).

In the bleaching process, hydrogen peroxide quickly oxidises the stained macromolecules and breaks them down into smaller fragments, which diffuse across the tooth surface, resulting in the whitening effect (Haywood, 1992; Goldstein & Garber, 1996).

In order to achieve satisfactory aesthetic results in a shorter period of time, the use of different concentrations of whitening agents for home and chairside bleaching has been proposed (Leonard *et al.*, 1998). The use of higher concentrations of carbamide peroxide promotes a release of higher amounts of hydrogen peroxide and allows whitening results to be achieved more quickly (Leonard *et al.*, 1998).

The enhancement of aesthetic appearance is not expected to occur at the risk of developing side effects on dental hard tissues. As bleaching procedures generally involve the direct contact of the whitening agents with tooth surfaces for an extensive period of time, alterations in enamel and dentine microhardness may occur.

Although some authors showed no changes in enamel microhardness (Murchinson *et al.*, 1992; Nathoo *et al.*, 1994), the majority of *in vitro* studies support that the oxidising reaction could alter the chemical structure of enamel after exposure to bleaching agents (Shannon *et al.*, 1993; Bitter, 1998; McCracken & Haywood, 1996; Rotstein *et al.*, 1996; Attin *et al.*, 1997; Perdigão *et al.*, 1998; Rodrigues *et al.*, 2001).

Bleaching agents could come into contact with dentine in areas of enamel defects or

abrasion and mainly on exposed root surfaces (Zalkind *et al.*, 1996). However, few studies were conducted to evaluate the effects of home-bleaching agents on dentine (Haywood *et al.*, 1990; Wandera *et al.*, 1994; Rotstein *et al.*, 1996; Zalkind *et al.*, 1996) and little is known about the consequences of the dentine exposure to higher concentrations of these oxidising products, and whether its long-period application could induce changes in tooth hardness. Moreover, the presence of saliva, which has a buffering and remineralising capacity, is expected to maintain the pH level of the oral cavity and increase or uphold the dentine mineral content (Featherstone *et al.*, 1986).

This *in vitro* study evaluated the dentine microhardness when exposed to different carbamide peroxide based bleaching agents for 42 days and the saliva effect in a post-treatment period.

METHODS AND MATERIALS

Experimental Design

The factors under study were Treatment Agents at seven levels (Nite White Excel 2Z 10%, Nite White Excel 2Z 22%, Opalescence 10%, Opalescence PF 20%, Rembrandt 10%, Rembrandt 22% and a placebo agent as a control) and Time at ten levels (0 - baseline - 8 hours, 7, 14, 21, 28, 35, 42 days of treatment and 7 and 14 days of post-treatment period, corresponding to 49 and 56 days from the beginning of the treatment).

This study used 15 experimental samples for each treatment agent made in 5 blocks, with 3 replicates each. As it was not possible to randomise the time intervals studied, a randomisation restriction was considered, characterising a factorial 7x10 *Split-plot* design (Montgomery, 1991).

Specimen Preparation

The study protocol was reviewed and approved by the local ethical committee. Forty freshly extracted, non-erupted third molars, were collected and immediately stored in 2% formaldehyde (pH 7.0). The crowns were removed approximately to the cementoenamel junction and the roots were longitudinally sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP, Brazil, 06454-920) used 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 to obtain slabs with a 3x3 mm standardised area. After sectioning was completed, all slabs were stored at 37±1°C in 100% relative humidity. The samples with stains or cracks, observed under a stereomicroscope used at X30 (Meiji Techno EMZ series, Saitama, Japan, 356), were discarded. A hundred and five dentine slabs were embedded individually in a self-curing polyester resin in a polyvinyl-chloride ring mould 2.1cm in diameter so that the external surface of the dentine fragment was exposed and left to polymerise .

After moulds were removed, specimens were polished in a water-cooled mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil, 01328-000) with aluminium oxide abrasive papers in a sequence of 600-, 1000- and 1200-grits (Carborundum/ 3M Dental Products, Sumaré, SP, Brazil, 13001-970) to obtain parallel planar surfaces for performing the Knoop microhardness test.

Treatment Agents

The specimens were randomly assigned to the seven experimental groups of 15 specimens each. All the materials used in the study are described in Table 1.

Exposure to Bleaching Materials

The dentine slabs were exposed to the treatment agents (experimental and placebo) for 8 hours per day during 42 days. During the remaining diary time (16 hours) they were stored in an

individual recipient with 13.5 ml of artificial saliva, consisting of a remineralization solution proposed by Featherstone *et al.* (1986) and modified by Serra & Cury (1992).

For each specimen an individual tray was manufactured, using a 0.4 mm thick ethyl vinyl acetate (EVA) polymer (BioArt Equipamentos Odontológicos Ltda., São Carlos, São Paulo, SP, Brazil, 13568-000) in a vacuum-forming machine (P7/ BioArt Equipamentos Odontológicos Ltda., São Carlos, São Paulo, SP, Brazil, 13568-000). A syringe was used to apply 0.02 ml of each treatment agent on the specimens' surfaces. Then, the samples were recovered with the trays and soaked in individual recipients with 13.5 ml of artificial saliva (pH = 7.0), at 37±1°C, for 8 hours. Next, the specimens were washed under running distilled and deionised water for 5 seconds and stored in 13.5 ml artificial saliva for the remaining dairy time (16 hours). These procedures were repeated for 42 days and the storage media was changed every 2 days.

Post-treatment Period

After 42 days of treatment, the specimens were kept in their individual recipients with 13.5 ml of artificial saliva, at 37±1°C, which was changed every two days. Knoop Microhardness tests were conducted 7 and 14 days after concluding the treatment period (corresponding to 49 and 56 days after the beginning of the treatment).

Microhardness Tests

Microhardness measurements were performed before the exposure to the treatment agents (*baseline*), during the agents' application (8 hours, 7, 14, 21, 28, 35, 42 days) and in the post-treatment period (7 and 14 days after concluding the treatment). A Knoop Microhardness testing machine (Future Tech – FM-1e, Tokyo, Japan, 140) was used to make three indentations on each specimen with a load of 10 g applied for 5 seconds.

Statistical Analysis

For each specimen, the average of the three Knoop Hardness Numbers was taken. A multi-factor Analysis of Variance (ANOVA) (α= 0.05) for *Split-plot* design was applied (repeated measurements at the same experimental unit). A study of the interaction among the factors analysed (Treatment Agent, Time and Block) was made. The interaction of particular interest was Treatment agent x Time. Multiple Comparisons Tukey's test (α= 0.05) was applied to identify differences in means within the factor Time, and a regression analysis was chosen to show the behaviour of dentine microhardness along the time intervals, within each level of the factor Treatment Agent. The analysis was performed with the Statgraphics Plus (Manugistics, Rockville, Maryland, USA, 20852) and SAS system 6.11 (SAS Institute Inc. Cary, NC, 27513-2414) software.

RESULTS

The data did not show either homogeneity of variances or normal distribution. In order to stabilise them, they were submitted to a root square transformation. The ANOVA for *Split-plot* verified a significant interaction between the Treatment Agent and Time (α= 0.05).

Table 2 shows the transformed mean Knoop microhardness values of each treatment agent at different time intervals and the results of the Tukey's test at a 5% level of significance. The statistical differences are shown by different upper cases at the right side of the mean (per column).

The Tukey's test revealed no significant differences for Treatment Agent up to the first day (8 h), but showed significant differences from the day 7 to the day 42 of treatment and in the post-treatment periods.

Regression analysis was used to demonstrate the behaviour of each Treatment Agent throughout and after the whitening procedures (Graphics 1-7). The groups REM 10% and 22%, OPA

10% and 20% and Control were fitted according to a quadratic function. For the groups NW 10% and 22% a linear regression was adjusted.

During the bleaching period, the agents NW 10% and 22% and OPA 20% did not differ from each other, showing constant microhardness values. There were no significant statistical differences among OPA 10% and REM 10% and 22%, which showed a significant reduction in microhardness after day 14 compared to the other agents. After bleaching procedures there was a tendency for dentine microhardness to increase for all groups.

DISCUSSION

The use of different agent concentrations for home bleaching is a common practice and has been frequently described in the literature (Haywood, 2000). Considering that the whitening process is based upon the ability of the bleaching agents to generate free radicals, which are extremely unstable and highly reactive, and that the breakdown of the organic matrix can occur (Goldstein & Garber, 1996), it is expected that higher concentrations of these oxidising agents could alter dentine microhardness.

The current study revealed that some bleaching agents containing as much as 20-22% carbamide peroxide did not reduce dentine microhardness, suggesting that mineral loss may not only be related to agents' concentration (Spyrides *et al.*, 2000) and the higher amounts of hydrogen peroxide released. Changes in dentine chemical and structural composition seems to occur after a repeated exposure to whitening agents and could be associated to its pH level or some of its compounds, such as fluoride ions, desensitising agents, carbopol or any other product (McCracken & Haywood, 1996).

Bleaching agents, mainly those of higher concentrations (NW 22%, OPA 20% and REM

22%), include desensitising agents, like nitrates, citrates or fluorides in an attempt to avoid detrimental effects. Oliveira *et al.* (2002) showed that 10% carbamide peroxide associated with desensitising agents, like potassium nitrate and strontium chlorate, could maintain or increase enamel microhardness submitted to bleaching treatment for 42 days. The authors also suggest that products containing these desensitising agents could increase microhardness values or react with the by-products of the carbamide reaction, enhancing microhardness over time.

The dentine exposure to NW 10% revealed higher microhardness values from day 14 to 35 compared to OPA 10% and REM 10% and showed constant microhardness values throughout the bleaching treatment. Dentine fragments treated with NW 22% and OPA 20% also showed constant microhardness values during and after the bleaching treatment. The presence of potassium nitrate and fluoride in NW 10%, NW 22% and OPA 20% might have contributed to the preservation of the dentine microhardness. Low levels of fluoride, when present during dentine demineralisation process, can markedly inhibit mineral loss, enhancing remineralization in a similar manner to that which occurs in dental enamel (Featherstone, 1994). A study by Wiesmann *et al.* (1998) suggests that potassium is involved in the process of dentine mineralisation, precipitating on the dentine matrix before calcium-phosphate association, which should explain the constant microhardness values observed in the specimens treated with all agents containing potassium nitrate. Furthermore, the storage in artificial saliva during the bleaching period was also supposed to contribute to mineral enhancement.

The agents NW 10% and 22%, even though described as carbamide peroxide bleaching agents, have only 8.10% of this whitening product and contain 0.73 and 5.05% of hydrogen peroxide, respectively. Although the released concentration of this oxidising agent is similar to those suggested as coming purely from carbamide peroxide whitening agents, the presence of hydrogen

peroxide is expected in carbamide peroxide degradation and could decrease pH level during bleaching procedures, as it releases hydrogen ions and perhydroxyl radicals (Goldstein & Garber, 1996). However, at the same time, in the presence of urea, a product of carbamide peroxide decomposition, the pH level is expected to rise and keep up for almost 2 hours (Leonard *et al.*, 1994a; Leonard *et al.*, 1994b). The present study suggests that low concentrations of hydrogen peroxide associated with carbamide peroxide could not decrease dentine microhardness.

The agent REM 22%, unlike the other 20-22% bleaching agents (OPA 20% and NW 22%), induced mineral loss during the treatment agent application. Reported as a low pH product, ranging from 4.9-6.8 (Shannon *et al.*, 1993), Rembrandt requires caution when considering the critical pH for dentine demineralisation (6.2-6.7) (Wefel, 1994; Hoppenbrouwers *et al.*, 1987). Different from NW 10% and 22%, which contain potassium nitrate, both REM 10% and 22% have sodium citrate, referred to as an ineffective desensitising agent due to its low capability of occluding dentine tubules (Collaert & Fisher, 1991). Furthermore, the presence of carbopol 940 and glycerine, or the possible interaction of some bleaching by-product with the dental hard tissues, might have induced the pH level decrease and, consequently, alterations to dentine mineral content.

When applying the placebo agent, there was a decrease in dentine microhardness, followed by its increase in the post-treatment period. The placebo agent consisted of a neutral pH product, containing glycerine and carbopol to provide equal hydration for the samples. Although glycerine and carbopol are considered inactive agents, it seems important to consider the effects of these products on dentine mineral content. As REM 10% and 22%, the placebo agent includes carbopol 940 in its composition and all these treatment agents revealed similar behaviour throughout the whitening period. A decrease in enamel microhardness, related to the treatment with a product containing carbopol, was reported by McCracken & Haywood (1996). This suggests that the bleaching-induced

changes in dental hard tissues might be associated with the compounds of some agents. To investigate this point, there is a need for further studies about the effect of these products separately on dentine microhardness.

A study conducted by Nathoo *et al.* (1994) evaluated the effects of dental hard tissues exposure to a commercial whitening system, showing that there were no changes on dentine microhardness values after 3 weeks of bleaching treatment. The present research revealed that, after a six-weeks bleaching treatment, OPA10% and REM 10%, as reported in a previous study (Freitas *et al.*, 2002), induced mineral loss even with the specimens' storage in artificial saliva. Although a solution that contains calcium and phosphate ions was employed to increase remineralization potential, and an attempt made to replicate the conditions of oral cavity (Featherstone *et al.*, 1986; Serra & Cury, 1992), there is still a need for further investigations on storage media for substituting human saliva.

Up to the first 8 hours of treatment none of the groups differed in microhardness values, but after this period the agents OPA 10% and REM 10% and 22% showed a mineral loss compared with the agents NW 10% and 20% and OPA 20%. Depending on the agent applied, dentine may show an increase or transitory decrease in microhardness. Even though products have the same carbamide peroxide concentration, as 10 or 20-22%, the addition of other compositional factors could influence their effects on the dentine surface. In the post-treatment period, artificial saliva showed a remineralising effect on the treated specimens. Although saliva maintained dentine microhardness values, the use of different bleaching agents for a six weeks treatment period should be prescribed with caution.

ACKNOWLEGMENT

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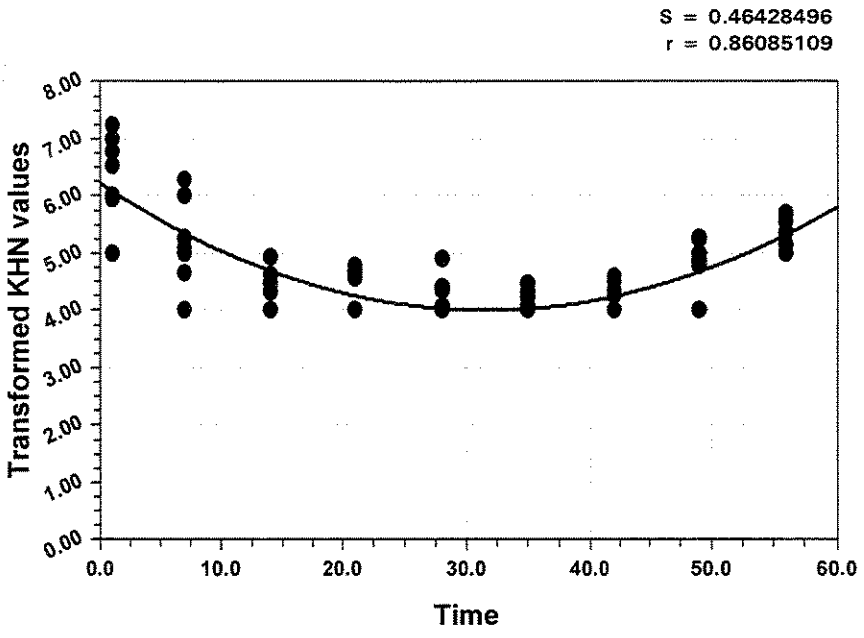
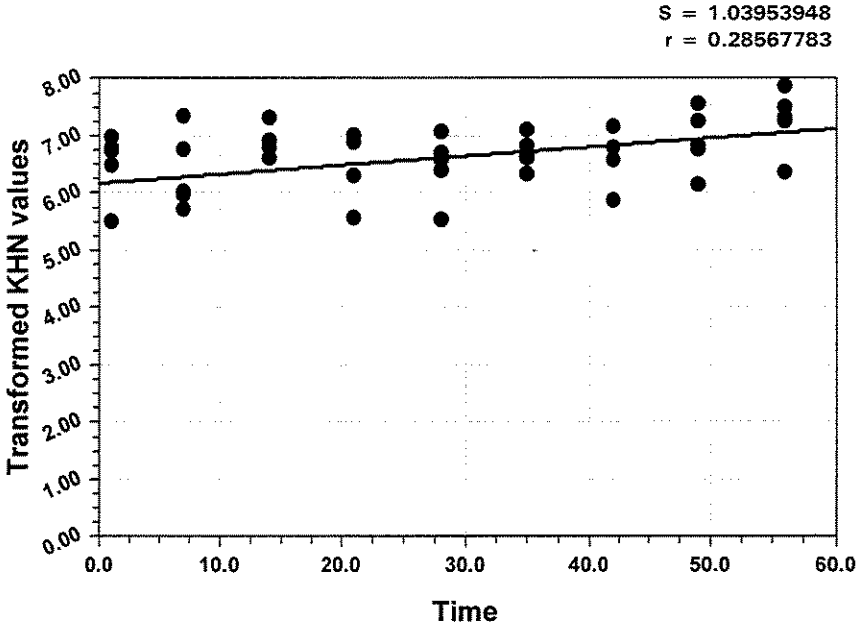
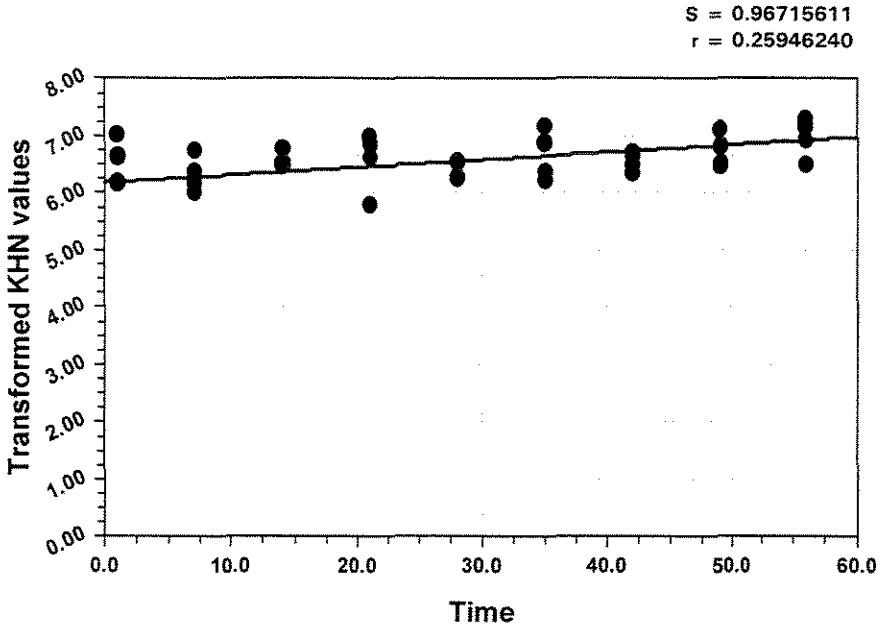


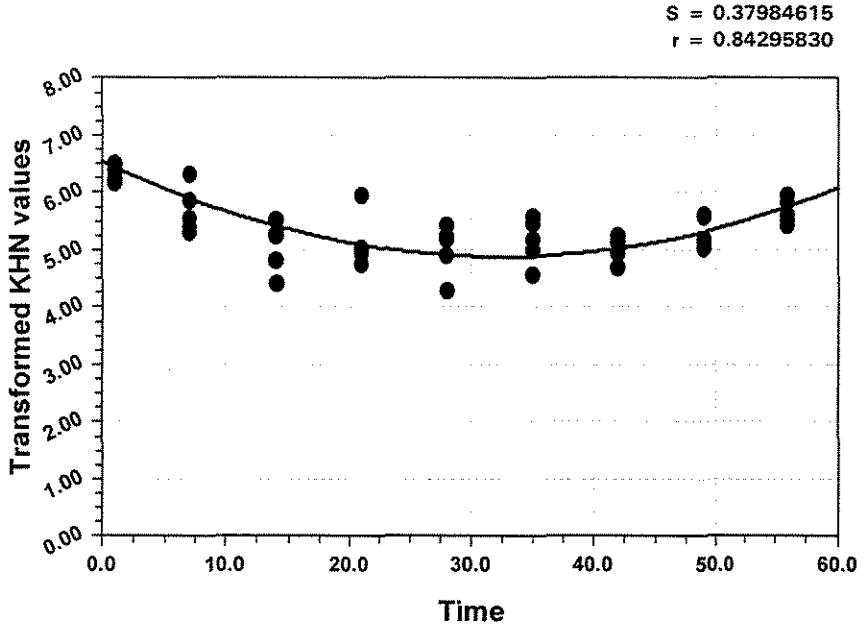
Graph 1. The root square-transformed microhardness values of the dentine exposed to the placebo agent were fitted according to a quadratic function.



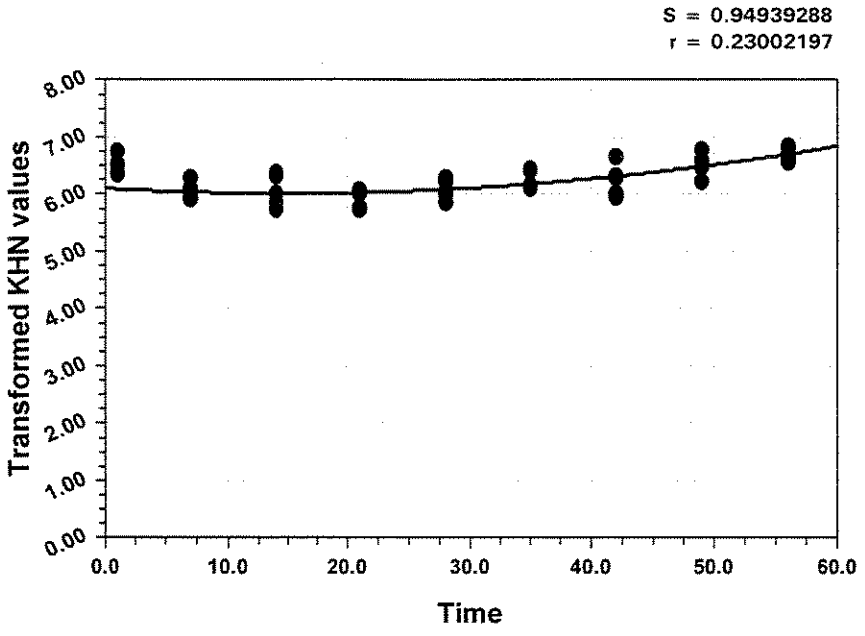
Graph 2. The root square-transformed microhardness values of the dentine exposed to the agent NW 10% were fitted according to a linear function.



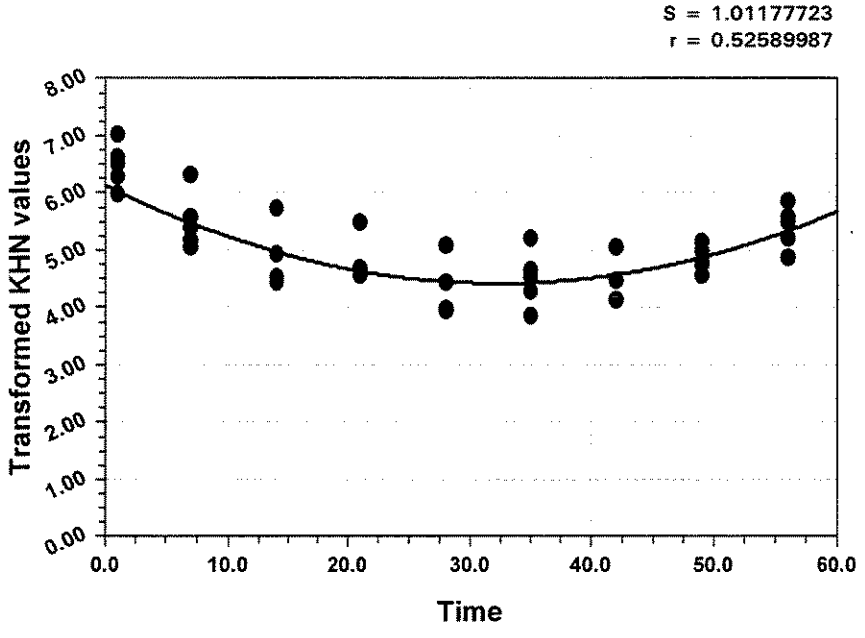
Graph 3. The root square-transformed microhardness values of the dentine exposed to the agent NW 22% were fitted according to a linear function.



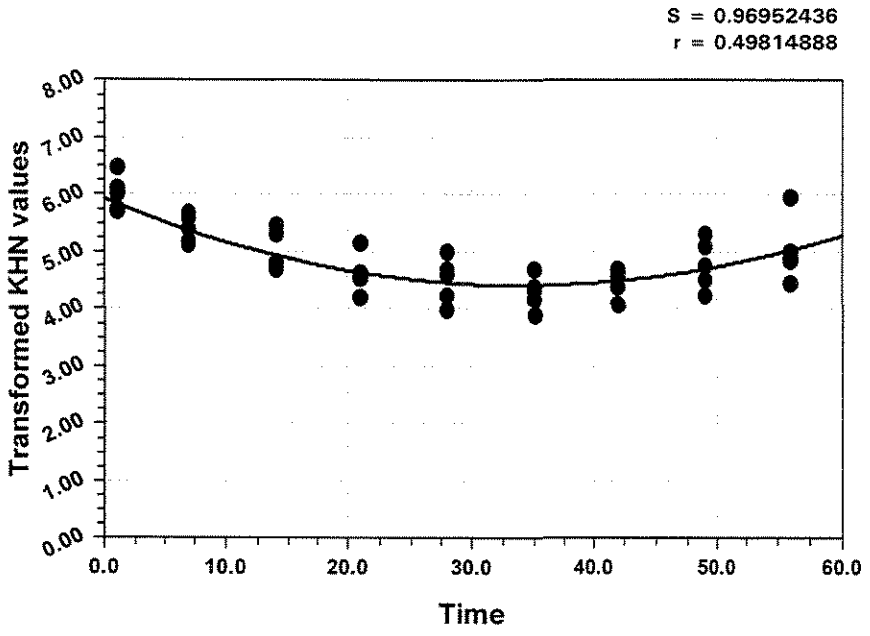
Graph 4. The root square-transformed microhardness values of the dentine exposed to the agent OPA 10% were fitted according to a quadratic function.



Graph 5. The root square-transformed microhardness values of the dentine exposed to the agent OPA 20% were fitted according to a quadratic function.



Graph 6. The root square-transformed microhardness values of the dentine exposed to the agent REM 10% were fitted according to a quadratic function.



Graph 7. The root square-transformed microhardness values of the dentine exposed to the agent REM 22% were fitted according to a quadratic function.

Table 1. Treatment agents applied on dentine surface

Treatment agent	Batch #	Manufacturer	Basic composition*
G1-Nite White Excel 10% 2Z [NW 10%]	01264021	Discus Dental	Propylene glycol, $C_3H_8O_3$, carbamide peroxide, silica, hydrogen peroxide, KNO_3, emulsifying wax NF, hydroxypropyl cellulose, flavour, deionised water, tetrapotassium pyrophosphate, dimethylpolysiloxane
G2-Nite White Excel 22% 2Z [NW 22%]	01236005	Discus Dental	Propylene glycol, $C_3H_8O_3$, carbamide peroxide, silica, hydrogen peroxide, KNO_3, emulsifying wax NF, hydroxypropyl cellulose, flavour, deionised water, KOH, dimethicone
G3-Opalescence 10% [OPA 10%]	403C	Ultradent	carbamide peroxide
G4-Opalescence PF 20% [OPA 20%]	41BM	Ultradent	Carbamide peroxide, ion fluoride, KOH
G5-Rembrandt 10% [REM 10%]	030372010	DenMat	$C_3H_8O_3$, $C_6H_5Na_3O_7$, carbamide peroxide, flavour, carbopol, triethanolamine
G6-Rembrandt 22% [REM 22%]	030372010	DenMat	$C_3H_8O_3$, $C_6H_5Na_3O_7$, carbamide peroxide, flavour, carbopol, triethanolamine
G7-Placebo [CON]	-	Proderma	carbopol 940, $C_3H_8O_3$

*As disclosed by the manufacturers

$C_6H_5Na_3O_7$= sodium citrate, $C_3H_8O_3$= glycerine , KOH = potassium hydroxide, KNO_3= potassium nitrate

Table 2. Root square-transformed Knoop microhardness means values and standard deviations (between parentheses) of dentine exposed to different agents during and after bleaching periods

Treatment Agents	Time (*in days*)									
	0	1	7	14	21	28	35	42	49	56
NW10	6.90 A	6.49 A	6.35 A	6.87 A	6.40 A	6.45 A	6.69 A	6.63 A	6.91 A	7.26 A
	(0.25)	(0.59)	(0.68)	(0.27)	(0.57)	(0.58)	(0.28)	(0.47)	(0.53)	(0.55)
NW22	6.90 A	6.54 A	6.31 A	6.57 A	6.64 A	6.39 A	6.72 A	6.59 A	6.77 A	7.02 A
	(0.30)	(0.36)	(0.28)	(0.12)	(0.50)	(0.15)	(0.40)	(0.16)	(0.26)	(0.31)
OPA20	6.90 A	6.51 A	6.07 AB	6.07 A	5.92 AB	6.09 A	6.26 A	6.24 A	6.52 A	6.72 A
	(0.33)	(0.15)	(0.15)	(0.27)	(0.17)	(0.18)	(0.16)	(0.29)	(0.20)	(0.12)
OPA10	6.91 A	6.36 A	5.69 AB	5.06 B	5.13 B	5.01 B	5.16 B	5.03 AB	5.31 B	5.66 B
	(0.25)	(0.15)	(0.41)	(0.43)	(0.47)	(0.45)	(0.39)	(0.22)	(0.28)	(0.21)
REM10	6.92 A	6.49 A	5.50 AB	4.82 B	4.81 B	4.38 B	4.51 BC	4.46 AB	4.85 B	5.40 B
	(0.26)	(0.39)	(0.50)	(0.55)	(0.38)	(0.47)	(0.50)	(0.39)	(0.23)	(0.38)
REM22	6.89 A	6.01 A	5.40 B	5.00 B	4.62 B	4.50 B	4.29 BC	4.46 B	4.78 B	5.03 B
	(0.20)	(0.32)	(0.25)	(0.35)	(0.34)	(0.40)	(0.30)	(0.25)	(0.43)	(0.56)
Control	6.84 A	6.50 A	5.32 B	4.60 B	4.69 B	4.42 B	4.27 C	4.46 B	5.01 B	5.50 B
	(0.14)	(0.56)	(0.61)	(0.23)	(0.07)	(0.30)	(0.15)	(0.14)	(0.24)	(0.23)

Different letters indicate significant statistical differences ($\alpha = 0.05$), within each column. Least significant difference = 0.8833

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CAPÍTULO 2

Monitoring of demineralized dentin microhardness throughout and after bleaching

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"Monitoring of demineralized dentin microhardness throughout and after bleaching"

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"Monitoring of demineralized dentin microhardness throughout and after bleaching"

ABSTRACT

Purpose: To evaluate the effect of six bleaching agents: Nite White [NW] 10% and 22% Excel 2Z (Discus Dental), Rembrandt [REM] 10% and 22% (DenMat), Opalescence [OPA] 10% and 20% (Ultradent) and a placebo agent on demineralized dentin microhardness at different time intervals. *Materials and Methods:* One hundred and five human dentin fragments (3x3mm) were embedded, planed and submitted to cariogenic challenges, composed of de and remineralization cycles. For 42 days, specimens were exposed to bleaching agents, consisting of applying them daily for 8 hours, removing and storing the specimens in artificial saliva for 16 hours. At the end of the bleaching treatment, specimens were kept in artificial saliva for 14 days. Knoop Microhardness tests were performed on specimens' surface before (baseline), during (8h, 7, 14, 21, 28, 35 and 42 days) and after bleaching procedures (7 and 14 days). *Results:* The ANOVA for split-plot showed significant effect on the interaction between bleaching agent and time (α=0.05). Tukey's test revealed no significant differences on demineralized dentin microhardness exposed to bleaching agents until day 7. Regression analyses demonstrated that NW 10% and 22% and OPA 10% and 20% increased dentin microhardness in different magnitudes, whereas REM 10% and 22% induced mineral loss during bleaching agent application, followed by microhardness recovery in the post-treatment period.

CLINICAL SIGNIFICANCE

The mineral content of demineralized dentin can be altered by different concentrations and trademarks of bleaching agents, but even during and mainly after the treatment, saliva may provide remineralizing effect.

INTRODUCTION

With the increased esthetic demand by patients, vital bleaching has become a treatment option for discolored teeth. Since the first report with regards to home bleaching technique[1], the dental professional rapidly recognized the benefits of the treatment and it has become a popular method of lightening teeth. In addition, agents with different concentrations have been introduced to obtain a whitening effect more quickly[2].

A bleaching agent, when in contact with saliva, breaks down into urea, ammonia, carbon dioxide and hydrogen peroxide[3]. The whitening effect is based on the ability of hydrogen peroxide to penetrate into enamel and dentin structure and degrade complex organic molecules, of high molecular weight, into lower molecular weights, which are lighter in color[3]. Dentin is more prone to be affected by the hydrogen peroxide in areas of enamel defects or abrasion and mainly on exposed root surfaces[4].

A study by Nyvad & others (1997)[5] indicates that even under conditions of daily plaque control, sound root surfaces are susceptible to changes in mineral distribution, which may lead to the formation of subsurface lesions, although these would not be clinically detectable. Under these conditions, it seems acceptable that a demineralized dentin could be exposed to bleaching products and that these oxidizing agents could induce changes in dentin microhardness.

Basting & others (2001)[6] showed that 10% carbamide peroxide does not seem to alter the microhardness of both sound and demineralized dentin when applied for three weeks. Until the development of the present study, no other reports about the long-term effect of both 10 and 20-22% carbamide peroxide bleaching agents on demineralized dentin were available in the literature.

The aim of this *in vitro* study was to evaluate the demineralized dentin microhardness throughout 42 days of exposure to different carbamide peroxide bleaching agents and at 7 and 14 days after concluding the treatment.

MATERIALS AND METHODS

Experimental Design

The factors under study were:

1. **Treatment agent** at six levels: Nite White (NW) 10% and 22% Excel 2Z[a], Opalescence (OPA) 10% and 20%[b], Rembrandt (REM) 10% and 22%[c] and a placebo agent as control;

2. **Time** at ten levels: 0 (baseline), 8 hours, 7, 14, 21, 28, 35, 42 days of treatment and 7 and 14 days of post-treatment period (which corresponds to 49 and 56 days from the beginning of the treatment)

This study was comprised of 15 experimental units for each treatment agent made in 5 blocks, with 3 replicates each. The randomized complete block design was used to reduce experimental error arising from known and controlled nuisance sources of variability[7]. The split-plot design was employed supported by repeated measurements of Knoop Microhardness taken on the surface of each specimen at each time interval. Figure 1 illustrates the setup of the experiment.

Dentin Preparation

The study protocol was reviewed and approved by the local ethical committee. Forty freshly extracted, non-erupted third molars were selected and immediately stored in 2% formaldehyde (pH 7.0). The crowns were removed approximately to the cement enamel junction and the roots were longitudinally sectioned with double-faced diamond disks used at a low motor speed to obtain 105 dentin fragments. The apical third was discarded and only the cervical region was used to obtain

specimens with a standardized area of 3x3mm. After sectioning, all fragments were stored at 37±1°C in 100% relative humidity, with the exception of those in which stains or cracks were observed under a stereomicroscope[d] used at 30X magnification. The 105 dentin fragments were embedded individually in a self-curing polyester resin in a ring mold so that the external surface of the dentin fragment was exposed and left to polymerize.

After molds were removed, the external surfaces of the dentin fragments were polished with a water-cooling mechanical grinder[e] with aluminum oxide disks in a sequence of 600-, 1000- and 1200-grits to obtain parallel planar surfaces for the Knoop microhardness test performance.

Cariogenic Challenge

Prior to the exposure to treatment agents, specimens were submitted to a pH-cycling regimen, as proposed by Featherstone & others (1986)[8] and modified by Hara & others (2000)[9]. The experimental units were immersed in 13.5 ml (1.5ml/mm^2) of demineralizing solution for 1 hour at 37±1 °C, followed by rinsing with distilled-deionized water and immersion in 13.5 ml of remineralizing solution for 23 hours at 37±1 °C. This protocol was applied during 3 consecutive days, until the beginning of the treatment.

Exposure to Treatment Agents

Fifteen specimens were randomly assigned into one of the six experimental or placebo (control) groups. The treatment agents used in this study are described in Table 1.

The dentin fragments were exposed to the treatment agents (experimental and placebo) for 8 hours per day during 42 days. During the remaining diary time (16 hours) they were kept in an individual recipient with 13.5 ml of artificial saliva, which consisted of a remineralizing solution proposed by Featherstone & others (1986)[8] and modified by Serra & Cury (1992)[10].

Using a syringe, 0.02 ml of each treatment agent were applied to the specimens' surfaces and individually recovered with trays made with a 0.4 mm thick ethyl vinyl acetate polymer in a vacuum forming machine[f]. Then, each experimental unit was soaked in recipients with 13.5 ml of artificial saliva (pH= 7.0), at 37±1 ºC for 8 hours. Next, the specimens were rinsed under running distilled-deionized water for 5 seconds and stored in 13.5 ml of artificial saliva for the remaining diary time (16 hours). These procedures were repeated for 42 days during which the storage media was changed every 2 days.

Post-treatment Period

After 42 days of treatment, the specimens were kept in their individual recipients with 13.5ml of artificial saliva, at 37±1 ºC for 2 weeks, which was also changed every two days. Knoop Microhardness tests were performed 7 and 14 days after concluding the treatment period (corresponding to 49 and 56 days after the beginning of the treatment).

Microhardness Tests

Microhardness measurements were performed before the exposure to the treatment agents (*baseline*), during the agents' application (8 hours, 7, 14, 21, 28, 35, 42 days) and at the post-treatment period (7 and 14 days after concluding the treatment). A Knoop Microhardness testing machine[g] was used to make three indentations on each specimen with a load of 10 g applied for 5 seconds.

Statistical Analysis

The statistical analysis of the data was made considering the average of the three Knoop Hardness Values, by a parametric method using a multi-factor Analysis of Variance (ANOVA) (α=0.05) for Split-plot design. A study of the interaction among the factors analyzed (treatment agent, time and block) was made. The interaction of particular interest was treatment agent x time.

Multiple Comparisons Tukey's test at the level of 5% of significance was used to check differences in means within the factor time, and regression analyses were applied to make inferences with regards to the effect of each bleaching agent throughout the different time intervals. Data analysis was performed with the SAS System 6.11[h] and Statgraphics Plus[i] software.

RESULTS

Due to the lack of homogeneity of variance, a logarithmic transformation was performed in order to stabilize the variance of the data. The ANOVA for split-plot verified significant effect on the interaction between treatment agent and time (α=0.05).

Table 2 shows the log-transformed means and standard deviations of Knoop microhardness values for each treatment agent at different time intervals and displays the results of the Tukey's test. The statistical differences are represented by different letters at the right side of the each mean (within column).

Within the factor time, the Tukey's test revealed no significant differences in demineralized dentin microhardness exposed to bleaching agents until day 7. On the remaining 35 days of treatment, the agents provided significant statistical differences in microhardness values, even in the post-treatment period, as shown in Table 2.

The regression analyses evaluated the microhardness of the demineralized dentin in consequence of its exposure to each one of the treatment agents throughout the bleaching and post-bleaching periods. The agents NW 10% and 22% and OPA 10% and 20% could be represented by linear functions (Graphs 2-5). By the comparison of the slope estimations, the products with higher concentrations (NW 22% and OPA 20%) exhibited a slighter increase in dentin microhardness than

the lower concentration (NW 10% and OPA 10%) counterparts, during and after the bleaching treatment.

The best curves to explain the effect of both REM 10% and REM 22% were quadratic (Graphs 6-7). These agents, likewise the control group (Graph 1), induced mineral loss during the bleaching procedures, but a microhardness recovery was observed in their post-treatment periods.

DISCUSSION

In areas presenting enamel defects or abrasion and exposed root surfaces, a shift in the oral equilibrium favoring demineralization over remineralization leads to dentin mineral loss[11]. As this early lesion, during a whitening procedure, could conceivably come into contact with bleaching agents, in this study the specimens were submitted to three pH cycles, based on the dynamic model of demineralization and remineralization proposed by Featherstone & others (1986)[8] and modified by Hara & others (2000)[9], in order to simulate the initial stage of root dentin caries development.

The regression analyses showed that, during and after the treatment period, NW 10% and 22% and OPA 10% and 20% exhibited an increase in microhardness values of the demineralized dentin. These findings might be associated with the remineralization induced by artificial saliva and fluorides, producing a more acid resistant surface[12]. Saliva seems to contribute to dentin microhardness enhancement because it is supersaturated with calcium and phosphate[13], and also due to its buffering ability[14].

Fluoride ions, during and after acid challenges, are supposed to inhibit dentin demineralization or increase its remineralization, respectively[15]. In fact, in consequence of OPA 20% application, demineralized dentin exhibited a more expressive increase in microhardness as compared to the specimens treated with NW 10%, NW 22% and OPA 10%, which does not contain

fluoride, as shown in Table 1.

The presence of desensitizing compounds such as potassium nitrate on NW 10%, NW 22% and OPA 20% may have also contributed to the preservation of the dentin microhardness. It is likely that this salt has precipitated on the dentin surface and increased its microhardness or reacted with the by-products of the bleaching reaction enhancing microhardness values throughout the treatment period. Oliveira & others (2002)[16] showed that 10% carbamide peroxide associated with desensitizing agents could maintain or increase enamel microhardness submitted to bleaching treatment for 42 days.

The bleaching agents NW 10% and 22% are described as having equivalent contents of carbamide peroxide. Actually, they present carbamide peroxide at 8.1% and also contain 0.73% and 5.05% hydrogen peroxide, respectively. Thus, it could be concluded that the percentage of hydrogen peroxide released is similar to those expected from 10 and 20-22% carbamide peroxide bleaching agents. Although hydrogen peroxide is reported to decrease pH level during bleaching procedures, in the presence of urea, another product of carbamide peroxide decomposition, the pH level is expected to increase[17]. Considering that the data obtained by NW 10% and 22% were not statistically different from OPA 10% and OPA 20%, it could be concluded that low concentrations of hydrogen peroxide associated with carbamide peroxide could not decrease dentin microhardness, probably because of the presence of urea.

On the other hand, REM 10% and 22% induced a transitory mineral loss during the bleaching agent application, which was recovered in the post-treatment period. Freitas & others (2002)[18] showed a significant decrease in sound dentin microhardness values when exposed to the same 10% carbamide bleaching agents. The bleaching agent Rembrandt has been reported as a low pH product, ranging from 4.9-6.8[19], relevant when considering the critical pH for dentin

demineralization (6.2-6.7)[11,20]. Although the Rembrandt technique profile advertises to pH 7.0, the presence of carbopol 940 and glycerin or the eventual interaction of some bleaching degradation product with the dental hard tissues, might have decreased pH level and, consequently, dentin microhardness.

The placebo agent applied on dentin surface decreased microhardness values, followed by its increase in the post-treatment period. It was consisted of a neutral pH glycerin and carbopol product, providing equal hydration for the samples. These unexpected results were possibly due to placebo's compounds, although they are considered inactive agents. As the groups treated with Rembrandt 10% and 22%, the dentin fragments exposed to the placebo agent revealed mineral loss during the whitening period, suggesting that such carbopol 940 could induce alteration in dentin structure. There is a report on the decrease of enamel microhardness, related to the treatment with a product containing carbopol, also suggesting that it could act as a barrier, inhibiting the artificial saliva from achieving or penetrating through the dental surface to promote remineralization[21]. However, further investigations are necessary to elucidate the effect of these agents on tooth properties.

The artificial saliva used to simulate oral conditions, which contains calcium and phosphate ions[8,22], was shown to play an important role in the obtained data. During and after the bleaching treatment, the recovery or overcoming of the microhardness values showed the remineralizing effect of the artificial saliva, which could be expected to be higher in *in vivo* conditions due to some factors such as salivary flow, the buffering capacity, oral hygiene[23] and the use of topical fluorides[24].

Throughout the bleaching treatment, depending on the agent applied, demineralized dentin may show an increase or transitory decrease in microhardness values. In the post-treatment period, artificial saliva presented a remineralizing effect on the bleached surfaces.

a. Discus Dental, Inc., Los Angeles, California, USA

b. Ultradent Products, Inc., South Jordan, Utah, USA

c. Den-Mat Corporation, Santa Maria, California, USA

d. Meiji Techno Emz Series, Saitama, Japan

e. Maxgrind/Solotest, São Paulo, SP, Brazil

f. P7/ Bioart Equipamentos Odontológicos Ltda., São Carlos, SP, Brazil

g. Future Tech – Fm-1e, Tokyo, Japan

h. SAS Institute Inc., Cary, NC

i. Manugistics, Rockville, Maryland, USA

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Table 1. Agents used in demineralized dentin surface treatment

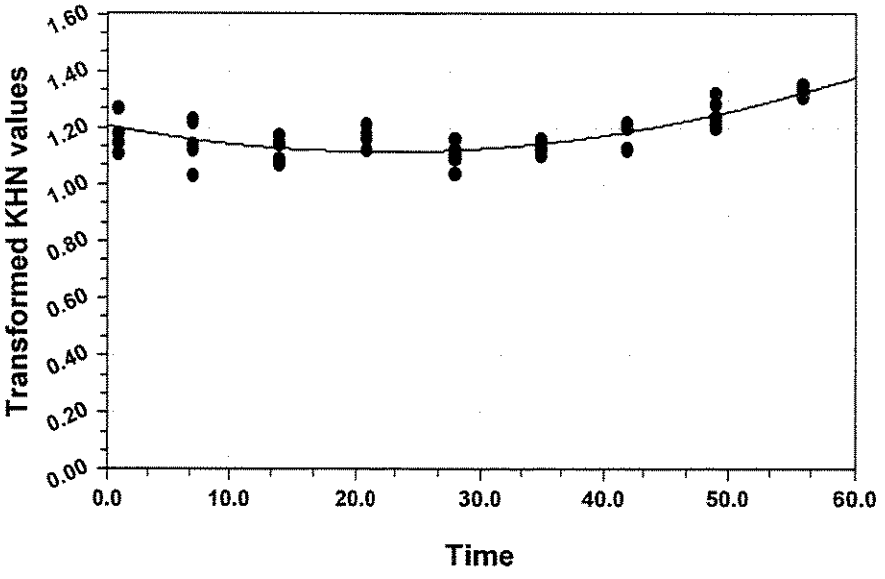
Treatment agent	Batch #	Manufacturer	Basic composition*
Placebo [Control]	-------	Proderma	1.2% carbopol 940, 5% $C_3H_8O_3$
Nite White Excel 10% 2Z [NW 10%]	01264021	Discus Dental	propylene glycol, $C_3H_8O_3$, carbamide peroxide, silica, hydrogen peroxide, KNO_3, emulsifying wax NF, hydroxypropyl cellulose, flavor, deionized water, tetrapotassium pirophosphate, dimethylpolysiloxane
Nite White Excel 22% 2Z [NW 22%]	01236005	Discus Dental	propylene glycol, $C_3H_8O_3$, carbamide peroxide, silica, hydrogen peroxide, KNO_3, emulsifying wax NF, hydroxypropyl cellulose, flavor, deionized water, KOH, dimethicone
Opalescence 10% [OPA 10%]	403C	Ultradent	carbamide peroxide
Opalescence PF 20% [OPA 20%]	41BM	Ultradent	carbamide peroxide, fluoride ion, KNO_3
Rembrandt 10% [REM 10%]	030372010	DenMat	$C_3H_8O_3$, $C_6H_5Na_3O_7$, carbamide peroxide, flavor, carbopol, triethanolamine
Rembrandt 22% [REM 22%]	030372010	DenMat	$C_3H_8O_3$, $C_6H_5Na_3O_7$, carbamide peroxide, flavor, carbopol, triethanolamine

*As disclosed by the manufacturers
$C_6H_5Na_3O_7$= sodium citrate, $C_3H_8O_3$= glycerin , KOH = potassium hydroxide, KNO_3= potassium nitrate

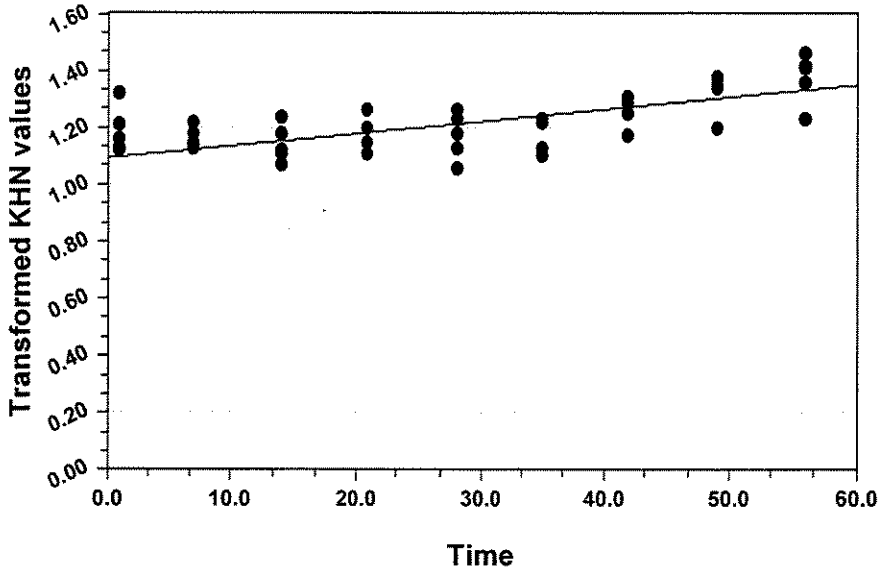
Table 2. Log-transformed Knoop microhardness means values and standard deviations (between parentheses) of the demineralized dentin exposed to different agents throughout and after bleaching

	Time (*in days*)																			
Treatment Agents	0		1		7		14		21		28		35		42		49		56	
Placebo	1.22 (0.03)	A	1.18 (0.06)	A	1.15 (0.08)	A	1.13 (0.04)	AB	1.16 (0.04)	AB	1.10 (0.04)	BC	1.13 (0.02)	CD	1.18 (0.05)	CD	1.25 (0.05)	BC	1.33 (0.01)	C
NW 10%	1.24 (0.06)	A	1.19 (0.08)	A	1.16 (0.03)	A	1.14 (0.07)	AB	1.18 (0.06)	AB	1.17 (0.08)	AB	1.18 (0.06)	BCD	1.26 (0.06)	BC	1.33 (0.08)	AB	1.38 (0.09)	ABC
NW 22%	1.20 (0.02)	A	1.18 (0.04)	A	1.21 (0.04)	A	1.21 (0.05)	A	1.22 (0.06)	A	1.26 (0.08)	A	1.30 (0.10)	A	1.38 (0.09)	A	1.40 (0.07)	A	1.43 (0.08)	AB
OPA 10%	1.19 (0.01)	A	1.12 (0.03)	A	1.12 (0.05)	A	1.11 (0.05)	B	1.14 (0.04)	AB	1.19 (0.09)	AB	1.21 (0.04)	ABC	1.23 (0.04)	BCD	1.29 (0.06)	BC	1.35 (0.08)	BC
OPA 20%	1.21 (0.03)	A	1.16 (0.05)	A	1.16 (0.05)	A	1.18 (0.07)	AB	1.18 (0.04)	AB	1.22 (0.06)	A	1.26 (0.05)	AB	1.32 (0.05)	AB	1.41 (0.04)	A	1.46 (0.02)	A
REM 10%	1.23 (0.08)	A	1.16 (0.05)	A	1.14 (0.07)	A	1.10 (0.06)	B	1.11 (0.06)	B	1.06 (0.04)	C	1.12 (0.06)	CD	1.16 (0.04)	D	1.23 (0.05)	C	1.31 (0.04)	C
REM 22%	1.23 (0.03)	A	1.17 (0.05)	A	1.18 (0.09)	A	1.17 (0.08)	AB	1.16 (0.07)	AB	1.10 (0.05)	BC	1.11 (0.05)	D	1.18 (0.06)	CD	1.22 (0.05)	C	1.30 (0.05)	C

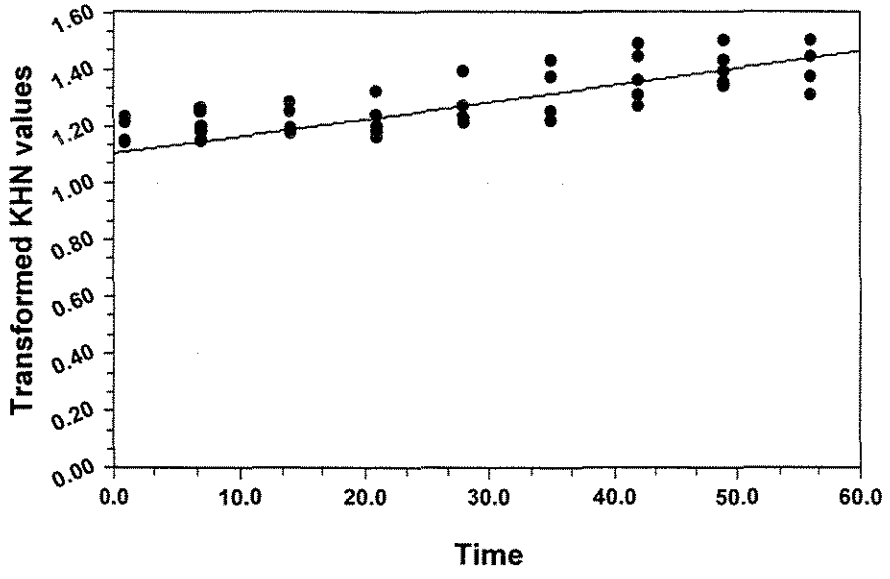
Different letters represent significant statistical differences ($p<0.05$), within each column. Least significant difference = 0.10



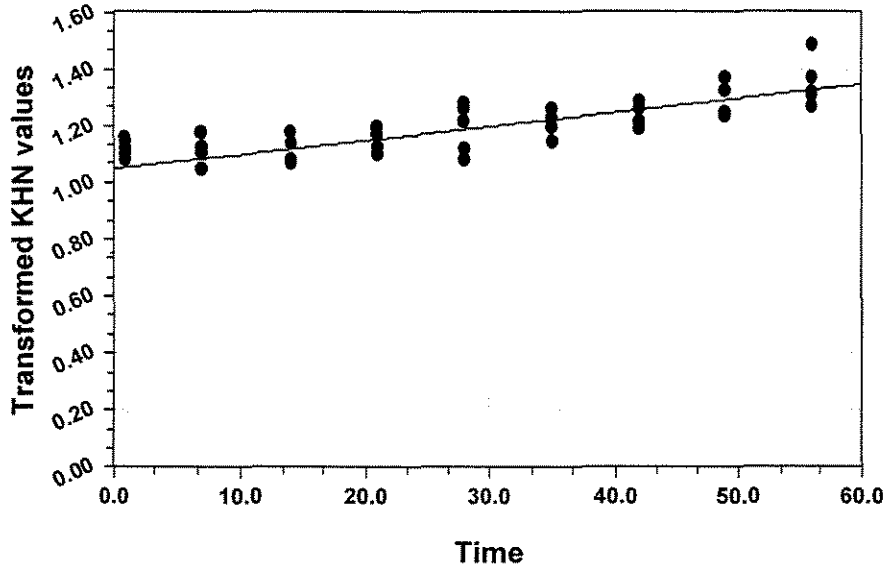
Graph 1. The log-transformed microhardness values of the demineralized dentin exposed to the placebo agent were fitted according to a quadratic function (r-square = 0.68)



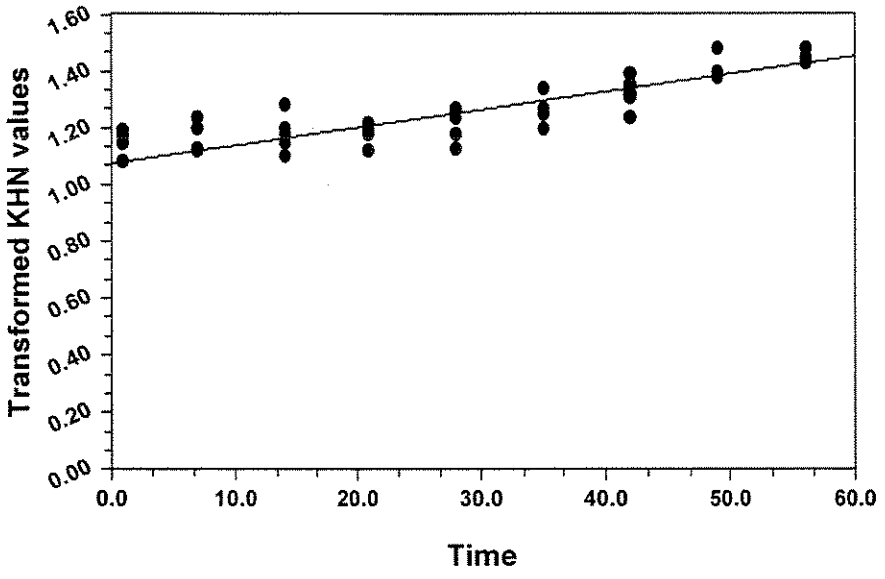
Graph 2. The log-transformed microhardness values of the demineralized dentin bleached with Nite White 10% were fitted according to a linear function (r-square = 0.30, slope = 0.003)



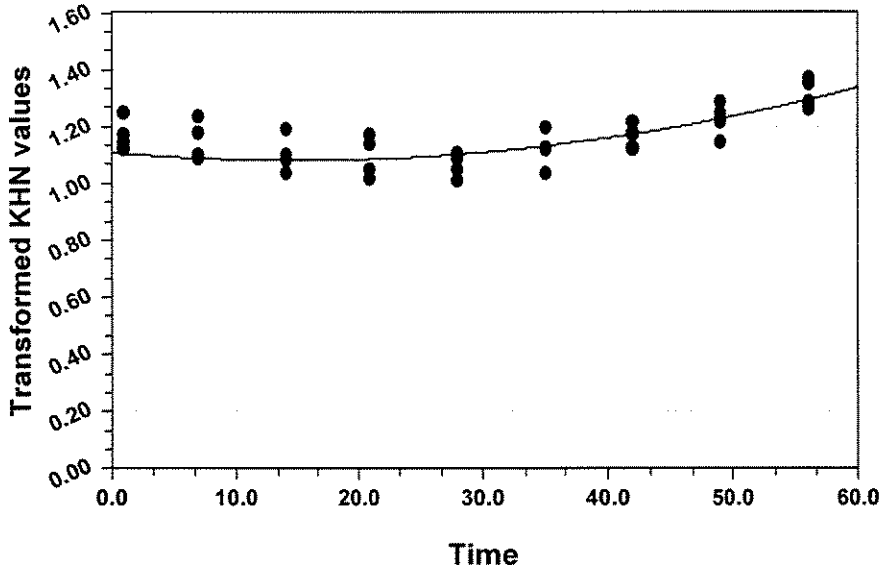
Graph 3. The log-transformed microhardness values of the demineralized dentin bleached with Nite White 22% were fitted according to a linear function (r-square = 0.63, slope = 0.004)



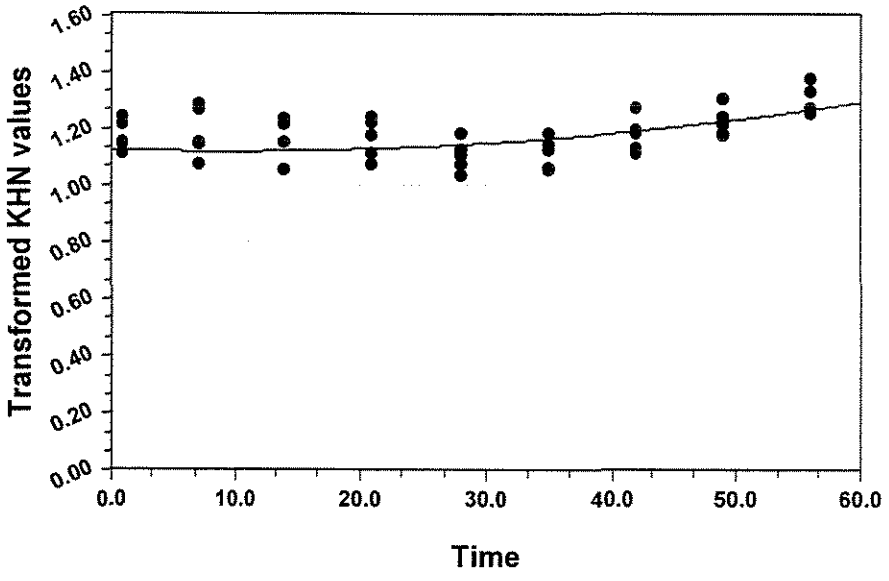
Graph 4. The log-transformed microhardness values of the demineralized dentin bleached with Opalescence 10% were fitted according to a linear function (r-square = 0.53, slope = 0.003)



Graph 5. The log-transformed microhardness values of the demineralized dentin bleached with Opalescence 20% were fitted according to a linear function (r-square = 0.68, slope = 0.005)



Graph 6. The log-transformed microhardness values of the demineralized dentin bleached with Rembrandt 10% were fitted according to a quadratic function (r-square = 0.62)



Graph 7. The log-transformed microhardness values of the demineralized dentin bleached with Rembrandt 22% were fitted according to a quadratic function (r-square = 0.39)

Figure 1.

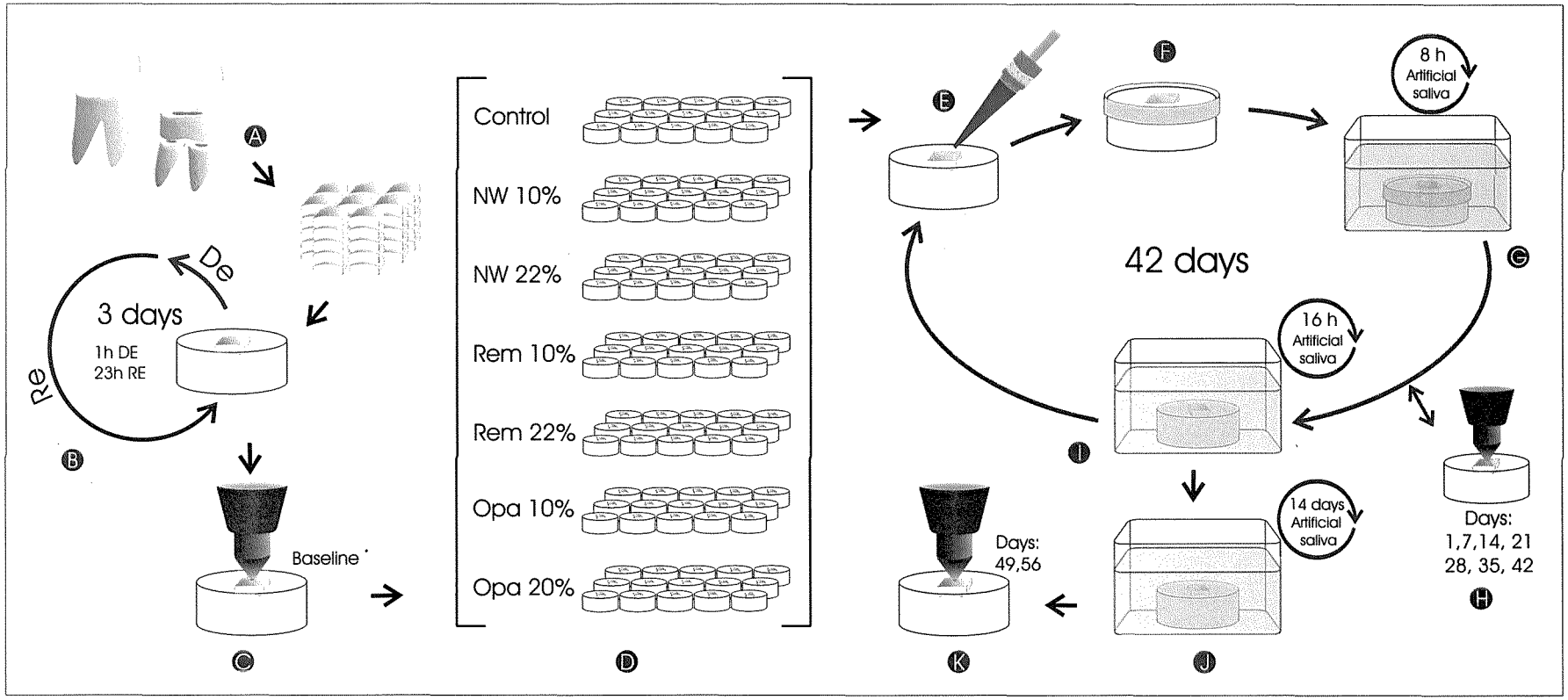

A
3 days
1h DE
23h RE
De
Re
B
Baseline
C
Control
NW 10%
NW 22%
Rem 10%
Rem 22%
Opa 10%
Opa 20%
D
E
F
8 h
Artificial
saliva
G
42 days
16 h
Artificial
saliva
I
14 days
Artificial
saliva
J
Days:
49,56
K
Days:
1,7,14, 21
28, 35, 42
H

Figure 1.

A. Dentin fragments preparation

B. Cariogenic challenge

C. Microhardness test (baseline)

D. Dentin fragments random assignment into the experimental and placebo groups

E. Application of the treatment agents on dentin surfaces

F. Specimens with the treatment agent and the customized tray

G. Specimens storage in artificial saliva for 8 hours a day

H. Microhardness test performed after the exposure to the treatment agents

I. Specimens storage in artificial saliva for 16 hours a day

J. Specimens storage in artificial saliva for 14 days after concluding the treatment

K. Microhardness test at the post-treatment period

CAPÍTULO 3

Dental mineral equilibrium during and after home- bleaching: a review

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DENTAL MINERAL EQUILIBRIUM

DURING AND AFTER HOME-BLEACHING: A REVIEW

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DENTAL MINERAL EQUILIBRIUM

DURING AND AFTER HOME-BLEACHING: A REVIEW

ABSTRACT

Background: Home bleaching techniques require that a peroxide-based bleaching gel be in contact with intraoral tissues and structures for a number of hours a day, for several days. During and after bleaching treatment, demineralization and remineralization events likely occur at the enamel surface and subsurface, affecting the tooth mineral content. *Purpose:* To review the effects of bleaching agents on the dental mineral equilibrium throughout the available literature. *Summary:* Mineral loss from dental hard tissues during bleaching treatment has been demonstrated by many studies. However, protective factors – mainly salivary components prevent excessive mineral loss and/or reestablish the mineral content after interruption of bleaching treatment.

CLINICAL SIGNIFICANCE

Studies show that home bleaching affects the mineral equilibrium at the enamel surface and subsurface. Understanding the factors related to variations in tooth mineral content during and after bleaching treatment is a key to clinical success, where excellence in esthetics should parallel oral health maintenance.

INTRODUCTION

During many years, esthetic treatment of vital discolored anterior teeth was only possible via invasive, irreversible restorative procedures. However, tooth whitening or bleaching techniques represent today a conservative treatment alternative for many discolored teeth. Among bleaching techniques, the dentist-supervised home bleaching technique, also known as nightguard vital bleaching, has become very popular due to its safety, effectiveness, and low cost[1].

Dentist-supervised home bleaching generally involves the use of either 1-10% hydrogen peroxide (HP) or 10-22% carbamide peroxide (CP). The exact mechanism by which home bleaching results in whiter (or less colored) teeth is not fully understood. In carbamide peroxide home bleaching, the CP breaks down into urea (2/3) and HP (1/3) when in contact with oral tissues and saliva[2]. The HP further breaks down into oxygen and water, while urea results in ammonia and carbon dioxide. CP diffuses through permeable enamel and dentin, oxidizing complex organic molecules that absorb light resulting in tooth darkening[2]. The organic compounds resulting from the oxidative reaction are less complex molecules that are partially or totally diffused from the tooth. The remaining compounds absorb less light, resulting in reduction or elimination of the discoloration[3] (Figure 1).

The effect of bleaching agents on enamel, dentin, and pulp tissues has been extensively researched, and is directly affected by bleaching agent concentration and contact time. Although studies have reported that home bleaching agents do not cause any enamel/dentin alterations[4-7], other studies claim morphological and compositional dentin and enamel changes as a result of CP-based bleaching [8-16].

Alterations on dental inorganic content appear to be of extreme clinical relevance, since they may reveal mineral loss (demineralization) or gain (remineralization) [17]. However, some bleaching

agents' ingredients or byproducts, as well as fluoride use and saliva contact have been reported to prevent or reduce mineral loss during and after bleaching treatment[13,15,16,18,19].

The purpose of this article is to review how and why the tooth mineral content may be altered during and after bleaching treatment.

BLEACHING AGENTS INGREDIENTS

Since home bleaching involves intimate contact between a bleaching agent and the tooth structure for a long period of time, studies have evaluated the effect of bleaching agents' active and inactive ingredients on enamel and dentin. Home bleaching agents typically contain one or more of the following ingredients: HP, CP, carbopol, glycerin, desensitizing agents, and/or fluoride (Figure 2).

Hydrogen Peroxide

The use of HP as a dental bleaching agent has been described for more than 70 years for both vital and nonvital teeth[20]. When in contact with saliva, HP breaks down into free radicals of hydrogen and perhydroxyl, the most potent free radical, which can oxidize stain-forming molecules[2]. Since HP is naturally secreted in large amounts in the oral cavity by salivary glands[21,22], it is not considered toxic when present in low levels[23]. In addition, despite being a strong oxidizing agent, HP is rapidly degraded in the oral environment by enzymatic and non-enzymatic systems[23].

High concentrations of HP (30-35%) were reported to alter enamel and dentin chemically and morphologically[10,11,24,25], probably due to the low pH resulting from HP's breakdown[22]. These products are used only for chairside or "power" bleaching, because the direct contact of this bleaching agent with the oral tissues might result on mucosa dehydration and irritation[22].

HP used for home bleaching is generally 1-10%. There are a limited number of reports on

side effects of home bleaching HP on tooth structure[26,27]. These studies support that low concentrations of HP do not alter enamel and dentin chemical/morphological structures.

Carbamide Peroxide

Formally introduced as a bleaching agent in 1989[28], 10% CP has been used as an oral antiseptic and cleanser since the late 1960s and is approved by the U. S. Food and Drug Administration for that purpose[29]. CP breaks down into urea and HP as mentioned earlier. HP is the active oxidizing agent in CP, and 10% CP correspond to approximately 3% HP. The CP degradation into HP is slower when carbopol is present[4].

Given the relatively slow rate of HP formation and its rapid degradation when in contact with saliva, very little HP would be present in the oral cavity from a properly fitted bleaching tray containing CP[23]. As a result, the side effects on tooth structure are not expected to be similar to those from HP apart.

Alterations on surface morphology of enamel treated with 10 % and 16% CP have been described[3,11,30]. Organic matrix degradation by free radicals generated from whitening agents may result in substantial loss of enamel proteins, which is related to the agents' acidic nature and/or concentration.

Although reports indicate that home bleaching induces *in vitro* mineral changes during whitening treatment[10,12,14,31], it has been considered safe[7] because the treated teeth are bathed in saliva, a remineralizing solution[16,19,32,33]. Additionally, various forms of fluoride can support remineralization[13]. Further studies are needed to evaluate the effects of other bleaching products ingredients and/or byproducts on enamel and dentin[3,11,16].

Carbopol

Carbopol is a synthetic cross-linked polymer of acrylic acid, used as a thickening agent to improve tissue adherence and to allow for a timed and sustained release of oxidizing agents in bleaching[34].

Besides its ability to slow down HP release without compromising the efficacy of the bleaching treatment, carbopol was reported to be possibly associated with reduction of enamel microhardness, as it could act as a demineralizing agent[12]. Carbopol can also form an impermeable barrier, inhibiting saliva from contacting enamel and dentin and promoting remineralization[15]. An increase in bleaching agents' thickness should not occur in detriment of mineral content. Caution is required when applying whitening products containing carbopol on teeth for a prolonged period of time.

Glycerin

Dental bleaching materials can vary by thickeners, flavors and other ingredients, including the vehicle, such as glycerin, dentifrice and glycol. Glycerin is used as a vehicle for many drugs, and is present in most bleaching agents. Glycerin can absorb water and in high concentrations may be dehydrating and irritating to mucous membranes[34]. As carbopol, glycerin may be related to alterations on the mineral content in enamel[15,35] and dentin[16]. It is hypothesized that as glycerin acts as a dehydrate product, it can reduce the fluid transport inside the tooth structure, which is extremely relevant to de/remineralization processes or to fluoride treatments.

Desensitizing agents

Desensitizing agents such as potassium nitrate and sodium citrate have been added to

bleaching products to reduce tooth sensitivity, a common side effect of bleaching treatments. This side effect is thought to be due to dehydration and to the transit of bleaching-related molecules through enamel and dentin into the pulp[36].

Potassium nitrate is an analgesic and anesthetic product claimed to prevents repolarization of the nerve fibers after the initial depolarization in the pain signal[37]. These agents could also react with CP byproducts and deposit on the tooth surface, enhancing microhardness over time[35]. Besides, it was shown that potassium was present in the pre-mineralized tooth surface and it could be responsible for attracting calcium and phosphate to the dental structure[38].

Other desensitizing agents such as sodium citrate do not prevent tooth mineral loss when added to bleaching agents[16]. Even though there are no studies confirming the actual relation between desensitizing agents and tooth mineral content, these products are expected to reduce side effects of whitening treatments without alteration on the tooth chemical and morphological structures.

Fluoride

Some commercial bleaching products contain fluoride as a desensitizing agent[37]. However, the most important role of fluoride seems to be its potential to inhibit demineralization and enhance remineralization. When present during acid challenges, fluoride may travel with the acids into the subsurface of the tooth and adsorb to the crystal surface protecting it against dissolution[39]. Considering the pH decreases during the first 5 minutes of bleaching[40], the presence of fluoride in the aqueous solution surrounding the tooth when the bleaching agent decomposes and produces hydrogen ions is relevant, since it could be integrated to the dental structure and provide a less soluble surface.

Fluoride-containing products such as dentifrices, mouthrinses, topical gels applied in the

dental office, and fluoridated water also contribute to uphold the enamel and dentin mineral content[39]. For this reason, some authors[3,13] recommend the combined application of remineralizing agents and fluoride rinses during and after bleaching treatments to avoid mineral loss, possibly reduce surface porosity, and tooth sensitivity. Even though bleaching procedures may alter the tooth's chemical and morphological structure, fluoride will impart a remineralizing effect. It is important to emphasize that minerals present in saliva will also contribute to the mineral equilibrium.

HYDROGENIONIC CONCENTRATION (pH)

Some bleaching agents present a pH ranging from 4.3 to 6.2[16,33], since the shelf life of most chemicals is extended with a low pH[2]. Because the critical pH for demineralization of enamel and dentin is 5.5[17] and 6.2-6.7[41,42], respectively, a bleaching agent with low pH has the potential to demineralize enamel and/or dentin.

Nowadays, otherwise, a large variety of bleaching agents can be found in the market including those with neutral pH, which use should be preferred, since they can prevent tooth demineralization. The ionization of buffered HP produces higher amounts of hydrogen and perhydroxyl, contributing to bleaching. The release of hydrogen could decrease the pH, increasing the risk of demineralization. However, urea, a byproduct of CP, has good buffer capacity[43], elevating the pH during bleaching[40,44]. Urea is also released by stimulated salivary glands, and is subjected to the same chemical reaction described above[40].

THE ROLE OF SALIVA

Saliva has an important role in upholding or recovering the tooth mineral content not only during bleaching treatment but also whenever the intraoral pH drops bellow the critical level (Figure

3). Saliva buffers acidic substances in the mouth, and favors enamel/dentin remineralization by providing mineral ions that replace those dissolved during demineralization[33,45].

The decrease on enamel microhardness reported in some *in vitro* studies[8,12] may be justified by extended exposure to the bleaching agent and by improper storage of the specimens. Studies using a remineralizing solution or saliva as storage media[13,16,19,33,46] showed bleaching treatment had little or no effect on enamel microhardness. Although fluoride may reduce potential mineral loss, saliva is essential to the intraoral mineral equilibrium, since it is saturated with calcium and phosphate.

The buffering capacity of saliva varies between a pH of 5.3 and 7.8, depending on salivary flow[47]. Hydroxyl radicals can be deactivated by non-enzymatic systems, including compounds with free hydroxyl groups, such as carbohydrates, that can trap hydroxyl radicals and reduce the potential for interaction with other cellular components[23]. The most important buffering component of saliva is sodium bicarbonate, which acts increasing the pH of the oral cavity by neutralizing acids and enhancing decomposition of HP[23]. Complex carbohydrates are also present in dental products such as dentifrices[23], which may facilitate the intraoral decomposition of HP. Saliva's buffering capacity is also associated with the presence of urea. Degradation of urea into ammonia and carbon dioxide is a major neutralizer of acid in saliva, since ammonia could rapidly react with free radicals of hydrogen[43].

Degradative enzymes present in saliva, such as peroxidase and catalase, constitute the primary means by which HP is regulated in the oral cavity. Both extracellular and intracellular peroxidases are responsible for the breakdown and regulation of HP, converting it into oxygen and water[48].

Ideally, *in vitro* studies should reproduce oral conditions, so results could be compared to clinical procedures. However, there is still no report on the *in vitro* use of a storage media that

contains the enzymes involved on the breakdown of HP for the evaluation of mineral content alterations during bleaching treatment.

Reduced salivary production did not adversely affect the rate of HP decomposition under normal conditions of use[23]. It has been proposed that peroxide-decomposing enzymes activity is increased to compensate for this condition[23]. Consequently, the side effects of bleaching agents on teeth of patients with reduced saliva flow may be similar to those with normal saliva flow.

CONCLUSIONS

A more thorough understanding of bleaching agents' and their ingredients' effect(s) on intraoral hard tissues will contribute to the decision-making process when it comes to select a product for a specific clinical application. *In vivo*, saliva and fluoride appear to benefit the tooth mineral integrity maintenance.

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FIGURES

Figure 1.

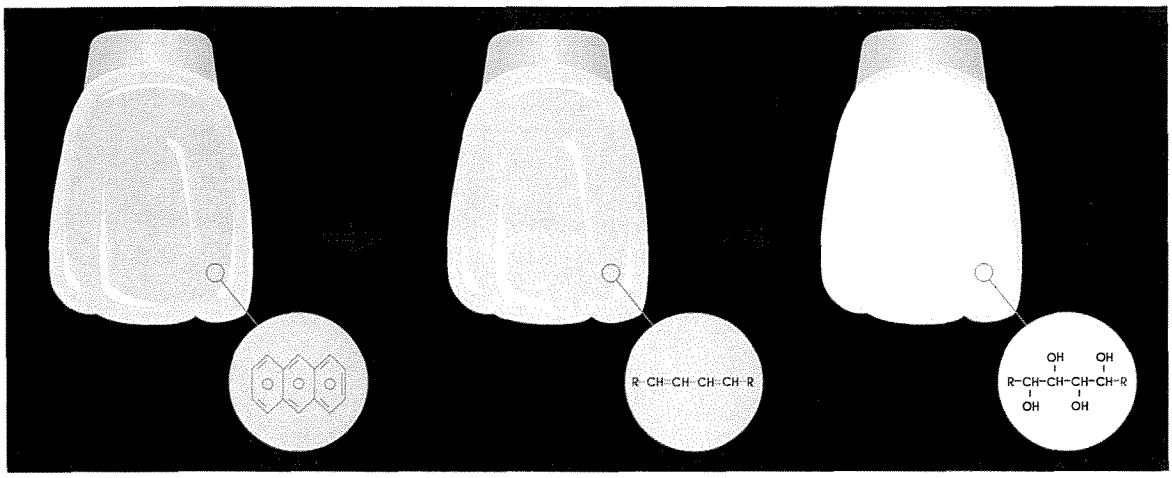

Figure 2.

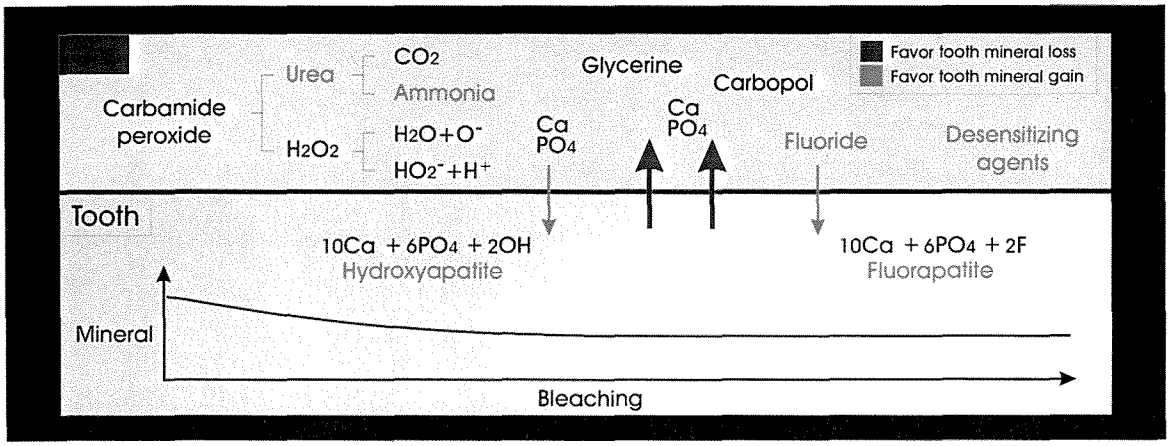

Figure 3.

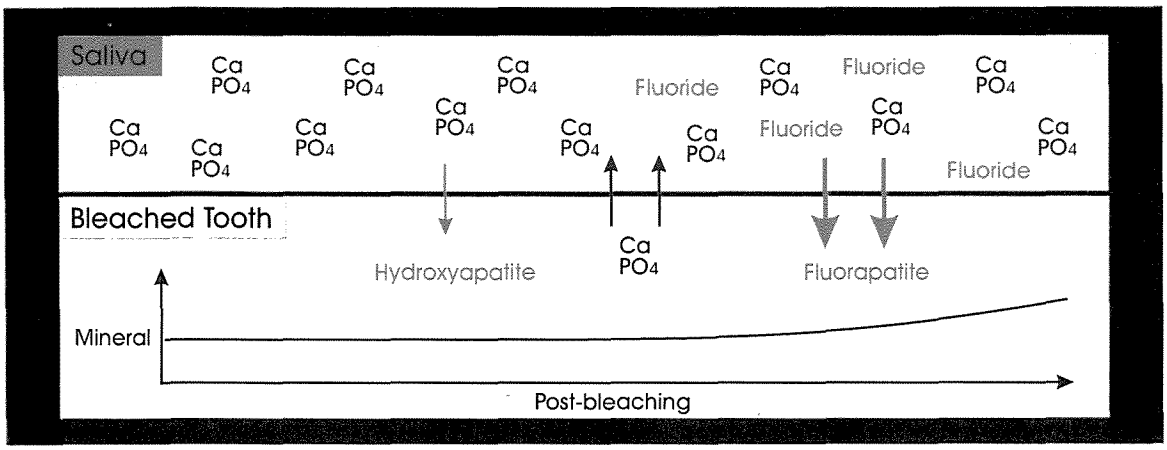

FIGURE LEGENDS

Figure 1. Degradation of complex organic molecules during the bleaching process.

Figure 2. Effects of bleaching agents ingredients on tooth mineral gain and loss.

Figure 3. Saliva and fluoride remineralizing effect at the post-bleaching period.

4. CONCLUSÕES GERAIS

1. Na dependência do agente clareador utilizado, a dentina humana hígida e desmineralizada pode apresentar um aumento ou uma redução transitória nos valores de microdureza, embora no período pós-tratamento a saliva apresente um efeito remineralizante.

2. Alterações no conteúdo mineral do esmalte e dentina ocorridos durante e após o tratamento clareador caseiro parecem estar relacionadas tanto aos componentes e às propriedades do agente clareador bem como à presença da saliva.

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ANEXOS

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito de agentes clareadores à base de peróxido de carbamida sobre a microdureza da dentina hígida e desmineralizada em diferentes tempos", sob o protocolo nº **104/2001**, da Pesquisadora ***PATRICIA MOREIRA DE FREITAS***, sob a responsabilidade da Profa. Dra. **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, 29 de novembro de 2001

We certify that the research project with title "Effects of carbamide peroxide bleaching agents on microhardness of sound and demineralized dentin at different times", protocol nº **104/2001**, 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 Resarch at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, November 29 2001

p/ Viviane Maia Barreto de Oliveira

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ANEXO 2

Journal of **Oral Rehabilitation**

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BRASIL

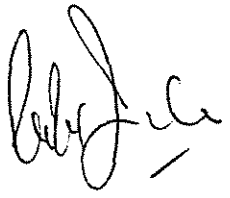
30th September 2002

Dear dr Serra

Re : " Dentine microhardness throughout and after whitening treatments "

Thank you for sending the above manuscript. We will write to you again on completion of the editorial review.

Yours sincerely,



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ANEXO 3

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Para : <mcserra@forp.usp.br>
Assunto : Manuscript Received
Data : Tue, 5 Nov 2002 14:09:01 -0500

Dear Dr. Serra:

I received your manuscript **"Monitoring of demineralized dentin microhardness throughout and after bleaching"** submitted to the ***American Journal of Dentistry***.

I will send the paper to two reviewers and will contact you immediately after I hear from them.

Sincerely,

Franklin Garcia-Godoy, DDS, MS
Editor, American Journal of Dentistry
Professor and Assistant Dean for Clinical Sciences
College of Dental Medicine
Nova Southeastern University
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ANEXO 4

JOURNAL OF ESTHETIC AND RESTORATIVE DENTISTRY

Official Publication of the American Academy of Esthetic Dentistry, Japan Academy of Esthetic Dentistry, Scandinavian Academy of Esthetic Dentistry, International Federation of Esthetic Dentistry, American Academy of Cosmetic and Adhesive Dentistry, Australian Society of Aesthetic Dentistry, Belgian Academy of Esthetic Dentistry

December 3, 2002

Dr. Mônica Campos Serra
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Departamento de Odontologia Restauradora – Dentística
Avenida do Café s/n – Monte Alegre
CEP: 14040-904
Ribeirão Preto, SP – Brazil

Dear Dr. Serra:

Thank you for your recent manuscript submission to the *Journal of Esthetic and Restorative Dentistry*, entitled, "Dental Mineral Equilibrium During and After Home-Bleaching: A Review." For reference purposes, the Tracking Number for your manuscript is: #120201. From this point, the manuscript will be subjected to several levels of review consistent with the peer review process. This process typically requires approximately 6-8 weeks, depending on the subject matter and the availability of reviewers, after which you will be apprised of the status of the manuscript relative to its acceptance for publication. If the submission is incomplete or deficient in any areas (e.g. missing abstract, legends, etc.), our Editorial Assistant, Ms. Betty Cates will contact you. It you have any questions regarding the status of your manuscript, please feel free to contact us.

I really appreciate your submitting this manuscript for our review. Only through the submission of high quality manuscripts can we hope to maintain a high standard of excellence for the *Journal of Esthetic and Restorative Dentistry*, making it the pre-eminent publication in the field of esthetic dentistry.

Sincerely,

Harald O. Heymann, D.D.S., M.Ed.
Editor-in-Chief

cc: Dr. Ed Swift, Associate Editor