CLAUDIA CIA WORSCHECH
CIRURGIÃ - DENTISTA

EFEITO DA ESCOVAÇÃO COM DENTIFRÍCIO ABRASIVO FLUORETADO E NÃO FLUORETADO SOBRE A SUPERFÍCIE DO ESMALTE DENTAL CLAREADO ATRAVÉS DE PERÓXIDO DE CARBAMIDA a 10% e 35%

Avaliação da rugosidade e dureza superficiais

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para a obtenção do Título de Doutora em Clínica Odontológica, Área de Concentração em Dentística.

PIRACICABA
2004

CLAUDIA CIA WORSCHECH
CIRURGIÃ - DENTISTA

EFEITO DA ESCOVAÇÃO COM DENTIFRÍCIO ABRASIVO FLUORETADO E NÃO FLUORETADO SOBRE A SUPERFÍCIE DO ESMALTE DENTAL CLAREADO ATRAVÉS DE PERÓXIDO DE CARBAMIDA a 10% e 35%

Avaliação da rugosidade e dureza superficiais

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para a obtenção do Título de Doutora em Clínica Odontológica, Área de Concentração em Dentística.

***Orientação**: Prof. Dr. Luis Roberto Marcondes Martins*

***Banca examinadora**: Prof. Dra. Fabiana Mantovani Gomes França*
Prof. Dr. José Augusto Rodrigues
Prof. Dr. José Roberto Lovadino
Prof. Dr. Luis Alexandre Maffei Sartini Paulillo

PIRACICABA
2004

Ficha Catalográfica

Worschech, Claudia Cia.
W897e Efeito da escovação com dentifrício abrasivo fluoretado e não fluoretado sobre a superfície do esmalte dental clareado através de peróxido de carbamida a 10% e 35%. / Claudia Cia Worschech. -- Piracicaba, SP : [s.n.], 2004.
xiii, 78 f. : il.

Orientador : Prof. Dr. Luis Roberto Marcondes Martins.
Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Dentística. 2. Esmalte dentário. 3. Dentifrício. I. Martins, Luis Roberto Marcondes. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Ficha catalográfica elaborada pela Biblioteca da Faculdade de Odontologia de Piracicaba - UNICAMP

Folha de Aprovação
Fornecida pela CCPG/FOP

Ao Adriano, meu Grande Amor, Companheiro e Amigo...
Meu exemplo de Força...de Fé...e de Vida...
cuja presença é fundamental em mim ...
Com ele compartilho todas as alegrias dessa conquista.....
Pelo entusiasmo com que participa da minha vida profissional e pessoal...

... a ele dedico este trabalho!!!!

e...ao Lucas,
nosso filho,
cuja
existência
faz de mim
uma mulher
plena e
preenche a
minha
alma!!!...

AGRADECIMENTOS

À Faculdade de Odontologia de Piracicaba, na pessoa do seu Diretor, Prof. Dr. Thales Rocha de Mattos Filho e do Prof. Dr. Mário Fernando de Góes (Diretor Associado).

Ao Prof. Dr. Pedro Luis Rosalen, coordenador do curso de pós-graduação e ao Prof. Dr. Roger William Fernandes Moreira, coordenador do Curso de pós-graduação em Clínica Odontológica.

Ao Prof. Dr. Mário Alexandre Coelho Sinhoreti, da Área de Materiais Dentários desta instituição, por ter gentilmente permitido a utilização do laboratório da Área, bem como os equipamentos necessários à realização desta pesquisa.

Ao Prof. Dr. José Roberto Lovadino, não somente por ser representante da Área de Dentística junto à subcomissão do programa de Clínica Odontológica, mas, especialmente, por ser um amigo querido e um incentivador, que muito contribuiu para a minha formação profissional e pessoal, estando sempre por perto, meus sinceros agradecimentos.

Ao Prof. Dr. Luis Roberto Marcondes Martins, pela oportunidade que me deu de continuar o curso de pós-graduação, sob sua orientação, pela confiança que me dedicou durante todos esses anos, pela firmeza e pela simplicidade com que se relaciona com as pessoas, pelo seu bom coração...e, por todas as suas atitudes...que me possibilitaram o aprimoramento de relações pessoais e profissionais.

Ao Prof. Dr. Luis Alexandre Maffei Sartini Paulillo, pela dedicação ao curso de pós-graduação, pela seriedade e responsabilidade com que desempenha o seu papel de professor, pela correção nas suas atitudes, pela transparência com que lida com as pessoas e

pelo seu tão criticado “senso crítico”. Muito obrigada por me orientar em todos os momentos mais difíceis que passei aqui...Você é um grande amigo!

Aos Profs. Drs. Cristiane Mariote Amaral, Caio Cezar Randi Ferraz e Mário Alexandre Coelho Sinhoreti, que, por participarem da banca examinadora do exame de qualificação, puderam melhorar a qualidade deste meu trabalho.

Aos demais professores da Área de Dentística: Giselle Maria Marchi Baron, Luis André Freire Pimenta, Marcelo Gianinni, que, de alguma maneira, puderam contribuir para a realização desse trabalho.

Ao José Augusto Rodrigues, meu colega de pós-graduação e dedicado amigo, agradeço a presença marcante durante a realização deste trabalho e à seriedade com que conduz o seu trabalho como pesquisador!

Aos amigos da pós-graduação: Alex José Sousa Santos, Fernão Hélio Campos Leite Júnior, Flávio Henrique Baggio Aguiar, Denise Sá Maia, Patrícia Freitas, que sempre com carinho me auxiliaram e dividiram comigo as tarefas infindáveis, proporcionando-me cumprir todos os compromissos...Muito obrigada! Especialmente, à Fabiana Mantovani Gomes França, colega de pós-graduação, dupla de clínica, amiga-irmã, com quem dividi cada momento do curso...agradeço a Companhia, a Força, a Fé e todos os outros ensinamentos de vida!!!

Aos meus pais, pela formação do meu caráter, pela minha formação religiosa, pelo sacrifício despendido na minha educação e por tornarem real o meu sonho de ser Dentista!!! .....Professora !!! e agora.....Doutora!!!

A DEUS, por iluminar o meu caminho, me tornar uma pessoa forte e estar sempre presente na minha vida!

" Só quem
se arrisca
a ir longe
demais
descobre o
quão longe
se pode
ir..."

ELLIOT

Quem espera que a vida seja feita de ilusão
Pode até ficar maluco, ou morrer na solidão
É preciso ter cuidado, pra mais tarde não sofrer
É preciso saber viver...

Toda pedra no caminho, você deve retirar
Numa flor que tem espinhos, você pode se arranhar
Se o Bem e o Mal existem, você pode escolher

É preciso saber viver...

No Novo Tempo, apesar dos castigos,
Estamos crescidos, estamos atentos,
Estamos mais vivos!!!

No Novo Tempo, apesar dos perigos,
De todos pecados, de todos enganos,
Estamos marcados!!!

Pra que nossa esperança
Seja mais que vingança
Seja sempre o caminho
Que se deixa de herança

No Novo Tempo, apesar dos castigos,
Estamos em cena, estamos nas ruas,
Quebrando as algemas!!!

No Novo Tempo, apesar dos perigos,
Agente se encontra cantado na praça,
fazendo pirraça
Pra sobreviver...
Pra sobreviver...
Pra sobreviver...

Pra que nossa esperança
Seja mais que vingança
Seja sempre o caminho
Que se deixa de herança

...Há Tempo para nascer e Tempo para morrer
Tempo para plantar e Tempo para arrancar a planta
Tempo para matar e tempo para curar
Tempo para destruir e Tempo para construir
Tempo para chorar e Tempo para rir
Tempo para gemer e Tempo para bailar
Tempo para atirar pedras e Tempo para recolher pedras
Tempo para abraçar e Tempo para se separar
Tempo para procurar e Tempo para perder
Tempo para guardar e Tempo para jogar fora
Tempo para rasgar e tempo para costurar
Tempo para calar e Tempo para falar
Tempo para amar e Tempo para odiar
Tempo para a guerra e Tempo para a Paz

Eclesiastes 3 $^{1-8}$

SUMÁRIO

RESUMO

O propósito deste trabalho *in vitro* foi avaliar a rugosidade e a dureza do esmalte dental humano exposto a 10% e 35% de peróxido de carbamida, em diferentes tempos e, submetido a diferentes tratamentos de limpeza superficial: G1- Não escovado; G2- Escovado com dentifrício abrasivo fluoretado; G3- Escovado com dentifrício abrasivo não fluoretado; G4- Escovado sem dentifrício. Cento e vinte fragmentos de esmalte de terceiros molares foram obtidos, usando um disco de diamante. Os fragmentos foram embutidos em resina de poliestireno e a superfície de esmalte de cada espécime foi polida através de discos e pastas abrasivas, em diferentes granulações, possibilitando a obtenção de superfícies planas e lisas. O clareamento foi feito na superfície do esmalte, durante 1 hora, uma vez por semana, nos grupos em que o Peróxido de Carbamida a 35% foi aplicado e, durante 6 horas, diariamente, nos grupos em que o Peróxido de Carbamida a 10 % foi utilizado e, esses tratamentos clareadores foram feitos durante 4 semanas, associados aos procedimentos de escovação, os quais eram executados, diariamente, durante 3 minutos. Um perfilômetro foi utilizado para medir os valores de rugosidade (Ra) e um microdurômetro foi utilizado para testar a dureza das amostras (Knoop) antes e após cada intervalo de 7 dias, desde o início do tratamento. Todas as unidades experimentais permaneceram em saliva artificial durante o tempo remanescente. O Peróxido de Carbamida a 10%, de forma isolada, não alterou a dureza ou a rugosidade do esmalte dental. O Peróxido de Carbamida a 10%, associado à escovação sem dentifrício, não alterou os valores de rugosidade do esmalte dental e reduziu os valores de microdureza superficial do esmalte dental. Escovação com dentifrícios abrasivos fluoretado e não fluoretado aumentou os valores de microdureza e de rugosidade do esmalte dental clareado através de Peróxido de Carbamida a 10% e somente o grupo escovado com flúor apresentou valores de dureza similares aos valores do baseline após 56 dias. O Peróxido de Carbamida a 35%, utilizado de forma isolada, reduziu os valores de microdureza superficial do esmalte dental, entretanto, não alterou a rugosidade superficial do mesmo. O Peróxido de Carbamida a 35%, associado à escovação sem dentifrício, reduziu os valores de microdureza superficial do esmalte dental e não alterou os valores de rugosidade do mesmo. Escovação com dentifrícios abrasivos fluoretado e não fluoretado aumentou os valores de microdureza e de rugosidade do esmalte dental clareado através de Peróxido de Carbamida a 35% .

ABSTRACT

The purpose of this study was evaluate superficial enamel dental roughness and microhardness when Carbamide peroxide 10% or 35% were used, in different time intervals and submitted to different superficial cleaning treatment: G1 – Not Brushed; G2 – brushed with abrasive fluoride dentifrice; G3 – Brushed with abrasive dentifrice without fuoride; G4 – Brushed with water. One hundred twenty fragments of molars were obtained and were polished and individually embedded in polystyrene resin. The specimens were polished with sandpaper of decrescent grit 400, 600 and 1000 and felt cloth with 6, 3, 1 and ¼ μm abrasive pastes. A perfilometer was used to obtain three measurements on the surface of each specimen and the mean of Ra value (μm) was determinate to baseline and 7, 14, 21, 28 days of treatment, and 7, 14, 21 and 28 days post treatment and microhardness tests were performed with a load of 25g for 5 s at for each time intervals. CP 10% used alone, did not alter the enamel microhardness and roughness values. CP 10% associated with brushing without dentifrice did not alter the enamel roughness values but decreased microhardness values. The groups, which received brushing with abrasive fluoride dentifrice or non-fluoride dentifrice, showed increase in microhardness and roughness values when CP 10% was used, only the group which received fluoride dentifrice showed values similar to baseline. CP 35% used alone, decreased microhardness superficial enamel values, however, did not alter superficial roughness in the same enamel. CP 35% associated with brushing without dentifrice, decreased the superficial microhardness enamel values. The groups, which received brushing with abrasive fluoride dentifrice or non-fluoride dentifrice, showed increase in microhardness and roughness values when CP 35% was used.

INTRODUÇÃO GERAL

A odontologia cosmética cresceu rapidamente nos últimos anos e, tratamentos não restauradores, para dentes com alteração de cor, foram desenvolvidos. O elevado nível de interesse pela aparência estética dos dentes aumentou a percepção dos pacientes em relação à cor dos mesmos e o clareamento dental tornou-se um dos tratamentos mais indicados e comuns, uma vez que oferece um caminho atrativo para embelezar os dentes de forma simplificada, com economia de tecido dental sadio e, muitas vezes, sem a necessidade de tratamento restaurador adicional (Haymann, 1997).

A técnica do clareamento dental caseiro foi descrita por Haywood & Heymann, em 1989, e empregava peróxido de carbamida a 10%, em uma moldeira individual, por 8 horas diárias, pelo período de 2 a 6 semanas.

O tratamento clareador apresenta uma série de vantagens, como o baixo custo e facilidade técnica e é descrito como uma alternativa segura e efetiva, para a maioria dos casos (Haywood & Heymann, 1989).

Agentes clareadores, contendo peróxido de carbamida, em baixas concentrações, são indicados para o tratamento caseiro, durante 6-8 horas diárias, enquanto que, para tratamento em consultório, novos sistemas, empregando concentrações mais elevadas, são utilizados por 1-2 horas diárias (Wille *et al.*, 2000).

A técnica de consultório apresenta vantagens em relação à técnica caseira como a diminuição do tempo do tratamento clareador e rápida satisfação dos pacientes. Além disso, o desconforto decorrente da utilização da moldeira, durante o tratamento caseiro, faz com que, muitos pacientes, prefiram realizar o tratamento em consultório (Broome, 1998; Mokhlis *et al.*, 2000; Papathanasious *et al.*, 2001).

Entretanto, o contato direto dos agentes clareadores com a superfície do esmalte, nas técnicas caseiras e de consultório, pode causar efeitos adversos ao esmalte dental (Rodrigues & Serra, 2001; Leonard *et al.*, 1998).

Sabe-se que o peróxido de carbamida se dissocia em peróxido de hidrogênio e uréia e a concentração de peróxido de hidrogênio aproxima-se a 1/3 do valor da concentração do peróxido de carbamida utilizado. Dez por cento de peróxido de carbamida

se dissocia em 3% de peróxido de hidrogênio e 7% de uréia (Goldstein & Garber, 1995). O produto dessa dissociação pode alterar a matriz orgânica do esmalte, devido à ação química dos radicais livres liberados pelo peróxido de hidrogênio. Embora a penetração do agente clareador, através dos tecidos dentais, possa causar alterações estruturais, esse efeito, sobre dentina e esmalte, é discutido de forma controversa (Potocknic *et al.*2000; Rodrigues, Basting *et al.*, 2001).

Alguns autores afirmam haver degradação superficial no esmalte após a exposição ao peróxido de carbamida a 10% ou a 35% e análises através de microscopia eletrônica de varredura demonstraram que a exposição da superfície do esmalte ao peróxido de carbamida a 10% proporcionou modificações significativas, sem um padrão definido, sugerindo dissolução os prismas (Hegedüs *et al.*, 1999)

Estudo de micro análises mostrou baixas concentrações de Ca e P no gel clareador, após a sua utilização, indicando perda de minerais do esmalte para o gel (Potocknic *et al.*, 2000). Essa perda de minerais ou desmineralização altera a dureza do esmalte (Featherstone, 1986).

Rodrigues *et al.,* em 2001 relataram que diferentes produtos clareadores, contendo a mesma concentração de peróxido de carbamida, são capazes de apresentar diferentes efeitos sobre o esmalte dental, podendo aumentar ou diminuir os valores de microdureza, em função do pH ao longo do tempo. Essa diminuição dos valores de microdureza do esmalte ocorre após a exposição aos agentes clareadores, *in vitro* (Basting *et al.*, 2001).

In situ, Rodrigues *et al.,* em 2003, também encontraram redução nos valores de microdureza superficial do esmalte exposto ao peróxido de carbamida a 10% e 37%, utilizados de forma isolada ou em associação, na técnica do clareamento em consultório e do clareamento caseiro.

Por outro lado, pacientes que se submetem ao clareamento dental, geralmente escovam os dentes por 3 ou 4 vezes ao dia e, algumas vezes, dentifrícios são utilizados para potencializar o efeito desses agentes clareadores, por conterem partículas abrasivas, as quais podem remover manchas extrínsecas do esmalte, através de desgaste (Isaacs *et al.,* 2000).

Logo após o tratamento, alterações são notadas no esmalte superficial e Attin *et al.,* 1997, recomendaram verniz ou enxaguatório, contendo flúor, após a exposição dos dentes ao peróxido de carbamida, na tentativa de remineralização do esmalte dental.

Assim, especialmente, os dentifrícios mais abrasivos têm um importante papel no processo de prevenção das manchas extrínsecas dos dentes (Isaacs *et al.,* 2000; Gerlach *et al.*, 2001), as quais têm sido associadas à ingestão de alimentos com corantes e bebidas como café ou refrigerantes tipo cola, entre outros, e, alguns abrasivos, contidos nesses cremes dentais, podem desgastar, de forma excessiva, o esmalte.

No tratamento em consultório, em que altas concentrações de peróxido de carbamida são utilizadas, por serem eficazes em diminuir o tempo de tratamento clareador, a utilização de dentifrícios abrasivos poderia desencadear prejuízos ainda maiores ao esmalte.

Entretanto, o uso regular do flúor representaria um importante método de prevenção da perda de mineral e de erosões causadas sobre o esmalte, devido ao uso de tratamentos de limpeza superficial, após a utilização de agentes clareadores (Tames *et al.*, 1998).

Devido à falta de evidência dos efeitos adversos causados no esmalte dental, pelos agentes clareadores, como o peróxido de carbamida, em baixas e altas concentrações, associados aos procedimentos de escovação dental com a utilização de dentifrícios abrasivos fluoretados ou não fluoretados propusemo-nos a realizar esta série de estudos *in vitro* que estão apresentados em quatro capítulos distintos.

CAPÍTULOS

Capítulo I **– Effect *in vitro* of 10% Carbamide Peroxide associated with Brushing and Dentifrices on Dental Enamel Microhardness at Different Time Intervals**
(submetido à avaliação - "The American Journal of Dentistry")

Capítulo II – ***In vitro* effect of toothbrush on Bleached Enamel with 35% Carbamide Peroxide at Different Treatment Intervals – Microhardness Evaluation**
(submetido à avaliação – "Operative Dentistry")

Capítulo III – **Effect of dentifrices on bleached surface enamel - roughness evaluation**
(submetido à avaliação - "The Journal of Prosthetic Dentistry")

Capítulo IV – **In vitro evaluation of human dental enamel surface roughness bleached with 35% carbamide peroxide submitted to abrasive dentifrice brushing**
(publicado na Pesquisa Odontológica Brasileira 2003; 17(4): 342-8)

Effect *in vitro* of 10% Carbamide Peroxide associated with Brushing and Dentifrices on Dental Enamel Microhardness at Different Time Intervals

Claudia Cia Worschech [1], José Augusto Rodrigues[1], Gláucia Maria Bovi Ambrosano[2], Luis Roberto Marcondes Martins[3]

1- DDS, MS, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

2- AGR ENG, MS, PhD, Professor, Department of Community Dentistry, School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

3- DDS, MS, ScD, Professor, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

Address: Faculdade de Odontologia de Piracicaba - UNICAMP
Departamento de Odontologia Restauradora - Dentística
Avenida Limeira, 901 - Areião
CEP: 13141-900
Piracicaba - SP
BRAZIL
Telephone: +55-19-3412-5337
FAX: +55-19-3412-5218
E-mail: caldri@terra.com.br

Effect *in vitro* of 10% Carbamide Peroxide associated with Brushing and Dentifrices on Dental Enamel Microhardness at Different Time Intervals

Objective: The purpose of this *in vitro* study was to evaluate the microhardness of human enamel exposed to a 10% carbamide peroxide bleaching agent (Opalescence 10%) at different times and with different superficial cleaning methods. **Materials and Methods**: Sixty fragments of human molar teeth with 4 x 4 mm were obtained using a diamond disc. The dental fragments were individually embedded in polystyrene resin in a ring mold. The specimens were sequentially polished to obtain flat surfaces. Microhardness tests were performed at baseline, and at 7, 14, 21, 28 days of treatment, and at 7, 14, 21, 28 days post treatment. The microhardness values were obtained using a microhardness tester with a load of 25g for 5 sec. Daily, the bleaching agent was applied on the surface of enamel human fragments for 6 hours. After that, the fragments received a cleaning surface treatment through brushing with fluoride and non-fluoride abrasive dentifrice, in a brushing machine. Than, the samples were stored in individual receptacles with a remineralization solution similar to artificial saliva for the remaining 18 hours. **Results:** The split plot analysis of variance and Tukey test revealed significant differences in microhardness values over time for enamel bleached and treated with different superficial cleaning methods. The control group G1 (control group) did not show differences in microhardness values all the time. The samples brushed with abrasive fluoride dentifrice (G2) showed an increase of microhardness values between the 7^{th} to the 28^{th} day, but at the end of the experiment, in the 56^{th} day, the microhardness values were similar to baseline values. The G3 (brushed with abrasive non fluoride dentifrice) showed increase of microhardness values between the 7^{th} e the 14^{th} days, but between the 28^{th} and the 56^{th} days these values keep constant and statistically higher than baseline values. The G4, which were brushed in water, did not show statistical differences among the bleaching test times but showed decrease in microhardness values between the 35^{th} to the 56 days (post treatment period).

CLINICAL SIGNIFICANCE

As routine, a dentifrice is used to clean teeth during bleaching treatment. However, bleaching treatment agents associated to abrasive dentifrices might contribute to tooth alterations and loss of mineral content. The evaluation of these effects must be known.

INTRODUCTION

The primary reason to patients brush their teeth daily is to achieve health and a good look. Today, a variety of different compounds are used to improve dentifrices formulations. It may offer a platform to prevent dental caries through fluorides, or sensibility through desensitizing agents, or stains using abrasives.

Dentifrice abrasives play an important role in the cleaning process preventing extrinsic stains deposition [1, 2] which have been associated with the ingestion of foods and caffeine, cola or color-containing beverages, wine, and tobacco products. However, intrinsic stains bonded within the structure of the tooth like in the oldest teeth cannot be remove neither by brushing nor by any abrasive process. Nevertheless, it can be easily reduced using a penetrating bleaching agent.

Bleaching of vital teeth has become one of the most popular and successful esthetic treatment in dentistry [3, 4]. It offers an attractive way to lighten teeth by a conservative, simplified, and economical technique without any additional restorative treatment [5].

These techniques generally use a hydrogen peroxide based material prepared into a thick gel. The most commonly bleaching agent is 10% carbamide peroxide used in a custom tray during the day or the nighttime[3, 6]. The bleaching agent diffuses through the interprismatic substance of the enamel breaking the highly pigmented carbon rings compounds converting them into chains, which are lighter in color [7, 6, 8].

As bleaching treatment involves direct contact of the whitening agent on the outer enamel surface for almost six hours per day, an extensive period of time, many studies have evaluated the adverse potential effects of these agents [9].

Scanning electron microscopic investigations demonstrated that the exposure of 10% carbamide peroxide to the surface of enamel was significantly altered and was not uniform in nature, some areas demonstrated little effect and other areas had serious

dissolutions of the surface [10, 7, 11, 12] and in some cases are similar to dental caries **.** Electron probe microanalysis showed low concentration of Ca and P in the bleaching gel after use, suggesting mineral loss from enamel to the bleaching agent [13].

Loss of mineral content or demineralization alters the microhardness of enamel. Rodrigues *et al.* [14] related that different bleaching materials with the same concentration of carbamide peroxide have different effects on the enamel, which could increase or decrease microhardness values. Other papers have reported decrease in the enamel microhardness and strength after exposure to bleaching agents *in vitro* [15, 7]**.** *In situ*, Basting *et al.,* 2001 [9] found alterations in enamel exposed to 10% carbamide peroxide while Shanon [16] did not find significant differences on the enamel microhardness after 4 weeks of exposure to 10% carbamide peroxide.

Little has been reported about *in situ* or *in vivo* alterations of external enamel structure after bleaching associated with toothbrushing, and other clinically relevant factors have not been taken into account.

The patients use, as routine, a dentifrice during bleaching treatment and it is well known that the use of abrasive dentifrices on the surface of intact enamel causes scratches or microwear [17]. Than, the use of bleaching agents might contribute to an excessive tooth wear and loss of mineral content associate with the abrasive effect of dentifrice.

The lack of conclusive evidence of the effects of bleaching agents associated with toothbrushing procedure on the enamel surface and the influence of saliva in its remineralization process still need be evaluated.

The aim of this study was to investigate the effects of brushing with abrasive dentifrice with or without fluoride after bleaching treatment with 10% carbamide peroxide on human dental enamel at different time intervals and in a post-bleaching treatment period.

MATERIALS AND METHODS

Experimental design

The factors under study were:

1. Surface Cleaning Treatments (in four levels): **GROUP 1** - Not brushed**; GROUP 2 -** Brushed with fluoride abrasive dentifrice; **GROUP 3 -** Brushed with abrasive dentifrice without fluoride and **GROUP 4 -** Brushed without dentifrice.
2. Time (in nine levels): *baseline, 7, 14, 21, 28* days of treatment and *7, 14, 21*, and *28* days after the end of the treatment, corresponding to the post treatment period.

The experimental units consisted of 60 sound human enamel fragments, randomly assigned to four different treatment groups (n=15). Knoop microhardness response was evaluated by quantitative methods. Three repeated measurements of Knoop microhardness were taken on the surface of each specimen at 7-days intervals.

Enamel Fragment Preparation

Thirty freshly extracted third molars were used. Immediately after extraction, the teeth were stored in TIMOL 1% solution (pH=7). The crowns were removed approximately in the cement enamel junction, and the roots were discarded. The crowns were longitudinally sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP-Brazil) used in a low motor speed (Kavo do Brasil – Joinvilhe SC- Brazil), to obtain 60 enamel fragments. The gingival and oclusal third of crown were discarded, and only the middle of the vestibular or lingual faces were used. Care was taken to leave the enamel fragments hydrated in this period. After sectioning was completed, specimens were soaked in distilled and deionized water at 37º C.

The enamel fragments had 4 X 4 mm. Fragments that showed stains or cracks were not used. The enamel fragments were embedded individually in a self-curing polyester resin in a polyvinyl chloride ring mold with 2.0 cm in diameter and the external surface of the enamel was exposed.

The molds were removed, and the external surfaces of the enamel fragments were leveled with a water-cooling mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil,

01328-000). Aluminum oxide disks were used in a sequential granulation of 400, 600, and 1,000 (Carburundum / 3M do Brazil Ltda). After that, these surfaces were polished with a felt cloth and especial abrasive pastes Top, Gold and Ram (Arotec Ind e Com Ltda / Brazil) with mineral oil coolant (LA, Arotec Ind and Com Ltda). These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests.

A standardized circular area of 12,56 mm^2 of exposed enamel was created on the specimens through adhesive paper and nail varnish, by covering the remaining enamel fragment with two coatings of nail varnish (Colorama, CEIL).

The enamel fragments were randomly assigned in 4 groups and submitted to different cleaning treatments G1 – Control; G2 brushed with fluoride abrasive dentifrice; G3 – brushed with non-fluoride abrasive dentifrice and G4 – Brushed with water.

Bleaching treatment

Carbamide peroxide bleaching agent (Opalescence 10% Ultradent Co., South Jordan, UT, USA) was evaluated. An individual tray was manufactured for each specimen using a 0.4 mm-thick flexible ethyl-vinyl acetate polymer (Bio-Art Equipment) placed in a vacuum-forming machine (P7, Bio-Art Equipment).

For the application of the treatment agent, a syringe was used to apply 0.02 ml [14, 18, 19, 20] of the bleaching agent to each specimen. The specimens were kept in individual receptacles with 18ml of remineralization solution similar to artificial saliva (Table 1), at 37ºC. The artificial saliva consisted of a remineralization solution proposed by Featherston *et al* in 1986, and modified by Serra and Cury in 1992.

After 6 hours, the specimens were taken out of the remineralization solution similar to storage media, and the trays were removed. The bleaching agent was washed out under running distilled and deionized water for 5 seconds.

During the remaining daily time (18 hours), the fragments were maintained in individual receptacles with 18,8 ml of remineralization solution similar to artificial saliva (pH 7.00) at 37ºC. The artificial saliva was changed daily.

After 28 days of bleaching treatment all the groups were kept only in remineralization solution similar to artificial saliva and this period, when the specimens did not receive more bleaching treatment, was called post-bleaching period.

Cleaning Surface Treatments

Soon after bleaching treatment, the samples were submitted to a cleaning surface treatment according to each group.

The G1 was not submitted to a cleaning treatment and it was immersed in the remineralization solution similar to artificial saliva after the end of bleaching treatment. This was the control group.

The groups treated with dentifrices were G2 brushed with fluoride abrasive dentifrice, G3 brushed with non-fluoride abrasive dentifrice.

The dentifrices used to cleaning surface treatment were marketed each two days to keep neutral pH. The dentifrices contained carbonates calcium as abrasive with fluoride to G2 and without fluoride to G3 (Table 2).

The dentifrices were mixed with distilled deionized water in a proportion of 3:1 (3 parts of water and 1 part of dentifrice) to form slurry. The slurries were prepared 20 minutes before the end of the bleaching treatment and each sample was treated with 20ml of the respective slurry. The slurries were agitated before they were placed inside the reservoir and cover the specimen.

The G4 received the cleaning treatment only with the toothbrush. This treatment was performed immersed in distilled deionized water.

The cleaning surface treatments were performed for 3 minutes daily. The samples were positioned in a brushing machine programmed to do 250 cycles per minute with a usual brushed force of 200g.

The brushing machine had a reciprocal action of an electric motor driven tooth-brushing machine. The toothbrushes with nylon multi-tufted brush have the heads sectioned and adapted in the brushing machine. Each sample was brushed with same brush heads.

After final brushing, the samples were removed from the recipients of the machine, washed through distillated water and stored in individual receptacles with 18 ml of

remineralization solution similar to artificial saliva (pH 7.00) at 37ºC until the next bleaching treatment.

After 28 days the bleaching treatment was finished and the post bleaching period was begun. At the post-bleaching period the surface cleaning treatment was performed daily in the same way.

Microhardness Testing

Microhardness measurements were performed prior to the bleaching treatment, to be used as control, and 7, 14, 21, 28 days after the start of bleaching treatment and at post treatment period as 7, 14, 21 and 28 days (corresponding to 35, 42, 49, and 56 days after the beginning of the bleaching treatment).

A microhardness tester machine (Future Tech, FM –1e) with a Knoop indentator was used. The long axis of the diamond was kept perpendicular to the enamel surface and a load of 25g was applied for 5 seconds. In each test time three indentations were made on each specimen. Since there were multiple test times (nine test times of three indentations each), specific sites to perform the indentations were randomly pre-established to each test time before the beginning of the experiment and they had nearly 500μm of distance from the other site.

Statistical Analysis

The average of the three indentations for each time period was used as the Knoop value for each specimen. The Knoop hardness Number (KHN) was obtained 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 involved a parametric method using repeated measures analysis of variance (ANOVA) followed by a Tukey's HSD hoc analysis (α=0.05).

RESULTS

The Analysis of Variance and Tukey test revealed significant differences for each cleaning treatment after bleaching agent had been applied, within each time interval ($p < 0.05$) The mean values are showed in Table 3, and the behavior of mean microhardness of enamel may be observed individually in graphic 1.

GROUP 1 (control group) was bleached and immersed in artificial saliva but did not show statistical difference between microhardness values from the beginning to the end of the treatment.

GROUP 2, submitted to bleaching and to cleaning treatment with an abrasive and fluoride dentifrice showed a significant statistical increase in microhardness values from the baseline to the 14th day. A significant statistical decrease in the microhardness values started at the 14th day to the 21th, but the 21th day had a value statistically similar to baseline. After that, the microhardness values had an increase statistically superior to baseline until the 35th day, after that a decrease in the microhardness values occurred. This decrease was continued but at the end of the experiment, in the 56th day, the microhardness values were similar to those in the 28th day and baseline values.

GROUP 3, bleached and treated with an abrasive dentifrice, showed results close to group 2. In this group occurred a statistical significant increase in the microhardness values from baseline to the 14th day followed by a statistical decrease in the microhardness values until the 21th day in which the value was statistically similar to baseline. From the 21th day to the 35th day the microhardness values showed an increase with statistically differences from baseline but statistical equal to all post-bleaching period.

GROUP 4, bleached and submitted to cleaning treatment with water, showed a constant decrease in the microhardness values during treatment and showed a non statistically significant decrease in microhardness values during bleaching period (from baseline to the 28th day). Although the values of microhardness in the post bleaching period, 35th to 56th days, were not statistically different, they showed a significant decrease from baseline values. The values from the 49th to the 56th days also differ from the 7th, 14th and 28th day microhardness values.

DISCUSSION

At home, vital bleaching is generally performed during nighttime. In the morning the first step of the patient is to remove the tray and brush their teeth. In this time, bleaching agent is washed out and some enamel is abraded depending on what kind of dentifrice is used.

An enamel wear evaluation was not the objective of this study, but it was to access the *in vitro* effect of dentifrices on enamel microhardness after exposition to 10% carbamide peroxide in a way close to clinical situations.

Penetration of bleaching agents into the tooth hard tissues can result in different changes in vital teeth [10, 7, 11, 12, 9, 23]. The increase porosity of enamel may be caused mainly by nascent oxygen released in the inner structure by hydrogen peroxide that penetrate along the enamel proteins (amelogenin and enamelin), because the mineralized, inorganic phase is much more compact than the organic one, and penetration through hydroxyapatite crystals is probably very low [24].

Others studies reported that enamel microhardness are reduced after prolonged exposition to 10% carbamide peroxide [15, 14, 7] and the effect of this reduction is associated to the abrasion [25]. In the literature, the influence of carbamide peroxide containing bleaching agents on enamel surface regarding microhardness and texture is controversially discussed [26, 15, 14, 7]. Rodrigues *et al.* related that there was microhardness alteration on the enamel when two different 10% carbamide peroxide were used and the alteration was a function of bleaching time and pointed out that different materials with the same concentration of carbamide peroxide have different effects on the enamel depending on the pH[14].

This study showed that, in the G1, there was no effect on the enamel microhardness after the application of carbamide peroxide after 28 days, this result agrees with Lopes *et al*, 2002, who showed that bleaching treatment conducted with two home bleaching agents had no adverse effects on enamel microhardness or on surface morphology[27]

The reduction in the microhardness is controversial and the effect of superficial cleaning treatments performed by patients during bleaching treatment has not been discussed yet.

The alteration of the enamel surface and the rate of change may be related to the calcification and fluoride content of the enamel [28]. Oral hygiene or drinking habits that may affect the enamel surfaces should be considered in the long-term use of bleaching agents [28].

The application of a fluoride toothpaste or mouth-rinse after exposing teeth to carbamide peroxide bleaching agents have been indicated in order to avoid demineralization or improve remineralization of the enamel after bleaching [23, 26].

Also, neutral pH of toothpaste may cause a deposition of considerable amounts of CaF_2. The effect of fluoride inhibits demineralization and possible surface change [29].

On the 56th day G1 (control group that was no brushed) and G2 (brushed with fluoride abrasive dentifrice) did not show differences when baseline values were analyzed. In the same period G3 (brushed with a non-fluoride abrasive dentifrice) showed high values of microhardness and G4 (toothbrushing with water) showed low values of microhardness when it was compared with baseline values.

ATTIN et al, 1997, related that application of a fluoride varnish or mouth-rinse is recommended after exposing teeth to carbamide peroxide bleaching agents in order to improve remineralization of the enamel [26].

The exposition of this enamel to neuter sodium fluoride solution 2% showed an increase of fluoride deposition as CaF_2 on the damaged enamel. This fact indicates that fluoride solutions may represent benefit to dental tissues, or reduce the superficial erosion caused on the enamel due to use of bleaching agents.[6] Another factor which may decrease the superficial erosion or remineralize the superficial enamel is saliva, which has a high potential for remineralization. It may be supposed that if some remineralization occurs, the fluoride abrasive dentifrice used (G2) removed mineral deposits on prism structures and subsequently exposed the fresh surface of the enamel.

In the same way, the non-fluoride abrasive dentifrice (G3) possibly removes some mineral precipitation or acts polishing eroded areas. Some authors have revealed the surface degradation and surface defects throughout SEM evaluation [7, 11, 12]. The wear of this degraded surface may expose fresh enamel with microhardness similar to non-bleached enamel.

In vitro, SEM analysis have demonstrated that the application of 10% carbamide peroxide on enamel surface causes morphological changes with an increase in porosity and erosions. Clinically, increase porosity allows the bleaching agent to easily penetrate through enamel and dentin and could explain the transitory dental sensitivity during its use. 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 [9].

The concomitant use of methods to inhibit the demineralization during tooth bleaching treatment might be useful. Home bleaching agents require professional supervision to ensure the proper application of bleaching agents, the use of the recommended amount of gel or paste, the correct length of treatment, and the prevention of adverse reactions [18].

CONCLUSION

According to the results and based on statistical analyses, it may be concluded that:

1. The isolated treatment with 10% carbamide peroxide for 6 hours a day for 28 days did not alter the enamel microhardness, for control group.
2. The enamel microhardness may increase during bleaching treatment when associated to brushing with an abrasive dentifrice.
3. The brushing without a dentifrice may decrease the enamel microhardness after bleaching treatment After 28 days of treatment).

REFERENCES

1 - Isaacs, RL, Bartizek RD, Owens, TS *et al*: Maintenance of tooth color after prophylaxis: comparison of three dentifrices. *J. Clin Dent*, 200; 112: 51-55.

2 - Gerlach, RW, Barker, ML, Hyde, JD *et al*: Effects of a tartar control whitening dentifrice on tooth shade in a population with long-standing natural stain. *J. Clin Dent*; 2001; 12: 47-50.

3 - Papathanasious, A, Bardwell, D. & Kugel, G.: A clinical study evaluating a new chairside and take-home whitening system. *Compendium*; 2001; 22: 289-297.

4 - Gürgan, S., Bolay, S. & Alaçam, R.: In vitro adherence of bacteria to bleached or unbleached enamel surfaces. *J. Oral. Rehabil*; 1997; 24: 624-627.

5 - Heymann, HO: Bleaching of vital teeth. *Quintessence. Int*; 1997; .28: 420-427.

6 - Kugel, G., Perry, RD, Hoang, E *et al*: Effective tooth bleaching in 5 days: Using a combined in-office and at-home bleaching system. *Compendium*; 1997; 18: 378-383.

7 - Smidt A, Weller D, Roman I, *et al.* Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *Am J Dent* 1998; 11: 83-85.

8 - Mokhlis, GR, Matis, BA & Cochran, MA: A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. *JADA*; 2000; 131:1269-1277.

9 - Basting, RT, Rodrigues, AL & Serra, MC: The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *In Situ*. *Oper. Dent*; 2001; 26: 531-539.

10 - Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. *J Prosthet Dent;* 1992; 67: 852-855.

11 - McGuckin RS, Babin JF& Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. *J Prosthet Dent*; 1992; 68:754-760.

12 - Gultz, J, Kaim, J, Scherer *et al*: Two in-office bleaching systems: A scanning electron microscope study. *Compendium*; 1999; 20: 965-968.

13 - Potocnik I, Kosec, L, Gaspersic, D.: Effect of 10% carbamide peroxide bçeaching gel on enamel microhardness, microstructure, and mineral content. *J. of Endod.*; 2000; 26: 203-206.

14 - Rodrigues JA, Basting RT, Rodrigues Jr AL, *et al*. Effects of 10% carbamide peroxide bleaching materials on enamel microhardness. *Am J Dent* 2001; 14: 67-71.

15 - Hachiya, Y, Takatsu, T, Hosoka, H *et al.* : A barniz to prevent etching unrestored enamel. *J. Prost. Dent*; 1985; .53: 46-50.

16 - Shannon H, Spencer P, Gross K, *et al.* Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. *Quintessence Int* ; 1993; 24: 39-44.

17 - Kuroiwa, M, Kodaka, T., Kuroiwa, M: Microstructural changes of human enamel surfaces by brushing with and without dentifrice containing abrasive. *Caries Res*; 1993; 27: 1-8.

18 - Freitas, PM, Basting RT, Rodrigues, AL *et al.*: Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence Int, 2002; 33: 370-375.*

19 - McCracken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996; 24: 395-398.

20 - Oliveira, R, Basting, RT, Rodrigues, JA *et al.*: Effects of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness. *Am. J. Dent* 2002; 15: "in press".

21 - Featherstone JDB, O'Really MM, Shariati M, *et al*. Enhancement of remineralization *in vitro* and *in vivo*. In: Leach AS. *Factors relating to demineralization and remineralization of the teeth*. Oxford: IRL; 1986: 23-34.

22 - Serra MC, Cury JA. The *in vitro* effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessence Int* 1992; 24: 39-44.

23 - Tames, D; Grando, J. &Tames, DR: Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida 10%. *Revista APCD*; 1998 ; 52: 145-149.

24 -Hegedüs, C, Bistey, T, Flóra-Nagy, *et al.*: An anatomic force microscopy study on the effect of bleaching agents on enamel surface. *J. of Dentistry*; 1999; 27: 509-515.

25 - Attin *et al*: Correlation of microhardness and wear in differently eroded bovine dental enamel : *Archs Oral Biol*.: 1997 : 42: 243-250.

26 - Attin T, Kielbassa AM, Schwanenberg M, *et al.* Effect of fluoride treatment on remineralization of bleached enamel. *J Oral Rehábil;* 1997; 24: 282-286.

27 - Lopes, GC, Bonissoni, L, Baratieri, LN *et al.*: Effect of bleaching agents on the hardeness and morphology of enamel. J.Esthet Restor Dent; 2002; 14: 24-30.

28 - Bitter NC, Sanders JL. The effect of four bleaching agents on enamel surface: A scanning electron microscopic study. *Quintessence Int;* 1993; 24: 817-824.

29 - Akal, N, Over, H, Olmez, A *et al.*: Effects of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel. *J Clin Pedriatr*. Dent; 2001; 25(4): 293-296.

TABLE 1: Artificial saliva compounds by PRODERMA PHARMACY (Piracicaba / Brazil)

ARTIFICIAL SALIVA pH=7.0	
COMPOUNDS	***Concentration***
Ca	1.5 mmol/l
KCl	50 mmol/l
PO_4	0.9 mmol/l
tri-hydroxymethil-aminomathan	20 mmol/l
distilled and deionized H_2O	qsp

*The artificial saliva compounds proposed by Featherstone et al, and modified by Serra and Cury

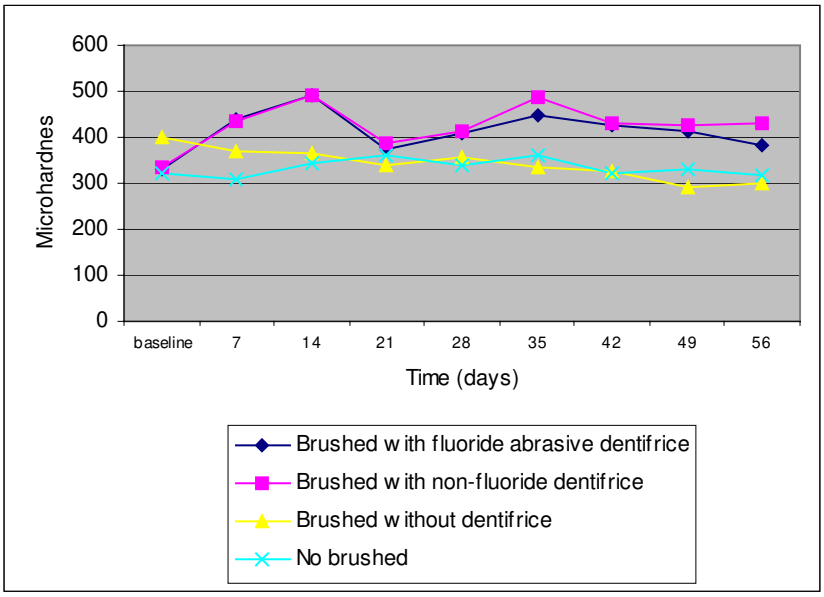
TABLE 2: Ingredients used to prepare the dentifrices by PRODERMA PHARMACY (Piracicaba / Brazil)

COMPOUNDS	**Toothpaste abrasive with fluoride**	**Toothpaste abrasive without fluoride**
Micronized calcium carbonate	52,5 %	52.5%
Glycerin	25 %	25%
Natrosol Gel	18%	18%
Sodium lauryl sulfate	2 %	2%
Sodium fluoride	-	0.16%
Distilled Water	qsp	qsp

Table 3. Mean Knoop microhardness values for each treatment agent at different time intervals

Period	Cleaning surface treatments							
	G1- No brushed (control)		G2- Brushed with fluoride dentifrice		G3- Brushed without non-fluoride		G4- Brushed without dentifrice	
	average	Standard deviation	average	Standard deviation	average	Standard deviation	average	Standard deviation
Baseline	323.9 a	86.1	330.9 e	93.2	334.2 d	80.5	398.6 a	169.4
7	308.4 a	76.3	438.4 abc	78.9	436.8 abc	60.1	370.5 ab	63.7
14	342.4 a	79.4	490.6 a	88.0	493.2 a	81.4	366.2 ab	38.3
21	361.8 a	52.7	373.7 de	59.2	387.2 cd	70.9	339.0 abcd	64.7
28	340.3 a	49.1	409.9 bcd	61.6	414.5 c	36.0	354.9 abc	60.2
35	359.6 a	62.8	447.7 ab	63.6	488.9 ab	102.8	335.1 bcd	71.6
42	321.5 a	64.3	427.5 bcd	106.5	429.7 bc	43.2	326.6 bcd	63.1
49	328.7 a	88.7	414.9 bcd	67.2	428.0 bc	57.8	290.8 d	65.8
56	318.6 a	57.6	382.2 cde	82.3	428.6 bc	55.4	299.2 cd	46.6

The Tukey test compared the difference among time intervals at the 5% level of significance ($p<0.05$). Equal letters indicate mean values that are not significant different (time comparisons for the same treatment agent).



Graphic 1: Mean Knoop microhardness of enamel fragments bleached and submitted to superficial cleaning treatment at different time intervals.

In vitro effect of toothbrush on Bleached Enamel with 35% Carbamide Peroxide at Different Treatment Intervals – Microhardness Evaluation

"ALTERATIONS IN BLEACHED ENAMEL AFTER BRUSHING" (running title)

Claudia Cia Worschech [1], José Augusto Rodrigues[1], Luis Roberto Marcondes Martins[2] Gláucia Maria Bovi Ambrosano[3]

1- DDS, MS, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.
2- DDS, MS, ScD, Professor, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.
3- AGR ENG, MS, PhD, Professor, Department of Community Dentistry, School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

Address: Faculdade de Odontologia de Piracicaba - UNICAMP
Departamento de Odontologia Restauradora - Dentística
Avenida Limeira, 901 - Areião
CEP: 13141-900
Piracicaba - SP
BRAZIL
Telephone: +55-19-3412-5337
FAX: +55-19-3412-5218
E-mail: caldri@terra.com.br

I*n vitro* effect of toothbrush on Bleached Enamel with 35% Carbamide Peroxide at Different Treatment Intervals – Microhardness Evaluation

"ALTERATIONS IN BLEACHED ENAMEL AFTER BRUSHING" (running title)

Summary

The purpose of this in vitro study was to evaluate the microhardness of human enamel submitted to different superficial cleaning methods after exposition to 35% carbamide peroxide bleaching agent. Sixty fragments of human molar teeth with 4 x 4 mm were obtained using a diamond disc. The dental fragments were embedded by polystyrene resin and sequentially polished to obtain flat surfaces. The bleaching agent was applied on the enamel surface for 1 hour/week. Daily the fragments received a cleaning surface treatment G1-not brushed, G2-brushed with a fluoride dentifrice, G3-brushed with a non-fluoride dentifrice, and G4-brushed without dentifrice. Than, they were stored in individual receptacles with a remineralization solution similar to artificial saliva for the remaining time. Microhardness tests were performed with a load of 25g for 5sec at baseline and 7, 14, 21, 28 days of treatment, and 7, 14, 21 and 28 days of post treatment. **Results:** Analysis of variance and the Tukey test revealed significant differences in microhardness values over time, for enamel bleached and treated with different superficial cleaning methods. G1 and G4 showed decrease in microhardness values, G2 and G3 showed an increase in microhardness values. The 35% carbamide peroxide may reduce the superficial enamel microhardness but when associated to abrasive dentifrices the enamel microhardness may be increased.

CLINICAL RELEVANCE

Habitually, a dentifrice is used to cleaning tooth during bleaching treatment but abrasive dentifrices might change the outer superficial enamel throughout wear and loss of mineral content during bleaching treatment.

INTRODUCTION

The most common agent used for tooth bleaching is carbamide peroxide, which is water soluble and unstable. In its decomposition hydrogen peroxide and urea are generated. Home bleaching is based in the application of low concentration of carbamide peroxide for a long period of time, during 3 to 4 weeks, and in-office bleaching uses high percentages of this agent in short periods (Wille, Combe, Pesun & others 2000).

The high concentration of carbamide peroxide in bleaching agents has the advantage of decreasing the treatment time and improves compliance with patients. However, the primary indication is to patients who fell uncomfortable using the tray during the day or night time and prefer to have their teeth bleached in-office (Broome, 1998); (Mokhlis, Matis, Cochran, 2000); (Papathanasious, Bardwell, Kugel, 2001).

The effectiveness of bleaching depends upon the cause of the stain: where, how deeply, and how long the stain has permeated in the structure of the tooth: and how well the bleaching agent can permeate into the source of the discoloration and remain there long enough to release deep stains (Akal, Over, Olmez *et al* 2001).

The in-office procedures have been used alone or in combination with abrasive materials like microabrasion techniques that remove the superficial stained enamel or dentifrices removing the superficial pigments (Kugel, Perry, Hoang *et al*, 1997).

This way, abrasive dentifrice play an important role supplementing and maintaining bleaching treatment as an additional cleaning process with the removal of stained pellicle and plaque (Isaacs, Bartizek, Owens *et al*, 2000).

Patients looking for tooth bleaching treatment generally brush their teeth 3 or 4 times daily with dentifrices that promote a "whitening effect". These dentifrices usually promote whitening by addition of abrasive particles that remove stains throughout enamel wear. There is a concern that abrasive dentifrices may be responsible for excessive superficial tooth wear and other complicating effects like tooth sensibility plaque (Isaacs, 2000).

The solely exposition of bleaching agents to the tooth hard tissues can result in different superficial changes in enamel (Bitter, 1992); (Smidt, Weller, Roman *et al*, 1998); (McGuckin, Babin, Meyer, 1992); (Gultz, Kaim, Scherer *et al*, 1999); (Basting, Rodrigues, Serra, 2001).

BITTER, 1992 and SMIDT, 1998 have showed degradation and surface defects formation after bleaching agents exposition to 10% carbamide peroxide and similar alterations were also observed to high concentrations as 35% carbamide peroxide (Oltu & Gurgan, 2000).

Enamel treated with bleaching agents also exhibited a small, but significant, decrease in abrasion resistance. This behavior was associated with an alteration of the organic matrix of enamel under the chemical action of hydrogen peroxide (Seghi & Denry, 1992). Regression analysis has showed a significant correlation between microhardness and toothbrushing abrasion of eroded enamel after exposure to erosive beverages (Attin, Koidl, Buchalla *et al*, 1997). BITTER & SANDERS, 1993 showed that the enamel exposed to bleaching agents had an increase in the porosity and in surface alterations during the time of exposure to the bleaching agent.

However, no studies reported the effect of bleached enamel after cleaning procedures such as toothbrushing with abrasive dentifrices or fluoride. For maintenance of oral health, we usually brush teeth daily, and toothbrushing is generally performed with a dentifrice. The dentifrice may contain abrasives that could cause abrasion to bleached sound enamel (Neves, Castro, Coutinho, et al, 2002) the aim of this study was to investigate the effects of different cleaning surface treatments, brushed with or without a fluoride abrasive dentifrice and without a dentifrice during and soon after bleaching treatment with 35% carbamide peroxide on human dental enamel surface.

METHOD AND MATERIALS

Experimental design

The factors under study were:

1. Surface Cleaning Treatments (in four levels): Not brushed **(GROUP 1 – control group);** Brushed with fluoride abrasive dentifrice **(GROUP 2);** Brushed with abrasive dentifrice without fluoride **(GROUP 3);** and Brushed without dentifrice and immersed only in distilled deionized water **(GROUP 4).**
2. Time (in nine levels): *baseline, 7, 14, 21, 28* days of treatment and *7, 14, 21, and 28* days after the end of the treatment, corresponding at post treatment period.

The experimental units consisted of 60 sound human enamel fragments, randomly assigned to different treatment groups (fifteen enamel fragments per group). Knoop microhardness response was evaluated by quantitative methods. Three repeated measurements of Knoop microhardness were recorded on the surface of each specimen at 7- days intervals.

Enamel Fragment Preparation

Thirty freshly extracted third molars were used. Immediately after extraction, the teeth were stored in thymol 1% solution (pH=7). The crowns were removed approximately to the cement enamel junction, and the rots were discarded. The crowns were longitudinally sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP-Brazil) used at a low motor speed (Kavo do Brasil – Joinvilhe SC- Brazil), to obtain 60 enamel fragments. The gingival and oclusal third of crown were discarded, and only the middle vestibular or lingual region were used. Care was taken to leave the enamel fragments hydrated in this period. After sectioning was completed, specimens were soaked in distilled and deionized water at 37º C.

Enamel fragments presented a 4 X 4 mm dimension and the ones presenting stains or cracks were not used. The enamel fragments were embedded individually in a self-curing polyester resin in a polyvinyl chloride ring mold 2.0 cm in diameter and the external surface of the enamel was exposed.

The molds were removed, and the external surfaces of the enamel fragments were leveled with a water-cooling mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil,

01328-000). Aluminum oxide disks were used in a sequential granulation of 400, 600, and 1,000 (Carburundum / 3 M do Brazil Ltda). After that, these surfaces were polished with a felt cloth and abrasive pastes with 6, 3, 1, and ½ μm and mineral oil coolant (Top, Gold; and Ram, Arotec Ind e Com Ltda / Brazil). These procedures were conducted to form parallel surfaces for the Knoop microhardness tester.

A standardized circular area of 12,56 mm^2 of exposed enamel was created on the specimens through adhesive paper and nail varnish, by covering the remaining enamel fragment with two coatings of nail varnish (Colorama, CEIL - Brazil).

Bleaching treatment

Carbamide peroxide bleaching agent (Opalescence 35% Ultradent Co., South Jordan, UT, USA) was used. The enamel fragments were exposed to the bleaching agent for 4 times and only one hour per week, during a period of 28 days.

For the application of the treatment agents, a syringe was used to apply 0.02 ml (Freitas, Basting, Rodrigues Jr & others, 2002; McCracken & Haywood, 1996; Rodrigues, Basting, Rodrigues Jr & others, 2001) of the agent to each specimen. The specimens were kept in individual receptacles with 18.8 ml of a remineralization solution similar to artificial saliva (Table 1), at 37ºC.

After 1 hour, the bleaching agent was washed out under running distilled and deionized water for 5 seconds. After that, they received the cleaning surface treatment. During the remaining daily time, the fragments were maintained in individual receptacles with 18.8 ml of a remineralization solution similar to artificial saliva (pH=7.00) at 37ºC. The remineralization solution was changed daily. The artificial saliva consisted of a remineralization solution proposed by Featherstone, O'Really, Shariati & others, 1986; and modified by Serra & Cury, 1992.

After 28 days of bleaching treatment (4 applications), all the groups were kept in remineralization solution for more 28 days to evaluate a post bleaching microhardness, At this period the specimens did not receive more bleaching treatment, but the cleaning surface treatment were still performed.

Cleaning Surface Treatment

After bleaching treatment, the samples were submitted to the cleaning surface treatments, daily. These treatments were performed once a day for 3 min with individual nylon multi-tufted brush heads in a brushing machine under an usual brushed force (200g) with 250 cycles per minute using a reciprocal action electric motor driven tooth-brushing machine, with exception of G1.

Group 1 (control group) was not submitted to a cleaning treatment and the samples remained immersed in the remineralization solution. G4 received the cleaning treatment only with the toothbrush with specimens immersed in distilled deionized water (without dentifrice).

The groups G2 and G3 were brushed with fluoride and a non-fluoride abrasive dentifrice, respectively. The dentifrices were marketed each two days to keep neutral pH. The dentifrices contained calcium carbonates as abrasive and fluoride was added in the G2 (Table 2).

The dentifrices were freshly prepared and agitated 20 minutes before cleaning surface treatments, in slurry, with 1 part of dentifrice to 3 parts of deionized and distilled water in weight. Each fragment of G2 and G3 were brushed immersed in 20 ml of respective slurry. After brushing, the samples were removed from the machine, washed throughout distillated water and replaced in the remineralization solution.

After 28^{th} day, the bleaching treatment was finished the post bleaching period had begun. During the post-bleaching period, the surface cleaning treatment was performed daily, in the same way.

Microhardness Tester

Microhardness measurements were performed prior to the bleaching treatment to be used as control and after 7, 14, 21, 28 days after the start of bleaching treatment and at post treatment period as 7, 14, 21, 28 days (corresponding to 35, 42, 49, and 56 days after the beginning of the bleaching treatment).

A microhardness tester machine (Future Tech, FM –1e) with a Knoop indentator was used. The long axis of the diamond was kept perpendicular to the enamel surface and a

load of 25g was applied for 5 seconds. In each time, three indentations were made on each specimen. Since there were multiple test times (nine test times of three indentations each), specific sites to perform the indentations were randomly pre-established to each test before the beginning of the experiment and they had nearly 500 μm of distance from the other site.

Statistical Analysis

The average of the three indentations for time period was used as the Knoop value to time to each specimen. The Knoop hardness number (KHN), was obtained 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 involved a parametric method using repeated measures analysis of variance (ANOVA) followed by a Tukey's HSD post hoc analysis (α=0.05).

RESULTS

The Analysis of Variance and Tukey test showed no significant differences among the groups before the beginning of the treatments (baseline). However, significant differences ($P<0.05$) were verified after the start of cleaning surface treatments, the groups 1 and 4 showed a decrease in the microhardness values compared to group 2 and 3. The mean values of microhardness are showed in table 3, and the behavior of enamel microhardness may be observed in graphic 1.

The group 1, bleached and not brushed, presented a decrease of microhardness in function of time. Before treatment this group was statistically similar to the others. However, there was a statistical significant decrease in the means of microhardness after 7 days. These values were statistically similar until the 28th day of treatment. The means values of microhardness obtained from the 35th to the 56th day statistically differed from median values of the 7th and the 14th day.

An increase in the microhardness values was noticed in the enamel fragments of groups 2 and 3. The group 2, brushed with a fluoride abrasive dentifrice, the increase in the microhardness values may be observed in the 7th day, but only in the 14th day this increase was statistically significant from baseline value. Between the 14th to the 35th day the means values showed a low decrease. In the 21th day these values were statistically similar to the 7th day and in the 35th day the microhardness value was also similar to baseline value. However, from the 42th to the 56th day the values started to increase and they became similar to the 14th day and statistically higher than baseline value.

The group 3, brushed with non-fluoride abrasive dentifrice, showed a microhardness increase closer to group 2. However, this increase was statistically different from baseline value in the 21th day. Between the 21th to the 28th day there was a decrease in the microhardness mean and the value in the 28th day was statistically similar to baseline. The means values obtained between the 35th to the 56th days had an increase and they were similar to the 14th and the 21th days and differed from baseline, 7th and 28th days.

The group 4, bleached and brushed without a dentifrice showed a decrease in the microhardness values. This decrease was statistically significant from the baseline value after the 14th day. Between the 14th and the 56th days the values of microhardness were statistically similar.

DISCUSSION

Penetration of bleaching agents into the tooth hard tissues can result in different changes in vital teeth (Basting *et al*, 2001); (Bitter, 1992); (Gultz *et al*, 1999); (McGuckin *et al*, 1992); (Smidt *et al*, 1998). The increase porosity of enamel may be caused mainly by nascent oxygen released in the inner structure by hydrogen peroxide that penetrate along the enamel proteins (amelogenin and enamelin), because the mineralized, inorganic phase is much more compact than the organic one, and penetration through hydroxyapatite crystals is probably very low (Hegedüs, Bistey, Flóra-Nagy *et al*, 1999).

In the literature the influence of carbamide peroxide on enamel surface with respect to microhardness and texture is controversially discussed (Attin, Kielbassa, Schwanenberg

et al, 1997); (Hachiya, Takatsu, Hosoka *et al*, 1985); (Rodrigues *et al*, 2001); (Smidt *et al*, 1998).

MCCRACKEN & HAYWOOD, 1996 showed that teeth exposed to 10% carbamide peroxide lost calcium, although this reduction of mineral content was not significant.

Teeth bleached and not brushed showed a decrease of microhardness in function of time, where teeth had a significant decrease in hardness on the surface of the enamel after 10% carbamide peroxide exposition (Akal *et al*, 2001).

The findings obtained in this study suggest that abrasive dentifrices may alter the enamel surface submitted to bleaching treatment. The values of microhardness were increased suggesting an abrasion or erosion exposing fresh enamel with microhardness non-altered by the bleaching agent. However, more studies must be done to evaluate and to quantify the surface wear throughout toothbrushing procedure.

The use of a fluoride dentifrice or mouth-rinse after teeth exposure to bleaching agents have been indicated in order to improve the remineralization of enamel (Attin, Koidl, Buchalla *et al*, 1997); (Tames, Grando & Tames 1998). However, the increase in enamel microhardness after the use of 35% carbamide peroxide associated to fluoride dentifrice (G2) in this study may not be totally attributed to a remineralization process due to the fluoride. Dentifrice brushing can remove mineral deposits on prism structures and subsequently exposed the fresh surface of the enamel to the oral environment (Kuroiwa, Kodaka, Kuroiwa, 1993).

The G3 was statistical similar to G2 all the times studied and G3 did not have fluoride in the dentifrice. The increase in the microhardness values in groups (G2 and G3) may be associated to the calcium carbonate abrasive. The use of abrasive-containing dentifrice also causes slight abrasion with microwear in the sound enamel (Kuroiwa & others, 1993).

The outer superficial enamel altered by bleaching agent could be weak and wear may have occurred in the groups G2 and G3. On the other hand, G1 had a decrease in the microhardness values. Nevertheless, the microhardness is still high in the post-bleaching period, which may be caused solely to the abrasive dentifrices.

The outer surface of the samples in the G1 had no cleaning surface treatment and the enamel altered by bleaching agent was not supposed to be removed and may have showed low microhardness values. However, in the post-bleaching period after 35th day, the microhardness values did not decrease and they were statistically similar but did not recover the baseline microhardness values, through the exposition to remineralization solution.

The G4 was a group that showed intermediate microhardness values to G1, G2, and G3. This group showed a decrease until the 14th day, after that it maintained a constant value of microhardness. This group received as cleaning superficial treatment only with brushing. The brushing in destilled deionized water may cause an initial alteration in the polished enamel surface demonstrated by a decrease in the microhardness until 14th day. After that, the brushing procedure was supposed not able to cause more abrasion and the microhardness values were statistically similar even in the post-bleaching period.

Another factor, which may decrease the superficial erosion or remineralize the enamel, is the presence of saliva, which has a high potential for remineralization. In this study a remineralization solution similar to natural saliva in the contents of Ca and P was used. This solution was proposed by FEATHERSTONE *et al* ,1986, and modified by SERRA and CURY,1992 and presents a higher potential remineralization effect. Also, neutral pH of this remineralization solution may inhibit demineralization and possible surface change. Furthermore, a combined *in vitro-in vivo* study demonstrated a decrease in the initial microhardness of enamel, followed by an increase in enamel microhardness due to a possible remineralization phenomenon provoked by saliva (Shannon, Spencer, Gross *et al*, 1993).

According to this study, is possible to suggest that a slight abrasion by brushing with abrasive dentifrices could remove the superficial layer of enamel and promote “new” superficial enamel with high microhardness values. Although the enamel hardness is increased it may be in function of tooth wear and this effect need be more studied to verify the advantages this abrasion brings to tooth enamel.

CONCLUSION

The application of 35% carbamide peroxide may reduce the superficial enamel microhardness and after 28th of the end of the treatment the microhardness values are still altered.

Carbamide peroxide in high concentration associated with abrasive dentifrices may increase the enamel microhardness even after the end of the bleaching treatment.

ACKNOWLEDGMENTS

We are pleasure to thank the ORALTECH PRODUCTS for donation of bleaching agents used in this study and AROTEC Ind e Co Ltda / Brazil for donation the felth cloth and abrasive pastes.

REFERENCES

Akal N, Over H, Olmez A, Bodur H. (2001) Effects of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel. *Journal of Clinical Pediatric Dentistry* **25(4):** 293-296.

Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. (1997) Effect of fluoride treatment on remineralization of bleached enamel. *Journal Oral Rehabilitation* **24:** 282-286.

Attin T; Koidl, U; Buchalla, W; Shaller, HG; Kielbassa, AM; Hellwig, E. (1997) Correlation of microhardness and wear in differently eroded bovine dental enamel. *Archives Oral Biology* **42:** 243-250.

Basting RT, Rodrigues AL, Serra MC (2001) The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *In Situ*. *Operative Dentistry* **26:** 531-539.

Bitter NC & Sanders JL. *(*1993) The effect of four bleaching agents on enamel surface: A scanning electron microscopic study. *Quintessence International* **24:** 817-824.

Bitter NC (1992) A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. *Journal Prosthetic Dentistry* **67:** 852-855.

Broome JC. (1998) At-home use of 35% carbamide peroxide bleaching gel: A case report. *Compendium* **19(8):** 826-829.

Featherstone JDB, O'Really MM, Shariati M, Brugler S. (1986) Enhancement of remineralization *in vitro* and *in vivo*. In: Leach AS. *Factors relating to demineralization and remineralization of the teeth*. Oxford: IRL, 23-34.

Freitas PM, Basting RT, Rodrigues Jr AL, Serra MC. (2002) Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence International* **33:** *370-375.*

Gultz J, Kaim J, Scherer W, Gupta, H. (1999) Two in-office bleaching systems: A scanning electron microscope study. *Compendium* **20:** 965-968.

Hachiya Y, Takatsu T, Hosoka, H*;* Fusayama*, T.* (1985) A Varnish to prevent etching unrestored enamel. J*ournal of Prostethic Dentistry* **53:** 46-50.

Hegedüs C, Bistey T, Flóra-Nagy, E; Keszthlyi, G; Jenei, A. (1999) An anatomic force microscopy study on the effect of bleaching agents on enamel surface. *Journal of Dentistry* **27:** 509-515.

Isaacs RL, Bartizek RD; Owens, TS; Walters, PA; Gerlach, RW. (2000) Maintenance of tooth color after prophylaxis: comparison of three dentifrices. *Journal Clinic Dentistry* **112:** 51-55.

Kurgel, G; Perry, RD; Hoang, E; Scherer, W (1997): Effective tooth bleaching in 5 days: Using a combined in-office and at-home bleaching system. Compendium **18 (4)**: 378-383.

Kuroiwa M, Kodaka T, Kuroiwa M (1993) Microstructural changes of human enamel surfaces by brushing with and without dentifrice containing abrasive. *Caries Research* **27:** 1-8.

McCracken MS, Haywood VB. *(*1996) Demineralization effects of 10 percent carbamide peroxide. *Journal of Dentistry* **24:** 395-398.

McGuckin RS, Babin JF, Meyer BJ (1992) Alterations in human enamel surface morphology following vital bleaching. *Journal Prosthetic Dentistry,* **68:** 754-760.

Mokhlis GR, Matis BA, Cochran MA. (2000) A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. *Journal of American Dental Association* **131:** 1269-1277.

Neves AN, Castro RA, Coutinho ET, Primo LG (2002) Microstructural analysis of demineralized primary enamel after in vitro toothbrushing. *Pesquisa Odontológica Br*asileira 16(2):137-143.

Oltu Ü & Gurgan S. (2000) Effects of three concentrations of carbamide peroxide n the structre of enamel. *Journal of Oral Rehabilitation* **27**: 332-340.

Papathanasious A, Bardwell D, Kugel G. (2001) A clinical study evaluating a new chairside and take-home whitening system. *Compendium* **22:** 294-297.

Rodrigues JA, Basting RT, Rodrigues Jr AL, Serra MC *(*2001): Effects of 10% carbamide peroxide bleaching materials on enamel microhardness. *American Journal of Dentistry* **14:** 67-71.

Seghi RR & Denry, I (1992) Effects of external bleaching on indentation and abrasion characteristics of human enamel *in vitro*. *Journal of Dental Research* **71(6):** 3140-1344.

Serra MC, Cury JA. *(*1992) The *in vitro* effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessence International* **23:** 143-7.

Shannon H, Spencer P, Gross K, Tira D. (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. *Quintessence International* **24:** 39-44.

Smidt A, Weller D, Roman I, Gedalia I. (1998) Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *American Journal of Dentistry* **11:** 83-85.

Tames D, Grando J. Tames DR (1998) Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida 10%. *Revista APCD* **52:** 145-149.

Wille T, Combe EC, Pesun IJ, Giles DW. (2000) Rheological characteristics of tooth bleaching materials. *Journal of Oral Rehabilitation* **27:** 1060-1063.

TABLE 1: The artificial saliva compounds proposed by Featherstone & others (1986) and modified by Serra & Cury (1992).

ARTIFICIAL SALIVA pH=7.0	
COMPOUNDS	***Concentration***
Ca	1.5 mmol/l
KCl	50 mmol/l
PO_4	0.9 mmol/l
tri-hydroxymethil-aminomathan	20 mmol/l
distilled and deionized H_2O	qsp

TABLE 2: Ingredients used to prepare the dentifrices by PRODERMA PHARMACY (Piracicaba / Brazil)

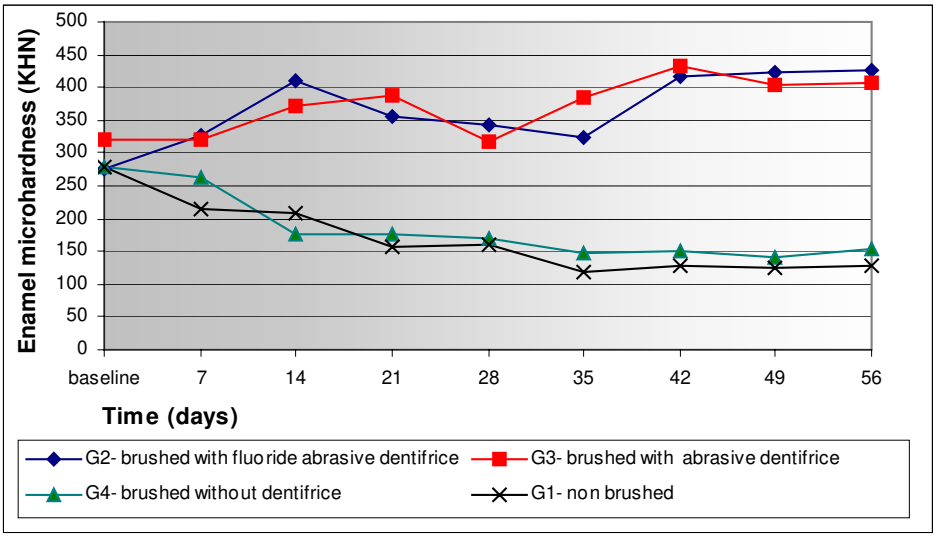
COMPOUNDS	**Toothpaste abrasive with fluoride**	**Toothpaste abrasive without fluoride**
Micronized calcium carbonate	52,5 %	52.5%
Glycerin	25 %	25%
Natrosol Gel	18%	18%
Sodium lauryl sulfate	2 %	2%
Sodium fluoride	-	0.16%
Distilled Water	qsp	qsp

Table 3. Mean Knoop microhardness values for each treatment agent at different time intervals

Period	Cleaning surface treatments							
	G1- No brushed (control)		G2- Brushed with fluoride dentifrice		G3- Brushed without non-fluoride		G4- Brushed without dentifrice	
	average	Standard deviation	average	Standard deviation	average	Standard deviation	average	Standard deviation
Baseline	279.5 A a	104.8	276.2 A d	79.7	320.3 A b	73.6	278.4 A a	107.8
7	213.6 C b	71.1	327.0 A cd	52.2	321.9 AB b	89.5	262.6 BC a	104.3
14	204.1 B b	52.7	411.8 A ab	64.9	370.9 A ab	87.0	176.8 B b	67.6
21	156.8 B bc	36.1	356.6 A bc	83.5	388.5 A a	85.9	177.7 B b	54.9
28	159.4 B bc	54.4	343.8 A c	77.6	315.9 A b	79.1	169.8 B b	48.8
35	119.1 B c	36.8	325.3 A cd	57.7	385.7 A a	71.2	145.9 B b	50.4
42	128.1 B c	47.5	417.3 A ab	57.9	433.4 A a	48.9	150.7 B b	56.2
49	124.4 B c	37.1	424.4 A a	62.8	402.4 A a	68.8	140.4 B b	47.2
56	126.9 B c	36.4	426.7 A a	40.9	405.5 A a	57.2	155.1 B b	73.4

The Tukey test compared the difference among time intervals at the 5% level of significance ($p<0.05$). Equal letters indicate mean values that are not significant different, capital letters are considered in horizontal and tiny letters are considered in vertical.

Graphic 1: Mean Knoop microhardness of enamel fragments bleached and submitted to superficial cleaning treatment at different time intervals.



EFFECT OF DENTIFRICES ON BLEACHED SURFACE ENAMEL - ROUGHNESS EVALUATION

Claudia Cia Worschech [1]; José Augusto Rodrigues[1]; Luis Roberto Marcondes Martins[2]; Luis Alexandre Maffei Sartini Paulillo[2]

1- DDS, MS, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

2- DDS, MS, ScD, Professor, Department of Restorative Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

Address: Faculdade de Odontologia de Piracicaba - UNICAMP
Departamento de Odontologia Restauradora - Dentística
Avenida Limeira, 901 - Areião
CEP: 13141-900
Piracicaba - SP
BRAZIL
Telephone: +55-19-3412-5337
FAX: +55-19-3412-5218
E-mail: caldri@terra.com.br

EFFECT OF DENTIFRICES ON BLEACHED SURFACE ENAMEL - ROUGHNESS EVALUATION

Dentifrice is used to cleaning tooth during bleaching treatment and, abrasive dentifrices might change the outer superficial enamel. The purpose of this *in vitro* study was to evaluate the roughness of human enamel exposed to 10% carbamide peroxide bleaching agent at different times and submitted to different superficial cleaning treatments, during 56 days. G1- not brushed; G2- Brushed with fluoride abrasive dentifrice; G3- brushed with a non-fluoride abrasive dentifrice; G4- brushed without a dentifrice. Sixty fragments of molar teeth with 4 x 4 mm were obtained and were individually embedded in polystyrene resin. The specimens were polished with sandpaper of decrescent grit 400, 600, and 1000 and felt cloth with 6, 3, 1 and ½ μm abrasive pastes. A perfilometer was used to obtain three measurements on the surface of each specimen and the mean of Ra value (μm) was determinated to initial and experimental roughness values. Bleaching was performed in the enamel surface for 6h daily. After that, each fragment received a cleaning surface treatment and was stored in artificial saliva for the remaining 18h. ANOVA and Tukey's HSD hoc analysis (α=0.05) revealed significant differences in roughness values over time, for enamel bleached and treated with different superficial cleaning methods**.** G1 and G4 showed no significant differences in roughness over time, G2 and G3 showed a significant increase in the surface roughness values. The solely use of 10% carbamide peroxide did not alter the enamel surface roughness but associated to abrasive dentifrices may result in significant increase of surface roughness.

INTRODUCTION

Home-applied carbamide peroxide bleaching agents are generally indicated for nighttime use during 6-8 hours. In the morning, the patients´ first precaution is to remove the tray and brush their teeth with dentifricies, which may be abrasive. During this

procedure, bleaching is washed away and some concern is expressed regarding enamel possible abrasion during toothbrushing on recent bleached enamel.

Penetration of bleaching agents into the tooth hard tissues can result in different changes in vital teeth [1, 2, 3, 4, 5]. However, the influence of carbamide peroxide containing bleaching agents on enamel surface with respect to microhardness or texture is controversially discussed. [6, 7, 8, 2] Some authors have revealed surface degradation and surface defects throughout SEM evaluation [1, 2, 3, 4].

ATTIN et al, 1997, showed that application of a fluoride varnish or mouth-rinse is recommended after exposing teeth to carbamide peroxide bleaching agents in order to improve remineralization of the enamel [6].

The use of powders, gels or pastes in oral hygiene dates back thousands of years. Dentifrices have been refined into complex formulations providing varied therapeutic and cosmetic benefits [9]. Abrasives dentifrices play an important role in the cleaning process but complicating factors include the recognition that cleaning effectiveness may not be solely related to abrasively, and concerns that some abrasives may contribute to excessive tooth wear [10, 11] .

Alterations of external bleached enamel structure after cleaning methods or toothbrushing procedure, with abrasive dentifrices with or without fluoride have not been evaluated yet. But, if teeth are daily brushed for maintenance of oral health, and brushing is generally performed with a dentifrice, which contains abrasives, this toothbrushing could cause stronger abrasion on some altered bleached sound enamel.

The lack of evidence of the effects of bleaching agents associate with tooth brushing procedure on the enamel surface and the influence of saliva to remineralize this surface determinated the issue of this study, which investigated the effects induced by brushing with abrasive dentifrice with and without fluoride after bleaching treatment, every day, on the enamel surface roughness.

METHOD AND MATERIALS

Experimental design

The factors under study were:

1 - Cleaning Surface Treatments (in four levels): Not brushed **(GROUP 1)**. Brushed with fluoride abrasive dentifrice **(GROUP 2)**. Brushed with abrasive dentifrice without fluoride **(GROUP 3)**. Brushed without dentifrice **(GROUP 4).**

2 - Time (in nine levels): baseline, 7, 14, 21, 28 days of treatment and 7, 14, 21, and 28 days after the beginning of the treatment, corresponding at post treatment period.

The experimental units consisted of 60 sound human enamel fragments, randomly assigned to different treatment groups (fifteen enamel fragments per group). Before bleaching treatments, a perfilometer (KOSAKA – Surf-Corder mod. 1700) was used to measure the initial surface roughness (baseline). Three repeated measurements were recorded on the surface of each specimen at each specimen at 7-days intervals (FIG. 1).

Enamel Fragment Preparation

Thirty freshly extracted human non-erupted third molars were used. Immediately after extraction, the teeth were stored in thymol 1% solution (pH= 7). The crowns were removed in the cement enamel junction, and the roots were discarded. The crowns were longitudinally sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP-Brazil) using a low motor speed (Kavo do Brasil – Joinvilhe SC- Brazil), to obtabin 60 enamel fragments. The gingival and oclusal third of crown were discarded, and only the middle vestibular or lingual sites were used. Care was taken to leave the enamel fragments hydrated in this period. Enamel fragments presented a 4 x 4 mm dimension and the ones presenting stains or cracks were not used. After sectioning was completed, specimens were soaked in distilled and deionized water at 37º C.

The 60 enamel fragments were embedded individually in a self-curing polyester resin in a polyvinyl chloride ring mold 2.0 cm in diameter so that the external surface of the enamel was exposed.

The molds were removed, and the external surfaces of the enamel fragments were leveled with a water-cooling mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil, 01328-000). Aluminum oxide disks were used in a sequential granulation of 400, 600, and 1,000 (Carburundum / 3 M do Brazil Ltda), and, after that they were polished with felt cloth, abrasive pastes 6, 3, 1, and ½ μm and a mineral oil coolant (Top, Gold, and Ram Arotec Ind e Com Ltda / Brazil). These procedures were conducted to form parallel surfaces for the roughness tester.

A standardized circular area 12,56 mm^2 of exposed enamel was created on the specimens using an adhesive paper by covering the remaining enamel fragment with two coatings of nail varnish (Colorama, CEIL). The enamel fragments were randomly assigned in 4 groups and the baseline values of surface roughness were obtained.

Bleaching treatment

Carbamide peroxide bleaching agent (Opalescence 10% Ultradent Co., South Jordan, UT, USA) was used. The enamel fragments were exposed to the bleaching treatment agents for six hours a day for a period of 28 days.

An individual tray, similar to the tray used by the patient during bleaching, was manufactured for each specimen using a 0.4 mm-thick flexible ethyl vinyl acetate polymer (Bio-Art Equipment) placed in a vaccum-forming machine (P7, Bio-Art Equipment).

For the application of the bleaching agent, a syringe was used to apply 0.02 ml [8, 12, 13] of each agent to each specimen. The specimens were individual closed in containers with 18.8 ml of remineralization solution similar to artificial saliva (pH 7.00) at 37ºC (Table 1). This solution consisted of a remineralization solution similar to a natural saliva in the Ca and P contents, proposed by Featherstone et al, 1986 [14] and modified by Serra and Cury in 1992 [15].

After 6 hours, the specimens were removed from the storage media, and the bleaching agent was washed out under running distilled and deionized water for 5 seconds.

During the remaining daily time (18 hours), the fragments were maintained in individual receptacles with 18.8 ml of remineralization solution at 37ºC. The remineralization solution was changed daily.

After the bleaching treatment period (28 days), when the specimens did not receive more bleaching treatment, all the groups were kept in remineralization solution for more 28 days but the cleaning surface treatment was still applied in the post-treatment period.

Cleaning Surface Treatment

Every day, after the bleaching treatment, the samples were submitted to a cleaning surface treatment according to the specifications of each group as follows: Group 1, control group, was not submitted to a cleaning treatment and the samples remained immersed in the remineralization solution after bleaching treatment.

Groups 2 and 3 received a cleaning surface treatment throughout marketed dentifrice containing carbonates calcium as abrasive with fluoride and without fluoride (Table 2) respectively. Group 4 received the cleaning treatment only with brushing in deioninezed distilled water.

Brushing procedures were performed once a day for 3 min during 28 days, at a brushing machine, under a usual brush force of 200g and 250 cycles per minute, in a freshly prepared slurry with 1 part of dentifrice to 3 parts of deionized and distilled water (50 g of toothpaste /150 g deionized and distilled every day. The brush heads were from the same brand of nylon multi-tufted toothbrushes and each product was brushed onto specimens with an individual brush heads. Sufficient slurry (20 ml) was placed in the reservoir until the specimen was completely covered.

The slurries were agitated for 20 min before they were placed inside the reservoir. The dentifrices were made each two days to keep the neutral pH. After final brushing, the samples were removed from the machine recipients, washed out by distillated water and maintained in individual receptacles at 37ºC until the next bleaching and brushing cycle.

Roughness Tester

Before bleaching treatments, a perfilometer (KOSAKA – Surf-Corder mod. 1700) was used to measure the initial surface roughness (baseline). Three measurements were recorded on the surface of each specimen, in different directions, and the mean Ra value (μm) was determinate for each specimen, at each interval of 7 days.

RESULTS

Statistical Analysis

Statistical analysis involved a parametric method using repeated measures analysis of variance (ANOVA) followed by a Tukey's HSD hoc analysis (α=0.05).

Baseline data were performed in order to verify the initial surface smoothness ($P<0.05$) and to contrast differences between the groups. The mean values of roughness are showed in table 3, and the behavior of enamel roughness may be observed in graphic 1.

At the baseline values all the groups showed similar statistical means however the groups submitted to cleaning surface treatments, the groups brushed with abrasives, (G2, G3) had statistically significant increase in the surface roughness in function of the time compared to control group (G1) and (G4) that was brushed without dentifrice. Specimen from control group (G1) stored in artificial saliva presented no increase on surface roughness mean values ($P> 0.05$) at the different time intervals.

The group 2 showed a statistically significant diferences with an increase in the surface roughness from the baseline to the 7^{th} day and to 14^{th} day. This increase was continual and the 21^{th} was statistically higher than baseline and 7^{th} day. After 21^{th} day the surface roughness was statistically similar until the end.

Group 3 showed that the surface roughness was increased but statistical diferecences may be noticed in the 14^{th} day, when roughness surface values was statistically higher than baseline value.

Similar to G1, the group 4 showed no statistical differences among the time intervals.

DISCUSSION

Patients who were submitted to tooth bleaching treatment generally are patients that brush their teeth 3 or 4 times daily. Sometimes it is recommended to use dentifrices with specific purposes like whitening or abrasion in order to improve the bleaching process. This attitude may be responsible for superficial tooth wear and other complicating factors [10] .

According some studies 10% carbamide peroxide bleaching agents alter enamel surface [16, 3, 17], as microhardess [8], or roughness surface [3]. However findings obtained in this

study show that carbamide peroxide caused no alterations on the surface enamel. Dentifrice abrasive plays an important role in this process [10] because the enamel was altered in G2 and G3 after cleaning treatments throughout abrasive dentifrices. There was an increase in those roughness values between the 14th and the 28th days and this values were kept high until the 56th day revealing high roughness values on the 28th days and after 28 days post treatment and these values were the highest and different from baseline values. However, the group 4 which was brushed with water (without dentifrice) showed no differences in roughness values similar to control group and it might suggest that abrasive dentifrice could alter the enamel surface.

The bleached enamel surfaces treated with abrasive dentifrices with or without fluoride were markedly altered in our study, it was difficult to say whether these changes were reversible or not. In clinical circumstances, the bleached surface is probably covered by acquired pellicle after a short period and this fact could protect the enamel surface

The use of rational and daily fluoride therapy, specially mouth rinsing with fluoride and neutral solutions, associated with fluoride dentifrices without abrasive can be an important method to decrease the superficial erosion caused into enamel due to use of cleaning superficial treatments after bleaching agents [18]. Another factor which may decrease the superficial erosion or to remineralize the enamel surface is saliva, which has a high potential for remineralization [19].

On the other hand, the use of abrasive-containing dentifrice causes enamel microwear [20]. This slight abrasion may alter the superficial layer of enamel and promote a "new" surface with major roughness values.

The oxidative process on enamel surface and pH of tooth-bleaching products have been considered as the main adverse effect on mineralized tissues after bleaching treatments and low concentrations of carbamide peroxide promote varying degrees of surface porosity and structural change, depending on bleaching agent [3, 16, 17, 21]. However no alteration was found in G1 and G4 that were treated with bleaching treatments, and were kept in remineralization solution.

Nevertheless, if urea and peroxides are capable of significantly attacking the enamel and weakening its structure, it is likely that tooth brushing could remove a certain amount of enamel from the surface [16] and alter superficial enamel.

CONCLUSION

This in vitro investigation showed that the solely use of 10% carbamide peroxide did not alter the enamel surface roughness but the cleaning treatments using brushing with dentifrices resulted in significant increase surface roughness.

REFERENCES

1- Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. *J Prost Dent* 1992: 67: 852-855.

2 - Smidt A, Weller D, Roman I, Gedalia, I.: Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *Am J Dent;* 1998, 11: 83-85.

3 - McGuckin RS, Babin JF& Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. *J Prosthet Dent* 1992:*,* 68:754-760.

4 - Gultz, J, Kaim, J, Scherer, W, Gupta, H: Two in-office bleaching systems: A scanning electron microscope study. *Compend* 1999; 20: 965-968.

5 - Basting, RT, Rodrigues, AL & Serra, MC: The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *In Situ*. *Oper Dent;* 2001; 26: 531-539.

6 - Attin T, Kielbassa AM, Schwanenberg M, Hellwig, E. Effect of fluoride treatment on remineralization of bleached enamel. *J Oral Rehabil* 1997: 24: 282-286.

7 - Hachiya, Y; Takatsu, T, Hosoda, H; Fusayama, T. A Varnish to prevent etching unrestored enamel. J *Prostet Dent* 1985:53: 46-50.

8 - Rodrigues JA, Basting RT, Serra, MC; Rodrigues Jr AL: Effects of 10% carbamide peroxide bleaching materials on enamel microhardness. *Am J Dent* 2001; 14: 67-71.

9 – White, D.J: Development of an improved whitening dentifrice based upon “stain specific soft silica” technology. J *of Clin Dent 2001 ,* v.12, n. 2, p. 25-29.

10 - Isaacs, RL, Bartizek RD: Owens, TS; Walters, PA; Gerlach, RW: Maintenance of tooth color after prophylaxis: comparison of three dentifrices. *J. Clinic Dent.* ; 2000; 112: 51-55.

11 – Gerlach, RW, Barker, ML, Hyde, JD,Jones, MB: Effects of a tartar control whitening dentifrice on tooth shade in a population with long-standing natural stain. *J Clin Dent;* XII, n. 2, 47-50.

12- Freitas, PM, Basting RT, Rodrigues, AL, Serra, MC.: Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence International 2002; 33: 370-375.*

13 - McCracken MS, Haywood VB.: Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996 ; 24: 395-398.

14- Featherstone JDB, O'Really MM, Shariati M, *et al*. Enhancement of remineralization *in vitro* and *in vivo*. In: Leach AS. *Factors relating to demineralization and remineralization of the teeth*. Oxford: IRL 1986; 23-34.

15 - Serra MC, Cury JA.: The *in vitro* effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quint Inter* 1992 ; 24: 39-44.

16- Hegedüs, C, Bistey, T, Flóra-Nagy, *et al.* An anatomic force microscopy study on the effect of bleaching agents on enamel surface. J *Dent* 1999: *27*: 509-515.

17 – Zalking, M, Arwaz, JR, Goldman A, Rotstein, I: *Esthet Dent Traumat*, 1996, v.12, p. 82-88.

18- Tames, D, Grando, J. & Tames, DR Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida 10%. *Revista APCD* 1998; 52: 145-149.

19 – Silverstone, LM: Remineralization Phenomena. *Caries Research* 1977; 11 (Supplement 1): 59-84.

20 - Kuroiwa, M, Kodaka, T., Kuroiwa, M: Microstructural changes of human enamel surfaces by brushing with and without dentifrice containing abrasive. *Caries Res* 1993; 27: 1-8.

21– Ernest, CP, Marroquin, BB, Willershausen- Zönnchen, B: Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel. *Quint Inter* 1996; v.27, n. 1, p.53-56.

TABLE 1: The artificial saliva compounds proposed by Featherstone et al, and modified by Serra and Cury.

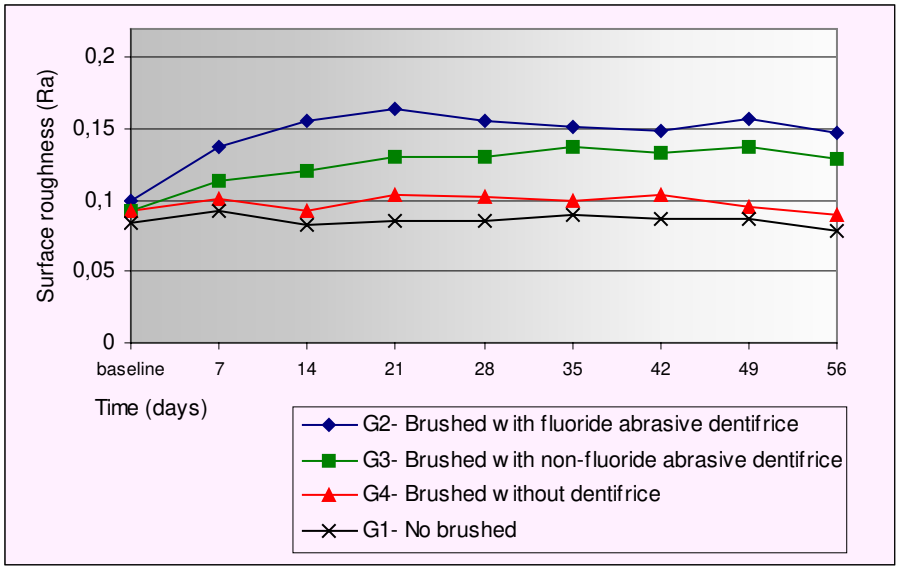
ARTIFICIAL SALIVA pH=7.0	
COMPOUNDS	***Concentration***
Ca	1.5 mmol/l
KCl	50 mmol/l
PO_4	0.9 mmol/l
tri-hydroxymethil-aminomathan	20 mmol/l
distilled and deionized H_2O	qsp

TABLE 2: Ingredients used to prepare the dentifrices by PRODERMA PHARMACY (Piracicaba / Brazil)

COMPOUNDS	**Toothpaste abrasive with fluoride**	**Toothpaste abrasive without fluoride**
Micronized calcium carbonate	52,5 %	52.5%
Glycerin	25 %	25%
Natrosol Gel	18%	18%
Sodium lauryl sulfate	2 %	2%
Sodium fluoride	-	0.16%
Distilled Water	qsp	qsp

Table 3. Surface roughness (Ra) values for each treatment at different time intervals

Period	Groups							
	No Brushed (control)		Brushing with fluoride		Brushing without fluoride		Brushing without toothpaste	
	means	Standard deviation	means	Standard deviation	means	Standard deviation	means	Standard deviation
Baseline	0.084 A a	0.026	0.099 A c	0.063	0.092 A b	0.041	0.092 A a	0.031
7	0.093 A a	0.026	0.138 A b	0.076	0.113 A ab	0.032	0.101 A a	0.028
14	0.082 B a	0.022	0.156 A ab	0.092	0.120 AB a	0.032	0.093 B a	0.030
21	0.085 B a	0.021	0.164 A a	0.104	0.131 AB a	0.035	0.103 B a	0.026
28	0.085 B a	0.021	0.155 A ab	0.099	0.130 AB a	0.033	0.102 B a	0.027
35	0.089 C a	0.024	0.152 A ab	0.085	0.138 AB a	0.044	0.100 BC a	0.025
42	0.087 B a	0.019	0.149 A ab	0.095	0.133 AB a	0.043	0.103 AB a	0.021
49	0.087 C a	0.024	0.157 A ab	0.089	0.137 AB a	0.043	0.095 BC a	0.017
56	0.079 C a	0.022	0.147 A ab	0.088	0.129 AB a	0.043	0.090 BC a	0.017



The Tukey test compared the difference among time intervals at the 5% level of significance ($p<0,05$). Equal letters indicate mean values that are not significant different, capital letters are considered in horizontal and tiny letters are considered in vertical.

Graphic 1: Mean roughness of enamel fragments bleached and submitted to superficial cleaning treatment at different time intervals.

In vitro evaluation of human dental enamel surface roughness bleached with 35% carbamide peroxide and submitted to abrasive dentifrice brushing

Avaliação *in vitro* da rugosidade superficial do esmalte dental humano clareado com peróxido de carbamida a 35% e submetido a escovação com dentifrícios abrasivos

Pesqui Odontol Bras 2003; 17(4): 342-8

Claudia Cia Worschech*

José Augusto Rodrigues*

Luis Roberto Marcondes Martins**

Gláucia Maria Bovi Ambrosano***

* DDS, MS, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

**DDS, MS, ScD, Professor, Department of Restorative Dentistry, Dentistry School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

***AGR ENG, MS, PhD, Professor, Department of community dentistry, School of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil.

ABSTRACT The aim of this *in vitro* study was to evaluate the surface roughness of human enamel bleached with 35% carbamide peroxide at different times and submitted to different superficial cleaning treatments G1- not brushed; G2- brushed with fluoride abrasive dentifrice; G3- brushed with a non-fluoride abrasive dentifrice; G4- brushed without a dentifrice. Sixty fragments of human molar teeth with 4x4mm were obtained using a diamond disc. The specimens were polished with sandpaper and abrasive pastes. A perfilometer was used to measure Ra values of the initial surface roughness and at each 7-day-intervals after the beginning of treatment. The bleaching was performed in the surface of fragments for 1 hour a week and the cleaning surface treatment for 3 minutes daily. The samples were stored in individual receptacles with artificial saliva. Analysis of variance and Tukey test revealed significant differences in surface roughness values to G2 and G3, that showed an increase in roughness over time, G1 and G4 showed no significant roughness differences. The bleaching with 35% carbamide peroxide did not alter the enamel surface roughness, but when the bleaching treatment was performed associated to brushing with abrasive dentifrices, there was a significant increase in roughness values.

Descriptors:
tooth bleaching; peroxide; dentifrice, toothbrushing

Resumo: O propósito deste estudo *in vitro* foi avaliar, em diferentes tempos, a rugosidade superficial do esmalte dental humano clareado com peróxido de carbamida a 35% e submetido a diferentes tratamentos superficiais de limpeza: G1- não escovado; G2- escovado com dentifrício fluoretado abrasivo; G3- escovado com dentifrício não-fluoretado abrasivo; G4- escovado sem dentifrício. Sessenta fragmentos de molares humanos com 4x4mm foram obtidos através do seccionamento com discos diamantados. Os espécimes foram polidos com lixas e pastas abrasivas. Um perfilômetro foi utilizado para determinar os valores de Ra ("roughness average") iniciais e, a cada intervalo de 7 dias, após o início do tratamento clareador. O clareamento foi realizado na superfície dos fragmentos por 1 hora, semanalmente e, os tratamentos superficiais, por 3 minutos diariamente. No restante

do tempo, os espécimes eram armazenados em receptáculos individuais com saliva artificial. A análise de variância e o teste tukey revelaram diferenças estatísticas significantes na rugosidade superficial em função do tempo, G2 e G3 demonstraram um aumento nos valores de rugosidade, G1 e G4 não apresentaram diferenças estatísticas. O clareamento com peróxido de carbamida a 35% não alterou a rugosidade superficial do esmalte humano, mas, quando associado ao tratamento superficial com abrasivos proporcionou um aumento significante da rugosidade superficial.
descritores: clareamento de dente, peróxidos; dentifrícios; escovação dentária

INTRODUCTION

Bleaching procedures have gained popularity with patients and dentists as conservative techniques to lighten natural teeth in order to improve the harmony of the smile. In- office vital bleaching has been used to introduce the patient to the bleaching process, to improve post bleaching cases or to function solely as a bleaching treatment[8]. This technique uses carbamide peroxide or hydrogen peroxide in concentrations higher than those used for at-home bleaching, and is carried out with rubber dam for 1 to 2 hours[8].

However, the exposure of tooth hard tissues to bleaching agents can result in microstructural changes in the enamel surface[3,8,13,18]. Some authors, throughout scanning electron microscopy (SEM) evaluation, have demonstrated demineralization, surface defects, and degradation of the sound enamel[3,8,13,18]. Fluoride therapy is strongly indicated to avoid this side effects[1] and one of the most useful method of fluoride application is the use of dentifrices.

On the other hand, abrasives dentifrices play an important role in the cleaning process by removing extrinsic stains and patients commonly use them during the bleaching treatment. Some complicating factors may be explained by acknowledging that cleaning effectiveness may not be solely related to abrasion, and there is concern that some abrasives may contribute to excessive tooth wear[7,11].

The alteration of the external enamel structure after bleaching treatment may be worst after cleaning with abrasive dentifrices as they might remove the degraded enamel an enhance the phenomena of erosion and wear.

However, to maintain oral health, teeth need to be daily brushed and there is a lack of evidences on the effects of bleaching agents combined with tooth brushing on the enamel surface as well as on its influence on enamel roughness. The goal of this study was to investigate the *in vitro* effects induced by in-office bleaching regimen combined with a brushing treatment with abrasive dentifrices with and without fluoride on the enamel surface roughness.

METHOD AND MATERIALS

Experimental design

The factors under study were *Cleaning Surface Treatment* in four levels G1- not brushed; G2- brushed with fluoride abrasive dentifrice; G3- brushed with abrasive dentifrice without fluoride; G4- brushed without dentifrice; and *Time* in nine levels: baseline, 7, 14, 21, 28 days of bleaching treatment and 7, 14, 21, and 28 days after the end of bleaching (post treatment period).

The experimental units were 60 sound human enamel fragments, randomly assigned into treatment groups (n=15). Six repeated measurements of surface roughness in roughness average (Ra) values were recorded on the surface of every specimen at each 7-days interval (figure 1).

Enamel Fragment Preparation

Since this study were designed to use human teeth, it was submitted to the Ethical Committee in Research at Piracicaba Dentistry School, State University of Campinas (UNICAMP) and it was approved in compliance with resolution CNS# 196/96 of the National Committee of Health/Health Department (Brazil).

Thirty freshly extracted human non-erupted third molars were used. Immediately after extraction, the teeth were stored in 1% thymol solution (pH=7). The roots were discarded and the crowns were longitudinally sectioned with double-faced diamond disks

(KG Sorensen, Barueri, SP-Brazil) using a low-speed handpiece (Kavo do Brasil, Joinville SC-Brazil), to produce 60 enamel fragments with 4x4mm from the middle buccal or lingual aspect. The fragments presenting stains or cracks were not used. After completing the sectioning, the specimens were soaked in distilled and deionized water at 37ºC.

The enamel fragments were embedded individually in a self-curing polyestyrene resin (BL 41110, Cromex, São Paulo, SP-Brazil) in a polyvinyl chloride ring mold with 2.0cm in diameter allowing the external surface of the exposed enamel.

The molds were removed, and in order to shape a plane enamel surface for roughness testing, the fragments' surface were leveled with a water-cooling mechanical grinder (Maxgrind/Solotest, São Paulo, SP, Brazil). Aluminum oxide disks were used in a sequential granulation of 400, 600, and 1,000 (Carburundum, 3M do Brazil Ltda), and, after that, they were polished with felt cloth and abrasive pastes 6, 3, 1, and ½ μm with a mineral oil coolant (Top, Gold, and Ram Arotec Ind e Com Ltda / Brazil).

On the specimens, a $13mm^2$ circular area of enamel was standardized for treatment with the bleaching agent. Circular adhesive papers were positioned on the enamel surface and the specimens were covered with two coats of nail varnish (Colorama, CEIL - Brazil), and after its cure, the adhesive papers were removed. The specimens were randomly assigned in 4 groups and the baseline values of surface roughness were obtained.

Bleaching treatment

A 35% carbamide peroxide bleaching agent (Opalescence Quik, Ultradent Co., South Jordan, UT, USA) was selected. The enamel fragments were exposed to the bleaching agent for one hour a week, during a 28 days period.

For the bleaching agent treatment, a graduated syringe was used to apply 0.02ml of bleaching agent on the enamel of each specimen[6,0,15] at 37ºC.

After 1 hour of exposure, the bleaching agent was washed out under running distilled and deionized water for 5 seconds. Then the specimens received the surface cleaning treatment and were placed in a freshly prepared remineralization solution that was changed daily. The remineralization solution was similar to natural saliva in terms of Ca and P contents, as proposed by Featherstone *et al*, 1986[5] and modified by Serra and Cury in

1992[16] composed by 1.5mmol/L of Ca, 50 mmol/L of KCl, 0.9 mmol/L of PO_4, and 20 mmol/L of tri-hydroxymethil-aminomathan at pH=7.0.

After 28 days, in the end of the bleaching treatment (4 applications), all groups were kept in a daily changed remineralization solution over 28 days still receiving the cleaning treatments.

Cleaning Surface Treatment

Everyday, after bleaching treatment, the specimens were submitted to the surface cleaning treatment according to each group's specifications, as follows: Group 1, control group, was not submitted to a cleaning treatment; Groups 2 and 3 received a surface cleaning treatment with a commercial dentifrice containing calcium carbonates as abrasives respectively with fluoride and without fluoride, respectively (Table 1); Group 4, received the brushing treatment without dentifrice.

Brushing procedures were performed for 3 minutes in a brushing machine, set at a usual brush force of 200g and 250 cycles per minute. The brush heads were from the same brand of nylon multi-tufted toothbrushes and each product was brushed onto specimens with an individual brush heads.

The dentifrices were freshly prepared in slurries (1 part of dentifrice to 3 parts of deionized and distilled water in weight). The slurries were agitated for 20 minutes before its use.

At the end of the cleaning surface treatment, the samples were removed from the machine's recipients, washed out with distillated water and maintained in individual receptacles at 37ºC until the next brushing cycle.

Surface Roughness Test

Before the bleaching treatments, a perfilometer (Surf-Corder mod. 1700, Kosaka, Tokio, Japan) was used to measure the initial surface roughness (baseline). Three different directions were used to perform six measurements on the surface of each specimen, with a cut off (λc) of 0.25mm in a velocity of 0,1mm/s (ISO 4228). The mean Ra value (μm) was determinate for each specimen, at each 7-days intervals.

RESULTS

Statistical analysis involved a parametric method using repeated measures analysis of variance (ANOVA) followed by a Tukey's HSD hoc analysis (α=0.05). The mean values of roughness are showed in table 2, and the behavior of enamel roughness may be observed in graphic 1.

Baseline data were performed in order to verify the initial surface smoothness ($P<0.05$) and to contrast differences between the groups. At the baseline values and the 7th day all the groups showed statistically similar means. The groups submitted to cleaning surface treatments presented a statistically significant increase in the surface roughness with time, as compared to control group (G1) that showed statistically similar means of surface roughness at different time intervals.

The Group 2 showed a statistically significant increase in the surface roughness from the baseline to the 14th day and from 14th day to 21st day. After the 21st day the values were statistically similar until the end of treatment with exception to 42nd day that showed the highest median value.

The mean values of roughness in Group 3 showed statistical increase from baseline to the 7th and 14th days to the 21st day, which were not different from each other. At the 21th day the roughness mean values increased and were tatisticaly different from the baseline, 7th and 14th day values. At the 28 st day roughness presented an increase until the 42 st day, wich diffred statistically from that of the 21 st day, and the roughness values showed a decrease but the values did not differ statistically from the 28 st day until the 56 st day.

The Group 4 showed statistically similar roughness values until the 28th day. The 35th day showed the highest roughness median value of this group which statistically differed those of the baseline, 7th, and 14th days. After the 35 st day these values decreased and the value on 42nd day statistically differed from those of the baseline and 7th day. The 49th and 56th days did not differ from all the values and also from the baseline values.

DISCUSSION

Patients who submit themselves to a tooth bleaching treatment generally are patients that brush their teeth 3 or 4 times a day to achieve health and beauty. Dentifrice are sometimes recommended for specific purposes, like cleaning or abrasion, in order to improve the bleaching process by removing superficial stains and polishing teeth. The most common abrasives these days are hydrated silica, dicalcium phosphate dihydrate, and calcium carbonate.[21] However, their abrasiveness may be responsible for superficial tooth wear and other complicating factors[11] and abrasion may be more severe when associated to bleaching treatment regimens.

Several studies have evaluated the effects of bleaching agents on tooth structure mainly using scanning electron microscopy. The use of 10% carbamide peroxide have produced slight surface modifications and the use of high concentrate agents indicated to in-office treatment has caused more severe alterations on the enamel microstructure[10,13,24]. The microhardness *in vitro* studies have demonstrated contradictory results, presenting mineral loss with the extended use of low pH agents, or increase in the mineral content due to demineralization and remineralization phenomena caused by the use of remineralization solutions and agents with neutral pH[1,15].

Porfilometric analyses are also conflicting. Titley *et al.* (1988)[20] found an apparent increase in surface porosities on enamel after the use of a 35% solution of hydrogen peroxide, McGuckin *et al.* (1992)[13] observed a slight increase in surface roughness after the use of 30% hydrogen peroxide, whereas, Wandera *et al.* (1994)[22] and Gürgan *et al.* (1997)[9] reported no surface alterations after bleaching with at-home agents as observed in the current study in G1.

However, the protocol employed in this study tried to be similar to an in-office-bleaching treatment performed in the oral cavity with the bleaching agent application followed by brushing and immersion in a solution similar to artificial saliva. The use of artificial saliva may decrease the superficial erosion and it might favor the remineralization of the enamel surface[17], the possible reason to observed results in G1. Nevertheless, the sole use of 35% carbamide peroxide for 1 hour weekly did not cause surface roughness alterations.

The aim of this study was to evaluate *in vitro* the effect of in-office bleaching associated to abrasive dentifrices brushing. The G1 may be considered a control group because it was not brushed and did not show alterations in enamel roughness during the experimental treatment times. The G4 was a second control group whose was not brushed with abrasive dentifrices and did not differ statistically from G1 demonstrating that had no effect on the bleached enamel surface roughness. However, the roughness tests were performed in the samples before and during experimental times, so that each specimen in a group could be considered as its own control. The G4 had a non-statistical increase from baseline to 28th day, which was statistically significant in the 35th day, suggesting a slight effect by the brushing during bleaching treatment; however, such effect was not significant in the 49th and 56th days.

The enamel surface roughness was increased after the surface cleaning treatment with abrasive dentifrices in G2 and G3. Although the roughness of these groups had been similar during all experiment time, the increase occurred from the 14th day for G2 and from the21st day for G3, up to the 42nd day, after that day the roughness kept high until the 56th day revealing higher roughness values than the baseline ones, G1, and G4.

Our results indicated that the fluoride presented in the G2 was not able to prevent the increase in the surface roughness and it may be supposed that some erosion and loss of enamel had occurred. Attin *et al.* (1997)[1] have shown that the application of fluoride solution cannot prevent, but it may reduce the loss of mineral from enamel during at-home bleaching treatment with 10% carbamide peroxide. According to Neves *et al*. (2002)[14] the appearances of enamel surfaces brushed with either a fluoridated or non-fluoridated dentifrice were quite similar. The same study showed that the control group, brushed with toothbrush and water showed a smooth surface caused by the tooth brushing treatment.

Nevertheless, the G4 roughness values were statistically similar to G1 (control group) all the studied times, G4 presented a surface roughness that ranked between the groups brushed with abrasive dentifrices (G2 and G3) and the control group (G1) strengthening the possible erosive effect from the brushing without the dentifrices.

On other hand, this study evaluated only one surface roughness parameter, the Ra. This parameter describes the overall roughness of a surface and it can be defined as the

arithmetical average value of all absolute distances of the roughness profile from the centerline within the measuring length. Although the Ra parameter is the most used parameter to evaluate roughness, other parameter may be used as a complementary data to obtain more information about the profile shape[23].

This study has been one of the first to evaluate the effects of abrasive dentifrice on enamel roughness after in-office bleaching and adverse effects were observed. Further research using others roughness parameters and in vitro and in vivo models are necessary to observe these adverse effects on the enamel surface.

The oxidative process involved in the main source of adverse effects on mineralized tissues during bleaching treatment[15]. Low concentrations of carbamide peroxide promote varying degrees of surface porosity and structural change, depending on the bleaching treatments[4,10,13,24]. The use of abrasive-containing dentifrice might result in enamel microwear[14,21]. The slight abrasion in this enamel may remove the superficial degraded layer and promote a "new" surface, even though, with high roughness values[14].

The bleached enamel surfaces treated with abrasive dentifrices with or without fluoride were markedly altered in our study. In spite of these results, the use of rational and daily fluoride therapy, specially mouth rinsing with fluoride and neutral solutions, associated with fluoride dentifrices without abrasives is an important method to prevent a possible erosion caused into enamel due to use of cleaning superficial treatments after bleaching agents[19].

CONCLUSION

This *in vitro* investigation showed that 35% carbamide peroxide did not alter the enamel surface roughness but, when the bleaching treatment was performed with cleaning treatments, through brushing with abrasive dentifrices, a significant increase in roughness values was observed.

REFERENCES

1. Attin T, Kielbassa AM, Schwanenberg M, Hellwig E Effect of fluoride treatment on remineralization of bleached enamel. J Oral Rehab. 1997; 24: 282-286.
2. Basting RT, Rodrigues AL, Serra MC: The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin In Situ. Oper Dent. 2001; 26: 531-539.
3. Bitter NC A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. J Prost Dent. 1992; 67: 852-855
4. Ernest CP, Marroquin BB, Willershausen-Zönnchen B Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel. Quintessence Int. 1996; 27(1): 53-56.
5. Featherstone JDB, O'Really MM, Shariati M, Brugler S. Enhancement of remineralization in vitro and in vivo. In: LEACH SA Factors relating to demineralization and remineralization of the teeth. Oxford, IRL, 1986.
6. Freitas PM, Basting RT, Rodrigues AL, Serra MC Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. Quintessence Int. 2002; 33: 370-375.
7. Gerlach RW, Barker ML, Hyde JD, Jones MB: Effects of a tartar control whitening dentifrice on tooth shade in a population with long-standing natural stain. J Clin Dent. 2001; 12(2): 47-50.
8. Gultz J, Kaim J, Scherer W, Gupta H. Two in-office bleaching systems: A scanning electron microscope study. Compendium 1999; 20(10): 965-969.
9. Gürgan S, Bolay S, Alacan R. Adherence of bacteria to bleached or unbleached enamel surfaces. J Oral Rehab. 1997; 24:624.
10. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An anatomic force microscopy study on the effect of bleaching agents on enamel surface. J Dent. 1999; 27(7): 509-515.
11. Isaacs RL, Bartizek RD, Owens TS, Walters PA, Gerlach RW. Maintenance of tooth color after prophylaxis: comparison of three dentifrices. J. Clinic Dent. 2001; 12: 51-55.

12. McCracken MS, Haywood VB Demineralization effects of 10 percent carbamide peroxide. J Dent. 1996; 24: 395-398.

13. McGuckin RS, Babin JF, Meyer BJ Alterations in human enamel surface morphology following vital bleaching. J Prosthet Dent. 1992; 68: 754-760.

14. Neves NA, Castro RA, Coutinho ET, Primo LG Microstructural analysis of demineralized primary enamel after in vitro tooth brushing. POB 2002; 16(2): 137-143.

15. Rodrigues JA, Basting RT, Rodrigues Jr AL, Serra MC. Effects of 10% carbamide peroxide bleaching materials on enamel microhardness. Am J Dent. 2001; 14: 67-71.

16. Serra MC, Cury JA The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quintessence Int. 1992; 24: 39-44.

17. Silverstone, LM: Remineralization Phenomena. Caries Res. 1997; 11 (supp1): 59-84.

18. Smidt A, Weller D, Roman I: Effect of bleaching agents on microhardness and surface morphology of tooth enamel. Am J Dent. 1998; 11: 83-85.

19. Tames D, Grando J, Tames DR. Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida 10%. Revista APCD. 1998; 52: 145-149.

20. Titley K, Torneck CD, Smith D. The effect of concentrated hydrogen peroxide solutions on the surface morphology of human tooth enamel. J Endod, 1988; 14(2):69-74.

21. Viscio D, Gaffar A, Fakhry-Smity S, Xu T. Present and future technologies of toot of tooth whitening. Compendium. 2000; 21(28): s36-s46.

22. Wandera A, Feigal RJ, Douglas WH, Pintado MR. Home-use tooth bleaching agents: An *in vitro* study on quantitative effects on enamel, dentin and cementum. Quintessence Int, 1994; 25(8):541-6.

23. Whitehead SA, Shearer AC, Watts DC, Wilson NHF. Comparison of methods for measuring surface roughness of ceramic. J Oral Rehabil. 1995; 22: 421-427.

24. Zalking, M; Arwaz, JR; Goldman A; Rotstein, I: Surface morphology changes in human enamel, dentin and cementum following bleaching: a scanning electron microscopy study. Endod Dent Traumatol. 1996; 12(2): 82-8.

Figure 1- Experimental design of the study

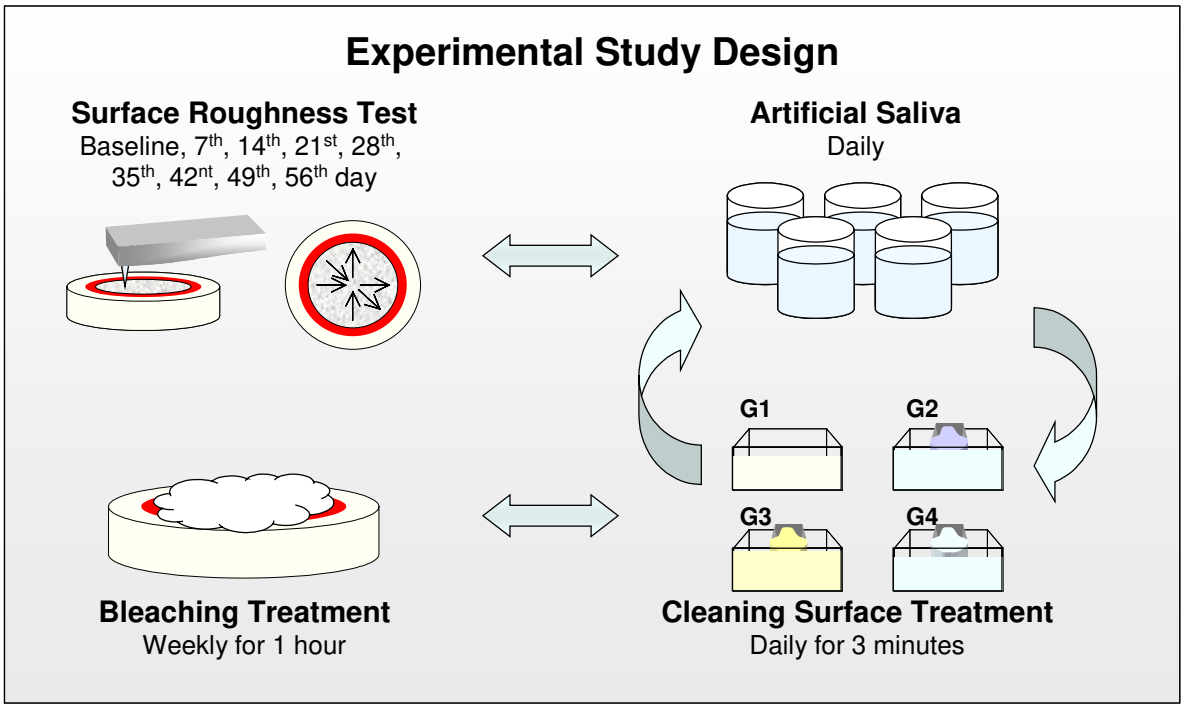


Table 1: Ingredients used to prepare the dentifrices by Proderma Pharmacy

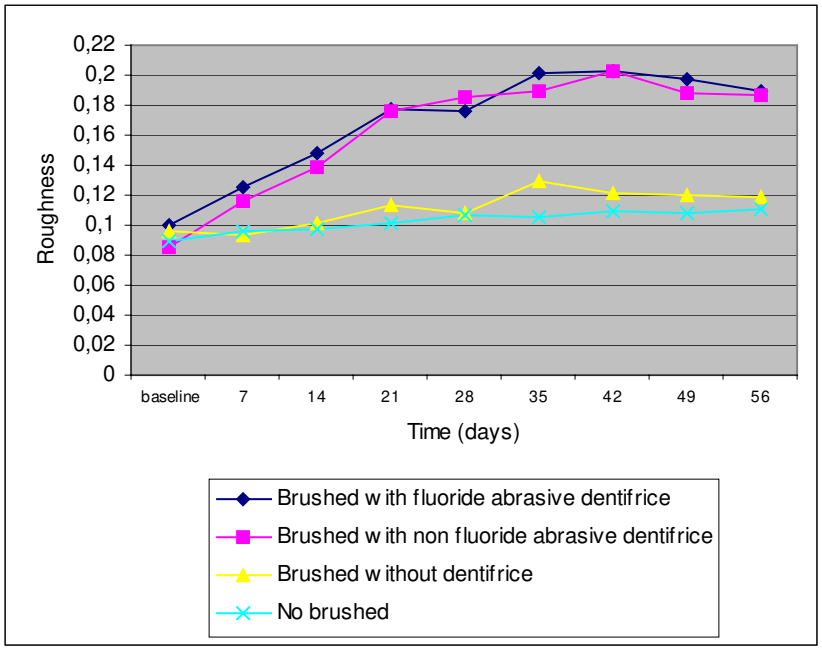
COMPOUNDS	Abrasive dentifrice with fluoride (G2)	Abrasive dentifrice without fluoride (G3)
Micronized calcium carbonate	52.5 %	52.5%
Glycerin	25 %	25%
Natrosol Gel	18%	18%
Sodium lauryl sulfate	2 %	2%
Sodium fluoride	0.16%	-
Distilled Water	qsp	qsp

Table 2. Means of surface roughness (Ra) and standard deviation (SD) values for each treatment agent at different time intervals

Period	Groups							
	G1-Non Brushed		G2-Brush with fluoride abrasive dentifrice		G3-Brush with abrasive dentifrice		G4-Brushing without dentifrice	
	means	SD	means	SD	means	SD	means	SD
Baseline	0.089 A a	0.033	0.100 A d	0.031	0.085 A d	0.031	0.096 A c	0.027
7	0.096 A a	0.037	0.125 A cd	0.030	0.116 A c	0.031	0.094 A c	0.029
14	0.098 B a	0.035	0.148 A c	0.045	0.138 AB c	0.052	0.101 AB bc	0.021
21	0.101 B a	0.037	0.177 A b	0.053	0.176 A b	0.063	0.114 B abc	0.032
28	0.107 B a	0.038	0.176 A b	0.064	0.185 A ab	0.082	0.108 B abc	0.033
35	0.105 B a	0.033	0.201 A ab	0.074	0.190 A ab	0.074	0.129 B a	0.039
42	0.109 B a	0.040	0.203 A a	0.067	0.203 A a	0.087	0.122 B ab	0.031
49	0.108 B a	0.044	0.198 A ab	0.053	0.188 A ab	0.081	0.120 B abc	0.044
56	0.111 B a	0.051	0.190 A ab	0.054	0.187 A ab	0.067	0.119 B abc	0.041

The Tukey test compared the difference among time intervals at the 5% level of significance ($p<0,05$). Equal letters indicate mean values that are not significant different, capital letters are considered in horizontal and tiny letters are considered in vertical.

Graphic 1: Mean roughness of enamel fragments bleached and submitted to superficial cleaning treatment at different time intervals



CONSIDERAÇÕES GERAIS

Por oferecer um caminho atrativo para otimizar a aparência dos dentes, de forma simplificada, preservando tecido dental sadio e, muitas vezes, sem a necessidade de tratamento restaurador adicional (Heymann, 1997)**,** o clareamento dental tornou-se um dos procedimentos mais indicados e comuns (Gürgan et al., 1997; Papathanasious et al., 2001; Oltu & Gurgan, 2000).

A despeito das alterações estruturais que podem ocorrer na camada superficial do esmalte, devido ao tratamento, a efetividade do clareamento depende da causa do escurecimento, da localização, profundidade e do tipo de estrutura dental acometida por ele (Akal *et al.,* 2001).

Assim, dentifrícios abrasivos têm um importante papel no processo de prevenção dessas manchas (Isaacs *et al.,* 2000; Gerlach *et al.*, 2001) e, dependendo do pH dos mesmos, pode ainda haver deposição de quantias consideráveis de CaF_2 sobre o esmalte (Akal *et al.,* 2001). Esse efeito pode inibir a desmineralização e a alteração da camada superficial do esmalte em um momento de desafio ácido e é possível que a escovação com dentifrícios remova esses depósitos minerais, expondo uma nova superfície ou camada de esmalte, no meio ambiente bucal (Kuroiwa *et al.*, 1993).

A superfície do esmalte clareado, submetida ao tratamento com dentifrícios abrasivos, com ou sem flúor, apresentou alterações significativas neste estudo, tanto em relação à dureza quanto em relação à rugosidade e é difícil afirmar se essas alterações são reversíveis, ou não.

O uso de dentifrícios ou enxaguatórios, após a exposição dos dentes aos agentes clareadores, é indicado como método de se facilitar a remineralização do esmalte alterado (Tames *et al.*, 1998), uma vez que o uso regular do flúor, especialmente sob a forma de enxaguatórios neutros ou associados a dentifrícios sem abrasivos, pode constituir um método importante para diminuir a erosão superficial do esmalte, após a exposição aos agentes clareadores (Tames *et al.*, 1998). Outro fator que favorece a remineralização do esmalte e reduz a erosão superficial é a presença de saliva, a qual apresenta um alto potencial para a remineralização (Featherstone *et al.,* 1986).

Neste estudo, foi utilizado, como saliva artificial, uma solução remineralizante similar à saliva natural. Essa solução foi proposta por Featherstone *et al.,* em 1986 e modificada por Serra & Cury, em 1992, e os dentifrícios abrasivos utilizados continham carbonato de cálcio e fluoreto de sódio que, embora possam reagir entre si, permitem a presença de flúor livre, capaz de remineralizar o esmalte (Eggert & Neubert, 1999). Essa composição foi escolhida por ser a única que testamos que mantinha um pH neutro e estável por um período de tempo. A manipulação dos dentifrícios nos permitiu a obtenção de fórmulas semelhantes em que o único ingrediente não comum era o fluoreto de sódio e isso proporcionou uma análise mais precisa da ação do flúor sobre o esmalte dental.

Verificou-se que a microdureza do esmalte no grupo controle, submetido ao clareamento com peróxido de carbamida a 10% e imerso na solução de saliva, não foi alterada e, mesmo ao longo de 28 dias de tratamento, não mostrou efeitos adversos.

Esse resultado concorda com Rodrigues *et al.*, em 2001, em que o mesmo tipo de agente clareador foi aplicado no esmalte dental humano por 28 dias e com Lopes *et al.*, em 2002, que não notaram efeitos adversos, tanto na dureza como na morfologia do esmalte, quando dois agentes clareadores foram utilizados (Lopes *et al.*, 2002).

Entretanto, um aumento nos valores de microdureza, após utilização de peróxido de carbamida a 10 ou a 35% foi observado nos grupos que receberam escovação com dentifrícios abrasivos com e sem flúor, assim, esse aumento nos valores de microdureza, pode não estar associado, necessariamente, ao processo de remineralização pelo flúor, pois o comportamento do grupo escovado sem flúor (G3) foi estatisticamente similar ao comportamento do grupo escovado com flúor (G2), em todos os tempos estudados.

O aumento nos valores de microdureza, nestes grupos (G2 abrasivo com flúor e G3, abrasivo sem flúor), pode estar associado ao abrasivo carbonato de cálcio utilizado, que pode ter causado discreta abrasão ou desgaste no esmalte superficial (Kuroiwa *et al.*, 1993) sugerindo que a camada mais superficial, alterada pelo agente clareador, poderia apresentar-se desorganizada, permitindo maior desgaste superficial, nos grupos G2 e G3.

Talvez, dentifrícios abrasivos tenham mesmo um papel importante nesse processo de desgaste (Isaacs *et al.,* 2000), uma vez que houve alteração na rugosidade

superficial do esmalte, após o tratamento clareador com peróxido de carbamida a 35%, seguido do tratamento de limpeza superficial, com dentifrício abrasivo fluoretado ou não fluoretado, em G2 e G3 respectivamente. Houve um aumento nos valores de rugosidade entre 14^{o} e o 28^{o} dias e esses valores mantiveram-se elevados até o 56^{o} dia. Entretanto, o G1 (grupo controle) não mostrou diferenças nos valores de rugosidade superficial, quando peróxido de carbamida foi utilizado a 10% ou a 35%; isso sugere que, referindo-se à alteração da lisura superficial do esmalte, a associação da escovação com o dentifrício abrasivo e gel clareador alterou a superfície e não o peróxido de carbamida utilizado de forma isolada.

Quando avaliamos os grupos controle (G1) e os resultados de microdureza, notamos haver diminuição nesses valores, quando utilizamos CP 35% que, embora apresente pH 6,5, pode não ter mantido esse valor de pH inicial ao longo do experimento, em função da dissociação dos seus componentes em contato com a saliva artificial, que, de certa forma, pode não ter possibilitado uma dissociação completa do peróxido de carbamida, uma vez que o mesmo quebra-se em peróxido de uréia e peróxido de hidrogênio e, para que o peróxido de uréia se dissocie em CO2 e amônia, que elevaria o pH da placa, deveria haver , além da água da saliva, enzimas como a peroxidase, responsáveis pela dissociação de peróxido de uréia. Assim, neste estudo, a saliva artificial, que contém somente eletrólitos e não contém enzimas pode não ter sido capaz de dissociar completamente o peróxido de uréia e, então, não ter proporcionado a elevação do pH da placa, ao longo do experimento, conforme comprovou Leonard, em 1994, em seus estudos *in vivo*.

Embora não tenhamos medido o pH dos géis ao longo do experimento, acreditamos ser esta uma explicação plausível para a diminuição dos valores de dureza superficial do grupo controle submetido à ação do CP 35%, ou seja, deve ter havido uma diminuição no pH do gel proporcionando a perda de mineral do esmalte.

Então, o acompanhamento do tratamento clareador por um profissional competente, tanto no sentido de orientar o paciente a reduzir o consumo de ácidos ou produtos comprovadamente erosivos imediatamente após a remoção das moldeiras com o agente clareador (escovação com dentifrício abrasivo, por exemplo), como para designar as

concentrações a serem indicadas, tempo de tratamento e riscos decorrentes do método, poderia permitir a obtenção de resultados estéticos favoráveis, com um mínimo possível de prejuízos para a estrutura dental (Tames *et al.*, 1998).

Neste aspecto, precisamos continuar estudando os efeitos do clareamento dental caseiro ou com a utilização de géis mais concentrados, associados a fontes de calor ou luz, capazes de proporcionar resultados estéticos aparentemente mais vantajosos e analisar se esses possíveis benefícios ocorrem em detrimento de alguma perda de estrutura mineral e definir até que ponto acelerar o tratamento clareador é mesmo necessário. Importante também é evitarmos a associação de produtos superficiais de limpeza que contenham alta abrasividade, capazes de remover ou alterar a superfície que está sendo tratada pelo gel. Nesta mesma linha de raciocínio pensamos que alimentos cítricos, corados, também possam interferir de alguma maneira, no resultado final do tratamento clareador, quando pensamos nas alterações de dureza superficial e/ou na deposição de corantes e bactérias em rugosidades que possam ser criadas durante o tratamento.

Embora tenhamos conhecimento dessas modificações temporárias que ocorrem na estrutura dental, julgamos ser o clareamento dental um tratamento seguro, se executado corretamente e, se acompanhado criteriosamente por um profissional habilitado. Isso permite-nos a escolha de um caminho conservativo, para solucionarmos problemas estéticos sem que precisemos lançar mão de procedimentos operatórios e/ou restauradores invasivos e isso, por si só, já é bastante atrativo.

CONCLUSÃO

De acordo com os resultados obtidos e baseados nas análises estatísticas, podemos concluir que:

- O Peróxido de Carbamida a 10%, de forma isolada, não alterou a dureza ou a rugosidade do esmalte dental.

- O Peróxido de Carbamida a 10%, associado à escovação sem dentifrício, não alterou os valores de rugosidade do esmalte dental e reduziu os valores de microdureza superfícial do esmalte dental.

- Escovação com dentifrícios abrasivos fluoretado e não fluoretado aumentou os valores de microdureza e de rugosidade do esmalte dental clareado através de Peróxido de Carbamida a 10% e após 56 dias, somente o grupo escovado com flúor mostrou valores de dureza similar aos valores iniciais.

- O Peróxido de Carbamida a 35%, utilizado de forma isolada, reduziu os valores de microdureza superfícial do esmalte dental, entretanto, não alterou a rugosidade superficial do mesmo.

- O Peróxido de Carbamida a 35%, associado à escovação sem dentifrício, reduziu os valores de microdureza superfícial do esmalte dental e manteve os valores de rugosidade superficial.

- Escovação com dentifrícios abrasivos fluoretado e não fluoretado aumentou os valores de microdureza e de rugosidade do esmalte dental clareado através de Peróxido de Carbamida a 35%.

REFERÊNCIAS*

Akal N, Over H, Olmez A, Bodur H. Effects of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel. *J Clin Pediatr Dent.* 2001; 25(4): 293-6.

Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. *J Oral Rehabil*. 1997; 24(4): 282-6.

Basting RT, Rodrigues Junior AL, Serra MC. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin in situ. *Oper Dent*. 2001; 26(6): 531-9.

Bitter NC, Sanders JL. The effect of four bleaching agents on enamel surface: A scanning electron microscopic study. *Quintessence Int.* 1993; 24: 817-24.

Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: a preliminary report. *J Prosthet Dent*. 1992; 67(6): 852-5.

Broome JC. At-home use of 35% carbamide peroxide bleaching gel: a case report. *Compend Contin Educ Dent*. 1998; 19(8): 824-9.

Eggert F, Neubert R. In vitro investigation of the liberation of fluoride ions from toothpaste compounds in a permeation model. *Eur J Pharm Biopharm*. 1999; 47(2): 169-73.

Featherstone JDB, O'Really MM, Shariati M, Brugler S. Enhancement of remineralization in vitro and in vivo. In: Leach SA. *Factors relating to demineralization and remineralization of the teeth*. Oxford: IRL; 1986.

Freitas PM, Basting RT, Rodrigues JA, Serra MC. Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals. *Quintessence Int*. 2002; 33(5): 370-5.

* De acordo com a norma da UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

Gerlach RW, Barker ML, Hyde JD, Jones MB, Cordero RE. Effects of a tartar control whitening dentifrice on tooth shade in a population with long-standing natural stain. *J Clin Dent*. 2001; 12(2): 47-50.

Goldstain RE, Garber DA. *Complete dental bleaching*. Chicago: Quintessence; 1995. p.13-83.

Gultz J, Kaim J, Scherer W, Gupta H. Two in-office bleaching systems: a scanning electron microscope study. *Compend Contin Educ Dent*. 1999; 20(10): 965-72.

Gurgan S, Bolay S, Alacam R. In vitro adherence of bacteria to bleached or unbleached enamel surfaces. *J Oral Rehabil*. 1997; 24(8): 624-7.

Hachiya Y, Takatsu T, Hosoda H, Fusayama T. A varnish to prevent etching unrestored enamel. *J Prosthet Dent.* 1985;53(1): 46-50.

Haywood VB, Heymann HO. Nightguard vital bleaching. *Quintessence Int*. 1989; 20(3): 173-6.

Haywood VB. History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. *Quintessence Int*. 1992; 23(7): 471-88.

Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent*. 1999; 27(7): 509-15.

Heymann HO. Bleaching of vital teeth. *Quintessence. Int*. 1997; 28: 420-7.

Isaacs RL, Bartizek RD, Owens TS, Walters PA, Gerlach RW. Maintenance of tooth color after prophylaxis: comparison of three dentifrices. *J Clin Dent*. 2001; 12(2): 51-5.

Kugel G, Perry RD, Hoang E, Scherer W. Effective tooth bleaching in 5 days: using a combined in-office and at-home bleaching system. *Compend Contin Educ Dent*. 1997; 18(4): 378-83.

Kuroiwa M, Kodaka T, Kuroiwa M. Microstructural changes of human enamel surfaces by brushing with and without dentifrice containing abrasive. *Caries Res*. 1993; 27(1): 1-8.

Leonard RH, Sharma A, Haywood VB. Use of different concentrations of carbamide peroxide for bleaching teeth: an in vitro study. *Quintessence Int*. 1998; 29(8): 503-7.

Lopes GC, Bonissoni L, Baratieri LN, Vieira LC, Monteiro S Jr. Effect of bleaching agents on the hardness and morphology of enamel. *J Esthet Restor Dent*. 2002; 14(1): 24-30.

McGuckin RS, Babin JF, Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. *J Prosthet Dent*. 1992; 68(5): 754-60.

Mokhlis GR, Matis BA, Cochran MA, Eckert GJ. A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. *J Am Dent Assoc*. 2000; 131(9): 1269-77.

Neves Ade A, Castro Rde A, Coutinho ET, Primo LG. Microstructural analysis of demineralized primary enamel after in vitro toothbrushing. *Pesqui Odontol Bras*. 2002; 16(2): 137-43.

Oltu U, Gurgan S. Effects of three concentrations of carbamide peroxide on the structure of enamel. *J Oral Rehabil*. 2000; 27(4): 332-40.

Papathanasiou A, Bardwell D, Kugel G. A clinical study evaluating a new chairside and take-home whitening system. *Compend Contin Educ Dent*. 2001; 22(4): 289-300.

Potocnik I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. *J Endod*. 2000; 26(4): 203-6.

Rodrigues JA, Basting RT, Serra MC, Rodrigues Junior AL. Effects of 10% carbamide peroxide bleaching materials on enamel microhardness. *Am J Dent*. 2001; 14(2): 67-71.

Rodrigues JA. *Efeito do clareamento de consultório associado ao clareamento caseiro sobre a microdureza do esmalte dental humano* [tese]. Piracicaba: UNICAMP/FOP; 2003.

Seghi RR, Denry I. Effects of external bleaching on indentation and abrasion characteristics of human enamel in vitro. *J Dent Res*. 1992; 71(6): 1340-4.

Serra MC, Cury JA. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessence Int*. 1992; 23(2): 143-7.

Smidt A, Weller D, Roman I. Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *Am J Dent.* 1998; 11: 83-5.

Tames D, Grando J, Tames DR. Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida 10%. *Rev Assoc Paul Cir Dent.* 1998; 52: 145-9.

Wille T, Combe EC, Pesun IJ, Giles DW. Rheological characteristics of tooth bleaching materials. *J Oral Rehabil*. 2000; 27(12): 1060-3.

ANEXOS

Original Message -----

From: Franklin Garcia-Godoy

To: caldri@terra.com.br

Sent: Thursday, April 24, 2003 3:12 PM

Subject: Paper received

Dear Dr.

I received your paper **Effect in vitro of 10% Carbamide Peroxide associated with Brushing and Dentifrices on Dental Enamel Microhardness at Different Time Intervals.**

I will send it to two revirewers for their comments and will contact you imemdiately after I hear from them

Sincerely,

Prof. Dr. Franklin Garcia-Godoy
Editor, American Journal of Dentistry
Professor and Assistant Dean for Research
Director, Clinical Research Center
Director, Biomaterials Research Center
College of Dental Medicine
Nova Southeastern University
3200 South University Drive
Fort Lauderdale, Florida 33328 - USA
Tel: (954) 262-7373
Fax: (954) 262-1782
E-mail: godoy@nova.edu

Original Message -----

From: Joan Matis

To: caldri@terra.com.br

Sent: Saturday, May 17, 2003 5:06 PM

Subject: Receipt of Manuscript

Dear Drs

Thank you for submitting your manuscript entitled *In Vitro Effect of Toothbrush on Bleached Enamel with 35% Carbamide Peroxide at Different Treatment Intervals – Microhardness Evaluation.* We appreciate your considering our journal for the publication of your work.

Copies of your paper will be sent to our referees for review. Once their reviews have been received and a decision has been made regarding publication, we will notify you. This process usually takes from ten to twelve weeks.

If you have any questions, please feel free to contact our office. Thank you again for submitting your manuscript to *Operative Dentistry*.

Sincerely

Joan Matis
Editorial Assistant/Subscription Manager
Operative Dentistry
email: jmatis@indy.rr.com
fax: USA 317-852-3162

DearDr.Worschech:

After some delay (the North Carolina office no longer exists) the manuscript entitled "Effect of dentifrices on bleached surface enamel: roughness evaluation," was forwarded to the Journal, has been received, and assigned number 15902. Please refer to this number in all correspondence relating to your article. All articles are considered in the order in which they were received.

Thank you for your interest in The Journal of Prosthetic Dentistry.

CarolA.Lefebvre,DDS,MS
Editor
CatherinePrysiazny
EditorialManager

JournalofProstheticDentistry
MCGSchoolofDentistry
AdministrationHall/AD1112
Augusta,GA30912-1255
Phone:(706)721-4558
Fax:(706)721-4571
Email: jpd@mcg.edu

Original Message -----

From: POB

To: caldri@terra.com.br ; gutojar@yahoo.com ; glaucia@fop.unicamp.br

Sent: Monday, December 08, 2003 10:44 AM

Subject: Correções Urgentes POB

Prezado autor

O artigo de sua autoria, protocolado sob o número 520, necessita de correções finais para que possamos dar continuidade ao processo de publicação do mesmo sem maiores atrasos. Citamos:

Atenciosamente

Comissão de Publicação da POB