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CAROLINA FRANÇA DE MEDEIROS MELO

RUGOSIDADE E MICRODUREZA DO ESMALTE CLAREADO COM PERÓXIDO DE CARBAMIDA A 10% E ESCOVADO COM DIFERENTES DENTIFRÍCIOS: ESTUDO *IN VITRO* E *IN SITU*

Profa. Dra. Ana Maria Spohr
Orientadora

PORTO ALEGRE
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BANCA EXAMINADORA

Orientadora: Profa. Dra. Ana Maria Spohr (PUCRS)

Prof. Dr. Alex José Souza dos Santos (UFRN)

Prof. Dr. Marcos Antonio Japiassú Resende Montes (UPE)

Profa. Dra. Julieta Gomes Tavares (PUCRS)

Profa. Dra. Luciana Mayumi Hirakata (PUCRS)

Dedico este trabalho...

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RESUMO

Foram realizados dois estudos, *in vitro* e *in situ* para avaliar a rugosidade e microdureza do esmalte clareado com peróxido de carbamida a 10% (PC10) e escovado com dentifrícios. Fragmentos de esmalte foram obtidos a partir de dentes humanos. A rugosidade e microdureza iniciais foram medidas. Para o estudo *in vitro*, estes fragmentos foram divididos em seis grupos (n=12): G1 – PC10 e escovado com um dentifrício regular (R); G2 – PC10 escovado com dentifrício clareador (W); G3 – PC10 escovado com dentifrício clareador com bicarbonato de sódio e peróxido de cálcio (BS); G4 – Placebo (PLA) + R; G5 – PLA+W; G6 – PLA+BS. Os fragmentos foram expostos a cada agente de tratamento por 6 horas e escovados com força de 200g por 250 ciclos/minuto durante 7 segundos. Os grupos foram armazenados à 37°C em saliva artificial. Após 21 dias foram feitas medidas finais de rugosidade e microdureza de cada fragmento. Para o estudo *in situ*, dois grupos de cinco voluntários receberam o PC10 e o PLA por 21 dias em diferentes sequências e em dois períodos distintos, utilizando um estudo crossover 2X3. Foram obtidos 180 fragmentos e distribuídos em 20 dispositivos removíveis intraorais (DRI). Cada DRI recebeu nove fragmentos divididos em três trios, nos quais foram escovados com três dentifrícios: R (Colgate máxima proteção anticáries) no centro do palato; W (Colgate total 12 whiteness gel) e BS (Colgate whitening Oxygen Bubbles) nas regiões direita e esquerda dos pré-molares, respectivamente. Ambos agentes de tratamentos foram aplicados por 8 horas durante a noite. Após as escovações, os voluntários usaram os DRI por, aproximadamente, 16 horas/dia. Para o segundo período (21 dias), novos DRI foram distribuídos e os voluntários receberam o agente de tratamento diferente daquele utilizado no primeiro período. As medidas de rugosidade e microdureza foram medidas antes e após cada período. De acordo com o teste t-Student pareado, *in vitro*, houve uma diminuição significante da microdureza para todos os grupos ($p<0,05$) e a rugosidade final foi estatisticamente maior que a final apenas para o G6 ($p=0.001$). *In situ*, a rugosidade final foi estatisticamente maior que a inicial e a microdureza final foi estatisticamente menor que a inicial ($p<0,05$).

De acordo com a ANOVA GLM, apenas os dentifrícios foram significantes ($p=0{,}037$) para a rugosidade superficial. No estudo *in vitro*, o agente PLA apresentou um aumento significativamente maior que o PC10 na microdureza do esmalte, enquanto que para o teste de Bonferroni, o dentifrício BS apresentou uma rugosidade significativamente maior que o W ($p<0{,}05$). Em relação a microdureza, com o PC10, os dentifrícios não diferiram entre si. Pode-se concluir, para o estudo *in vitro,* que a associação do PC10 com dentifrícios R, W e BS por 21 dias não alterou significativamente a rugosidade, mas diminuiu significativamente a microdureza do esmalte. Para o estudo *in situ*, a associação do peróxido de carbamida a 10% com os dentifrícios BS, W e R causou um aumento significativo na rugosidade e uma diminuição significativa na microdureza superficial do esmalte. O dentifrício BS apresentou a maior rugosidade, seguido por W e R.

Palavras chave: Odontologia, Dentes – Clareamento, dentifrícios, Peróxido de carbamida, Dentes – esmalte, Rugosidade, Microdureza, Saliva artificial.

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LISTA DE ABREVIAÇÕES, SIGLAS E SIGNIFICADOS

PUCRS	Pontifícia Universidade Católica do Rio Grande do Sul
Toothpaste R	Colgate máxima proteção anticáries
Toothpaste W	Colgate Total 12 Whiteness gel
Toothpaste BS	Colgate Whitening Oxygen Bubbles Fluoride Toothpaste
PC10	Peróxido de carbamida a 10%
PLA	Placebo
VHN	Vickers Hardness Number
µm	Micrometro
µg	Micrograma
mm	Milímetro
mm^2	Milímetro quadrado
cm	Centímetros
ANOVA GLM	Análise de variância: Modelo linear Geral
α	Nível de significância
P	Valor de probabilidade
et al.	Abreviação de et alii (e outros)
ml	Mililitro

SUMÁRIO

INTRODUÇÃO GERAL

A introdução do peróxido de carbamida como agente clareador caseiro (HAYWOOD; HEYMANN, 1989) causou um grande impacto na Odontologia estética, uma vez que esse tratamento 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 (GOLDSTEIN, 1997). Por estas características, a técnica de clareamento caseiro ficou popular entre pacientes e dentistas. A técnica original envolve a aplicação do agente clareador em uma moldeira de acetato por 6 a 8 horas, à noite, durante 2 a 6 semanas (HAYWOOD; HEYMANN, 1989).

O peróxido de carbamida se degrada em peróxido de hidrogênio a 3% e uréia a 7%. Estas substâncias são obtidas após a dissociação dos produtos de clareamento em contato com a saliva e fluidos orais. A uréia se degrada em amônia e dióxido de carbono. O peróxido de hidrogênio, devido a sua instabilidade e fácil decomposição em água e oxigênio, penetra através dos poros de esmalte e dentina até produzir um efeito mais claro no dente (GOLDSTEIN; KIREMIDJIAN-SCHUMACHER, 1993). Agentes clareadores afetam a luminosidade dental através da decomposição do peróxido em radicais livres, que quebram as grandes moléculas pigmentadas de esmalte e dentina em moléculas menores, tornando-as menos pigmentadas. Este é o momento em que o clareamento chegou ao fim, ponto de saturação (GOLDSTEIN E GARBER, 1995).

Por se tornar uma técnica segura e efetiva (HAYWOOD, 2003), fabricantes vem colocando no mercado uma grande variedade de novos produtos, desde géis clareadores a dentifrícios que prometem deixar os dentes mais claros.

O hábito de escovar os dentes é a prática de higiene bucal mais comum para evitar o aparecimento de lesões de cáries. A escovação normal com o uso de dentifrícios produz mínimo desgaste na superfície do esmalte que não vai causar danos significantes durante a vida (ADDY; HUNTER, 2003). A adição de agentes abrasivos aos dentifrícios é importante para facilitar a remoção de depósitos encontrados na superfície dos dentes (DAWSON et al., 1998). Os dentifrícios clareadores encontrados no mercado possuem partículas abrasivas em sua composição, como carbonato de cálcio, sílica hidratada, bicarbonato de sódio e óxido de alumínio (MEYERS et al., 2000). A adição de substâncias clareadoras, como os peróxidos, proporciona um efeito clareador moderado devido à liberação de oxigênio (RITTER, 2002).

Assim, dentifrícios que prometem ação clareadora estão sendo usados como agentes potencializadores do tratamento clareador. Diante da grande variedade de dentifrícios ao alcance dos pacientes, é importante saber se o tipo de dentifrício causará algum prejuízo ao esmalte dental durante o tratamento clareador caseiro.

Algumas alterações na superfície do esmalte dental podem ser resultado da ação dos agentes clareadores como o peróxido de carbamida a 10%. Esses efeitos se apresentam como depressões rasas, aumento da porosidade e leve erosão (BITTER, 1992), mas podem ser observadas também alterações morfológicas severas na superfície do esmalte como aumento da rugosidade dessa estrutura (PIMENTA *et al.,* 2003), as quais podem ser responsáveis pela diminuição da microdureza do esmalte (RODRIGUES *et al.*, 2001).

Estudos *in vitro* têm relatado que o clareamento com peróxido de carbamida a 10% não alterou a microdureza (MCCRACKEN; HAYWOOD, 1995), nem a rugosidade superficial do esmalte humano (ÇOBANKARA et al., 2004). No entanto, quando esse agente clareador está associado a dentifrícios abrasivos através de escovações simuladas, houve um aumento significativo da rugosidade superficial (WORSCHECH, 2006).

A interação *in vivo* entre saliva e esmalte é um fator que, geralmente, não é incorporado em pesquisas *in vitro* (CIMILLI; PAMEIJER, 2001). A saliva tem o poder de limpeza, capacidade tampão e habilidade de remineralização (THYLSTRUP; FEJERSKOV, 1998) que pode prevenir os efeitos de desmineralização dos agentes clareadores (JUSTINO; TAMES; DEMARCO, 2004). Trabalhos *in situ* têm sido feitos para simular condições de efeito real dos tratamentos clareadores no meio bucal, avaliando microdureza (ARAÚJO et al., 2003; MULLER ARCARI et al., 2005), rugosidade (BASTING et al., 2007) e morfologia superficial (JUSTINO; TAMES; DEMARCO, 2004), mas ainda não foi avaliado o desgaste produzido por diferentes dentifrícios durante o tratamento clareador caseiro.

Este estudo teve o objetivo de avaliar *in vitro* e *in situ* o efeito do peróxido de carbamida a 10% associado a diferentes dentifrícios durante o tratamento clareador sobre a microdureza e rugosidade superficial do esmalte.

CAPÍTULO 1

ROUGHNESS AND MICROHARDNESS OF ENAMEL BLEACHED WITH 10% CARBAMIDE PEROXIDE AND BRUSHED WITH DIFFERENT TOOTHPASTES: IN VITRO STUDY

"ALTERATIONS IN BLEACHED ENAMEL AFTER BRUSHING WITH THREE DIFFERENT TOOTHPASTES " (running title)

Carolina França de Medeiros Melo, DDS, DMD, PhD Student, Department of Restorative Dentistry, School of Dentistry, PUCRS.

Address: Rua Luzitana, 441/601, Higienópolis, Porto Alegre, RS, Brazil, ZIP 90.520-080, e-mail: carolfmedeiros@gmail.com

Ana Maria Spohr*, DDS, DMD, PhD, Associate Professor, Department of Prosthodontics, School of Dentistry, PUCRS.

***Correspondence should be addressed to:**

Dra. Ana Maria Spohr

PUCRS - Av. Ipiranga 6681, prédio 6 – Faculdade de Odontologia

Porto Alegre, RS, Brazil Zip 90619900

Fax: +55 51 3320-3626

e-mail: anaspohr@terra.com.br

ROUGHNESS AND MICROHARDNESS OF ENAMEL BLEACHED WITH 10% CARBAMIDE PEROXIDE AND BRUSHED WITH DIFFERENT TOOTHPASTES: IN VITRO STUDY

Clinical Relevance

Because of the large variety of whitening toothpastes available and knowing that the patient may use these products to potentiate the whitening treatment, dentists should be aware whether the toothpaste would be harmful to the patient's enamel during home bleaching treatment.

SUMMARY

This in vitro study evaluated the combined effect of 10% carbamide peroxide with three toothpastes used during bleaching treatment on microhardness and surface roughness of enamel. Fragments (4mm x 4mm x 2mm) were obtained from 18 human teeth. The initial roughness and microhardness were measured. The fragments were randomly divided into six groups (n=12): G1 - 10% carbamide peroxide (PC10) and brushing with regular toothpaste (R); G2 - PC10 and whitening toothpaste (W); G3 – PC10 and whitening toothpaste with baking soda and peroxide (BS); G4 – Placebo (PLA)+R; G5 (PLA+W); G6 (PLA+BS). The fragments were daily exposed to each treatment agent for six hours and the brushing procedures were performed for seven seconds, with a brushing force of 200g and 250 cycles per minute. The groups were kept in artificial saliva at 37°C until the next cycle of treatment agent and brushing procedures. After 21 days, the final roughness and microhardness measurements of each fragment were obtained. According to paired Student's t-test, there was a significantly decrease in microhardness for all groups ($p<0.05$). The final roughness was statistically higher than the initial roughness only in the group treated with G6 ($p=0.001$). According to ANOVA GLM and Bonferroni tests, PLA presented a significantly higher roughness than PC10, and toothpaste BS presented a statistically higher roughness ($p<0.05$) than W. Toothpaste R did not differ statistically from W and BS ($p>0.05$). For PC10, the toothpastes did not differ among them in relation to microhardness ($p>0.05$). According to paired Student's t-test, toothpastes R and W were statistically different for both treatments. The association of PC10 with toothpastes R, W and BS for 21 days did not significantly alter the enamel surface roughness, but there was a significant decrease in the enamel microhardness.

Keywords: Dental bleaching, toothpastes, 10% carbamide peroxide, artificial saliva, in vitro study, enamel, microhardness, surface roughness.

INTRODUCTION

The simplicity, low cost and efficiency of nightguard vital bleaching treatment continues to fascinate a public that desires to improve its smile with whiter teeth. The use of 10% carbamide peroxide in the nightguard bleaching treatment was introduced in 1989 by Haywood & Heymann.[1] Since then, a great deal has been published about the effect of bleaching on the dental enamel surface.

Some in vitro research pointed out no significant alterations in the enamel microhardness,[2-3] or enamel roughness[4]. However, when 10% carbamide peroxide was associated with abrasive toothpastes by simulated tooth brushing, there was a significant increase in the enamel surface roughness. [5]

The primordial role of toothpastes is to clean the largest number of tooth surfaces. This cleaning involves the elimination of plaque and food residues.[6] Regular brushing with toothpastes is safe and does not harmfully affect enamel.[7] Over the last few years, manufacturers have developed new products in order to meet the expectations of patients and consumers. Therefore, abrasive components such as calcium carbonate, sodium bicarbonate, peroxides, hydrated silica, and aluminum oxide have been added to the toothpastes to increase their cleaning power and to whiten the teeth.[8] Whitening toothpastes that contain small quantities of abrasive particles or peroxides may abrade the enamel surface if excessively used.[9]

Thus, toothpastes that promise whitening action have been used as agents to potentiate bleaching treatment. In view of the large variety of whitening toothpastes available to the patient and consumers, it is important to know whether the toothpaste would cause any harm to the patient's enamel when combined to nightguard vital bleaching treatment.

The aim of this study was to evaluate the in vitro effect of 10% carbamide peroxide associated with different types of toothpastes used during bleaching treatment on the microhardness and surface roughness of enamel.

METHODS AND MATERIALS

Experimental design

The factors under evaluation were treatment agents at two levels (experimental and control) and fluoride toothpastes at three levels (Colgate Máxima Proteção Anticáries, Colgate Total 12 Whiteness Gel and Colgate Whitening Oxygen Bubbles Fluoride). The specifications of the materials are listed in Table 1.

The experimental units consisted of 72 fragments of sound human enamel, randomly assigned to six different groups (n=12). The variables were surface roughness and microhardness. The schematic illustration of the experimental sequence is shown in Figure 1.

Preparation of the enamel fragments

Thirty-six freshly extracted human third molars were scraped to remove any remaining soft tissues, polished with pumice slurry and kept in 0.5% chloramine for 24 hours. After this period, the teeth were stored in deionized water at room temperature until required for use. The use of extracted human teeth followed a protocol that was reviewed and approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul – PUCRS.

The root of each tooth was mounted in self-cured acrylic resin (Clássico – Dental Products, São Paulo, SP, Brazil). Each tooth was cut along the long axis on the buccal, lingual, mesial and distal surfaces using a water-cooled diamond disc (Labcut 1010 low-speed diamond saw, Extec Corp, Enfield, CT, USA) to obtain fragments of enamel measuring approximately $16mm^2$ (4mm X 4mm X 2mm), without any stains or cracks. The fragments were steam sterilized for 20 minutes at 121^o C.

The sectioned fragments of enamel were fixed in acrylic resin and the upper surface of the samples was then manually ground and polished with 1500-, 2000- and 2500-grit carbide abrasive papers (Carborundum, 3M do Brazil Ltda., Sumaré, SP, Brazil) to create a flat surface. They were immersed in containers with deionized water. Before the experimental phase, the initial measurements of surface roughness and microhardness of each fragment were obtained.

Experimental phase – In vitro conditions

The specimens were randomly divided into six groups (n=12): G1 – 10% carbamide peroxide (PC10) and brushed with a regular toothpaste (R); G2 – PC10 and brushed with a whitening toothpaste (W); G3 – PC10 and brushed with a whitening toothpaste with baking soda and peroxide (BS); G4 – Placebo (PLA) and brushed with R; G5 – PLA and brushed with W; G6 – PLA and brushed with BS.

The specimens were exposed to each treatment agent for six hours a day for a period of 21 days. A syringe was used to apply 0.02 ml of treatment agent on each specimen, according to the group. A 10% carbamide peroxide bleaching agent (Opalescence PF, Ultradent Products, Inc., South Jordan, Utah, USA) was used in the first three groups and a placebo agent (Carbopol gel, Pharmacus, Porto Alegre, RS, Brazil) was used in the remaining groups. After daily treatment agent application, the specimens were rinsed under running deionized water for five seconds.

All samples from each group were individually bonded (Superbonder Loctite, Henkel Ltda., São Paulo, SP, Brazil) to the center of the acrylic plate (5.3cm X 2.5cm X 0.5cm). This set was put into an acrylic reservoir to start brushing procedures. A homogeneous solution of each toothpaste (slurry) was freshly prepared every day with a mixture containing toothpaste that was weighed on an analytical balance (AG 204, Mettler/Toledo, São Paulo, SP, Brazil), and deionized water in a ratio of 1:2.[6] Sufficient slurry (20ml) was placed in the acrylic reservoir to completely cover the set of specimens.

Brushing procedures were performed once a day for 21 days, with a brushing machine developed by Ideia – Institute of Research and Development and Dental Materials Department (PUCRS, Porto Alegre, RS, Brazil). The toothbrush heads (Colgate Classic, Colgate-Palmolive Indústria e Comércio Ltda., São Bernardo do Campo, SP, Brazil) were fixed onto the handle of the brushing machine, allowing the toothbrush head to be aligned parallel to the surface of the specimens. The specimens were brushed for seven seconds each, with a brushing force of 200g and 250 cycles per minute. After daily brushing procedures, the specimens were rinsed under running deionized water for five seconds.

During the remaining time, the groups were kept in containers with an artificial saliva at 37ºC until the next cycle of treatment agent and brushing procedures. The artificial saliva (Salivan®, APSEN Farmacêutica S/A, São Paulo, SP, Brazil) used was changed weekly. During the weekends these groups were kept in containers with artificial saliva at 37 ºC.

Microhardness test

The Vickers microhardness number (VHN) was measured with a microhardness tester (HMV-2, Shimadzu, Tokyo, Japan). Three indentations were made in the central region of each fragment under a 100g load for five seconds in each experimental period: initial (before the bleaching treatment) and final (after the bleaching treatment). The final VHN value of each fragment was the arithmetic mean of the three measurements.

Surface roughness test

The mean enamel roughness (Ra, μm) of each tooth was measured with a roughness tester (SL-201, Mitutoyo Surftest Analyzer, Tokyo, Japan) before and after each treatment period. In the center of the fragment surface, three different traces were recorded for each fragment with a 0.25 cut-off. The Ra value of each specimen was the arithmetic mean of the three measurements.

Statistical Analysis

The surface microhardness and roughness results were submitted to the Kolmogorov-Smirnov test to verify the normal distribution of the variables. The Student's t-test was performed for comparison between the initial and final roughness and microhardness values. This was followed by the Analysis of Variance (ANOVA GLM) in a 2 X 3 factorial scheme (treatment agents X toothpastes) and Bonferroni test. The initial roughness and microhardness readouts were used as covariables to verify the interference of these in the final readouts. For all tests, groups were considered statistically different at $\alpha = 0.05$. All statistical analyses were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

According to the Student's t-test, the final roughness was statistically higher than the initial roughness only in the group treated with placebo and BS toothpaste (p=0.001) (Figure 2). The final microhardness was statistically lower than the initial microhardness for all groups ($p<0.05$) (Figure 3).

According to ANOVA GLM, treatment factor (p=0.00) and toothpaste factor (p=0.890) were significant for surface roughness. The interaction between the factors (p=0.06) and the initial roughness (p=0.890) were not significant. According to Bonferroni, the placebo agent (0.16 μm) presented a significantly higher roughness value than the bleaching agent (0.12 μm) (Table 2). The toothpaste BS (0.16 μm) presented a statistically higher final roughness ($p<0.05$) than toothpaste W (0.13 μm). Toothpaste R (0.14 μm) did not differ statistically from toothpastes W and BS ($p>0.05$) (Table 2).

For Vickers microhardness, the initial value (p=0.240) was not significant. The treatment factor (p=0.005), toothpaste factor (p=0.010), and the treatment and toothpaste interaction (p=0.001) were significant. According to Bonferroni

(Table 3), for the placebo treatment, the toothpastes R (327 VHN) and BS (303 VHN) presented statistically higher final microhardness ($p<0.05$) when compared with toothpaste W (222 VHN). For bleaching treatment, the toothpastes did not differ among them ($p>0.05$). As SPSS does not make multiple comparisons for the effect of the interaction, Student’s t-tests (Table 3) were performed within each toothpaste for the treatments. Toothpastes R and W were statistically different for the bleaching and placebo treatments. Toothpaste BS did not differ statistically for the treatments ($p>0.05$).

DISCUSSION

Whitening toothpastes are recommended for maintaining the color of the teeth after bleaching treatment because they are developed for removing stains from tooth surfaces. For this purpose, they have small quantities of peroxides or abrasive particles in their composition.[9] The abrasive agents found in toothpastes are calcium carbonate, hydrated silica, sodium bicarbonate, sodium tripolyphosphate, aluminum oxide[8], and a mixture of these components. The addition of whitening substances such as peroxides provides a moderate whitening effect due to the oxygen release.[9] The presence of these agents in toothpastes is important to facilitate the removal of residues found on tooth surfaces.[10] Nevertheless, the abrasiveness of toothpastes must be moderate to avoid the excessive wear of the enamel and dentin surfaces.[11]

A 50 μm deep demineralization was observed below the enamel surface in an in vitro study after 10% carbamide peroxide bleaching.[12] Other in vitro studies that used only 10% carbamide peroxide presented an increase in enamel roughness, but this alteration was not significant.[4,13-14] On the other hand, the interaction between bleaching agents and toothpastes may exert a negative influence on the human enamel surface.

Most of the studies evaluate the separate effect of 10% carbamide peroxide[2,4,14-19] and toothpastes[6,8,20-22] on dental enamel. Few studies evaluate the combined effect of those two treatments to simulate realistic conditions.[7,21] Whether a patient is under bleaching treatment or not, toothbrushing is considered a daily oral hygiene practice. Therefore, the aim of the present study was to focus on the combination effect of the bleaching agent with different toothpastes. Given the methodology used, it was not possible to determine, however, which factor mostly affected the surface roughness and microhardness of enamel. The goal of the present study was the combined effect of both treatments.

There were no statistical differences among the groups in the initial roughness and microhardness analyses. This result was satisfactory, because it showed homogeneity at the beginning of the experiment, as no treatment had yet been performed on the enamel surfaces.

In the present study, the groups that received 10% carbamide peroxide associated with toothpastes presented an increase in enamel surface roughness, although this result was not statistically significant. Nevertheless, Worschech and others[5] verified a statistically significant increase in the enamel surface roughness when 10% carbamide peroxide was associated with toothpaste containing calcium carbonate as the abrasive agent, with and without fluoride. Quantitative differences between the studies in regards to the enamel roughness could be due to a series of variables, such as the brushing time used in the studies. Worschech and others[5] used brushing for 180 seconds daily, at a speed of 250 cycles/minute for 56 days. According to Sexton & Philips,[25] for each brushing session in a certain area, the individual performs 15 brushing cycles. Thus, the present study reproduced the number of cycles daily performed by an adult. For this purpose, a simulated brushing machine was used, with a speed of 250 cycles/minute. In this sense, two daily brushing procedures resulted in 30 cycles with a total duration of 7 seconds, for 21 days.

Irrespective of the toothpaste used, the placebo agent caused a statistically higher surface roughness (0.16 µm) than carbamide peroxide (0.12 µm). In addition to the effect of the toothpastes, this increased roughness could be justified by the fact that the placebo gel was composed of carboxypolymethylene polymer (carbopol). In an in situ study by Rodrigues and others[19] a significant decrease in enamel microhardness was verified when the carbopol-based placebo was applied. The authors suggested a destructive effect of carbopol on enamel surface. Although studies have correlated carbopol to enamel microhardness, it is believed that it could also influence the surface roughness of this substrate. Nevertheless, the difference of only 0.04 µm in the mean surface roughness of enamel between placebo and 10% carbamide peroxide treatments might not be clinically relevant.

The group treated with placebo and brushed with BS toothpaste was the only group that presented a significant increase in enamel surface roughness. This result could be explained by the sum of the destructive effect of carbopol contained in the placebo, and by the composition of the BS toothpaste. This toothpaste has a combination of abrasive agents (hydrated silica and sodium bicarbonate) and calcium peroxide as bleaching agent. This increased surface roughness might have influenced the statistical analyses leading to a statistical significance for the toothpaste factor.

Increased and significant enamel surface roughness was found for the toothpaste BS (0.16 µm) compared to toothpaste W (0.13 µm). Nevertheless, it is believed that a 0.03 µm difference would not be clinically relevant. Toothpaste R (0.14 µm) did not differ from the toothpastes BS and W. Toothpaste W contains hydrated silica which is an intermediate abrasive agent[6] and toothpaste R has calcium carbonate which is a polishing agent with a lower abrasive power.[26] The combination of abrasive agents contained in the toothpaste BS probably caused the greatest surface roughness. Moreover, no differences in surface roughness were observed among the groups treated with 10% carbamide peroxide associated with toothpastes (Figure 2). This finding highlights the similar behavior found for the toothpastes when associated with the bleaching gel.

According to Bollen, Lambrechts & Quirynen,[27] surface roughness above 0.20 µm leads to dental plaque accumulation. None of the toothpastes studied presented roughness above this value.

A significant decrease in enamel microhardness was observed for all groups when 10% carbamide peroxide was associated with brushing using toothpastes. In vitro studies have shown that the application of 10% carbamide peroxide alone (not associated with brushing procedures) did not cause changes in the enamel microhardness.[14,15,28] However, there is no consense in the scientific literature regarding the harmful or beneficial effects of bleaching agents on the enamel microhardness. Attin and others[29] evaluated 50 studies (with a total of 166 enamel microhardness measurements) and reported a decrease in enamel microhardness after bleaching in 51% of the measurements. No decrease in microhardness was found in 49% of the measurements.

In the 10% carbamide peroxide groups, the combination with toothpaste R yelded the lowest enamel microhardness (285 VHN) followed by the toothpaste W (328 VHN), and toothpaste BS (334 VHN). No statistical significance was found among those three groups. Within the toothpastes used in this study, only toothpaste BS contains a bleaching agent (calcium peroxide). Since bleaching agents may cause mineral loss in the enamel surface,[30] it was expected that this toothpaste together with the bleaching gel would lead to a decrease in enamel microhardness. However, the toothpaste BS associated with the bleaching gel presented the highest enamel microhardness, suggesting that the bleaching agent contained in the toothpaste did not significantly affect enamel microhardness. Therefore, the different compositions of the toothpastes were not a significant factor in the enamel microhardness when associated with 10% carbamide peroxide.

For the placebo groups a significant decrease in enamel microhardness was observed when it was associated with the toothpastes. This is probably due to the destructive effect of carbopol on enamel surface, since this acidic polymer may cause enamel demineralization[31]. The abrasive agents contained in the toothpastes may also be responsible for the decrease in enamel microhardness in all groups (bleaching and placebo). The abrasive agents remove part of the mineral on the enamel surface.[32,33] This mineral loss wears out the enamel leading to a proximity to dentin and, consequently, decrease in microhardness. For the placebo treatment, the toothpastes R (327 VHN) and BS (303 VHN) presented statistically higher final microhardness than the toothpaste W (222 VHN). According to the composition of toothpaste BS, which contains two abrasive agents and a bleaching agent, the lowest surface microhardness value was already expected.

Between treatments (Table 3), toothpaste W and BS presented a lower microhardness for placebo when compared to 10% carbamide peroxide. Statistical difference was found only for toothpaste W. The abrasives contained in these toothpastes might have had more abrasive effect on enamel when associated with the placebo, which is carbopol-based. Moreover, carbopol may act as an impermeable barrier on enamel thus blocking the penetration of the artificial saliva and, consequently, avoiding the recovery of the initial enamel microhardness values.[34] This decrease in enamel microhardness when using cabopol-based placebo has been previously reported.[19,34-35] Another factor that may have contributed to the change in microhardness is the absence of fluoride in the placebo gel. Attin and others[36] revealed that the contact of fluoride with the tooth surface was important for the enamel remineralization process and the increase in surface microhardness. In relation to toothpaste R, there was an opposite result, because the microhardness of enamel was lower for the treatment with 10% carbamide peroxide in comparison with placebo. It is difficult to find a plausible explanation for this result, as well as for the fact that toothpaste BS did not have a lower microhardness. Although care was taken to control the variables of simulated brushing, one cannot discard the possibility of the influence of some confounding variables. The action of the brushes, the force and speed of brushing, and the energy required to maintain the abrasive particles of the slurry in suspension are important variables that may affect the determination of dental tissue loss. In relation to these variables, the homogeneity of the slurry is considered one of the most difficult variables to control.[33]

Further research should focus on the association of other bleaching agents with different toothpastes since the material composition may lead to distinct results.

CONCLUSIONS

The association of 10% carbamide peroxide with toothpastes R, W, and BS for 21 days did not significantly alter the enamel surface roughness. Nevertheless, there was a significant decrease in the enamel microhardness and there was no statistical difference among the toothpastes.

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Table 1: Composition and manufacturer of each treatment agent and toothpastes.

Treatment Agents/Toothpaste	Composition	Manufacturer
Opalescence PF	10% carbamide peroxide, 0,5% potassium nitrate, 0,11% w/w fluoride, water (pH~6.5)	Ultradent Products, Inc – Utah, USA
Carbopol Gel	Carbopol	Pharmacus, Porto Alegre, RS, Brazil
Regular toothpaste: Colgate Máxima Proteção Anticáries, identified by “R”.	Calcium Carbonate, water, Sorbitol, Sodium Lauryl Sulfate, Sodium Monofluorophosphate (1450 ppm fluorine), flavor, Cellulose gum, Tetrasodium Pyrophosphate, Sodium Silicate, Sodium Saccharin, Methylparaben, Propylparaben*.	Colgate-Palmolive Indústria e Comércio Ltda., São Bernardo do Campo – SP, Brasil
Whitening fluoride toothpaste: Colgate Total 12 Whiteness Gel identified by “W”.	Water, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, PVM/MA Copolymer, flavor, Carrageenan, Sodium Hydroxide, Sodium Fluoride (1450 ppm fluorine), Triclosan, Sodium Saccharin, CI 77891, CI 77019, CI 42090*.	Colgate-Palmolive Indústria e Comércio Ltda., São Bernardo do Campo – SP, Brasil
Whitening Fluoride Toothpaste with Baking Soda and Peroxide: Colgate Whitening Oxygen Bubbles Fluoride Toothpaste identified by “BS”.	Glycerin, Hydrated Silica, Propylene glycol, water, sodium bicarbonate, Sodium Monofluorophosphate (0.14% w/v fluoride ion), Pentasodium Triphosphate, Tetrasodium Pyrophosphate, Sodium Lauryl Sulfate, flavor, Sodium Hydroxide, Sodium Saccharin, Carrageenan, Cellulose gum, calcium peroxide, titanium dioxide*.	Colgate/Palmolive Company New York, NY, USA
Artificial Saliva	Carmellose Sodium (10ml), Vehicle (1ml): Sorbitol, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, potassium acid fosfate, methylparaben, water.	Salivan®, APSEN Farmacêutica S/A, São Paulo, SP, Brazil.

*The manufacturer does not indicate the percentage of each component

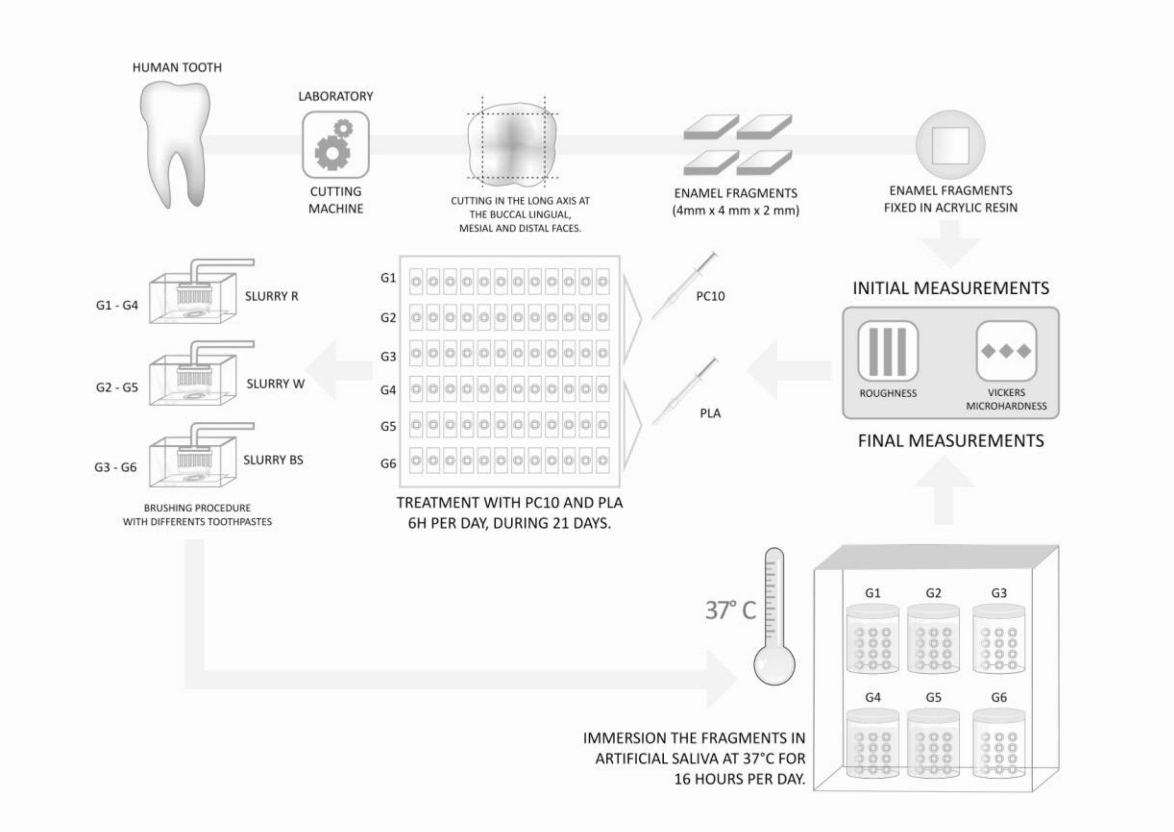
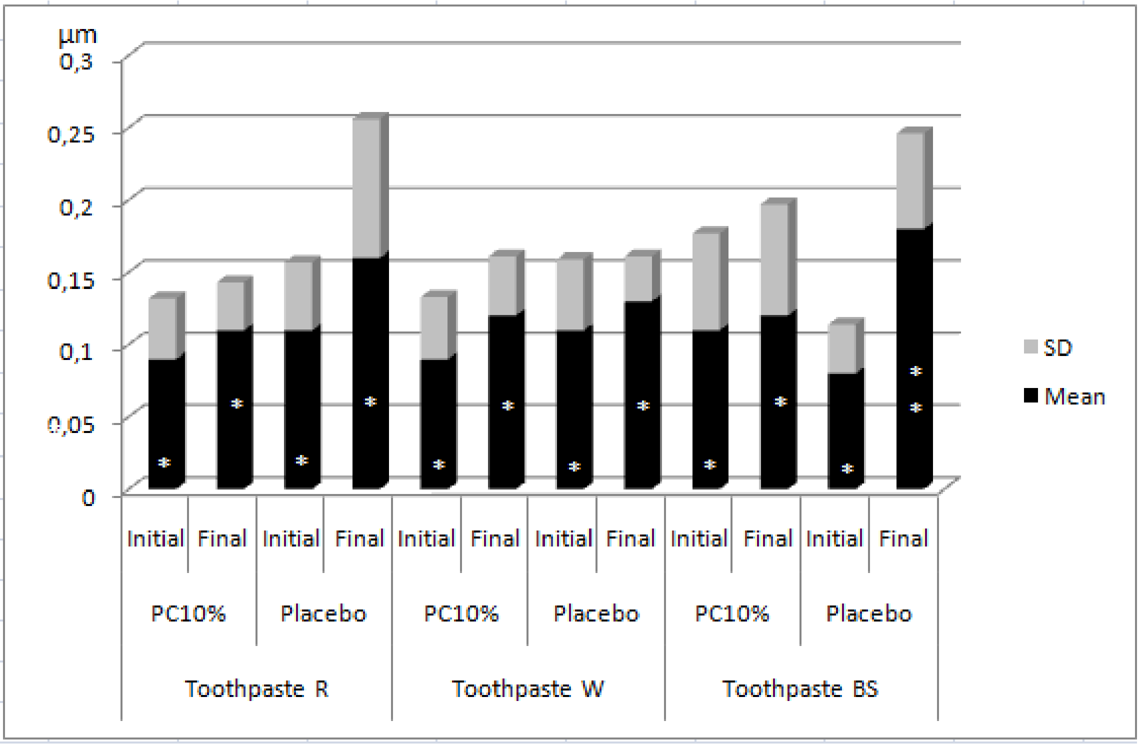
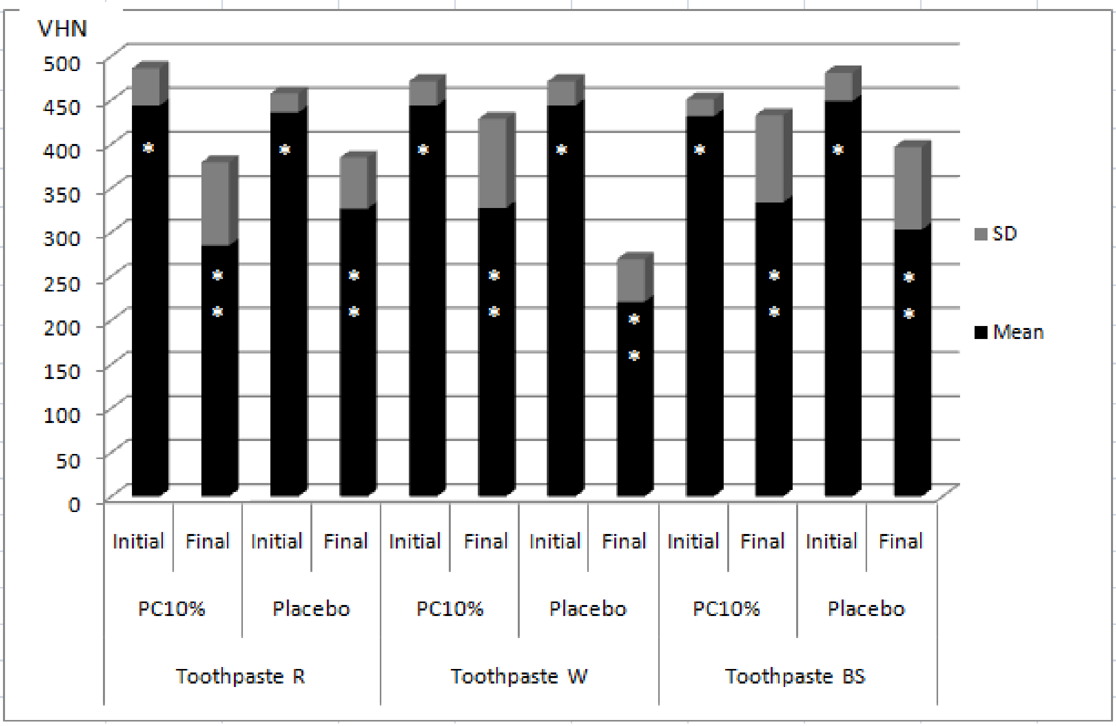


Figure 1: Schematic illustration of the experimental sequence.



Means followed by the same amount of signal (*) did not differ statistically according to paired Student's t-test (α = 0.05).

Figure 2: Mean values of enamel surface roughness (µm)



Means followed by the same amount of signal (*) did not differ statistically according to paired Student's t-test (α = 0.05).

Figure 3: Mean values of enamel vickers microhardness (VHN).

Table 2: Final surface roughness mean values.

Treatments	Mean (µm)	SD	Toothpastes	Mean (µm)	SD
PC10	0.12[a]	0.058	**R**	0.14[ab]	0.071
PLA	0.16[b]	0.070	**W**	0.13[a]	0.044
			BS	0.16[b]	0.081

Different lowercase in the column indicate statistically significant difference according to Bonferroni test (p<0.05).

Table 3: Final vickers microhardness mean values.

	Toothpastes		
	R	**W**	**BS**
Treatments	Mean (VHN)	Mean (VHN)	Mean (VHN)
PC10	285 Aa	328 Ab	334 Aa
PLA	327 Ab	222 Ba	303 Aa

Different capital letters in the same LINE indicate statistically significant difference for the Bonferroni test ($p < 0.05$). Different lowercase in the same COLUMN indicate statistically significant difference for the paired Student's t-test ($p < 0.05$).

CAPÍTULO 2

ROUGHNESS AND MICROHARDNESS OF ENAMEL BLEACHED WITH 10% CARBAMIDE PEROXIDE AND BRUSHED WITH DIFFERENT TOOTHPASTES: IN SITU STUDY

Carolina França de Medeiros Melo[a], Ana Maria Spohr [b,*]

[a] DDS, DMD, PhD Student, Department of Restorative Dentistry, School of Dentistry, PUCRS.

[b] DDS, DMD, PhD , Associate Professor, Department of Restorative Dentistry, School of Dentistry, PUCRS.

***Correspondence should be addressed to:**

Dra. Ana Maria Spohr

PUCRS

Av. Ipiranga 6681, prédio 6 – Faculdade de Odontologia

Porto Alegre, RS, Brazil

Zip 90619900

Fax: +55 51 3320-3626

e-mail: anaspohr@terra.com.br

ROUGHNESS AND MICROHARDNESS OF ENAMEL BLEACHED WITH 10% CARBAMIDE PEROXIDE AND BRUSHED WITH DIFFERENT TOOTHPASTES: IN SITU STUDY

ABSTRACT

Objectives: This in situ study evaluated the association effect of 10% carbamide peroxide bleaching and three toothpastes used during bleaching treatment on the microhardness and surface roughness of enamel.

Methods: Two groups of five volunteers received the bleaching (10% carbamide peroxide) and the placebo agents for 21 days in different sequences and in two distinct phases in a crossover 2X3 study. The experimental units consisted of 180 fragments (4mm X 4mm X 2mm) of human enamel distributed among twenty intraoral removable acrylic appliances. Each appliance received nine fragments, divided into three triplets which were brushed with different toothpastes: R (Colgate Máxima Proteção Anticáries) in the center of the palate; W (Colgate Total 12 Whiteness Gel) and BS (Colgate Whitening Oxygen Bubbles Fluoride Toothpaste) in the right and left premolar areas, respectively. Both treatments agents were applied for approximately eight hours during the night. After brushing with toothpastes, the volunteers used the intraoral appliance for about 16 hours/day, to simulate clinical conditions. After a washout period (15 days), new appliances were distributed and the volunteers were crossed over to the alternate agent for 21 days. Surface roughness (Ra) and microhardness (100g/5s) were measured before and after each phase.

Results: According to the paired Student's t-test, the final roughness was statistically higher than the initial roughness, and final microhardness was statistically lower than the initial microhardness ($p < 0.05$). According to ANOVA GLM, only the toothpaste factor was significant ($p = 0.037$) for roughness. Factors and interactions were not significant for microhardness.

Conclusion: The association of PC10 with the toothpastes BS, W, and R caused a significant increase in enamel roughness and a significant decrease in enamel microhardness. Toothpaste BS presented the highest roughness, followed by W and R. PC10 associated with R, W or BS toothpastes caused equivalent decrease in the enamel microhardness.

Keywords: dental bleaching, toothpastes, 10% carbamide peroxide, in situ study, enamel, microhardness, surface roughness

1. Introduction

The introduction of 10% carbamide peroxide used in the home bleaching technique[1] caused great impact on esthetic Dentistry because it was considered a safe, effective[2], and economical technique. This treatment could help the patients whiten their teeth without the need for additional restorative treatment.[3]

The effects of 10% carbamide peroxide on the dental enamel surface have been extensively studied. Some in vitro research pointed out no significant changes in the microhardness[4,5] or surface roughness[6] of this tooth substrate. However, when 10% carbamide peroxide is associated with abrasive toothpaste by simulated tooth brushing, a significant increase in the surface roughness of enamel can be found.[7]

In vitro studies do not generally simulate the in vivo interaction between saliva and enamel.[8] This is an important factor to be considered due to the remineralizing power of saliva that may prevent the demineralizing effect of bleaching agents.[9] At present, in situ studies have been simulated the real effect of bleaching treatments on the oral environment, where the microhardness,[10,11] surface roughness[12] and surface morphology[8,9] can be evaluated. However, the wear caused by different types of toothpastes during bleaching treatment has not yet been evaluated.

The toothbrushing routine is the most common oral hygiene practice to prevent the manifestation of caries lesions. The whitening toothpastes found on the market contain abrasive particles in their composition, which remove stains from the enamel surface. The whitening toothpastes with peroxide, however, contain a low concentration of substances capable of releasing oxygen to produce a small teeth whitening effect.[13] Therefore, in view of the large variety of whitening toothpastes over the counter and having in mind that patient may use these products to potentiate the whitening treatment, it seems important to identify which type of toothpaste may be used during bleaching.

The aim of this study was to evaluate in situ the effect of 10% carbamide peroxide associated with different types of toothpastes used during bleaching treatment on the microhardness and surface roughness of enamel.

2. Materials and Methods

2.1. Selection of volunteers

Ten volunteers, females between the ages of 22 to 42 were selected from the Dental School of PUCRS, Porto Alegre, Brazil. The experimental procedures were performed with the informed consent of the subjects, after approval of the research protocol by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul – PUCRS. Exclusion criteria for participating in this study were: use of any type of medication likely to interfere with salivary secretion, use of fixed or removable partial dentures or orthodontic appliances, pregnancy or breastfeeding, smokers or subjects with dentin sensitivity, bulimia and esophageal reflux.

2.2. Experimental design

This double-blind experiment was performed in two periods with a two-week (15 days) washout period. Ten volunteers were randomly divided into two groups (n=5). Each group received either the bleaching agent (10% carbamide peroxide) or the placebo agent for 21 days in different sequences and in two distinct periods in a crossover 2x3 study. The factors under evaluation were: a) treatment agents at two levels: experimental and control; b) fluoride toothpastes at three levels: Colgate Máxima Proteção Anticáries (R), Colgate Total 12 Whiteness Gel (W) and Colgate Whitening Oxygen Bubbles Fluoride (BS). The specifications of the materials are listed in Table 1.

The experimental units consisted of 180 human enamel fragments. Ninety fragments were randomly distributed among ten intraoral removable acrylic appliances for each phase. All volunteers underwent treatment with the bleaching agent for 21 days, then for another 21 days with the placebo agent. The quantitative response variables were surface roughness and microhardness. The schematic drawing of this study is shown in Figure 1.

2.3. Preparation of the enamel fragments

Forty-five freshly extracted human third molars were scraped of any remaining soft tissues, polished with pumice slurry and kept in 0.5% chloramine for 24 hours. After this period, the teeth were stored in deionized water.

The root of each tooth was mounted in self-cured acrylic resin (Clássico – Dental Products, São Paulo, SP, Brazil). Each tooth was cut along the long axis on the buccal, lingual, mesial and distal surfaces using a laboratory cutting machine Labcut 1010 (Extec, London, England) with a low-speed water-cooled diamond saw (Extec, London, England) to obtain enamel fragments measuring approximately $16mm^2$ (4mm X 4mm X 2mm), with no stains or cracks.

The sectioned enamel pieces were manually ground and polished with 1500-, 2000- and 2500-grit carbide abrasive papers (Carborundum/3M do Brazil Ltda., Sumaré, SP, Brazil) to create a flat surface. The enamel fragments were steam sterilized for 20 minutes at 121^oC. Steam sterilization is the most effective method to avoid bacterial contamination and does not change the mineral content of the teeth.[14] After this, the enamel fragments were stored in deionized water at room temperature until required. Before fabricating the removable appliances, the surface roughness and microhardness measurements of each piece were obtained.

2.4. Intraoral appliance preparation and mounting of the fragments

Intraoral removable acrylic appliances were made using the models (Plaster type III, Rutenium, Rio de Janeiro, RJ, Brazil) obtained from alginate impressions (Jeltrate II, Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brasil) taken from the volunteers. Each appliance received nine enamel fragments, divided into sets of three. They were fixed side by side, in the center of palate, right and left premolar areas on the lingual side of each appliance.

2.5. Experimental phase - In situ conditions

The volunteers received a “research kit” with toothbrush (REACH® Essencial Junior, Johnson&Johnson, São José dos Campos, SP, Brazil), three different toothpastes kept in syringes identified by letters (“R”, “W” and “BS”), treatment agent and operating instructions. A fluoride-free toothpaste, PHILLIPS (GlaxoSmithKline do Brasil Ltda., Rio de Janeiro, RJ, Brazil), was used by the volunteers for daily oral hygiene during the experimental phase.

In the first phase (21 days), five volunteers applied the bleaching agent whereas the other five volunteers applied the placebo agent on the appliance out of the mouth for about eight hours during the night. The volunteers were blind as regards which agent they were using. In the morning and at night, they were instructed to clean the intraoral appliance under running water, followed by brushing the fragments: Triplet “R” (in the center of palate area) with toothpaste “R”; Triplet “W” (right premolar area) with toothpaste “W” and Triplet “BS” (left premolar area) with toothpaste “BS”. The toothpaste was placed on the toothbrush and rubbed in back and forth movement cycles for 30 seconds, twice a day.

After brushing, the volunteer inserted the intraoral appliance in the mouth for about 16 hours to simulate clinical conditions and the effects of saliva on bleached enamel. During this period, the appliances were allowed to be removed only during meals and for oral hygiene purposes.

After 21 days of the first experimental phase, the fragments were removed from the acrylic resin with tungsten carbide instruments (KOMET do Brazil Ltda., São Paulo, SP, Brazil). Volunteers were then submitted to a washout period of 15 days to eliminate the residual effects of the previously applied treatment. Subsequently, they received the second intraoral appliance and a new “research kit” for experimental phase 2. This time the volunteers used the treatment agent they had not received in experimental phase 1 for another 21 days. In the end of this last phase, the fragments were removed with tungsten carbide instruments.

2.6. Microhardness test

The Vickers hardness number (VHN) was measured with a HMV hardness tester (Shimadzu, Tokyo, Japan). Three indentations were made on the center of each fragment under a 100g load for 5 seconds in each experimental period: initial (before the treatments) and final (after the treatments). The VHN value of each fragment was the arithmetic mean of the three measurements.

2.7. Surface roughness test

The mean enamel roughness (Ra, µm) of each tooth was measured with a roughness tester SL-201 (Mitutoyo Surftest Analyzer, Tokyo, Japan) before and after each treatment period. In the center of the fragment surface, three different traces were recorded for each specimen with a 0.25 cut-off. The Ra value of each specimen was the arithmetic mean of the three measurements.

2.8. Statistical analysis

The microhardness and surface roughness results were submitted to the Kolmogorov Smirnov test to verify the normal distribution of the variables. The paired Student's t-test was performed for comparison between the initial and final roughness and microhardness values. This was followed by the Analysis of Variance (ANOVA GLM) and Bonferroni test. Each patient was considered a block, in a factorial scheme 2 X 3 (treatment agents X toothpastes). The initial roughness and microhardness readouts were used as co variables to verify the interference of these in the final readouts. For all tests, groups were considered statistically different at $\alpha = 0.05$. All statistical analyses were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

3. Results

According to the paired Student's t-test, the final roughness was statistically higher than the initial roughness (Figure 2), and final microhardness was statistically lower than the initial microhardness (Figure 3) ($p<0.05$).

According to ANOVA GLM, the treatment factor ($p=0.438$), the interaction between the treatment and toothpaste factors ($p=0.369$), as well as the initial roughness ($p=0.138$) were not significant. The toothpaste factor ($p=0.037$) and the effect of patients (block) ($p=0.003$) were significant. According to the Bonferroni test, the toothpaste BS (0.20μm) presented statistically higher surface roughness ($p<0.05$) than toothpaste R (0.15 μm). Toothpaste W (0.18 μm) did not differ statistically ($p>0.05$) from toothpastes BS and R.

Regarding Vickers microhardness, the treatment factor ($p=0.076$), the toothpaste factor ($p=0.070$), the interaction between treatment and toothpaste factors ($p=0.410$), as well as the initial microhardness ($p=0.06$) were not significant. Only the effect of patients was significant ($p=0.001$).

4. Discussion

The addition of abrasive agents to toothpastes is important to facilitate the removal of debris from tooth surfaces.[15] Regular brushing with the use of toothpaste is a safe oral hygiene method and does not affect human enamel.[16] However, patients who are submitted to bleaching treatment may use whitening and abrasive toothpastes to increase the treatment efficacy, and this association could be harmful to dental enamel.

It is believed that this in situ study is the first to evaluate the effect of 10% carbamide peroxide associated with three different fluoride toothpastes on the microhardness and surface roughness of human enamel. The 10% carbamide peroxide was chosen because it is the bleaching agent most frequently found on the market[17] and has been present in the majority of publications on home bleaching over the last 20 years.[18]

In relation to different methodologies, in situ models have advantages in comparison with in vitro models, because the presence of human saliva and acquired pellicle provide remineralization and protection to the enamel surface.[9,19] Two different methodologies using the in situ model to simulate realistic clinical conditions for nightguard vital bleaching treatments were found in the literature. The first methodology used fragments of enamel and dentin fixed on the buccal surface of sound molars and/or premolars with the aid of phosphoric acid, adhesive and composite resin, and a custom tray for bleaching agent application. Although this technique is frequently used,[12,20,21] it presents some disadvantages for the volunteer, such as the use of the adhesive system and resin composite on sound enamel, and the need for polishing the enamel at the end of the experiment. Moreover, the area that receives the fragment bonding is not bleached, and it could generate a stain on the buccal surface of the tooth. In the present study, the bleaching treatment and brushing were performed on human enamel fragments fixed onto removable palatine acrylic appliances, outside the oral medium, thus being a less invasive method. These removable plates have also been used by Justino et al.[9] and Araújo Jr et al.[10] Furthermore, the crossover model was used[20,21] because it is less expensive and statistically more powerful.[22]

Each volunteer in this study was considered a statistical block so that the differences between them would be minimized. The differences include the biologic factors: salivation levels, buffer capacity and saliva composition, in addition to diet and force during brushing.[21] Nevertheless, in the final roughness and microhardness analyses, the effect of patients (block) was significant. It means there was natural variability among the volunteers, even using exclusion criteria. Thus, as individuals are naturally different, it is expected that this effect would be significant. On the other hand, there were no statistical differences in both surface roughness and microhardness in the initial analyses. This result was satisfactory because it showed homogeneity in the beginning of the experiment.

The great majority of the published studies evaluate the separate effect of 10% carbamide peroxide[4,5,6,8,9,10,11,23] and toothpastes[24-28] on dental enamel. Few studies are realistic enough to evaluate the combined effect of those two treatments.[7,21] Considering that tooth brushing is a daily oral hygiene practice, whether patient is under bleaching treatment or not, the aim of the present study was to focus only on the combination effect of the bleaching agent with different toothpastes. Given the methodology used, it was not possible to determine, therefore, which factor mostly affected the surface roughness and microhardness of enamel. The goal of the present study was the combined effect of both treatments only.

According to the results, the combined effect of 10% carbamide peroxide and toothpastes significantly increased the enamel surface roughness. The bleaching gel used (Perfect Bleach) has a pH of 6 and is carbopol-free. Studies have reported that carbopol-free 10% carbamide peroxide with pH close to neutral does not affect enamel surface roughness.[6,23] However, when associated with toothpastes, this bleaching agent significantly increased the enamel surface roughness in an in vitro study.[7]

The enamel roughness was also increased for the placebo associated with toothpastes groups. The placebo gel consisted of a carboxypolymethylene polymer (carbopol). This placebo was similar to bleaching agent in appearance and consistency and could not be easily identified by the volunteers.[11] The same placebo gel was used in other studies.[11,12,20,21] However, it has been suggested that the carbopol contained in the placebo gel may also harmfully affect the dental enamel.[11] Therefore, it can be assumed that the increased enamel surface roughness found in the placebo groups may be due to the effect of carbopol and abrasive agents contained in the toothpastes.

The toothpaste factor presented significant difference in surface roughness. Toothpaste BS, composed of hydrated silica, calcium peroxide, and sodium bicarbonate created significantly rougher enamel, when compared with toothpaste R, which contains calcium carbonate that is a polishing agent with less abrasive power.[29] Sodium bicarbonate contributes to stain removal,[30] calcium peroxide provides a whitening effect due to the oxygen release, and hydrated silica is an intermediate abrasive agent.[24] The combination of abrasive agents in a single toothpaste could explain the significant increase in enamel surface roughness with toothpaste BS. According to Haywood[31] there is a group of whitening toothpastes that are more abrasive than regular toothpastes, thus the excessive use of abrasives combined with more vigorous brushing not only acts on stain removal, but may also promote exaggerated enamel wear.

Toothpaste W, with hydrated silica, presented no statistical difference from toothpastes BS and R. Wülknitz[24] evaluated the abrasiveness and cleaning capacity of different toothpastes. The author considered the hydrated silica an intermediate abrasiveness agent, and it was more efficient in removing stains from enamel and dentin when compared with other abrasives. The result obtained for toothpaste C may be due to the moderate abrasiveness of hydrated silica, placing this abrasive agent in an intermediate position.

In accordance with Bollen, Lambrechts e Quirynen,[32] surface roughness above 0.20 µm lead to dental plaque accumulation. None of the toothpastes studied presented roughness above this value. Despite the statistical difference between the roughness created by the toothpaste BS (0.20 µm) and the toothpaste R (0.15 µm), it can be assumed that a difference of 0.05 µm would not be a clinical issue.

Several authors have demonstrated that the microhardness of enamel does not change after exposure to 10% carbamide peroxide under a variety of in vitro conditions.[5,33,34] McCracken e Haywood[35] reported that 10% carbamide peroxide caused a 1.06 $\mu g/mm^2$ calcium loss on enamel without clinical significance. In the present study, however, the association of 10% carbamide peroxide with toothpastes caused a significant decrease in microhardness. The same decrease in microhardness could be observed in the placebo groups.

The toothpaste factor was not significant, which highlights that the different compositions of the toothpastes did not affect the enamel microhardness. In relation to the composition, all toothpastes used in the present study contained fluoride. Watanabe et al.[36] evaluated whitening toothpastes with and without fluoride, and non-fluoride toothpastes exhibited a great mineral loss, while the fluoride toothpastes presented the cariostatic effect expected due to the remineralization process. Furthermore, brushing with fluoride toothpaste helps to prevent the decrease in surface microhardness during bleaching treatment.[37] It could be assumed that the absence of fluoride in toothpastes could result in lower surface microhardness as well as higher roughness values of enamel. Besides that, the presence of fluoride in the 10% carbamide peroxide gel could have had a positive influence on the result of this research. Attin et al.[38] revealed that the contact of fluoride with the tooth surface was important for the enamel remineralization process and the increase in surface microhardness.

In this in situ study, the presence of saliva may have played an important role on the results. The combination of an in situ and in vitro study to evaluate the effect of three 10% carbamide peroxide agents on the microhardness of enamel indicated that the effect of bleaching gel may be modified by the action of human saliva on enamel remineralization.[8] Some studies suggested that saliva could revert some mineral loss caused by bleaching treatment.[5,8,11]

In an overall analysis of all groups, the mean initial microhardness was 420 VHN, with a decrease of 95 VHN in 21 days after combining the treatments with toothpastes. It is not known whether this decrease is clinically significant or not. Although the enamel microhardness was not evaluated some weeks after the end of the bleaching treatment, it is believed that human saliva may have led to enamel microhardness recovery due to its remineralization capacity.[39]

Within the clinical significance of the present study is the evidence that enamel microhardness and surface roughness are altered when 10% carbamide peroxide bleaching is associated with toothbrushing using the toothpastes BS, R, and W. Moreover, it can be assumed that an individual could use the toothpastes BS, R, and W while bleaching his teeth with 10% carbamide peroxide with no clinical significance on enamel microhardness and surface roughness related to the choice of the toothpaste. It means that one could use a whitening toothpaste (BS and W) instead of a regular toothpaste (R) in a regular basis.

5. Conclusions

- 10% carbamide peroxide associated with toothpastes BS, W and R caused a significant increase in enamel surface roughness and a significant decrease in enamel microhardness;
- Toothpaste BS presented the highest surface roughness, followed by W and R;
- 10% carbamide peroxide associated with R, W or BS toothpastes caused similar decrease on enamel microhardness.

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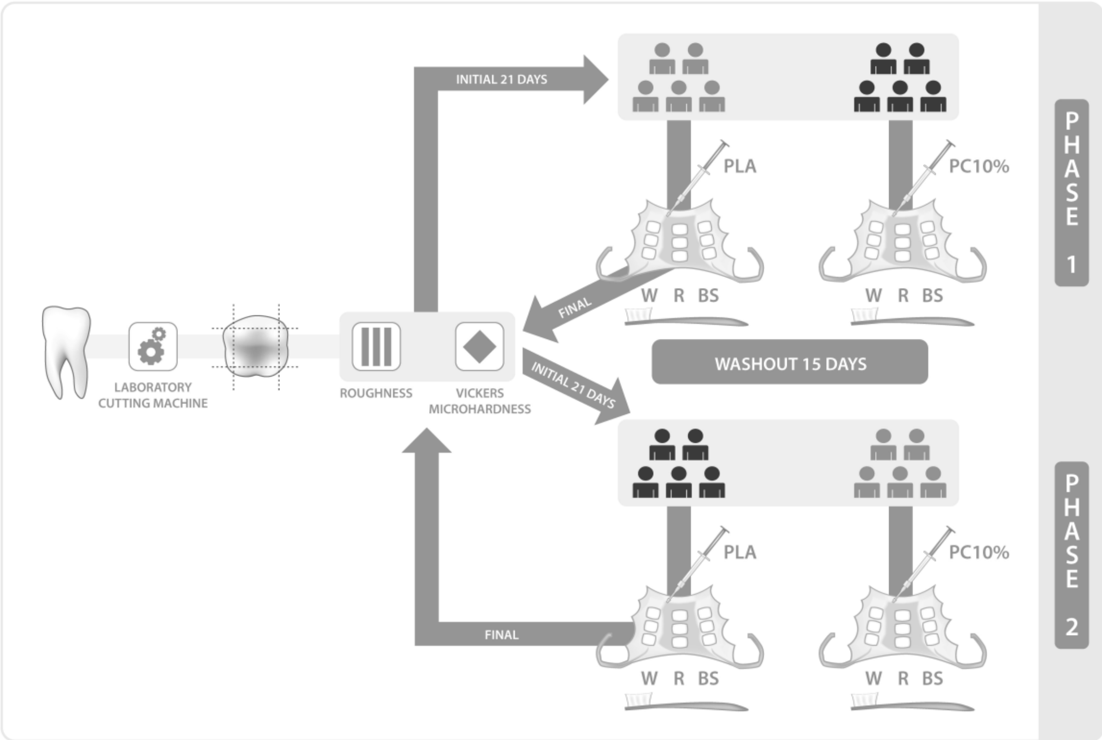
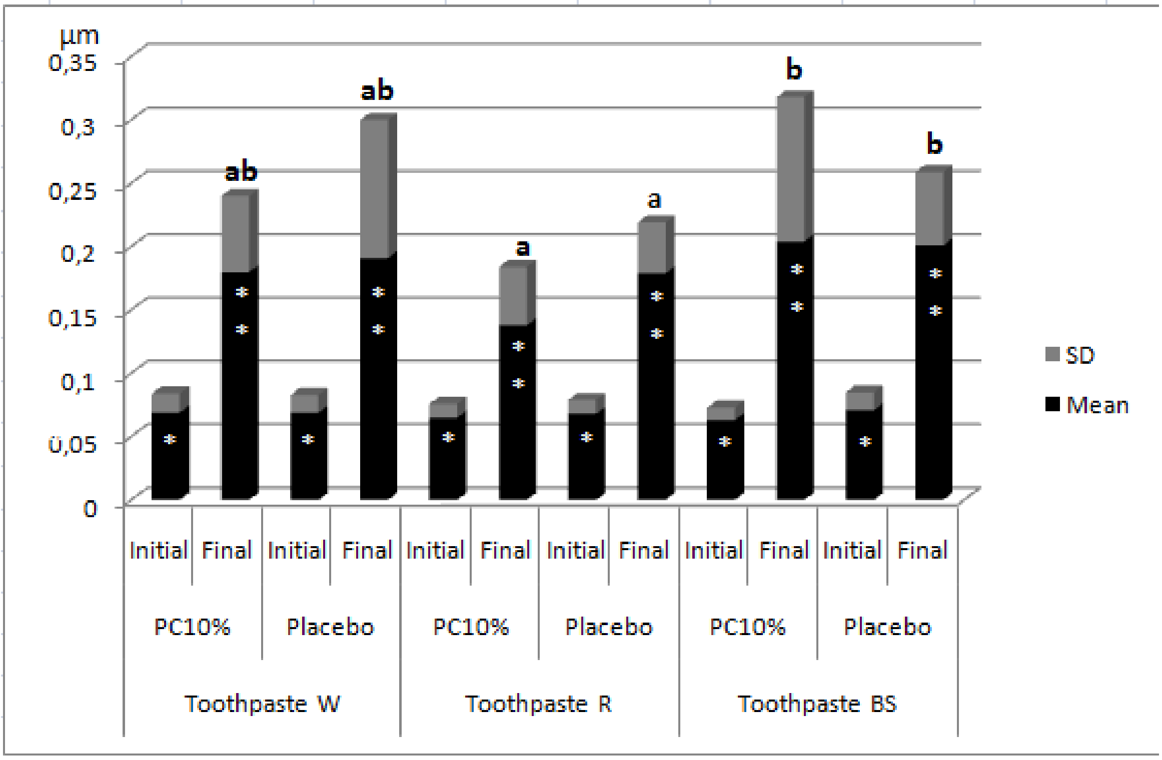


Figure 1: Schematic Drawing of the experimental design – Each human tooth was cut along the long axis of the buccal, lingual, mesial and distal surfaces using a laboratory cutting machine with a low-speed water cooler diamond saw to obtain enamel fragments (4mm X 4mm X 2mm). Each fragment was measured with a computerized roughness tester and a HMV hardness tester before and after each treatment period. In the first phase (21 days), while five volunteers applied the bleaching agent, the other five volunteers applied the placebo agent on the intraoral appliance. They brushed the fragments in the center of palate area with toothpaste R, right premolar area with toothpaste W and left premolar area with toothpaste BS. After 21 days, they were submitted to a washout period of 15 days. After this they received the second intraoral appliance for experimental phase 2. This time the volunteers used the treatment agent they had not received in experimental phase 1 for another 21 days. In the end of this phase, the fragments were removed with tungsten carbide instruments.

Table 1: Composition and manufacturer of each treatment agent and toothpastes.

Treatment Agents/Toothpaste	Composition	Manufacturer
Perfect Bleach	10% carbamide peroxide, potassium, menthol, fluoride* (pH~6.0).	VOCO – Cuxhaven, Germany
Carbopol Gel	Carbopol	Pharmacus, Porto Alegre, RS, Brazil
Regular toothpaste: Colgate Máxima Proteção Anticáries, identified by “R”.	Calcium Carbonate, water, Sorbitol, Sodium Lauryl Sulfate, Sodium Monofluorophosphate (1450 ppm fluorine), flavor, Cellulose gum, Tetrasodium Pyrophosphate, Sodium Silicate, Sodium Saccharin, Methylparaben, Propylparaben*.	Colgate-Palmolive Indústria e Comércio Ltda., São Bernardo do Campo – SP, Brazil
Whitening fluoride toothpaste: Colgate Total 12 Whiteness Gel identified by “W”.	Water, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, PVM/MA Copolymer, flavor, Carrageenan, Sodium Hydroxide, Sodium Fluoride (1450 ppm fluorine), Triclosan, Sodium Saccharin, CI 77891, CI 77019, CI 42090*.	Colgate-Palmolive Indústria e Comércio Ltda., São Bernardo do Campo – SP, Brazil
Whitening Fluoride Toothpaste with Baking Soda and Peroxide: Colgate Whitening Oxygen Bubbles Fluoride Toothpaste identified by “BS”.	Glycerin, Hydrated Silica, Propylene glycol, water, sodium bicarbonate, Sodium Monofluorophosphate (0.14% w/v fluoride ion), Pentasodium Triphosphate, Tetrasodium Pyrophosphate, Sodium Lauryl Sulfate, flavor, Sodium Hydroxide, Sodium Saccharin, Carrageenan, Cellulose gum, calcium peroxide, titanium dioxide*.	Colgate/Palmolive Company New York, NY, USA

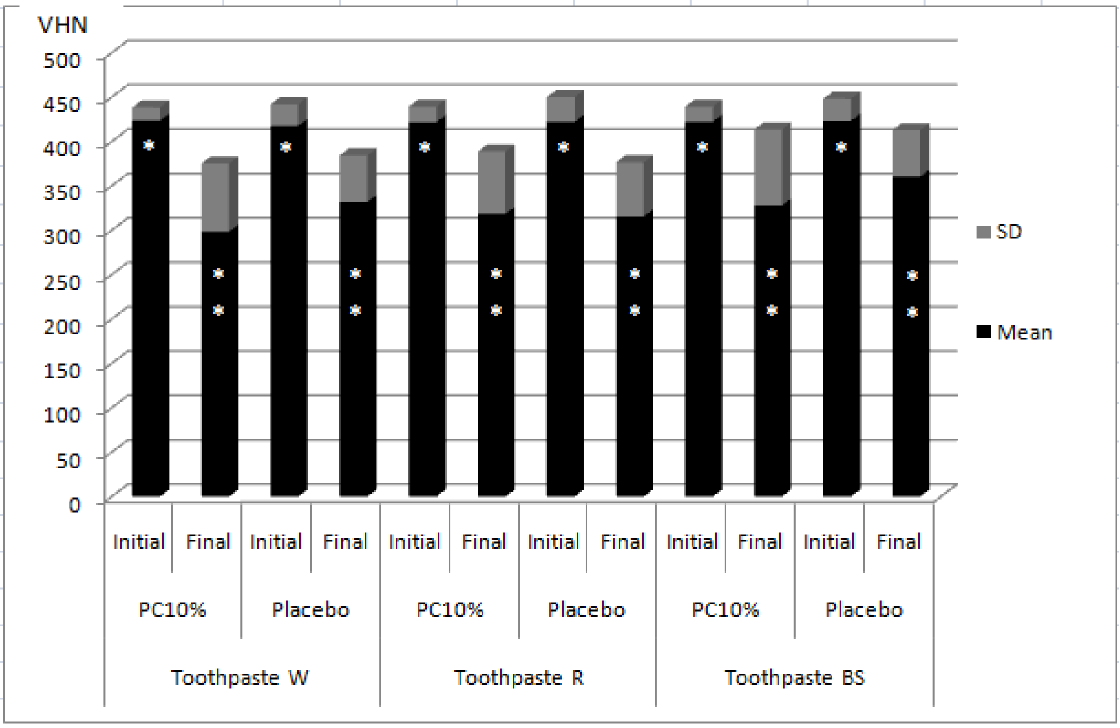
*The manufacturer does not indicate the percentage of each component



Means followed by the same amount of signal (*) did not differ statistically according to paired Student's t-test (α = 0.05).

Means followed by same letters did not differ statistically according to Bonferroni test (α = 0.05).

Figure 2 – Mean values of enamel surface roughness (µm).



Means followed by the same amount of signal (*) did not differ statistically according to paired Student's t-test (α = 0.05).

Figure 3 – Mean values of enamel Vickers microhardness (VHN).

DISCUSSÃO GERAL

Metodologias *in vitro* e *in vivo* são utilizadas para avaliar os efeitos dos agentes clareadores sobre a superfície do esmalte dental. Estas diferentes metodologias podem influenciar os resultados encontrados nos estudos. A interação *in vivo* entre saliva e esmalte é um fator que, geralmente, não é incorporado em pesquisas *in vitro* (CIMILLI; PAMEIJER, 2001). A saliva tem o poder de limpeza, capacidade tampão e habilidade de remineralização (THYLSTRUP; FEJERSKOV, 1998) que pode prevenir os efeitos de desmineralização dos agentes clareadores (JUSTINO; TAMES; DEMARCO, 2004).

O peróxido de carbamida a 10% foi escolhido por ser o agente clareador mais encontrado no mercado (HAYWOOD, 1992) e estar presente na maioria das publicações sobre clareamento caseiro nos últimos 20 anos (KIHN, 2007). Como controle, foi utilizado placebo, que consistiu de gel a base de carbopol (polímero carboxipolimetileno), pelo fato de sua aparência e consistência serem similares ao gel clareador (RODRIGUES *et al*., 2005).

Algumas pesquisas *in vitro* não apontaram alterações significantes na microdureza (McCRACKEN; HAYWOOD, 1995; LOPES *et al.*, 2002) nem na rugosidade (ÇOBANKARA *et al*., 2004) do esmalte dental. Entretanto, quando o peróxido de carbamida a 10% foi associado a dentifrícios abrasivos através da escovação simulada, houve um aumento significante da rugosidade superficial do esmalte dental (WORSCHECH, 2006). Atualmente, trabalhos *in situ* têm sido feitos simulando condições reais dos tratamentos clareadores no meio bucal, avaliando microdureza (ARAÚJO, *et al.*, 2003; MÜLLER ARCARI *et* *al.*, 2005), rugosidade (BASTING *et al*., 2007) e morfologia superficial (TÜRKÜN, *et al.*, 2002; JUSTINO; TAMES; DEMARCO, 2004).

A limpeza do maior número de superfícies do dente é a função primordial dos dentifrícios. Esta limpeza envolve a eliminação de placa e depósitos advindos de resíduos alimentares (WÜLKNITZ, 1997). A escovação normal com o uso de dentifrícios é segura e não afeta o esmalte dental humano (ADDY; HUNTER, 2003). Os agentes abrasivos encontrados nos dentifrícios são carbonato de cálcio, sílica hidratada, bicarbonato de sódio, óxido de alumínio (MEYERS *et al*., 2000) e a mistura entre eles. A adição de substâncias clareadoras como os peróxidos proporcionam um efeito branqueador moderado devido à liberação de oxigênio (RITTER, 2002). O efeito destes agentes sobre os dentifrícios é facilitar a remoção de depósitos encontrados na superfície dos dentes (DAWSON *et al*., 1998). A abrasividade dos dentifrícios deve ser moderada para evitar o desgaste excessivo da superfície do esmalte e da dentina (JOINER *et al*., 2008).

O presente trabalho *in vitro* e *in situ* estudou o efeito de três diferentes dentifrícios fluoretados associados ao peróxido de carbamida a 10% na microdureza e rugosidade superficial do esmalte dental humano.

Para as duas metodologias, as análises iniciais de rugosidade e microdureza não apresentaram diferenças estatísticas entre os grupos. Este resultado foi satisfatório, porque mostrou homogeneidade no início de cada experimento, pois ainda não havia sido realizado nenhum tratamento nas superfícies de esmalte.

Os dois estudos realizados apontaram aumento da rugosidade superficial do esmalte quando comparadas as leituras iniciais das finais, porém este aumento foi significativo apenas para o estudo *in situ*.

No estudo *in situ,* foi observado que o peróxido de carbamida a 10% associado aos dentifrícios causou aumento significativo na rugosidade superficial do esmalte. O gel clareador Perfect Bleach apresenta pH 6 e não contém carbopol. Estudos mostraram que o peróxido de carbamida a 10% sem carbopol e com pH próximo da neutralidade não influenciou significativamente na rugosidade do esmalte (ÇOBANKARA *et al.,* 2004; MORAES *et al.,* 2006). No entanto, quando o peróxido de carbamida foi associado a dentifrícios, houve aumentou significante na rugosidade do esmalte em um estudo *in vitro* (WORSCHECH *et al*., 2006). Este resultado corrobora com o presente estudo *in situ*.

A rugosidade do esmalte também aumentou para os grupos dos placebos associados aos dentifrícios. O carbopol (polímero carboxipolimetileno) foi o gel placebo utilizado. Ele foi escolhido por apresentar aparência e consistência similares ao agente clareador, tornando difícil sua identificação por parte dos voluntários (RODRIGUES et al., 2005). O mesmo gel placebo foi utilizado em outros estudos (BASTING *et al.*, 2001; BASTING *et al.*, 2007; FARAONI-ROMANO *et al.,* 2009). Rodrigues *et al*. (2005) sugeriram um efeito destrutivo do carbopol na superfície de esmalte. Dessa forma, supõe-se que, nos grupos placebo, houve aumento da rugosidade do esmalte devido à possível ação do carbopol e dos agentes abrasivos dos dentifrícios.

O fator dentifrício foi significante na rugosidade superficial do esmalte no estudo *in situ*. Os dentifrícios utilizados nesta pesquisa foram: Colgate máxima proteção anti-cáries (R), Colgate Total 12 Whiteness Gel (W) e Colgate Whitening Oxygen Bubbles (BS). Este último, composto de sílica hidratada, peróxido de cálcio e bicarbonato de sódio, deixou o esmalte significativamente mais rugoso, quando comparado ao dentifrício R, que possui o carbonato de cálcio, um agente de polimento de menor poder abrasivo (ANDRADE JUNIOR, 1998). O bicarbonato de sódio contribui para a remoção de manchas (MANDEL, 1998), o peróxido de cálcio proporciona um efeito branqueador devido à liberação de oxigênio e a sílica hidratada é um agente abrasivo intermediário (WÜLKNITZ, 1997). A combinação de agentes abrasivos em um único dentifrício, como o dentifrício BS, pode explicar este aumento significante da rugosidade da superfície do esmalte. Segundo Haywood (1996), há um grupo de dentifrícios clareadores que são mais abrasivos que os dentifrícios regulares. Desta forma, o uso excessivo de abrasivos combinado à escovação mais vigorosa não somente atua na remoção de manchas, como também pode promover um desgaste exagerado do esmalte.

O dentifrício W, com sílica hidratada, não apresentou diferença estatística dos dentifrícios BS e R no estudo *in situ*. Wülknitz (1997) avaliou a abrasividade e poder de limpeza de diferentes dentifrícios. Este autor considerou a sílica hidratada um agente abrasivo intermediário, sendo mais eficiente na remoção de manchas do esmalte e dentina quando comparado a outros abrasivos. O resultado obtido, neste estudo, para o dentifrício W pode ter ocorrido devido à abrasividade moderada da sílica hidratada.

De acordo com Bollen, Lambrechts e Quirynen (1997) rugosidades acima de 0,20 µm favorecem o acúmulo de placa bacteriana. No presente estudo, nenhum dos dentifrícios associados com os tratamentos causou rugosidade acima desse valor. Apesar de haver diferença estatística entre o dentifrício BS (0,20 µm) e o dentifrício R (0,15 µm), acredita-se que a diferença de apenas 0,05 µm não teria significância clínica.

Após a exposição ao peróxido de carbamida a 10%, estudos *in vitro* não demonstraram mudanças na microdureza do esmalte (MURCHISON; CHARLTON; MOORE, 1992; SEGHI; DENRY, 1992; LOPES *et al.,* 2002). O estudo de McCracken e Haywood (1996) mostrou que o peróxido de carbamida a 10% provocou perda de 1,06 µg/mm^2 de cálcio no esmalte, a qual não seria clinicamente significante. Contudo, no presente estudo *in situ*, a associação do peróxido de carbamida a 10% com dentifrícios causou uma redução significante na microdureza. Para os grupos placebo também houve redução significante na microdureza do esmalte.

O fator dentifrício não foi significante, evidenciando que as diferentes composições dos dentifrícios não tiveram influência na microdureza superficial do esmalte. No presente estudo *in situ*, foram utilizados apenas dentifrícios fluoretados. Watanabe *et al.* (2005) avaliaram dentifrícios clareadores com e sem flúor. Os dentifrícios não fluoretados apresentaram uma grande perda mineral, enquanto os fluoretados exibiram um efeito cariostático devido ao processo de remineralização. Além disso, a escovação com dentifrícios fluoretados ajuda a prevenir uma diminuição na microdureza durante o tratamento clareador (WIEGAND; SCHREIER; ATTIN, 2007). Pode-se supor que a ausência de fluoretos nos dentifrícios pode resultar em valores baixos de microdureza e valores altos de rugosidade do esmalte. Assim, a presença do flúor no gel de peróxido de carbamida a 10% pode ter tido uma influência positiva nos resultados desta pesquisa. Attin *et al.* (2006) revelaram que o contato do flúor com a superfície do dente foi importante na remineralização do esmalte e aumento da microdureza.

Pelo fato de ser um estudo *in situ*, provavelmente a saliva teve influência importante nos resultados. A combinação de um estudo *in situ* e *in vitro,* para avaliar o efeito de três diferentes géis de peróxido de carbamida a 10% na microdureza do esmalte, indicou que o efeito do gel clareador pode ser modificado pela ação da saliva humana no processo de remineralização do esmalte (SHANNON *et al*., 1993). Outros estudos também concordam que a saliva pode reverter a perda mineral provocada pelo tratamento clareador (LOPES *et al.*, 2002; RODRIGUES *et al*., 2005).

Para o presente estudo *in vitro*, os grupos que receberam peróxido de carbamida a 10% associado aos dentifrícios apresentaram aumento da rugosidade superficial do esmalte, embora este resultado não tenha sido estatisticamente significativo. No entanto, no estudo *in vitro* de Worschech *et al.* (2006), também foi observado aumento na rugosidade superficial do esmalte quando associado o peróxido de carbamida a 10% com dentifrício contendo carbonato de cálcio como agente abrasivo com e sem flúor, sendo que este aumento foi estatisticamente significante. Diferenças quantitativas na rugosidade superficial do esmalte podem decorrer de uma série de variáveis, como o tempo de escovação utilizado nos estudos. Worschech *et al.* (2006)

utilizaram escovações diárias por 180 segundos, com velocidade de 250 ciclos/minuto, durante 56 dias. De acordo com Sexton e Philips (1951), para cada sessão de escovação, em determinada área, o indivíduo realiza 15 ciclos de escovação. Dessa forma, o presente estudo *in vitro* tentou reproduzir o número de ciclos realizados diariamente por uma pessoa adulta. Para isso, foi utilizada uma máquina de escovação simulada com velocidade de 250 ciclos/minuto, sendo que duas escovações diárias resultaram em 30 ciclos com duração total de 7 segundos, durante 21 dias.

Independentemente do dentifrício usado, o agente placebo provocou um maior valor de rugosidade (0,16 µm) se comparado ao peróxido de carbamida a 10% (0,12 µm). Uma justificativa para este achado, além do próprio efeito dos dentifrícios, é o fato do gel placebo ser composto de carbopol. Um estudo *in situ* de Rodrigues et al. (2005) verificou redução significante na microdureza do esmalte quando aplicado placebo a base de carbopol. Os autores sugeriram um efeito destrutivo do carbopol sobre a superfície do esmalte. Apesar dos estudos relacionarem o carbopol com a microdureza do esmalte, acredita-se que o mesmo também possa influenciar na rugosidade superficial deste substrato. No entanto, a diferença de apenas 0,04 µm na média de rugosidade superficial do esmalte não teria uma diferença clínica importante.

O grupo tratado com placebo e escovado com o dentifrício BS foi o único que apresentou um aumento significante na rugosidade superficial do esmalte. Este resultado poderia ser explicado pela soma do efeito destrutivo do carbopol presente no gel placebo, pela ausência de flúor no placebo e pela composição do dentifrício BS, que possui uma combinação de agentes abrasivos: sílica hidratada e bicarbonato de sódio e ainda um agente clareador, o peróxido de cálcio. Possivelmente esse maior valor de rugosidade influenciou a análise estatística, de forma que o fator dentifrício foi significante.

O dentifrício BS (0,16 µm) causou rugosidade do esmalte estatisticamente superior ao dentifrício W (0,13 µm). No entanto, acredita-se que a diferença de apenas 0,03 µm não teria significância clínica. O dentifrício R (0,14 µm) não diferiu estatisticamente dos dentifrícios BS e W. Este último contém sílica hidratada que é um agente abrasivo intermediário (WÜLKNITZ, 1997) e o dentifrício R possui o carbonato de cálcio que é um agente de polimento de menor poder abrasivo (ANDRADE JUNIOR *et al.*, 1998). Possivelmente a combinação de agentes abrasivos contidos no dentifrício BS provocou maior rugosidade superficial no esmalte (0,16 µm). Além disso, analisando somente os grupos do peróxido de carbamida a 10% associado aos dentifrícios, observa-se que não houve uma diferença numericamente importante na rugosidade entre os grupos, evidenciando um comportamento similar dos dentifrícios quando associados com o peróxido de carbamida.

Uma redução significante na microdureza foi observada para todos os grupos quando o peróxido de carbamida a 10% foi associado a escovações com diferentes dentifrícios. Estudos *in vitro* têm observado que a aplicação desse agente clareador isoladamente, excluindo os procedimentos de escovação, resultou em mudanças na microdureza do esmalte (MURCHISON; CHARLTON; MOORE, 1992; LOPES *et al.*, 2002; MORAES *et al.,* 2006). Porém, ainda não há um consenso estabelecido na literatura sobre o possível efeito dos agentes clareadores de reduzir ou não a microdureza do esmalte.

Um estudo conduzido por Attin *et al.* (2009) avaliou 50 publicações com 166 medições de microdureza do esmalte, das quais 51% mostraram uma redução da microdureza comparado ao *baseline* imediatamente após o clareamento, e 49% das aferições não evidenciaram redução na microdureza.

Os resultados do presente estudo *in vitro* mostraram que, para o tratamento com o peróxido de carbamida a 10% associado a escovações, a menor microdureza foi obtida com o dentifrício R (285 VHN), seguido do dentifrício W (328 VHN) e o dentifrício BS (334 VHN), não havendo diferença estatística entre esses valores. O dentifrício BS é o único que contém um agente clareador na composição (peróxido de cálcio). Pelo fato dos agentes clareadores terem capacidade de causar uma perda mineral da superfície do esmalte (McCRACKEN; HAYWOOD, 1996), esperava-se que o dentifrício BS, associado ao gel clareador, causaria menor microdureza do esmalte. No entanto, esse dentifrício teve o maior valor de microdureza, sugerindo que o agente clareador contido no dentifrício não teve efeito significante na microdureza do esmalte. Portanto, as diferentes composições dos dentifrícios não foram um fator significante na microdureza do esmalte quando associado ao peróxido de carbamida a 10%.

Para os grupos placebo também foi observada uma redução significante na microdureza do esmalte quando associado aos dentifrícios. Provavelmente, isto se deve ao efeito destrutivo do carbopol na superfície do esmalte, uma vez que este polímero acídico pode causar desmineralização do esmalte (VAN DER REIJDEN *et al.*, 1997). O efeito abrasivo dos dentifrícios também poderia ser uma das causas para a redução da microdureza nos grupo tratados tanto com o placebo quanto com o peróxido de carbamida. Os abrasivos removem parte da superfície mineral do esmalte, desgastando esse substrato (PICKLES *et al*., 2005; PARRY *et al*., 2008), levando a uma maior proximidade da dentina e, consequentemente, à redução da microdureza. Para o tratamento placebo, os dentifrícios R (327 VHN) e BS (303 VHN) apresentaram valores de microdureza superficial finais estatisticamente maiores que o dentifrício W (222 VHN). De acordo com a composição do dentifrício BS, que possui dois agentes abrasivos, esperava-se que o mesmo apresentasse o menor valor de microdureza superficial.

Entre os tratamentos, os dentifrícios W e BS apresentaram valores de microdureza superficial menores para o tratamento placebo quando comparados ao tratamento com peróxido de carbamida a 10%, havendo diferença estatística significante apenas para o dentifrício W. Possivelmente os abrasivos contidos nesses dentifrícios tiveram efeito mais abrasivo sobre o esmalte em associação ao placebo por este ser à base de carbopol. Além disso, o carbopol pode agir como uma barreira impermeável, inibindo a penetração da saliva artificial no esmalte e evitando a recuperação dos valores normais de microdureza (BASTING; RODRIGUES; SERRA, 2003). Uma redução da microdureza, após o tratamento com placebo a base de carbopol, foi observada por alguns autores (BASTING; RODRIGUES; SERRA, 2003; RODRIGUES *et al.*, 2005; BASTING; RODRIGUES; SERRA, 2005). Outro fator que pode ter influenciado na microdureza é a ausência de flúor no placebo. Attin *et al.* (2006) revelaram ser importante o contato do flúor na superfície do dente para o processo de remineralização do esmalte e aumento da microdureza superficial. Em relação ao dentifrício R, este apresentou um resultado controverso devido aos valores de microdureza terem sido menores para o tratamento com o peróxido de carbamida a 10% em comparação ao placebo. É difícil encontrar uma explicação plausível para este achado, assim como para o fato do dentifrício BS não ter tido o menor valor de microdureza superficial. Embora cuidados tenham sido tomados para controlar as variáveis da escovação simulada, não se pode descartar a possibilidade da influência de algumas variáveis confundentes. A ação das escovas, a força e velocidade de escovação, e a energia necessária para manter as partículas abrasivas do *slurry* em suspensão são variáveis importantes que podem afetar a determinação da perda de tecido. A respeito dessas variáveis, a homogeneidade do *slurry* é considerada uma das mais difíceis de ser controlada (PARRY *et al*., 2008).

Diversos fatores podem influenciar na rugosidade e microdureza do esmalte durante o tratamento clareador. Portanto, sugerem-se mais estudos para determinar a influência de outros fatores sobre essas variáveis dependentes.

CONCLUSÕES

Respeitando as limitações destes estudos:

1) Os resultados *in situ* sugerem que:

- O peróxido de carbamida a 10% associado aos dentifrícios R, W ou BS causou aumento significativo na rugosidade superficial do esmalte e redução significativa na microdureza superficial do esmalte;

- O dentifrício BS apresentou a maior rugosidade superficial, seguido do W e R;

- A combinação do uso do peróxido de carbamida a 10% com os dentifrícios R, W ou BS provocou uma redução similar da microdureza superficial do esmalte.

2) Os resultados *in vitro* sugerem que:

- A escovação com os dentifrícios R, W e BS, durante 21 dias de tratamento clareador caseiro, não alterou a rugosidade superficial do esmalte, mas provocou alterações significativas na microdureza superficial do esmalte;

- A escovação com o dentifrício BS apresentou valores de rugosidade superficial finais significativamente maiores que a escovação feita com o dentifrício W;

- Durante o tratamento clareador caseiro, os dentifrícios R, W e BS se comportaram da mesma forma para a microdureza superficial do esmalte.

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ANEXOS

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 03 de dezembro de 2007

O Projeto de: Tese

Protocolado sob nº: 0071/07

Intitulado: Estudo in situ e in vitro do efeito do peróxido de carbamida 10% associado a dentrifrícios na dureza e rugosidade superficial do esmalte

Pesquisador Responsável: Profa. Dra. Ana Maria Spohr

Pesquisadores Associados: Carolina França de Medeiros

Nível: Doutorado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *09 de novembro de 2007.*

Este projeto deverá ser imediatamente encaminhado ao CEP/PUCRS

2007.

Profa. Dra. Marília Gerhardt de Oliveira
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 209
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900
Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

CEP/PUCRS

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM PESQUISA

Ofício 1531/07-CEP

Porto Alegre, 26 de dezembro de 2007.

Senhor(a) Pesquisador(a)

O Comitê de Ética em Pesquisa da PUCRS apreciou e aprovou seu protocolo de pesquisa registro CEP 07/04055, intitulado: "**Estudo in situ e in vitro do peróxido de carbamida 10% associado a dentrifícios na dureza e rugosidade superficial do esmalte".**

Sua investigação está autorizada a partir da presente data.

Relatórios parciais e final da pesquisa devem ser entregues a este CEP.

Prof. Dr. José Roberto Goldim
COORDENADOR DO CEP-PUCRS

Ilmo(a) Sr(a)
Profa Ana Maria Spohr
N/Universidade

PUCRS
Campus Central
Av. Ipiranga, 6690 – 3ºandar – CEP: 90610-000
Sala 314 – Fone Fax: (51) 3320-3345
E-mail: cep@pucrs.br
www.pucrs.br/prppg/cep

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
BANCO DE DENTES HUMANOS

Porto Alegre, 05 de dezembro de 2007.

Ao Comitê de Ética em Pesquisa da PUCRS:

O Banco de Dentes Humanos da Faculdade de Odontologia da PUCRS declara que fornecerá 90 terceiros molares humanos necessários ao desenvolvimento do projeto de pesquisa intitulado "Estudo *in situ* e *in vitro* do efeito do peróxido de carbamida a 10% associado a dentifrícios na dureza e rugosidade superficial do esmalte" sob responsabilidade dos pesquisadores Carolina França de Medeiros e Ana Maria Spohr.

Os dentes somente serão liberados após aprovação do projeto pelo Comitê de Ética em Pesquisa da PUCRS.

Prof. Dr. João Batista Blessmann Weber
Responsável pelo Banco de Dentes da FO-PUCRS

Av. Ipiranga, 6681 - Prédio 6
Porto Alegre - RS
Fone: (51)3320-3562
E mail: odontologia@pucrs.br

CONSENTIMENTO LIVRE E ESCLARECIDO

Convidamos você a participar de uma pesquisa para estudar o efeito de um agente clareador, o peróxido de carbamida a 10%, associado a diferentes cremes dentais, sobre a dureza e rugosidade superficial do esmalte dental.

Se você decidir participar desta pesquisa, nós inicialmente realizaremos uma moldagem da sua arcada superior com alginato, para obtermos um modelo em gesso. Sobre este modelo, será confeccionado um dispositivo intrabucal (parecido com aqueles aparelhos removíveis que são utilizados por pacientes em tratamento ortodôntico). Neste aparelho intrabucal serão fixados nove fragmentos de esmalte obtidos de dentes extraídos de outras pessoas, os quais serão esterilizados adequadamente em autoclave, não fornecendo nenhum risco de contaminação. Antes de deitar-se a noite, e com o aparelho intrabucal fora da boca, você deverá aplicar o produto clareador sobre os fragmentos dentários e deixar este agindo toda a noite. Ao levantar-se, pela manhã, você deverá remover o gel clareador, limpar o aparelho e escovar os fragmentos dentários com diferentes tipos de cremes dentais. Terminado este procedimento, você deverá colocar o aparelho na boca e permanecer com o mesmo todo o dia. É permitida a remoção do aparelho para alimentar-se e para realizar o procedimento de higiene rotineira (escovação dos seus dentes), o qual será feito com um creme dental não fluoretado fornecidos pelos pesquisadores. O procedimento de escovação dos fragmentos de esmalte será realizada duas vezes ao dia (manhã e antes de dormir). Na primeira etapa você precisará utilizar o aparelho intrabucal durante três semanas (21 dias). Passado este período, você receberá um “tempo de descanso” de duas semanas, iniciando então a segunda etapa do estudo. Nesta segunda etapa, será fornecido um novo aparelho intrabucal, com as mesmas características do anterior, e serão realizados os mesmos procedimentos, também durante 21 dias. A pesquisa consta de dois momentos porque iremos verificar a ação de dois produtos diferentes. Nenhum dos produtos ou dispositivos utilizados na pesquisa fornecem qualquer tipo de risco ou malefício para os seus dentes, oclusão ou saúde em geral. Todos os materiais serão fornecidos pelos pesquisadores.

Se você participar deste estudo, você pode nos ajudar a verificar se os cremes dentais presentes no mercado podem fornecer algum tipo de alteração significativa ao esmalte dental quando é realizado um procedimento de clareamento.

A sua participação é confidencial. Caso publicarmos os resultados em uma revista, você não será identificado. Sua decisão de participar do estudo é voluntária e você é livre para escolher não participar do estudo ou parar a qualquer momento. Caso você escolha não participar ou parar os testes, isto não afetará qualquer futuro atendimento na Faculdade de Odontologia da PUCRS.

Se você tiver alguma pergunta ou dúvida, sinta-se a vontade para perguntar. Caso tiver perguntas mais tarde, poderá entrar em contato com a Profa. Ana Maria Spohr pelo telefone (51) 9995-0465 ou com a aluna Carolina França de Medeiros pelo telefone (51) 8110-1996.

Nós lhe daremos uma cópia assinada deste formulário. Sua assinatura indica que você decidiu participar desta pesquisa e que você leu e entendeu as informações acima explicadas a você.

__________________________________ ______________________________

Assinatura do participante Assinatura da testemunha

__________________________________ ______________________________

Assinatura da pessoa obtendo o consentimento Data Hora

____

Nome e cargo da pessoa obtendo o consentimento

INSTRUÇÕES PARA OS VOLUNTÁRIOS

- Você utilizará a placa durante todo o dia, por 21 dias, removendo somente para alimentar-se, higienizar-se e durante o período de aplicação do gel. Durante os 21 dias de uso da placa sua higiene bucal será realizada apenas com o dentifrício PHILLIPS por não conter flúor. Porém, no período de *wash-out* você poderá voltar a utilizar seu dentifrício.

- Antes de dormir, remova a placa da boca e lave em água corrente. Faça a escovação de cada trio de fragmentos da seguinte forma:

- Trio da esquerda: escove com a pasta “BS” por 30 segundos e lave em água corrente;

- Trio central: escove com a pasta “R” por 30 segundos e lave em água corrente;

- Trio da direita: escove com a pasta “W” por 30 segundos e lave em água corrente.

- Aplique pequena quantidade de gel sobre todos os fragmentos dentários. Deixe o gel aplicado na placa, fora da boca, durante toda a noite, aproximadamente 8 horas. Ao levantar, remova o gel em água corrente e realize novamente a escovação dos fragmentos.

- Recoloque a placa em boca durante todo o dia, por aproximadamente 16 horas.

- A escovação dos fragmentos deverá ser feita duas vezes ao dia (ao levantar no período da manhã e antes de dormir).

- Tenha cuidado para não trocar as pastas adequadas para cada trio de fragmentos.

- Qualquer dúvida não hesite em ligar para Carolina (51-81101996).