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Avaliação do desgaste superficial do esmalte e da dentina radicular submetidos ao tratamento clareador: *in vitro* e *in situ*

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Tese apresentada à Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo, para obtenção do título de Doutor em Odontologia.

Área de concentração: Odontologia Restauradora - Dentística
Orientadora: Profa. Dra. Mônica Campos Serra

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FOLHA DE APROVAÇÃO

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“O verdadeiro amor não se conhece por aquilo que exige, mas por aquilo que oferece.”

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“O sucesso consegue-se com decisão, confiança, persistência; e não, com desânimo, indecisão, lamúrias.”

Paderewski

“ A gente pode morar numa casa mais ou menos, numa rua mais ou menos, numa cidade mais ou menos, e até ter um governo mais ou menos.

A gente pode dormir numa cama mais ou menos, comer um feijão mais ou menos, ter um transporte mais ou menos, e até ser obrigado a acreditar mais ou menos no futuro.

A gente pode olhar em volta e sentir que tudo está mais ou menos...

Tudo bem!

O que a gente não pode mesmo, nunca, de jeito nenhum...

é amar mais ou menos, sonhar mais ou menos, ser amigo mais ou menos, namorar mais ou menos, ter fé mais ou menos, e acreditar mais ou menos.

Senão a gente corre o risco de se tornar uma pessoa mais ou menos."

Chico Xavier

RESUMO

FARAONI-ROMANO, J.J. **Avaliação do desgaste superficial do esmalte e da dentina radicular submetidos ao tratamento clareador: *in vitro* e *in situ*.** 2008. 59f. Tese (Doutorado) - Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2008.

Devido a alterações químico-estruturais causadas pelo clareamento, os substratos dentais poderiam tornar-se mais susceptíveis a perda tecidual, principalmente se expostos a desafios erosivo/abrasivos. Desta forma, o presente estudo teve como objetivos: 1) analisar *in vitro*, se o esmalte e a radicular dentina clareada com diferentes agentes e concentrações, apresenta uma maior susceptibilidade ao desgaste, quando submetido a ciclos de erosão e abrasão; 2) comparar o efeito da aplicação de um agente clareador a base de peróxido de carbamida a 10% a um placebo no desgaste do esmalte e da dentina radicular, através de um modelo *in situ*. Os resultados do estudo *in vitro* mostraram que, independentemente do agente usado, o clareamento não aumentou o desgaste do esmalte frente a episódios erosivo-abrasivos. Na dentina, o desgaste foi dependente do agente clareador aplicado. Baseado no protocolo *in situ* adotado, o peróxido de carbamida a 10% não causou maior desgaste superficial no esmalte, mas aumentou a perda de tecido dentinário comparado ao placebo. Pode-se concluir que, em termos de desgaste superficial, o esmalte não foi afetado pelo tratamento clareador, enquanto a dentina mostrou-se mais susceptível. Assim, sugerem-se cuidados adicionais na seleção do agente clareador em situações clínicas que apresentam dentina radicular exposta.

Palavras-chave: Clareamento de dente. Erosão de dente. Abrasão dentária. Corrosão dentária. Desgaste. *In vitro*. *In situ*. Esmalte dentário. Dentina.

ABSTRACT

FARAONI-ROMANO, J.J. **Evaluation of enamel and root dentin surface wear submitted to bleaching treatment: in vitro and in situ.** 2008. 59f. Thesis (Doctoral) - Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2008.

Due to the chemical and microstructural alterations caused by bleaching, the dental substrates can become more susceptible to tissue loss, mainly if exposed to erosive/abrasive challenges. Therefore, the present study had the following objectives: 1) to analyze *in vitro*, if enamel and root dentin that had been bleached with different agents and concentrations, were at increased risk of wear when submitted to cycles of erosion and abrasion; 2) to compare the effect of the application of a 10% carbamide peroxide bleaching agent to a placebo on wear of enamel and root dentin, through an *in situ* model. The results of the *in vitro* study showed that independent of agent used, the bleaching demonstrated no increase in the wear of enamel when exposed to the erosive-abrasive episodes. In dentin, the wear was dependent on the bleaching agent applied. Based on the *in situ* protocol adopted, the 10% carbamide peroxide did not cause higher wear on the enamel, but increased the wear of the root dentin compared to the placebo. It could be concluded that in terms of superficial wear, enamel was not affected by bleaching treatment, while dentin showed to be more susceptible. Thus, additional caution is suggested in the choice of the bleaching agent when root dentin is exposed.

Keywords: Tooth bleaching. Tooth erosion. Tooth abrasion. Dental etching. Wear. *In vitro*. *In situ*. Dental enamel. Dentin.

SUMÁRIO

1. INTRODUÇÃO GERAL

A crescente valorização da estética, associada aos conceitos modernos da Odontologia minimamente invasiva, tem tornado o clareamento dental um dos tratamentos mais realizados na área da Odontologia estética e um dos mais desejados pelos pacientes.

Na dependência da causa do manchamento dental, diagnosticado através de um exame clínico minucioso e uma anamnese detalhada, a técnica de clareamento dental, se indicada, dependerá da vitalidade ou não dos dentes. Em relação aos dentes vitais, as técnicas mais difundidas são as de consultório e a caseira supervisionada pelo dentista, também conhecidas por *in-office* e *at-home*, respectivamente. As principais diferenças entre estas técnicas são relativas à concentração do produto clareador, tempo e modo de aplicação.

O clareamento de consultório requer a realização de isolamento absoluto ou aplicação de uma barreira gengival. Emprega-se alta concentração de peróxido de hidrogênio, que varia de 35 a 50%, ou peróxido de carbamida na concentração de 35 a 40%. No clareamento de consultório, os resultados não dependem da cooperação do paciente quanto ao uso do gel. Por outro lado, a principal desvantagem desta técnica é a sensibilidade dental relatada pela maioria dos pacientes devido à alta concentração utilizada, e a irritação gengival no caso de um isolamento inadequado.

O clareamento caseiro supervisionado pelo dentista, foi introduzido em 1989 por Haywood e Heymann, e tem tido ampla aceitação. É uma técnica considerada prática, segura e efetiva, com efeito clínico comprovado, que requerer reduzido tempo clínico de consultório, além de oferecer menor custo para os pacientes. O agente clareador mais utilizado nesta técnica é o peróxido de carbamida, especialmente na concentração 10%, embora, produtos à base de peróxido de hidrogênio e carbamida em diferentes concentrações, estão sendo lançados no mercado. O paciente aplica o agente numa moldeira, e a utiliza durante algumas horas do dia ou da noite por um período de 2 a 6

semanas, sob supervisão do cirurgião dentista. É indicada para o clareamento de vários dentes ou arcadas completas. A sensibilidade dental é menos freqüente, mas quando ocorre é mais branda que na técnica de consultório devido à concentração aplicada.

Apesar do clareamento dental ser considerado um procedimento seguro e efetivo, seus efeitos têm sido extensamente estudados no esmalte e em menor proporção no tecido dentinário quanto às alterações na micromorfologia superficial, no conteúdo mineral e na microdureza. No entanto, há poucos relatos do efeito dos peróxidos na perda tecidual do esmalte e nenhum para dentina radicular.

A perda de tecidos duros por desgaste pode ter como conseqüência, danos irreversíveis aos pacientes, como problemas funcionais e estéticos, além de sensibilidade dentinária, necessitando muitas vezes de tratamento restaurador. O processo de desgaste dental pode ser considerado um fenômeno complexo e multifatorial, por ser o resultado da interação entre os processos de abrasão e erosão, os quais podem potencializar o desgaste nos tecidos dentais duros.

Para verificar se o clareamento dental influencia o desgaste erosivo-abrasivo do esmalte e da dentina radicular, foi delineado e desenvolvido o estudo *in vitro*: *"Erosive/abrasive wear of enamel and dentin submitted to different bleaching treatments"* (Capítulo 1).

Comparado aos estudos laboratoriais, os estudos *in situ* refletem resultados mais próximos à situação clínica, devido aos aspectos biológicos presentes (composição, fluxo e capacidade tampão da saliva, além de formação de película adquirida) e comportamentais (diferentes hábitos alimentares, de higienização e presença de flúor). Alguns estudos já demonstraram alterações na microdureza e na rugosidade do esmalte após o clareamento dental, porém é desconhecido o efeito do agente clareador no desgaste superficial dos tecidos dentais duros. Desta forma, foi conduzido o trabalho: "Effect of a 10% carbamide peroxide on wear resistance of enamel and dentin: *in situ* study" (Capítulo 2).

2. PROPOSIÇÃO

Este estudo, composto por dois artigos científicos, teve como objetivo geral avaliar o desgaste superficial do esmalte e da dentina radicular submetidos ao clareamento dental. Os objetivos específicos foram:

- verificar, *in vitro*, se o clareamento com diferentes agentes empregados na técnica caseira e de consultório incrementa o desgaste erosivo-abrasivo do esmalte e da dentina radicular (Capítulo 1);

- avaliar, através de um modelo experimental *in situ,* o efeito da aplicação de um agente clareador a base de peróxido de carbamida a 10% comparado a um placebo, no desgaste do esmalte e da dentina radicular (Capítulo 2).

3. CAPÍTULOS

3.1. CAPÍTULO 1

SUBMETIDO À PUBLICAÇÃO NO PERIÓDICO JOURNAL OF DENTISTRY (ANEXO A)

EROSIVE/ABRASIVE WEAR OF ENAMEL AND DENTIN SUBMITTED TO DIFFERENT BLEACHING TREATMENTS

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SHORT TITLE: Wear of enamel and dentin after bleaching treatment

KEYWORDS: enamel, root dentin, at-home bleaching, in-office bleaching, erosion, abrasion, surface wear

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SUMMARY

Objectives: To evaluate the erosive/abrasive wear of enamel and dentin previously submitted to bleaching with different agents.

Methods: Slabs of bovine enamel and dentin (3x3x2mm) were embedded in polyester resin in a ring mold, ground flat and polished. Half of the specimen surface was covered with nail varnish to obtain a reference surface. According to a randomized complete block design, 10 enamel and 10 dentin slabs were allocated to be bleached with carbamide peroxide-containing agents (CP) 10%, 15% or 37%; 35% hydrogen peroxide agent (HP); or exposed to artificial saliva (control). After bleaching treatment, specimens were submitted to 10 erosive/abrasive cycles, each one composed of immersion in an acid drink (Sprite Light) or distilled water, exposure to artificial saliva, and simulation of brushing strokes. Surface wear was evaluated profilometrically.

Results: For enamel, ANOVA did not demonstrate significant interaction between the factors under study ($p=0.1861$) or significant statistical difference by the use of different bleaching agents ($p=0.3702$), whereas the groups exposed to the acidic drink presented higher wear ($p=0.0001$). For dentin, ANOVA showed interaction between erosive/abrasive cycles and bleaching ($p=0.0491$). Tukey's test revealed that the bleached root dentin, except the CP37%, which did not differ from the control, showed higher wear when submitted to erosive/abrasive challenges. CP10% and CP15% had the highest wear and HP35% showed an intermediate wear.

Conclusions: Bleaching did not increase the erosive/abrasive wear of enamel irrespective of the agents tested. However, bleaching can increase the wear of root dentin facing erosive/abrasive challenges, depending on the gel used.

INTRODUCTION

With the current interest in esthetic dentistry, vital bleaching has become the most frequently used treatment modality for improving dental appearance.[1] Basically, two techniques have been proposed for bleaching of vital teeth: at-home and in-office bleaching or even a combination of both.[2] At-home bleaching typically uses a relatively low-concentrated whitening agent in a custom tray for a certain number of hours per day or night, for at least two weeks.[3] In-office bleaching usually uses high concentration of whitening agents,[2,3] for shorter periods. Ten percent carbamide peroxide is the most frequently chosen product for home bleaching,[1] due to its safety and effectiveness.[4] However, agents having 15-16%, 20-22% and 35-38% concentrations of carbamide peroxide have been launched on the market to obtain whitening more quickly.[4]

Owing to the direct contact of the agent with the outer enamel surface and sometimes with the dentin surface, *in vitro* studies have evaluated the effects of different concentrations of carbamide and hydrogen peroxide on dental hard tissues, being the results contradictory. Some studies have reported no alterations in microhardness,[5,6] surface roughness[7-10] and chemical composition[12] of dental substrates. Conversely, in other publications it was found that whitening agents have been capable of altering the microhardness,[13-15] surface roughness[16-18] and chemical composition[17,19] of dental tissues. In addition, increased susceptibility to brushing abrasion has been shown.[20]

People's lifestyle has markedly changed in the last decades with the increase in the total amount and frequency of consumption of acidic foods and drinks, mainly the consumption of soft drinks, an extrinsic factor of dental erosion,[21] which may provide removal and softening of the surface and also dissolution of mineral content.[21] Moreover, abrasion resistance of dental hard tissues softened by erosion is lower than that of sound tissues,[22-24] which may contribute to wear. The prevalence of dental erosion has increased in recent years,[21] at the same time that bleaching procedures have gained popularity.[8,25] However, with exception of the studies of Burgmaier et al.,[26] Sulieman et al.,[25] Pretty et al.,[27] who evaluated erosion, no research tested soft drink, considered one of the most consumed beverage.[21] In

addition, and most importantly, no report verified the effect of erosion on bleached root dentin.

This in vitro study aimed to evaluate the erosive/abrasive wear of enamel and root dentin previously submitted to bleaching with different agents.

MATERIALS AND METHODS

Experimental design

The factors under study were bleaching agents at five levels: four experimental groups (Table 1) and artificial saliva, as the control of the bleached groups; and erosive/abrasive challenge at two levels: Sprite Light and distilled water, as control. The experimental units consisted of 100 bovine enamel slabs and 100 bovine root dentin slabs, randomly assigned to be bleached with one of the five agents (n = 10). This study consisted of a 5x2 factorial design, performed in accordance with a complete block design. The response variable was the wear depth (micrometers) evaluated profilometricaly.

Preparation of dental slabs

Fifteen freshly extracted bovine incisors were used in the study. After extraction, the teeth were kept in 0.1% thymol (pH = 7.0), and then submitted to a soft-tissue debriment with periodontal curettes and cleaned with a slurry of pumice in a webbed rubber cup in a slow-speed handpiece. The roots were separated from their crowns in the cementoenamel junction using a low speed water-cooled diamond saw.[a] Enamel slabs (3 x 3 x 2 mm) were obtained from the middle third of the buccal surface, while dentin slabs (3 x 3 x 2 mm) were cut from the cervical third of the root surface (Fig. 1A). The slabs were embedded in self-curing polyester resin[b] in a ring mold, exposing the external dental surfaces. After 24 h, specimens were ground flat and polished with 400-, 600- and 1200-grit aluminum oxide papers using a water-cooled mechanical grinder.[c] Final polishing was performed with a 6-μm

diamond abrasive paste[d] (Fig. 1B). The experimental units were placed into an ultrasonic cleaner[e] with distilled water for 10 min to remove polishing debris.

Half of the specimens surface was covered with nail varnishf, exposing an area of 1.5 x 1.5 mm of each slab (Fig. 1C). This procedure ensured the presence of reference surfaces when measuring the depth of the erosion/abrasion grooves. Specimens were stored at 37 ± 0.5ºC in 100% relative humidity.

Bleaching treatment

Three carbamide peroxide bleaching agents of different concentrations and one hydrogen peroxide were evaluated (Table 1). The control group was mock-treated with artificial saliva, consisting of a remineralizing solution that was proposed by Featherstone *et al*[29] and modified Serra & Cury[30].

A volume of 0.02 mL of each bleaching agent or remineralizing solution was applied to the exposed area of each specimen, following the manufacturer's instructions (Table 1). Then, samples treated with the bleaching agents CP10%, CP15% and remineralizing solution were covered by an individual tray,[11] manufactured using a 1 mm thick flexible ethyl vinyl acetate polymer in a vacuum tray-forming machine[g],[11,13-15] and individually immersed in 8 mL of distilled water at 37ºC. At the end of the bleaching period, specimens were rinsed with distilled water for 10 s. For CP37% and HP35%, the specimens were kept dry during the bleaching procedure to simulate rubber dam isolation (in-office treatment). Between bleaching sessions, all specimens were immersed in individual containers with 8 mL of remineralizing solution (pH = 7.0) at 37ºC (Fig. D).

Erosive treatment and brushing-abrasion

After 21 days of bleaching treatment, specimens were cycled through the regimen based on the description of Attin et al.[23] The adopted protocol was as follows: each specimen was individually exposed to 10 mL of erosive soft drink Sprite Light[h] or distilled water as control for 5 min; rinsed with distilled water for 5 s; exposed to 10 mL of remineralizing

solution for 1 min. Then, the samples were positioned in a brushing simulator[i], equipped with 10 reciprocating arms, each holding the head of a soft-bristled toothbrush[j]. Briefly, specimens were submitted to a linear toothbrush abrasion movement of 40 strokes, with a rate of 4.5 strokes per second. The load applied was 400 g at 37± 0.5ºC to simulate the oral condition. The abrasive slurry was prepared by mixing dentifrice[k] and distilled water at a ratio of 1:3 by weight, respectively.[31] The experimental units were cycled through this regimen 10 times consecutively (Fig. E).

Wear depth measurements

To determine wear, the nail varnish was removed from the specimens with acetone-soaked cotton wool,[23,27] exposing the untreated reference areas. The specimens were ultrasonically cleaned for 10 min. The wear depth was measured using the profilometer[l] equipped with a diamond stylus of 2 μm radius (Fig. F). Five profilometric traces at a constant speed of 0.1 mm/s and a load of 0.7 mN, perpendicular to the brushing direction, were performed on each specimen, as proposed by Faraoni-Romano et al.[31] The average of these five measurements was used as the wear depth value for each specimen.

Statistical analysis

The assumptions of homogeneity of variance and normal distribution were checked by Hartley's test and Shapiro-Wilks test, respectively. Data were evaluated using two-way analyses of variance (ANOVA) with a significance level of 5%. The Tukey's test was applied to detect significant differences. The software SAS 6.11[m] was used to perform the statistical analyses.

RESULTS

Since for both enamel and root dentin, the dependent variable did not show homogeneity of variance data were log-transformed. Table 2 shows the original means

(standard deviations), expressed in micrometers, and the statistical comparisons of erosive/abrasive wear depth of each dental substrate according to the bleaching agent used.

For enamel, ANOVA ($\alpha = 0.05$) applied to the transformed data did not demonstrate significant effect of the interaction of bleaching and erosive/abrasive challenge ($p = 0.1861$). No significant difference was noticed among the depth of enamel loss provided by using either the bleaching agents or artificial saliva ($p = 0.3702$). There was significant effect for the erosive/abrasive challenge factor ($p = 0.0001$), with the highest wear being observed for the groups exposed to the erosive/abrasive challenge.

For root dentin, ANOVA ($\alpha = 0.05$) applied to the transformed data demonstrated a significant interaction of bleaching and erosive/abrasive challenge ($p = 0.0491$). Increased wear were detected for those groups submitted to the erosive/abrasive challenge with the acidic beverage. Tukey´s test ascertained that the lowest wear depth values were shown after using CP37%, while CP10% and CP15% resulted in the highest depth of dentin loss values. HP35% showed an intermediate result.

DISCUSSION

In this study, bovine enamel and root dentin were submitted to at-home and in-office bleaching with different concentrations and agents, combined or not with erosive/abrasive challenge. In order to reproduce the clinical condition, specimens bleached with at-home technique (10% or 15%) were covered by a custom tray[11,13-15] and the gel remained in contact with the dental tissues for 3 h and were immersed in distilled water at 37ºC to promote the dissociation of carbamide peroxide. For the in-office technique (37% or 35%), agents were applied to the specimens with a microbrush, and individual trays were not used. The slabs were kept dry, simulating rubber dam isolation.[20] The at-home bleaching was applied for 21 days, while the in-office bleaching was performed 3 times, with a 7- day bleaching interval between each section. At the bleaching treatment intervals, the samples of

all groups were exposed to remineralizing solution to simulate the protective benefits of saliva.[8,11,13-15]

Afterwards, erosion-like lesions on enamel and root dentin were induced by the simulated consumption of dietary acids. Although the citric acid has already showed to be effective in the formation of these lesions,[25-27] a soft drink was used in the present study, because it is a beverage ready for consumption and easily available. Among the soft drinks, Sprite Light was chosen in function of presenting low pH (2.9),[32] low mineral concentration and high titratable acidity compared with other drinks.33 Besides, it contains citric acid that has chelating properties able to bind to calcium and dissolve tooth mineral.[32] The model alternating storage in Sprite Light (5 min) and remineralizing solutions (1 min) followed by toothbrush abrasion was based, in part, on that described by Attin et al.[23] which may reflect a situation in the oral cavity observed during consumption of erosive soft drinks.[23] To represent a higher acidic challenge, the number of cycles was increased to 10. After the erosive challenge, samples were kept in a remineralizing solution, which presents inorganic components such as calcium, phosphate, among others (Table 1), and was the same solution used during bleaching treatment.

In an attempt to reproduce in vitro brushing abrasion, an automatic toothbrushing device was used, designed to simulate the brushing abrasion under a situation close to that of the oral conditions. The number of brushing strokes, speed and load applied during the adult toothbrushing[33] was incorporated in the design of abrasive challenge of specimens.

To measure the loss of dental hard tissue induced by erosive/abrasive challenges, some techniques have been applied, such as microradiography,[27,34] profilometry,[27,34] scanning electron microscopy,[34] atomic force microscopy, nano and microhardness tests, iodide permeability test, confocal laser scanning microscopy and chemical analysis.[34] Among these, surface profilometry was chosen because it is a method that allows quantitative assessment, with good reproducibility, being considered a well-established and suitable technique.[34] In addition, it can be adopted for surface loss measurement with high precision,

provided that tissue loss exceeds about 0.4 μm,[34] as the values obtained in this study (Table 2).

Increase of wear was observed after the specimens had been submitted to soft drink, previously to abrasion, confirming the efficacy of the protocol employed. The present results are corroborated by the authors Burgmaier et al.,[26] Sulieman et al.[25] Pretty et al.,[27] although they have used different protocols, with regard to the type of acid, its concentration and time of exposition.

Irrespective of the contact with the acidic beverage, no significant difference occurred between bleached and unbleached enamel, in agreement with the reports of Burgmaier et al.[26] Sulieman et al.[25] and Pretty et al.[27] Although previous studies have evaluated response variables different from wear, no superficial alterations on enamel structure due to at-home[3,5,6,7,10,11] and/or in-office[3,8,9,26] bleaching agents were found. This finding seems to be related to the storage in remineralizing solution between bleaching treatment,[5-11,25,27] short exposure time to agents,[6,8,10,11] pH,[25-27] and to the bleaching agents composition.[5,7,9,10]

With respect to wear of root dentin, to the authors' knowledge, no studies involving the association of the erosive treatment on pre-bleached dentin by at-home or in-office technique has yet been reported. The difference in the wear values of dentin between eroded and uneroded groups can be probably attributed to the composition and structure of this tissue.[35] Within these characteristics, the carbonate content and the small crystals of dentin, which provide more surfaces available for acid attack, make its mineral more soluble.[36] In fact, in the current experiment, the root dentin exposed to the acidic beverage showed higher surface wear than the specimens exposed to distilled water, for all bleaching agents.

The wear observed for root dentin varied depending on the bleaching agent used. The highest wear depth was observed for CP10% and CP15%, although these gels present lower concentration of hydrogen peroxide (3.6% and 5.4%, respectively[2]) compared to CP37% (13,3%[2]) and HP35%. This can be ascribed to the higher contact time and the frequency of exposition of CP10% and CP15% agents. In general, high concentrations of CP and HP are applied for short periods of time,[2,3] as accomplished in this study (Table 1).

Another factor that could have contributed to wear is the pH of the agents used. Considering that the pH of CP10% and CP15% are lower (Table 1) than the critical pH of root dentin (approximately 6.7) demineralization may have occurred. The information about the pH of the CP37% and HP35%, reported to range from 6.0 to 7.0, according to the manufacturer (Table1), does not allow the investigation of its influence in the results. Despite the concentration and pH, wear of the specimens treated with CP37% and HP35% could have been minimized by the extended contact time with the remineralizing solution, due to the reduced time of application of the bleaching agent per week (Table 1).

It must be assumed that this study was performed in vitro, not involving the biological factors, such as composition, flow rate, buffer capacity of saliva and salivary pellicle formation, as in the in situ/in vivo studies.

CONCLUSIONS

The agents used in the at-home and in-office technique did not increase the erosive/abrasive wear of enamel. However, it is suggested that caution is needed in the choice of the bleaching agent in clinical situations that root dentin may be in contact with the bleaching gel, since, depending on the agent applied, an increase in wear can occur if erosive/abrasive challenges are present.

a. Isomet 1000, Buehler, Lake Bluff, IL, USA
b. Milflex Indústria Química Ltda., São Bernardo do Campo, SP, Brazil.
c. Struers A/S, Rodovre, Denmark.
d. Arotec Ind e Com Ltda, Cotia, SP, Brazil.
e.T1440D, Odontobrás Ltda., Ribeirão Preto, SP, Brazil.
f. Colorama-Maybelline, Procosa Produtos de Beleza Ltda., São Paulo, SP, Brazil.
g. P7/ Bio-Art Equip Odontológicos Ltda., São Carlos, SP, Brazil.
h. Companhia de Bebidas Ipiranga, Ribeirão Preto, SP, Brazil.
i. MSEt, Marcelo Nucci-ME, São Carlos, SP, Brazil.
j. Colgate Classic, Colgate-Palmolive Co., São Bernardo do Campo, SP, Brazil.

k. Colgate Cavity Protection Gel, Colgate-Palmolive/Kolynos do Brazil Ltda.,Osasco, SP, Brazil.
l.Surfcorder SE-1700, Kosaka Corp, Tokyo, Japan.
m. SAS Institute Inc., Cary, NC, USA.

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Table 1. Bleaching treatments tested, basic composition and their application protocol.

Agent	Code	Basic composition	Application protocol	pH
Remineralizing solution (control)	---	calcium, phosphate, potassium chloride, hydroxymethyl-aminomethane	3-hour daily application, for 21 days	7,0
Rembrandt Gel Plus* (10% carbamide peroxide)	CP10%	Glycerin, carbamide peroxide, sodium citrate, carbopol, triethanolamine, flavor	3-hour daily application, for 21 days	6.23[φ]
Rembrandt Xtra Comfort* (15% carbamide peroxide)	CP15%	Glycerin, carbamide peroxide, sodium citrate, carbopol, triethanolamine, flavor	3-hour daily application, for 21 days	6.11[φ]
Whiteness Super[†] (37% carbamide peroxide)	CP37%	37% carbamide peroxide, neutralized carbopol, glycol, deionized water	3 consecutive exposures for 20 min each, at 1-week interval, for 21 days	6.0-7.0[ψ]
Whiteness HP[†] (35% hydrogen peroxide)	HP35%	33%-37% hydrogen peroxide, thickener, red colour, glycol, deionized water	2 consecutive exposures for 15 min each, at 1-week interval, for 21 days	6.0-7.0[ψ]

* Oral B Laboratorios, Belmont, CA, USA.
[†] FGM Produtos Odontológicos Ltda., Joinvile, SC, Brazil.
[φ] Information taken from Price et al. (2000)[28]
[ψ] Information taken from manufacturer's web site

Table 2. Original means (standard deviations) of wear depth (μm) for enamel and dentin submitted to each bleaching agent and to erosive drink.

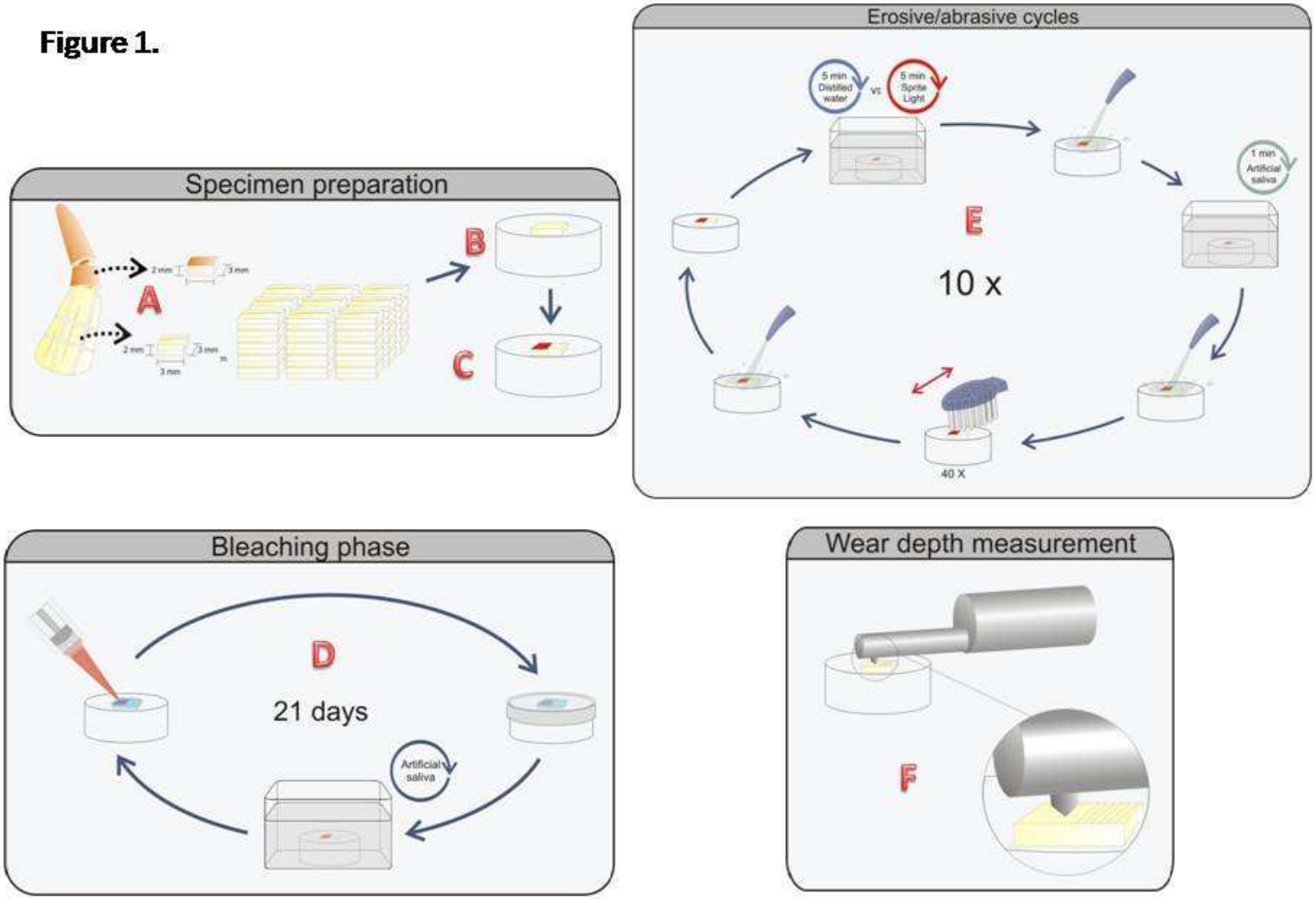
Bleaching treatment	Enamel		Root dentin	
	Control (distilled water)	Acidic beverage[ж]	Control (distilled water)	Acidic beverage[ж]
Control (Artificial saliva)	0.41 (0.20) A a	4.14 (0.81) A b	0.81 (0.23) A a	3.58 (0.53) A b
CP10%	0.40 (0.17) A a	3.30 (0.68) A b	0.97 (0.29) A a	5.02 (1.01) B b
CP15%	0.37 (0.13) A a	4.14 (0.76) A b	0.93 (0.24) A a	5.91 (0.72) B b
CP37%	0.41 (0.11) A a	3.21 (1.07) A b	0.81 (0.26) A a	3.46 (0.74) A b
HP35%	0.45 (0.14) A a	3.79 (0.90) A b	0.77 (0.10) A a	4.54 (0.88) AB b

[ж] Sprite Light

Means followed by the same capital letter are not statistically different ($p \leq 0.05$) within the same column (bleaching treatment) for each substrate.

Means followed by the same lower case are not statistically different ($p \leq 0.05$) within the same line (erosive challenge) **FOR EACH SUBSTRATE.**

Figure 1.


Specimen preparation
A
B
C
2 mm
3 mm
Erosive/abrasive cycles
5 min Distilled water
vs
5 min Sprite Light
1 min Artificial saliva
E
10 x
40 X
Bleaching phase
D
21 days
Artificial saliva
Wear depth measurement
F

LEGENDS

Figure 1. Diagram of the experimental set-up: (A) enamel and root dentin slabs (3x3x2 mm) were cut from bovine incisors; (B) the sections were embedded in polyester resin in a ring mold, ground flat and polished. (C) Half of the specimen surface was covered with nail varnish to obtain a reference surface. (D) Bleaching treatment with tray (PC10%, PC15% and control - every day) or without tray (PC37% and PH35% - once a week), for 3 weeks. (E) Cycles of acidic challenge and brushing abrasion were performed 10 times. (F) After removal of nail varnish, wear depth was measured using the profilometer.

3.2. CAPÍTULO 2

SUBMETIDO À PUBLICAÇÃO NO PERIÓDICO DENTAL MATERIALS (ANEXO B)

Effect of a 10% cabamide peroxide on wear resistance of enamel and dentin: *in situ* study.

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Short title: Wear of dental hard tissues after i*n situ* bleaching

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ABSTRACT

Objectives: This triple-blind, 2x2 crossover *in situ* study, compared the effect of home bleaching treatment with a 10% carbamide peroxide and a placebo on wear of bovine enamel and root dentin.

Methods: Thirty slabs of each substrate (2 x 3 x 2 mm) were selected for each phase, after flattening and polishing procedures and microhardness test. Two thirds of the surface area of slabs was covered for measuring the wear depth. After a 7-day lead-in period, one specimen of each substrate was randomly fixed on the facial surface of each one of 30 subject's upper second premolars. Then, 15 volunteers bleached their maxillary arch with the carbamida peroxide gel for a two-week period, while the remainders used a placebo agent. After a 1-week washout period, a new set of enamel and root dentin slabs were bonded to the premolars and volunteers were crossed over to the alternate agent for 14 days. The effect of the bleaching agent on the enamel and root dentin wear was measured with a profilometer.

Results: For enamel, ANOVA did not demonstrate significant difference between the wear provided by the placebo and the bleaching agent (p=0.3713), but showed difference for dentin (p=0.0346). Tukey's test revealed that the dentin treated with bleaching agent presented higher wear than the placebo.

Significance: The bleaching did not increase the wear of enamel, but caused higher wear on the root dentin.

Keywords: bleaching, 10% carbamide peroxide, surface wear, *in situ* study, enamel, root dentin.

INTRODUCTION

Nowadays, appearance of the teeth is of great importance to patients that often seek esthetic treatments related to dental discoloration. Among treatments, tooth bleaching has attracted the interest of patients and dentists because it represents a non-invasive option and is relatively simple to carry out [1]. At-home bleaching technique, in particular, received worldwide acceptance when described in 1989 by Haywood e Heymann [2]. Ten percent carbamide peroxide is the bleaching agent most used in this technique [2] as it is considered safe and effective [3]. As whitening of vital teeth generally involves direct and frequent contact of the bleaching agent with the outer enamel surface and sometimes the dentin for an extensive period of time, *in vitro* studies have evaluated the effects of carbamide peroxide on dental hard tissues. Changes were observed in surface texture [4-8], mineral content [9-12], chemical composition [6,13,14], and loss by toothbrushing abrasion [15].

Additionally, some *in situ* studies, that represent an intermediate stage between *in vitro* studies and clinical trials [16], have investigated the effects of bleaching treatment on microhardness of dental enamel [13,17-19] and dentin [18,20]. Surface morphology [13,21], roughness [21] and calcium content on enamel [13] were also observed. However, the effect of whitening agents on wear of enamel and dentin, by means of an *in situ* model, has still not been evaluated.

Considering that the *in vitro* bleaching of enamel with 10% carbamide peroxide caused demineralization up to 50 µm below the surface [10], or that could reduce the hardness up to 150 µm [9], it could be assumed that the dental substrate would be subject to occurrence of wear. In fact, Wiegand et al.[15] already showed that bleaching increase the substrate loss by toothbrushing. Nevertheless, no *in situ* study validated the alteration and the increase of wear of enamel and dentin.

As in the *in situ* studies the remineralization potential of human saliva can minimize the adverse effects of bleaching agents on enamel and dentin [18], there are doubts if the bleaching gel may increase the susceptibility to wear of dental tissues.

Therefore, this *in situ* study was conducted to evaluate the effect of home bleaching treatment based 10% carbamide peroxide on wear of enamel and root dentin.

MATERIALS AND METHODS

Ethical Aspects and volunteers

The protocol of this study was reviewed and approved by the Ethics Committee of the Faculty of Dentistry of Ribeirão Preto, USP (process nº 2004.1.834.58.6). Thirty volunteers (28 females and 2 males, aged 19-43 years) who fulfilled the inclusion criteria (normal saliva flow, absence of dental caries and/or periodontal disease, willing to perform bleaching treatment following the research schedule) without violating the exclusion criteria (use of orthodontic appliances, presence of fixed or removable denture, pregnant or nursing women, smokers and dentin sensitivity) took part in this study after signing an informed, written consent (Resolution nº 196 from National Health Council, Brazil, 1996).

Experimental design

This triple blind study followed a two-period crossover design. Each phase lasted for 14 days and a washout period of one week between each phase. The factor under study was bleaching treatment at two levels: one experimental group (10% carbamide peroxide) and one control group (placebo). The volunteers were randomly divided into two groups of 15, and each group received the bleaching or the placebo agent in different sequence, in two distinct periods (bleaching agent-placebo; placebo-bleaching agent). The experimental units consisted of 60 bovine enamel slabs and 60 bovine root dentin slabs, randomly assigned to the 30 volunteers (one enamel slab and one dentin slab per each phase). Each volunteer was considered a statistical block. The response variable was the wear depth (μm) evaluated profilometricaly. The steps of this experiment are shown in Figure 1.

Preparation of dental slabs

Sixty freshly extracted bovine incisors were cleaned to remove tissue remnants and stored in 0.1% thymol (pH = 7.0). The roots were separated from their crows in the cementoenamel junction using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Enamel slabs (3 x 2 x 2 mm) were obtained from the middle third of the vestibular surface, while dentin slabs (3 x 2 x 2 mm) were cut from the cervical third of the root surface. Dental slabs were fixed with stick wax in acrylic resin cylinders and the upper surface of the samples was then flattened and serially polished with 400-, 600- and 1200-grit Al_2O_3-abrasive papers and with 0.3- and 0.05 µm alumina polishing suspensions (Alpha and Gamma Micropolish, Buehler, Lake Bluff, IL, USA, BA1003) cloths in a water-cooled mechanical grinder (Struers A/S, Rodovre, Denmark). To remove polishing debris, specimens were placed in an ultrasonic cleaner (T1440D, Odontobrás Ltda., Ribeirão Preto, SP, Brazil) with distilled water for 10 min. A stereomicroscope (Nikon 88286, Tokyo, Japan) at 40x magnification was employed to select and discard samples that presented pits or cracks. Enamel and root dentin sections were then gas sterilized (ethylene oxide).

Selection of enamel and root dentin slabs

After sterilization, microhardness measurements were performed on sound substrates, through a Knoop indenter (HMV-2, Shimadzu, Kyoto, Japan), under a 50-g load for enamel and 25-g load for root dentin applied for 30 and 10 s, respectively. Four indentations, located 500 µm from the margin of the dental slab and 250 µm apart, were made and the surface microhardness (SMH) was calculated for each specimen. A total of 60 slabs of enamel and 60 slabs of dentin were selected based on the mean values obtained for each dental substrate (enamel and root dentin).

Preparation of dental slabs for the experimental phase

In order to ensure the presence of reference surfaces (area unexposed to the bleaching agent, the brushing strokes and the oral environment) when measuring the depth

of the abrasion grooves, a thin layer of composite resin was applied over the two ends of surface of the specimens, based on the description of Amaechi et al.[22], leaving a window of 1 x 2 x 2 mm in their central area. The resin composite (Herculite XRV, Kerr, Emigsville, PA, USA, 308832) was light-cured for 40 s and polished with aluminum oxide discs (Sof-Lex - Pop-On, 3M, Sumaré, SP, Brazil, 08095). Specimens were stored at 37 ± 0.5ºC in 100% relative humidity.

Tray Preparation

Upper and lower dental arch impressions were taken with alginate using a stock tray and stone cast molds were made. On the molds, vestibular reservoirs were generated with one coat of nail varnish on all teeth, except on the second premolars where reservoirs (3 x 4 x 3 mm) were prepared with resin composite corresponding to the specimen that would be fixed in the volunteers' teeth. Two trays were manufactured for each volunteer using a 1 mm thick flexible ethyl vinyl acetate polymer in a vacuum tray-forming machine (P7/ Bio-Art Equip Odontológicos Ltda., São Carlos, SP, Brazil).

Preparing the volunteers for the Experimental Phase

In the pre-experimental period or run-in phase, which lasted one week, each volunteer received a toothbrush (Oral-B Indicator Plus 35, Gillette do Brasil Ltda., Manaus, AM, Brazil) and a fluoride dentifrice (Colgate Cavity Protection, Colgate-Palmolive Ltda.,Osasco, SP, Brazil, AO).

A complete prophylaxis was performed on each patient and the initial color of the teeth was determined by Vita scale (Wilcos do Brasil Indústria e Comércio Ltda, Petrópolis, RJ, Brazil).

Experimental Phase

The 30 volunteers were randomly divided into two groups of 15. First, the tray was tested, and when necessary, adjustments were performed. After, two dental specimens - one

of enamel and one of dentin – were randomly fixed to the vestibular surfaces of the superior second premolars (if absent, the upper first premolars) of each patient. The base of the specimens and the area corresponding to the place to fix it in the vestibular surface of the superior second premolar were acid etched with phosphoric acid. In the sequence, an adhesive system (Single Bond, 3M, Sumaré, SP, Brazil, 7KK) and a resin cement (Rely-X, 3M, Sumaré, SP, Brazil, FCFN) were applied following the manufacturer's instructions. The slab was positioned on the correct area, the excess of the cement was removed and light-cured. The volunteers were instructed on how the bleaching treatment would be performed, the manner to apply the bleaching gel (Whiteness Perfect 10%, FGM, Joinville, SC, Brazil, 16FEV06) or placebo gel (Whiteness Perfect, FGM, Joinville, SC, Brazil, 16FEV06) in tray, how to clean the tray after removing it from the mouth and keep it in a container provided. In the experimental Phase 1, Group 1 applied the bleaching agent, while Group 2 applied placebo agent in the tray and wore it during the night for about 8 hours. The volunteers were blind as to which agent they were using.

After the bleaching period (14 days), the specimens were carefully removed with orthodontic pliers and the residual cement was removed with resin polishing carbide burs (Jet, Beavers Dental, ON, Canadá, 2389949) and aluminum oxide discs (Sof-Lex - Pop-On, 3M, Sumaré, SP, Brazil, 08095).

Volunteers were submitted to a washout period of one week, with the aim to eliminate the residual effects of the treatment previously applied. Then, they received new toothbrushes and dentifrices and the dental hygiene technique was reinforced.

In the experimental Phase 2, two other dental specimens - one of enamel and one of dentin – were randomly fixed in the same way as that used for experimental Phase 1. New trays were distributed to the volunteers to eliminate any possible residues left by the previously applied agent. In this phase, the Group 1 applied the placebo agent, while Group 2 used bleaching agent for another two weeks. The specimens were removed in the same manner that in the Phase 1. After finishing the experimental Phase 2, the volunteers began

the bleaching treatment in the mandibular arch. At the end of the bleaching treatment, photographs were taken and esthetic restorations were replaced when necessary.

Wear depth measurements

To determine wear, the composite resin was removed from the specimens. As the acid etching/adhesive system was not used, the composite resin was carefully and gently detached from the enamel and dentin surface of the sections, exposing the untreated reference areas. The specimens were cleaned ultrasonically for 10 min. The wear depth, on both tissues, was measured using the profilometer (Surfcorder SE-1700, Kosaka Corp, Tokyo, Japan) equipped with a diamond stylus of 2 μm radius. Five profilometric traces at a constant speed of 0.1 mm/s and a load of 0.7 mN, perpendicular to the brushing direction, were performed for each specimen. The average of these five measurements was used as the wear depth value for each specimen.

Statistical analysis

Period and *carryover* effects were determined by the Student's t test. The data were statistically evaluated using two-way analyses of variance (ANOVA) with a significance level of 5%. Tukey's test was applied where significant differences were detected. The software Statgraphics Plus (Statgraphics Plus Software, Manugistics, Rockville, MD, USA) was used to perform the statistical analyses.

RESULTS

The Student's t tests verified that the effects of period ($p = 0.99$ for enamel and $p = 0.15$ for dentin) and carryover ($p = 0.44$ for enamel and $p = 0.36$ for dentin) were not statistically significant. Table 2 shows the means and standard deviations of enamel and dentin root wear.

For enamel, ANOVA did not demonstrate significant difference between placebo and bleaching agents ($p = 0.3713$). For root dentin, there was a significant difference between

bleaching and placebo agents (p = 0.0346). Tukey's test revealed that the placebo agent provided lower wear than the bleaching agent.

DISCUSSION

Different protocols of *in situ* studies have been used to assess the effects of bleaching treatment on dental hard tissues. Among the described protocols in the literature, most of them adopted the use of a removable palatal appliance containing the slabs of enamel [13,17,19] or dentin [20,23]. Some disadvantages about these protocols may be identified such as the difficulty to ensure that all volunteers followed it suitably; the fact that these protocols did not represent the erosive and abrasive challenge that occurs in the oral cavity, as the appliance was removed during meals and hygiene procedures; and the abrasive effect that the tongue can exert on dental tissues, increasing the overall loss of tooth substance [24]. Moreover, some disadvantages were also observed in relation to the bleaching treatment performed. In the studies of Araujo et al. [17], Maia et al.[19] and Arcari et al. [20] that employed the same methodology for enamel [17,19] and dentin, it is impossible to reject the probability of contact of the bleaching gel with the control specimens or, to avoid the residual effect of bleaching gel in the control group, considering that the slabs are present in the same device. In other researches, the control specimens were removed from the appliance and kept in artificial saliva, while the specimens were bleached in the mouth [23]; or the bleaching treatment was performed outside the oral cavity and then placed in the mouth of volunteers [13].

In the present study, an *in situ* model was chosen to simulate the bleaching treatment, as closely as possible of the clinical situation, avoiding these above mentioned problems. The adopted protocol was similar to Basting et al. [18], in which the specimens of enamel and dentin were fixed on the facial surface of the maxillary second premolars. Another important aspect related to this study was the crossover experimental design. In this experimental design, each volunteer performed the treatment with bleaching gel and placebo gel, in different periods, with a washout of one week to eliminate the residual effect between

the treatments. Thus, each volunteer was considered one complete statistical block, eliminating the different habits among them [25], such as diet, toothbrushing force, and biological factors including flow rate, buffering capacity and composition of saliva.

Ten percent carbamide peroxide agent was used to represent the gel most commonly used in home-bleaching. In this research, special care was taken in the choice of control group, represented by a placebo, which was prepared by the same industry of the bleaching agent. Thus, the placebo presented the same appearance, color, flavor, consistency and composition - with exception of the 10% carbamide peroxide. Problems related to the manipulation of placebo by pharmacy, that in general, contain glycerin, carbopol and their associations were avoided, once these components already demonstrated change in the microhardness of dental tissues [26]. The protocol of bleaching treatment was performed according to manufacturer's instructions.

A platform of composite was constructed on the two ends of the selected slabs and then fixed on the teeth of the volunteers, with the aim of serving as a reference area to allow latter evaluation of dental loss, and also providing protection of the exposed area against the attrition of soft tissues, as proposed by Amaechi & Higham, [22]. Unlike this study, the slabs of enamel and dentin used in the present experiment were obtained from bovine teeth, due to the fact that they present similar chemical composition to human teeth [27]. Moreover, bovine teeth are easily obtained and manipulated, in function of their large size.

Among the different methods used to assess the loss of dental hard tissue, profilometry was chosen because it is a method of high precision [28] and permits the measurements of wear in a relatively simple way [29]. Few studies in the literature have evaluated the wear after bleaching treatment.

With respect to enamel, the result of current study did not reveal a significant difference between the wear provided by 10% carbamide peroxide and the placebo. This finding corroborates a previous *in vitro* study in which 10% carbamide peroxide applied by same period of this study did not affect the enamel wear produced by an oral wear simulator in comparison with untreated specimens [30]. Contradicting these findings, a difference in

wear of the enamel bleached with the same concentration of carbamide peroxide and submitted to cycles of toothbrushing abrasion was observed. Despite the bleached group differing from unbleached, the magnitude of wear has been considered clinically irrelevant [15]. In fact, the mean value obtained was around 0.207 μm, wear considered very small compared to values this *in situ* study (Table 2). The reports of Attin et al. [9] and Efeoglu et al. [10], have demonstrated that the 10% carbamide peroxide are not only able to penetrate and diffuse through enamel, but also into it. Thus, demineralization and reduction of subsurface microhardness up to 50 μm after 15 days bleaching [10] and 150 μm below the surface of the enamel after 10 days bleaching [9] was observed. Possible explanations for these alterations are the time of application of the bleaching gel [15], insufficient remineralizing period to repair subsurface microstructural defects [9] and the result of the uncontrolled reaction of the peroxide radical [10]. As consequence of these surface and subsurface changes, enamel could be more susceptible to abrasive wear. However, due to the biological factors of saliva – flow, buffering capacity, acquired pellicle, composition – and to pH value of the bleaching agent does not affect the enamel [31], the increase of wear was not verified in this study.

Some *in situ* investigations also are in agreement with the absence of difference between bleaching treatment and control in terms of microhardness [13,17], micromorphology [13,21] and content of calcium [13]. In contrast with these findings, alterations on the microhardness [18] and surface roughness [21] have been observed, probably because of the prolonged contact between the bleaching gel and the dental tissue [18,21]. Moreover, the carbopol present in the placebo agent and in bleaching agent may have caused changes in dental structure microhardness and in its mineral content [21].

With regards to root dentin, the specimens exposed to the bleaching gel exhibited higher wear value than the placebo gel. This finding may be attributed to the composition and structure of this tissue [32], which possess higher organic content than enamel [27], besides to present higher porosity and solubility [27,33]. Other important aspect to consider is the critical pH for the root dentin which is approximate 6.7 [33]. In the current experiment,

although the pH of both gels presented lower value than that established as critical for root dentin, the pH of the placebo was higher than the pH of the bleaching agent (Table 1). Thus, during demineralization, a loss of mineral from the tissue occurs and, together with brushing, may increase dentin surface wear. Changes on the dentin after the use of 10% carbamide peroxide were also observed on surface morphology [8], microhardness [11,12] and mineral content [14] of *in vitro* studies and on the microhardness of an *in situ* study [20]. Probable explanations for these alterations are the low pH [11,12], composition of the bleaching agents [12] and protocol of the bleaching treatment [8,14]. On the other hand, in some *in vitro* studies [9,34,35] there were no changes neither on the surface [35] and subsurface [9] microhardness nor on the morphology and surface roughness of dentin [34], owing to the short-term regimens [34,35] and neutral pH [9]. The *in situ* researches of Basting et al. [18,21], also did not show significant difference on microhardness and surface roughness, probably due to the pH of bleaching agent used (6.7).

CONCLUSION

According to the data obtained in this study, the at-home bleaching performed with 10% carbamide peroxide *in situ* did not affect the enamel in terms of wear, but caused higher wear on the root dentin.

Bleaching treatment may be considered harmless to enamel wear. Nevertheless, if patients present exposed dentin, the professional must take additional care, in relation to the choice of bleaching agent and clinical protocol employed.

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TABLES

Table 1. Bleaching treatments tested, their application protocol and pH.

Agent	Code	Basic Composition[ψ]	Application protocol	pH	Batch #
Whiteness Perfect (10% carbamide peroxide)	CP10%	10% carbamide peroxide, neutralized carbopol, glycerol and distilled water	8-hour daily application, for 14 days	6.14[φ]	16FEV06
Placebo (Whiteness Perfect)	PLA	neutralized carbopol, glycerol and distilled water	8-hour daily application, for 14 days	6.28[φ]	16FEV06

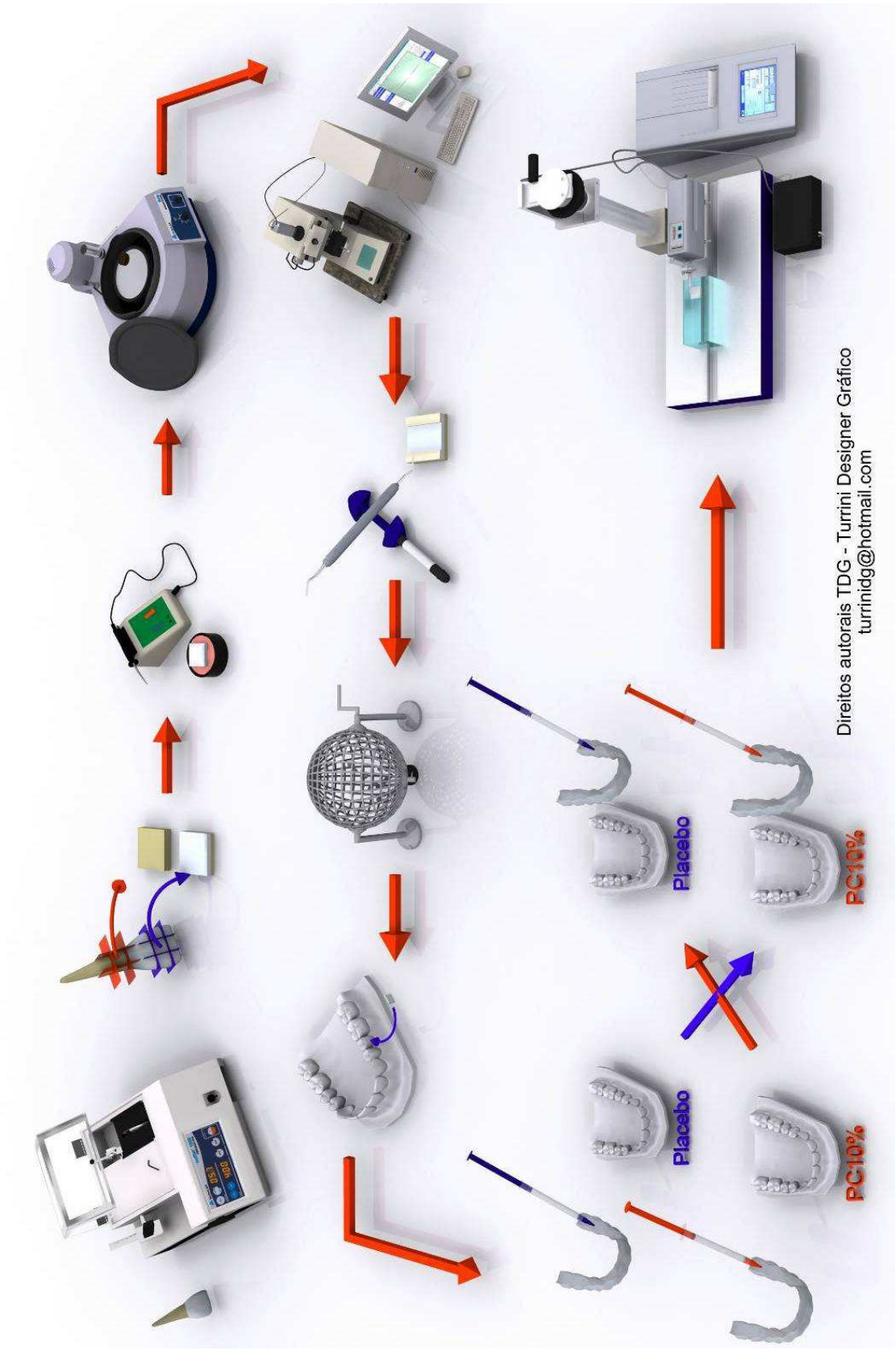
[φ] Measured with mPA Tecnopon (mPA Tecnopon, MS Tecnopon Equipamentos Especiais Ltda., Piracicaba, SP, Brazil)

Table 2. Means (standard deviations) of surface wear (µm) of enamel and root dentin exposed to 10% carbamide peroxide and to placebo agents.

Substrate	Enamel	Root dentin
CP 10%	1.78 (0.83)	2.67 (1.31)
Placebo	1.59 (0.83)	2.08 (1.07)

Means connected by brackets did not differ from one another ($\alpha = 0.05$; l.s.d. = 0.55) for the same dental substrate.

FIGURE 1.


Placebo
PC10%
Placebo
PC10%
Direitos autorais TDG - Turrini Designer Gráfico
turrinidg@hotmail.com

CAPTIONS

Schematic drawing of the experimental set-up: Using a low speed water-cooled diamond saw, bovine enamel and root dentin slabs (3 x 2 x 2 mm) were obtained. Samples were fixed with stick wax in acrylic resin cylinders, and ground flat and polished in a water-cooled mechanical grinder. Slabs were pretested using a microhardness tester. Two thirds of the surface area of specimens was covered with resin composite, for measuring the depth of wear. The specimens were randomly fixed on the facial surface of the maxillary second premolars of 30 volunteers. The volunteers were divided into 2 groups of 15: in the first experimental phase, the Group I received the bleaching agent and the Group II received the placebo, for a period of two weeks. In the second phase, after a wash-out of 1 week, the Group I received the placebo and the Group II received the bleaching agent, characterizing a 2 x 2 cross-over study. The composite resin was removed from the specimens and wear depth was assessed in profilometer, in relation to the reference area.

4. CONCLUSÃO GERAL

De acordo com os resultados obtidos e considerando o delineamento adotado para os estudos, pode-se concluir que:

- O clareamento *in vitro*, realizado com agentes clareadores caseiros e de consultório, não aumentou o desgaste erosivo/abrasivo do esmalte, em relação aos substratos não clareados. No entanto, para a dentina, na dependência do agente utilizado, o clareamento pode aumentar o desgaste frente a episódios erosivo-abrasivos.

- O clareamento *in situ* com peróxido de carbamida a 10%, não aumentou o desgaste do esmalte, mas proporcionou maior desgaste da dentina radicular.

- Em termos de desgaste superficial, o esmalte não foi afetado pelo tratamento clareador, mas, na dependência do agente empregado, a dentina pode ser afetada. Portanto, aconselha-se uma seleção criteriosa do agente clareador, em situações clínicas em que houver dentina radicular exposta.

ANEXOS

ANEXO A - Comprovação de envio para publicação do artigo referente ao Capítulo 1

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Dear Dr. Faraoni-Romano,

Your submission entitled "EROSIVE/ABRASIVE WEAR OF ENAMEL AND DENTIN SUBMITTED TO DIFFERENT BLEACHING TREATMENTS" has been received by the Journal of Dentistry

You will be able to check on the progress of your paper by logging on to Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/jjod/.

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ANEXO B - Comprovação de envio para publicação do artigo referente ao Capítulo 2

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Ms. Ref. No.: DEMA-D-08-00061
Title: Effect of a 10% cabamide peroxide on wear resistance of enamel and dentin: in situ study.
Dental Materials

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ANEXO C - Aprovação do Comitê de Ética

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FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
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Avenida do Café, s/nº - Telefone: (016) 602-3970
14040-904 - Ribeirão Preto - SP - Brasil
Fax: (016) 633-0999

CEP/00205/FORP/180205
MLCK/mlck

Prezada Professora,

Ref. Processo nº 2004.1.834.58.6

De ordem da Senhora Vice-Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em sua 56ª Sessão realizada no dia 18 de fevereiro de 2005, deliberou **aprovar** o Projeto de Pesquisa envolvendo seres humanos intitulado: **"Efeito de um peróxido de carbamida a 10% na resistência ao desgaste do esmalte e da dentina: estudo *in situ*"**, e que será desenvolvido por Vossa Senhoria, na Faculdade de Odontologia de Ribeirão Preto, devendo o atestado para publicação final, ser expedido pelo Comitê de Ética em Pesquisa, após a entrega e aprovação do Relatório Final pelo referido Comitê.

Na oportunidade, solicitamos entregar na Seção de Expediente e Protocolo com o formulário preenchido pelo pesquisador responsável, o **Relatório Parcial** no dia **30 de agosto de 2006** e o **Relatório Final** no dia **28 de fevereiro de 2008.**

Atenciosamente,

Lúcia Kühl

Maria Lúcia Câmara Kühl
Secretária do Comitê de Ética em Pesquisa

Ilma Sra.
Profa. Dra. MÔNICA CAMPOS SERRA
Professora Associada do Departamento de Odontologia Restauradora - FORP/USP

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