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Original Research

Microhardness and Roughness of Enamel Bleached with 10% Carbamide Peroxide and Brushed with Different Toothpastes: An *In Situ* Study

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Abstract:

Background: This *in situ* study evaluated the roughness and microhardness of enamel bleached with 10% carbamide peroxide (PC10) and brushed with different toothpastes.

Materials and Methods: Two groups of volunteers received PC10 and placebo agents for 21 days in two phases in a crossover 2 × 3 study. Fragments of human enamel were distributed among intraoral removable appliances (IRA). Nine fragments, divided into three triplets, were used in each IRA, and these were brushed with toothpastes R (Colgate), W (Colgate Total 12 Whiteness Gel) or BS (Colgate Whitening Oxygen Bubbles Fluoride). Treatments agents were applied for 8 h overnight. After brushing, the volunteers used the IRA for about 16 h/day. After a washout period, new IRAs were distributed and the volunteers were crossed over to the alternate agent for 21 days. Roughness and microhardness were measured before and after each phase.

Results: According to the paired Student's *t*-test, roughness of enamel increased and microhardness decreased ($P < 0.05$). According to analysis of variance generalized linear models, only the toothpaste factor was significant ($P = 0.037$) for roughness.

Conclusion: Enamel microhardness and surface roughness are altered when PC10 bleaching is associated with tooth brushing using toothpastes BS, R, and W.

Key Words: Dental bleaching, enamel, microhardness, surface roughness, toothpastes

Introduction

The introduction of 10% carbamide peroxide (PC10) in home-bleaching technique[1] had a major impact on esthetic dentistry because it was considered a safe, effective,[2] and economical technique. This treatment could help patients whiten their teeth without the need for additional restorative treatment.[3]

The effects of PC10 on the dental enamel surface have been studied extensively. Some research *in vitro* indicated no significant changes in the microhardness[4,5] or surface roughness[6] of this tooth substrate. However, when PC10 was associated with abrasive toothpaste by simulated tooth brushing, a significant increase in the surface roughness of enamel was found.[7]

In vitro studies do not generally simulate the *in vivo* interaction between saliva and enamel.[8] This is an important factor for consideration because of the remineralizing power of saliva that may prevent the demineralizing effect of bleaching agents.[9] At present, *in situ* studies have simulated the real effect of bleaching treatments on the oral environment and hence that 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 tooth brushing routine is the most common oral hygiene practice for prevention of caries lesions. The whitening toothpastes on the market contain abrasive particles, which remove stains from the enamel surface. 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 over the counter whitening toothpastes and having in mind that patients may use these products to potentiate 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 PC10 associated with different types of toothpastes used during bleaching treatment on the microhardness and surface roughness of enamel.

Materials and Methods

Selection of volunteers

Ten female volunteers between the ages of 22 and 42 years were selected from the Dental School of Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil. The experimental procedures were undertaken with the understanding and written consent of the volunteers, after approval of the research protocol by the Research Ethics Committee of the 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 individuals with dentin sensitivity, bulimia, and esophageal reflux.

Experimental design

This double-blind experiment was performed in two periods with a 2 week (15 days) washout period. Ten volunteers were randomly divided into two groups ($n = 5$). Each group received either the bleaching agent (PC10) or the placebo agent for 21 days in different sequences and in two distinct periods in a crossover 2 × 3 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 10 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 details of this study are outlined in Figure 1.

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 h. The teeth were then 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, UK) with a low-speed water-cooled diamond saw (Extec, London, UK) to obtain enamel fragments measuring approximately 4 mm × 2 mm × 2 mm 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 min at 121°C. Steam sterilization is the most effective method of avoiding bacterial contamination and does not change the mineral content of the teeth.[14] The enamel fragments were then stored in deionized water at room temperature until required. Before fabricating the removable appliances, the surface roughness and microhardness of each piece were measured.

Intraoral appliance preparation and mounting of the fragments

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

Experimental phase: In situ conditions

The volunteers received a research kit with a toothbrush (Reach® Essencial Junior, Johnson & Johnson, São José dos Campos, SP, Brazil), three different toothpastes kept in syringes identified by the 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 and five volunteers applied the placebo agent

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, confidence intervals (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

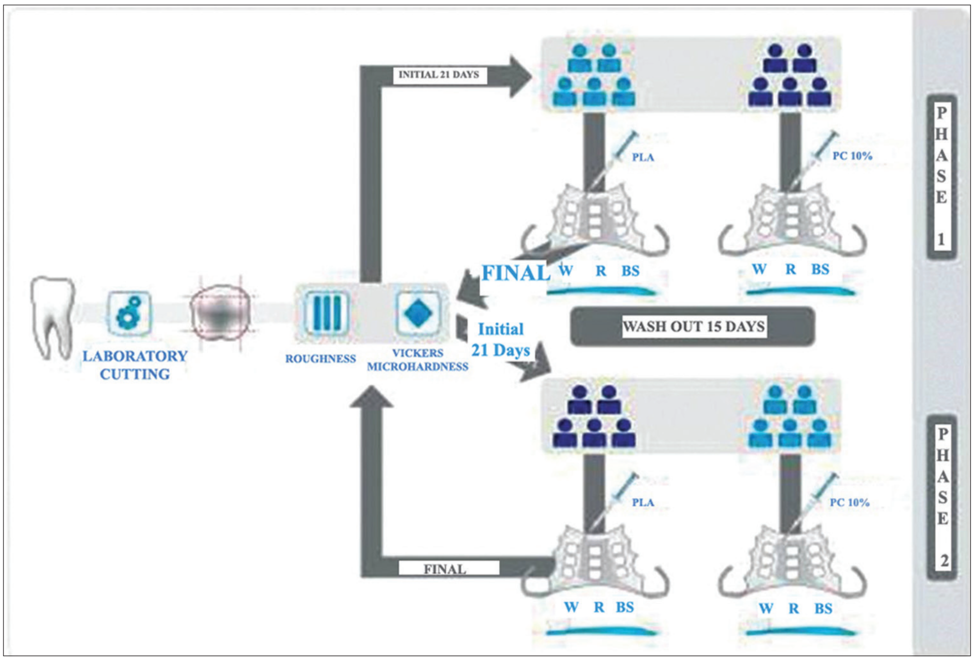

Figure 1: Schematic drawing of the experimental design-each human tooth was cut along the long axis of the buccal, lingual, mesial and distal surfaces using a laboratory cutting machine with a low-speed water cooler diamond saw to obtain enamel fragments (4 mm × 4 mm × 2 mm). Each fragment was measured 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.

to the appliance out of the mouth for about 8 h overnight. 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, and then brush the fragments: Triplet R (in the center of the palate area) with toothpaste R; triplet W (right premolar area) with toothpaste W; triplet BS (left premolar area) with toothpaste BS. The toothpaste was placed on the toothbrush and rubbed in using back and forth movement cycles for 30 s, twice a day.

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

After the first experimental phase of 21 days, the fragments were removed from the acrylic resin with tungsten carbide instruments (Komet do Brazil Ltda., São Paulo, SP, Brazil). The volunteers were then subjected 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. At the end of this phase, the fragments were removed with tungsten carbide instruments.

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 100 g load for 5 s in each experimental period: Initial (before the treatments) and final (after the treatments). The VHN value of each fragment was the arithmetic mean of 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 specimen with a 0.25 cut-off. The R_a value of each specimen was the arithmetic mean of three measurements.

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 generalized linear models (ANOVA GLM) and Bonferroni tests. Each volunteer was considered a block, in a factorial 2 × 3 scheme (treatment agents × toothpastes). 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 paired Student's *t*-test, the final roughness was statistically higher than the initial roughness (Figure 2), and the 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

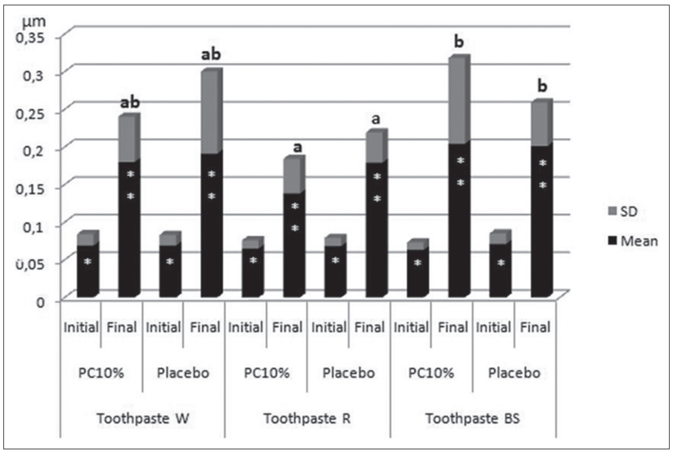

Figure 2: Mean values of enamel surface roughness (µm)– Means followed by the same amount of signal (*) did not differ statistically according to paired Student's *t*-test (α = 0.05). Means followed by same letters did not differ statistically according to Bonferroni test ($\alpha = 0.05$).

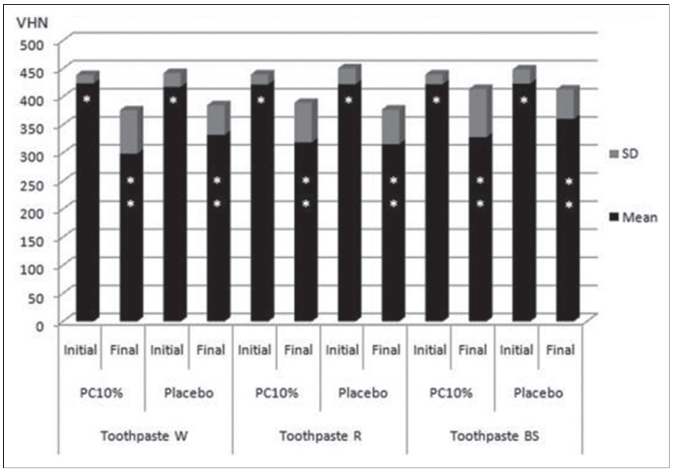

Figure 3: Mean values of enamel Vickers microhardness– Means followed by the same amount of signal (*) did not differ statistically according to paired Student's *t*-test ($\alpha = 0.05$).

($P = 0.369$), as well as the initial roughness ($P = 0.138$) were not significant. The toothpaste factor ($P = 0.037$) and the effect of the volunteers (block) ($P = 0.003$) were significant. According to the Bonferroni test, the toothpaste BS (0.20 µm) 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 HMV, 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 the volunteers was significant ($P = 0.001$).

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 undergoing bleaching treatment may use whitening and abrasive toothpastes to increase the treatment efficacy, and this association could be harmful to dental enamel.

This *in situ* study evaluated the effect of PC10 associated with three different fluoride toothpastes on the microhardness and surface roughness of human enamel. The PC10 was chosen because it is the bleaching agent most frequently found on the market,[17] and has been reported in the majority of publications on home bleaching in the last 20 years.[18]

In relation to different methodologies, *in situ* models have advantages compared 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 night guard 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, and were thus a less invasive method. These removable plates have also been used by Justino *et al.*[9] and Araujo *et al.*[10] Furthermore, a 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 the volunteers (block) was significant. This means that there was natural variability among the volunteers, even using exclusion criteria. Thus, as individuals are naturally different, it was 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 at the beginning of the experiment.

Most published studies have evaluated the separate effect of PC10[4-6,8-11,23] and toothpastes[24-28] on dental enamel. Few studies have evaluated the combined effect of those two treatments.[7,21] Considering that tooth brushing is a daily oral hygiene practice, whether the patient is undergoing 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 which factor most 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 PC10 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 PC10 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 the toothpaste 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 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 a significant difference in surface roughness. Toothpaste BS, composed of hydrated silica, calcium peroxide, and sodium bicarbonate created significantly rougher enamel compared with toothpaste R, which contains calcium carbonate, a polishing agent with less abrasive power.[29] Sodium bicarbonate contributes to stain removal,[30] calcium peroxide provides a whitening effect due to 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 that hydrated silica was an intermediate abrasive agent, and was more efficient in removing stains from enamel and dentin compared with other abrasives. The result obtained for toothpaste W may be due to the moderate abrasiveness of hydrated silica, placing this abrasive agent in an intermediate position.

In accordance with Bollen *et al.,*[32] surface roughness above 0.20 μm leads to dental plaque accumulation. None of the toothpastes studied presented roughness above this value. Despite the statistical difference between the roughness created by toothpaste BS (0.20 μm) and 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 shown that the microhardness of enamel changes after exposure to PC10 under a variety of *in vitro* and *in situ* conditions.[33,34] However, McCracken and Haywood[35] reported that PC10 caused a 1.06 $\mu g/mm^2$ calcium loss on enamel without clinical significance. In the present study, the association of PC10 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 greater mineral loss; the fluoride toothpastes presented were found to have the cariostatic effect expected as a result of the remineralization process. Furthermore, brushing with fluoride toothpaste helps to prevent the decrease in surface microhardness during bleaching treatment.[37] The absence of fluoride in toothpastes could result in lower surface microhardness as well as higher roughness values of enamel. In addition, the presence of fluoride in the PC10 gel could have had a positive influence on the results 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 PC10 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 have 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 PC10 bleaching is associated with tooth brushing using toothpastes BS, R, and W. Moreover, it can be speculated that an individual could use toothpastes BS, R, and W, while bleaching his teeth with PC10 with no clinical significance on enamel microhardness and surface roughness related to the choice of the toothpaste.

Conclusions

- PC10 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.
- PC10 associated with R, W or BS toothpastes caused similar decreases in enamel microhardness.

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