Bleaching Agents with Varying Concentrations of Carbamide and/or Hydrogen Peroxides: Effect on Dental Microhardness and Roughness

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ABSTRACT

Purpose: To evaluate the effect of low and highly concentrated bleaching agents on microhardness and surface roughness of bovine enamel and root dentin.

Methods: According to a randomized complete block design, 100 specimens of each substrate were assigned into five groups to be treated with bleaching agents containing carbamide peroxide (CP) at 10% (CP10); hydrogen peroxide (HP) at 7.5% (HP7.5) or 38% (HP38), or the combination of 18% of HP and 22% of CP (HP18/CP22), for 3 weeks. The control group was left untreated. Specimens were immersed in artificial saliva between bleaching treatments. Knoop surface microhardness (SMH) and average surface roughness (Ra) were measured at baseline and post-bleaching conditions.

Results: For enamel, there were differences between bleaching treatments for both SMH and Ra measurements ($p = 0.4009$ and $p = 0.7650$, respectively). SMH significantly increased ($p < 0.0001$), whereas Ra decreased ($p = 0.0207$) from baseline to post-bleaching condition. For root dentin, the group treated with CP10 exhibited the significantly highest SMH value differing from those groups bleached with HP18/CP22, HP7.5, which did not differ from each other. Application of HP38 resulted in intermediate SMH values. No significant differences were found for Ra ($p = 0.5975$). Comparing the baseline and post-bleaching conditions, a decrease was observed in SMH ($p < 0.0001$) and an increase in Ra ($p = 0.0063$).

Conclusion: Bleaching agents with varying concentrations of CP and/or HP are capable of causing mineral loss in root dentin. Enamel does not perform in such bleaching agent-dependent fashion when one considers either hardness or surface roughness evaluations.

CLINICAL SIGNIFICANCE

Bleaching did not alter the enamel microhardness and surface roughness, but in root dentin, microhardness seems to be dependent on the bleaching agent used.

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INTRODUCTION

With careful diagnosis, appropriate treatment planning, and attention to technique, bleaching may represent a more conservative and safe means to lighten discolored teeth. Currently, a broad range of bleaching agents containing varying concentrations of carbamide peroxide (CP) and/or hydrogen peroxide (HP) is available.[1]

Although at-home bleaching with 10% CP (CP10) has become a standard technique for teeth whitening,[1,2] in-office technique has emerged because highly concentrated products can promote faster whitening.[3] Although both bleaching techniques have been considered effective,[1,4,5] enamel and dentin may show morphological and chemical alterations, especially when in-office bleaching agents are used.[6–9]

Alterations on enamel surface topography have been observed after application of at-home bleaching[6,10–13] and in-office bleaching.[6,9,14,15] A rough appearance was also detected on dentin after either technique.[6] Contradicting these findings, other studies reported that neither at-home[16–20] nor in-office whitening products[17,21] changed the enamel surface texture. It has also been found that dentin does not show increased roughness following at-home bleaching.[13,19]

In terms of microhardness, there have been conflicting results in the literature. Whereas some studies showed that at-home bleaching decreased enamel[22–24] and dentin[25] microhardness, others did not observe any detrimental effects.[11,12,20,23,26,27] For in-office bleaching techniques, results have also been controversial. In some investigations authors have reported a reduction in enamel[8,24,26,28] and dentin[28] microhardness, whereas in another study, no alteration in dentin microhardness was detected.[8]

Despite the number of publications devoted to the investigation of bleaching effects, there is still a need for further studies to help increase our understanding of how dental tissues are affected and, if so, under what conditions. Toward this goal, this in vitro study was designed to test the hypothesis that microhardness and surface roughness of enamel and root dentin would be affected by the application of bleaching agents with varying concentrations of CP and/or HP.

MATERIALS AND METHODS

Experimental Design

The factor under study was bleaching treatment, evaluated at five levels, as listed in Table 1. Experimental units were 100 enamel slabs and 100 root dentin slabs, obtained from bovine teeth. The experimental layout followed a randomized complete block design. The specimens were randomly allocated to one of the five treatments ($N = 20$). The response variables were the Knoop surface microhardness (SMH) and average surface roughness (Ra).

Preparation of Dental Slabs

Two weeks after extraction, bovine incisors were cleaned to remove any remaining tissue and kept in 10% formalin solution (pH = 7.0). Teeth were sectioned at the cemento-enamel junction using a low-speed water-cooled diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA) separating the root from the coronary portion. Enamel and root dentin slabs ($4 \times 4 \times 2$ mm) were obtained from the middle third of the facial surface and from the cervical third of the root surface, respectively. Enamel sections were flattened under water cooling on a polishing machine (DU-9U2; Struers A/S, Rodovre, Denmark) with 400, 600, and 1,200 grit Al_2O_3 papers, whereas for root dentin sections only the last two grits were used. Slabs were then polished with 0.3- and 0.05-μm alumina suspensions (Alpha Micropolish II and Gamma Micropolish; Buehler Ltd.) on cloths (G-cloth, Buehler Ltd.). To remove the polishing debris,

TABLE 1. CHARACTERIZATION OF THE BLEACHING TREATMENTS TESTED.

Group	Product (manufacturer)	Bleaching agent	Treatment	Mean pH†
CP10	Platinum Overnight (Colgate-Palmolive Ltd., Campo, SP, Brazil)	10% Carbamide peroxide	8 hours per day for 21 days (in a tray)	5.9†
HP7.5	Day White 2 (Discus Dental Inc., Culver City, CA, USA)	7.5% Hydrogen peroxide	1 hour per day for 21 days (in a tray)	6.9†
HP38	Opalescence Xtra Boost (Ultradent, Products Inc., South Jordan, UT, USA)	38% Hydrogen peroxide	15 minutes per week for 3 weeks (no tray)	7.0‡
HP18/CP22	White Speed In-Office Fast Pack (Discus Dental Inc., Culver City, CA, USA)	18% Hydrogen peroxide and 22% Carbamide peroxide*	30 minutes per week for 3 weeks (in a tray)	4.7§
UN	Unbleached	—	Artificial saliva	7.0

*Equivalent to 35% carbamide peroxide, according to the manufacturer.
†Information taken from Price et al.[31]
‡Information taken from manufacturer's web site (http://www.ultradent.com/products/instructions/opal_boost.pdf).
§Information supplied for the Dental Discus Quality Control Department, Los Angeles, California.

specimens were placed into an ultrasonic cleaner (T1440D; Odontobrás Ltd., Ribeirão Preto, São Paulo, Brazil) with deionized water for 10 minutes. A stereomicroscope (Nikon 88286; Nikon, Tokyo, Japan) at 40× magnification was utilized to discard samples that presented stains or cracks. Specimens were kept at 37 ± 0.5°C in 100% relative humidity until SMH measurements were performed.

Microhardness Tests

A microhardness tester (HMV-2; Shimadzu, Kyoto, Japan) with a Knoop indenter was used to measure the SMH of 130 specimens of each dental substrate (enamel and root dentin). For enamel, a 25-g load was applied, whereas root dentin was indented with a 10-g load. For both substrates, indentation time was 20 seconds. Indentations were performed at five locations spaced 500 µm apart (500 µm from the edge) of each slab. A total of 100 out of the 130 sectioned pieces of enamel and root dentin were selected and then coated with wax, except for their outer surface. SMH values of the selected slabs were considered the baseline SMH. After bleaching treatments, five indentations were performed on the opposite margin of each specimen, in the same way as for the baseline measurements, and the average was considered the post-bleaching SMH value.

Surface Roughness Measurements

A profilometer (Surfcorder SE 1700; Kosaka Corp., Tokyo, Japan) was utilized to measure Ra. Measurements were carried out with a diamond stylus of 2-µm diameter, which traversed the surface at a 0.1-mm/second constant speed with a 0.7-mN force. Six tracings in four different directions were performed and recorded before and after the bleaching treatment for each specimen and the averages considered baseline and post-bleaching Ra values.

Bleaching Treatment

Dental slabs were randomly assigned to one of the five groups, as specified on Table 1. To better simulate clinical conditions,

TABLE 2. MEANS OF MICROHARDNESS (SURFACE MICROHARDNESS) FOR ENAMEL AND ROOT DENTIN FOLLOWING BLEACHING TREATMENTS (α = 0.05).

Group	Enamel		Root dentin	
	Baseline	Post-bleaching	Baseline	Post-bleaching
10% CP	213 (37)^{a}	^{A}248 (38)^{b}	34.0 (5.5)^{a}	^{A}31.5 (8.9)^{b}
18% HP/22% CP	217 (36)^{a}	^{A}239 (42)^{b}	34.5 (5.4)^{a}	^{B}25.2 (7.6)^{b}
38% HP	212 (36)^{a}	^{A}234 (50)^{b}	37.6 (5.4)^{a}	^{AB}26.4 (6.7)^{b}
7.5% HP	211 (38)^{a}	^{A}227 (39)^{b}	33.2 (5.7)^{a}	^{B}23.4 (7.2)^{b}
UN	231 (41)^{a}	^{A}253 (43)^{b}	37.1 (5.2)^{a}	^{B}25.1 (6.9)^{b}

Standard deviations are in parentheses.
Statistically different means are indicated by different lower case letters (within same line) and different capital letters (within same column).
CP = carbamide peroxide; HP = hydrogen peroxide; UN = unbleached.

modified trays were manufactured for each dental slab (except for UN and HP38 groups) in a vacuum tray-forming machine (P7/Bio-Art Equip Odontológicos Ltd., São Carlos, São Paulo, Brazil) using a 1-mm thick flexible ethyl vinyl acetate polymer (Bio-Art Equip Odontológicos Ltd.). A volume of 0.02 mL of bleaching agent was applied with a syringe on each dental specimen, which was then individually covered with a tray in the presence of 1 mL of artificial saliva (pH = 7.0) at 37 ± 0.5°C (Table 1). The bleaching agents were applied on dental specimens according to the manufacturers' instructions (Table 1). During bleaching intervals, specimens were individually maintained in 2 mL of artificial saliva at 37 ± 0.5°C, which was changed daily. For the HP38 group, slabs remained dry during the bleaching procedure to simulate rubber dam isolation. For the unbleached (UN) group, slabs were maintained in artificial saliva at 37 ± 0.5°C, which was changed daily. The artificial saliva used during all the experiment consisted of a remineralizing solution composed of calcium (1.5 mmol/L), phosphate (0.9 mmol/L), potassium chloride (150 mmol/L), hydroxymethyl-aminomethane (20 mmol/L) at pH 7.0 that was proposed by Featherstone and colleagues,[29] and modified by Serra and Cury.[30] The experiment lasted for 3 weeks.

Statistical Analysis

The SMH and Ra means were calculated at baseline and after the bleaching treatments. All statistical procedures were performed with Statgraphics Plus software (Manugistics, Rockville, MD, USA) at a significance level of $\alpha = 0.05$. Analyses of variance (ANOVA) were used to check significant differences between SMH and Ra for each substrate at the post-bleaching condition. Tukey's tests were used when there were significant differences between pairs of means. A paired *t*-test was computed to check for significant differences between baseline and post-bleaching values.

RESULTS

Means and standard deviations are shown in Tables 2 and 3.

For the enamel, ANOVA revealead no significant differences for microhardness ($p = 0.4009$) and for surface roughness ($p = 0.7650$) after treatment with different bleaching agents (Tables 2 and 3). Paired *t*-tests demonstrated a significant increase in the post-bleaching SMH values ($p < 0.0001$) and a significant decrease in Ra values ($p = 0.0207$).

For the root dentin, ANOVA showed significant differences for microhardness ($p = 0.0054$) (Table 2). Tukey's test showed that CP10 exhibited the significantly

TABLE 3. MEANS OF ROUGHNESS (Ra) FOR ENAMEL AND ROOT DENTIN FOLLOWING BLEACHING TREATMENTS (α = 0.05).

Group	Enamel		Root dentin	
	Baseline	Post-bleaching	Baseline	Post-bleaching
10% CP	0.1006 (0.0258)[a]	[A]0.0950 (0.0280)[b]	0.1070 (0.0264)[a]	[A]0.1218 (0.0406)[b]
18% HP/22% CP	0.0967 (0.0254)[a]	[A]0.0949 (0.0221)[b]	0.1079 (0.0245)[a]	[A]0.1054 (0.0174)[b]
38% HP	0.1072 (0.0281)[a]	[A]0.1009 (0.0316)[b]	0.1000 (0.0154)[a]	[A]0.1171 (0.0434)[b]
7.5% HP	0.0982 (0.0279)[a]	[A]0.0919 (0.0242)[b]	0.1054 (0.0335)[a]	[A]0.1117 (0.0394)[b]
UN	0.1056 (0.0406)[a]	[A]0.0999 (0.0340)[b]	0.1071 (0.0198)[a]	[A]0.1230 (0.0464)[b]

Standard deviations are in parentheses.

Statistically different means are indicated by different lower case letters (within same line). Equal capital letters (within same column) indicate means that are not significantly different.

CP = carbamide peroxide; HP = hydrogen peroxide; UN = unbleached.

highest SMH value differing from those groups bleached with 18% HP/22% CP and 7.5% HP, which did not differ from each other. Application of 38% HP resulted in intermediate SMH values. ANOVA demonstrated no significant difference among treatments for Ra values ($p = 0.5975$, Table 3). Paired *t*-tests revealed a significant decrease in SMH values ($p < 0.0001$, Table 2) and a significant increase in Ra values ($p = 0.0063$, Table 3).

DISCUSSION

The findings of this experiment confirmed in part the hypothesis that microhardness and surface roughness of enamel and root dentin would be affected by the application of bleaching agents with varying concentrations of CP and/or HP. This is because data supported the tested hypothesis only for root dentin SMH data. At the post-bleaching condition, SMH values were reduced in a bleaching agent-dependent fashion. Despite SMH and Ra values significantly changed for enamel from baseline to post-bleaching condition, the effects of bleaching agents did not differ from each other or from the untreated control group. The same was observed for Ra measurements obtained for root dentin.

After bleaching, enamel SMH values increased, corroborating a previous study of our group.[32] This result may be ascribed to the remineralizing solution used as an artificial saliva, which may have masked possible alterations in enamel microhardness, resulting in no difference among agents. Despite the use of the same remineralizing solution, in another study,[23] CP10 agents caused increase or reduction in enamel microhardness, depending on the trademark used (Opalescence—increased, and Rembrandt—decreased). It has been suggested that, besides the remineralization potential exerted by artificial salivas, some gel composites may provide remineralizing action.[12] Conversely, in other investigations, no change in enamel microhardness was detected after using bleaching agents containing CP10[12,26,33] or 6% HP.[20] This may be explained by the different pH[12,33] and composition[26] of the bleaching agents, to the nature of the artificial salivas used[12,20,33] and to shorter periods utilized for bleaching treatment.[20,33] There are also reports of decreased microhardness of enamel following treatment with HP[7,8,12,24,26] or CP.[22,24] This alteration seems to be related to the fact that specimens were stored in 100% relative humidity,[7,22,24] exposed for a long time to bleaching agents,[8,12] or received 37% phosphoric acid prior to bleaching.[26]

There was a decrease in root dentin SMH values (i.e., a mineral loss) from baseline to post-bleaching condition. This result is corroborated by other investigations,[7,25,28] which also verified a decrease in dentin microhardness following bleaching with 10, 15, and 35% CP agents, and 30 and 35% HP products. In effect, root dentin is more susceptible to demineralization, which occurs in solutions with a pH lower than 6.7.[34] Another important observation is that HP attacks both organic and mineral components of dentin.[35] Destruction of the organic components is mainly because of the oxidizing ability of HP, whereas changes in the mineral components are probably because of its acidity.[35] In this current research, three of the utilized bleaching agents, CP10, 7.5% HP, and 18% HP/22% CP, have been reported to have acidic pH (Table 1), whereas 38% HP has a neutral pH. Although the pH has been related to dentin demineralization, it seems that it does not play a major role in this study. In fact, CP10 presented high microhardness values in spite of its low pH value. The differences in root dentin SMH values caused by the bleaching treatments might be explained by the composition of the bleaching agents, as previously demonstrated.[36] One unexpected result was that the UN group exhibited reduction in microhardness. A possible explanation for this observation may be the pH of the remineralizing solution. Although it had been prepared with pH 7.0, a slight decrease in pH may have occurred over time, which would have been enough to cause demineralization of root dentin. Enamel would not have been affected by this supposed pH change, as its critical pH is around 5.5.

At the post-bleaching phase, enamel Ra values decreased, but no differences were verified among treatments. In some previous studies, no alterations were found either on surface roughness of enamel treated with 10,[18,19] 15[19], or 35%[21] CP agents nor on topography of enamel bleached with CP10 products,[12,16,17] or 6[20] and 30% HP agents,[17] probably because of the remineralizing effect exerted by the artificial saliva used. Conversely, an in situ report showed that surface roughness of enamel treated with home-use CP agent differed from enamel treated with placebo.[13] This may be due to carbopol, which causes changes in enamel mineral content.[25] However, these alterations did not cause changes in micromorphology of enamel, showing that surface alterations would not be perceptible clinically. There are also in vitro investigatons that have found an increase in enamel roughness after bleaching with 35% HP,[14] and morphological changes on enamel following application of CP10 bleaching gels,[6,10,11] and of 3[12] and 30% HP-containing products.[6] It may be speculated that alterations on enamel topography revealed by those studies were because of the long exposure time that specimens had been kept in contact with bleaching agents[6,9,11,12,14] and to the fact that no remineralizing solutions had been used.[6,9–11]

In contrast to enamel, root dentin showed an increase in surface roughness at the post-bleaching condition. This may be ascribed to the fact that root dentin is more soluble than enamel.[34] The increase in surface roughness suggests loss of substance that was confirmed by the microhardness alterations. As for enamel, no differences were observed among any bleached and the UN group. An in situ research report also did not show significant difference between treatments on morphology and on surface roughness, probably because of the pH of bleaching agent used (pH 6.7). Moreover, intra-oral challenges, such as abrasion and erosion, may have masked the effect of the bleaching gel. Similar results, observed by scanning electron microscopy (SEM), revealed severe changes such as roughening and an etched appearance of the dentin exposed to CP10 or 30% HP.[6] Another study found no alterations in surface roughness on dentin submitted to 10 and 15% CP,[19] probably because both at-home treatments were applied for 4 hours over 7 days, a time span shorter than the one used in this study. However, the SEM observations demonstrated slight morphological alterations on the surface of the dentin, such as removal of the smear layer and opened tubule orifices, which may be because of an etch-like effect of CP agents on the organic structure of dentin.[19]

CONCLUSIONS

In view of the results, it is concluded that bleaching agents with varying concentrations of CP and/or HP are capable of causing mineral loss in root dentin. Enamel does not perform in such bleaching agent-dependent fashion when one considers either hardness or surface roughness evaluations.

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