Effects of various hydrogen peroxide bleaching concentrations and number of applications on enamel

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Abstract

Aim: To evaluate the effects of three hydrogen peroxide (HP) concentrations on enamel mineral content (MC), following three peroxide applications and using three methods to measure the MC.

Methods: Forty samples were obtained from bovine incisors and randomly divided into 4 groups (n=10). The control group remained untreated, while the experimental groups were tested for three HP concentrations (10%, 35% and 50%). The HP gel was applied 3 times on the enamel surface for 30 min per application and the samples were analyzed after each application. The MC of the enamel was determined before and after bleaching using Fourier transform (FT-Raman) spectroscopy and micro energy-dispersive x-ray fluorescence spectrometry (µEDXRF). The calcium (Ca) lost from the bleached enamel was quantified with an atomic absorption spectrometer (AAS). The data were analyzed statistically by ANOVA, Tukey and Dunnett’s tests (p<0.05).

Results: The FT-Raman showed a decrease in MC for all bleaching treatments, without influence of the different HP concentrations or the number of applications. µEDXRF did not detect any changes in MC.

Conclusions: Ca loss was observed by the AAS, with no difference among the three HP concentrations. The FT-Raman and AAS analyses detected MC reduction and Ca loss after HP bleaching.

Keywords: dental enamel; bleaching agents; hydrogen peroxide.

Introduction

Tooth bleaching is a well-accepted method of treating stained and discolored teeth. The techniques comprise the application of a strong oxidizing agent as an active ingredient on a tooth surface to achieve a whitening effect¹. The bleaching mechanism is based on the decomposition of peroxides into free radicals, which react with the organic pigment molecules, transforming them into smaller and less pigmented compounds². Bleaching agents designed for professional use only contain a high concentration of peroxides (30% to 40%), while the active ingredient of patient-applied (at-home) tooth bleachers, present at lower concentrations, are carbamide peroxide (10% to 20%) and hydrogen peroxide (3% to 7.5%)³.

Since the introduction of at-home bleaching in 1989 by Haywood and
Heymann et al., many studies have evaluated the effects of hydrogen and carbamide peroxides on mineralized and dental pulp tissues. A number of studies have reported some important side effects, such as increased roughness, decreased microhardness, reduction of enamel and dentin strength, color changes and significant decrease in the calcium (Ca), phosphate or carbonate concentration in enamel. On the other hand, some authors have found no significant adverse effects following the bleaching.

Such differences in the adverse effects on enamel and dentin caused by bleaching agents are due to the large number of bleaching protocols, peroxide types and concentrations, and evaluation methods. Regarding the methods, microhardness, scanning electron and polarized light microscopy, micro energy-dispersive x-ray fluorescence spectrometry (µEDXRF), Fourier transform-Raman (FT-Raman) spectroscopy and atomic absorption spectrometer (AAS) have been used to determine the adverse effects resulting from bleaching techniques.

The objective of this study was to analyze the effect of different hydrogen peroxide (HP) concentrations (10%, 35% and 50%) after 1, 2 and 3 applications on an enamel surface, comparing three methodologies commonly used to evaluate the changes in the mineral contents of teeth. The research hypothesis was that HP promotes alterations in the mineral content of enamel regardless of the peroxide concentration and the number of peroxide applications.

Material and methods

Tooth preparation and experimental groups

Forty bovine incisors having the same age were used in this study. After extraction, the teeth were cleaned and stored in 0.1% thymol solution at 4 °C for 30 days. They were then submitted to manual debridement with a periodontal curette to remove organic debris. The root portion was removed with a diamond saw 2 mm below the dentin-enamel junction.

The enamel surface of all samples was flattened using 600-grit Al₂O₃ abrasive paper and polished with 1000- and 1200-grit aluminum oxide abrasive papers to remove irregularities from the bovine enamel surface. The enamel surfaces of all teeth were coated with 2 layers of nail varnish in 0.1% thymol solution at 4 ºC for 30 days. They were then submitted to manual debridement with a periodontal curette. The root portion was removed with a diamond saw 2 mm below the dentin-enamel junction.

The teeth were randomly divided into 4 groups (n=10), according to the HP concentration (10%, 35% or 50%) and an unbleached control group. The teeth from the control group were kept in distilled water at 37 °C.

FT-Raman spectroscopy

Spectra of the teeth before (baseline) and after enamel treatments (bleaching or control) were obtained using a FT-Raman spectrometer (RFS 100/S; Bruker, Karlsruhe, Germany). All spectra were processed by adjusting five Raman vibrational stretching modes: 430 cm⁻¹ (p1), 449 cm⁻¹ (p2), 586 cm⁻¹ (p3), 1043 cm⁻¹ (p4) and 1073 cm⁻¹ (p5). The p1–p3 represent phosphate modes and p4 and p5 are carbonate vibrations (type B carbonate). For the qualitative and semiquantitative spectral analysis, the spectra were corrected at baseline and then normalized to the 960 cm⁻¹ peak. The area of each band was obtained by Gaussian shapes. Microcal Origin Software (Microcal Software, Northampton, MA, USA) was used for calculating the integrated areas of the peaks for the unbleached and bleached samples. The averages of integrated areas of the evaluated Raman peaks (p1 to p5) were calculated for the baseline and treated data.

µEDXRF spectrometry analysis

The semi-quantitative elemental analyses of calcium (Ca) (Ca wt.%) and phosphorus (P) (P wt.%) were conducted by an energy-dispersive micro x-ray fluorescence spectrometer, model µEDX 1300 (Shimadzu, Kyoto, Japan), equipped with a rhodium x-ray tube and a Si (Li) detector cooled by liquid nitrogen (N₂) and coupled to a computer system for data processing. The voltage in the tube was set at 15 kV, with an automatic adjustment of the current and a beam diameter of 50 μm. Three spectra from each specimen were collected before and after bleaching treatments. The measurements were performed with a count rate of 100 s per point (live time) and a dead time of 25%. The energy range of the scans was 0.0 to 40.0 eV. The equipment was adjusted using a certified commercial reagent of stoichiometric hydroxyapatite [Aldrich, synthetic Ca₁₀(PO₄)₆(OH)₂, grade 99.999%, lot 10818HA] as reference. The measurements were collected under fundamental parameters of characteristic x-ray emissions of Ca and P, and the elements O and H were used as a chemical balance. The energy calibration was performed using the internal standards of the equipment, as previously reported.

Bleaching procedures

The bleaching agents were prepared using a rate of 10 parts of HP (Dinâmica Reagentes Analíticos, São Paulo, SP, Brazil) to 1 part of thickener (Carbopol Últrez 10; Proderma, Piracicaba, SP, Brazil), and pH adjusted to 6.0, according to a pilot study. A 2-mm thick layer of HP gel (0.2 g) was applied 3 times on the enamel surface for 30 min per application. Between applications, the samples were rinsed with 5 mL distilled and deionized water; this rinse was kept for AAS analysis.

Atomic absorption spectrometer

The rinse (HP and water) was evaluated using AAS. After each HP application, a standardized amount of water (5 mL) was used for rinsing and removing the peroxide gel from the enamel surface. The Ca concentration in the solution was measured using AAS (Varian Spectra AA50). Before the sample analysis, the equipment was calibrated with Ca standards ranging from 0.2 to 2.0 μg/mL.

The concentrations of Ca in the peroxide gels were previously analyzed and were not found in significant amounts. Each analysis used lanthanum solution to reduce the interference of phosphate in the calcium analysis.

Data analysis

The SAS software system (SAS Institute, Cary, NC, USA) was used for data analysis, with the significance set at 5%. Data from the FT-RS analysis were analyzed by two-way ANOVA and Tukey’s test. EDXRF data were analyzed by two-way ANOVA and Dunnett’s test to compare the treated groups with the control group. AAS data were analyzed by two-way ANOVA, Tukey’s test and Dunnett’s test.

Results

The selected range of Raman spectra from the phosphate and carbonate main vibrational modes of enamel after 1, 2 and 3 applications of HP are shown in Figures 1 to 3, respectively. The Raman spectra of the inorganic content did not show obvious reduction in intensity after treatments, compared with the non-treated and control group (Figures 1-3). The peaks at 430 and 449 cm\(^{-1}\) were associated to the \(\nu_2\mathrm{PO}_4^{3-}\) modes. The peak at 586 cm\(^{-1}\) was attributed to phosphate \(\nu_4\mathrm{PO}_4^{3-}\) vibrations and the peak 960 cm\(^{-1}\) was related to \(\nu_1\mathrm{PO}_4^{3-}\) vibration. The peaks at 1043 and 1073 cm\(^{-1}\) were attributed to bending and stretching modes of carbonate (\(\mathrm{CO}_3^{2-}\)), respectively (Figures 1-3).\(^{21}\)

Table 1 presents the means and standard deviations (SD) of the integrated area of the Raman peaks for the enamel before and after treatments. Two-way ANOVA revealed statistically significant differences for evaluation time (\(p = 0.0386\)). However, there was no significant difference between the HP gel concentrations (\(p = 0.2144\)) or the number of applications (\(p = 0.8982\)).

Table 2 exhibits the mean Ca, P and Ca/P ratio of enamel at baseline and after treatment, obtained by \(\mu\)EDXRF analysis. There was no statistically significant difference among the peroxide concentrations (\(p = 0.1344\)), number of applications (\(p = 0.1951\)), evaluation times (\(p = 0.4420\)) or the control group compared with the bleached experimental groups (\(p = 0.6259\)).

The results of AAS are depicted in Table 3. All experimental bleached groups differed from the unbleached control group, according to Dunnett’s test (\(p < 0.05\)).
Table 1 – Mean (standard deviation) of the integrated area of Raman peaks (p1 to p5) at baseline and after bleaching.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before bleaching (baseline)</th>
<th>After bleaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>67.8 (4.1) A a</td>
<td>65.4 (3.6) A a</td>
</tr>
<tr>
<td>10% HP + 1 application</td>
<td>68.0 (7.5) A a</td>
<td>56.0 (5.8) B a</td>
</tr>
<tr>
<td>10% HP + 2 applications</td>
<td>67.0 (8.2) A a</td>
<td>56.4 (5.7) B a</td>
</tr>
<tr>
<td>10% HP + 3 applications</td>
<td>63.8 (2.4) A a</td>
<td>58.7 (2.4) B a</td>
</tr>
<tr>
<td>35% HP + 1 application</td>
<td>70.3 (8.4) A a</td>
<td>61.5 (5.6) B a</td>
</tr>
<tr>
<td>35% HP + 2 applications</td>
<td>67.9 (8.6) A a</td>
<td>58.4 (8.8) B a</td>
</tr>
<tr>
<td>35% HP + 3 applications</td>
<td>71.2 (4.1) A a</td>
<td>60.5 (8.3) B a</td>
</tr>
<tr>
<td>50% HP + 1 application</td>
<td>69.4 (9.8) A a</td>
<td>56.7 (3.6) B a</td>
</tr>
<tr>
<td>50% HP + 2 applications</td>
<td>69.3 (4.1) A a</td>
<td>59.8 (7.5) B a</td>
</tr>
<tr>
<td>50% HP + 3 applications</td>
<td>69.2 (6.6) A a</td>
<td>60.6 (4.8) B a</td>
</tr>
</tbody>
</table>

Means followed by different letters, capital letters in rows and lowercase letters in columns, are statistically different by Tukey's test (p<0.05).

Table 2 – Means of element content in wt% (standard deviation) in enamel surface.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ca (mean ± SD)</th>
<th>P (mean ± SD)</th>
<th>Ca/P (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatments</td>
<td>After treatments</td>
<td>Before treatments</td>
<td>After treatments</td>
</tr>
<tr>
<td>Control Group</td>
<td>30.7 (7.8)</td>
<td>32.3 (7.7)</td>
<td>15.6 (3.9)</td>
</tr>
<tr>
<td>10% HP + 1 application</td>
<td>34.0 (5.8)</td>
<td>32.8 (3.9)</td>
<td>17.3 (2.5)</td>
</tr>
<tr>
<td>10% HP + 2 applications</td>
<td>30.1 (3.9)</td>
<td>28.9 (6.1)</td>
<td>16.5 (2.8)</td>
</tr>
<tr>
<td>10% HP + 3 applications</td>
<td>33.9 (2.4)</td>
<td>31.7 (2.6)</td>
<td>17.7 (1.2)</td>
</tr>
<tr>
<td>35% HP + 1 application</td>
<td>34.0 (7.8)</td>
<td>30.4 (2.8)</td>
<td>17.5 (5.9)</td>
</tr>
<tr>
<td>35% HP + 2 applications</td>
<td>37.2 (3.7)</td>
<td>31.6 (7.1)</td>
<td>19.1 (1.9)</td>
</tr>
<tr>
<td>35% HP + 3 applications</td>
<td>34.7 (5.1)</td>
<td>33.2 (4.4)</td>
<td>17.9 (1.7)</td>
</tr>
<tr>
<td>50% HP + 1 application</td>
<td>32.7 (1.0)</td>
<td>35.3 (1.9)</td>
<td>17.4 (0.7)</td>
</tr>
<tr>
<td>50% HP + 2 applications</td>
<td>35.6 (4.0)</td>
<td>33.9 (3.2)</td>
<td>19.1 (1.9)</td>
</tr>
<tr>
<td>50% HP + 3 applications</td>
<td>30.4 (3.9)</td>
<td>33.0 (2.2)</td>
<td>17.5 (6.3)</td>
</tr>
</tbody>
</table>

Means followed by different letters, capital letters in rows and lowercase letters in columns are statistically different by Tukey's test (p<0.05). Means followed by asterisk (*) differ from control group by Dunnett's test (p<0.05).

Table 3 – Concentration of calcium (µg/mL) in hydrogen peroxide (HP) gels and rinsing water from the bleached enamel surfaces.

<table>
<thead>
<tr>
<th>HP Concentration</th>
<th>1 Application</th>
<th>Number of Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>142.6 (27.5) Aa*</td>
<td>128.7 (44.6) Aa*</td>
</tr>
<tr>
<td>35%</td>
<td>131.8 (17.7) Aa*</td>
<td>149.5 (25.4) Aa*</td>
</tr>
<tr>
<td>50%</td>
<td>120.1 (28.0) Aa*</td>
<td>102.5 (20.8) Aa*</td>
</tr>
</tbody>
</table>

Means followed by different letters, capital letters in rows and lowercase letters, in columns are statistically different by Tukey's test (p<0.05). Means followed by asterisk (*) differ from control group by Dunnett's test (p<0.05).

HP concentration had no influence on Ca loss in any of the applications (p = 0.0884). The first and second applications of HP released significantly higher values of Ca in the rinse compared with the third (p < 0.0001).

**Discussion**

µEDXRF, FT-Raman and AAS are versatile and nondestructive techniques that allow use of the same samples for the various types of dental enamel analysis. These methods provide precise concentrations of Ca, phosphate and carbonate and the Ca/P ratio, as shown in the Results section. The hypothesis that HP promotes alterations in the mineral content of enamel regardless of peroxide concentration or number of peroxide applications was accepted only after using the FT-Raman and AAS methods.

FT-Raman analysis showed that HP decreased the integrated area of Raman peaks of enamel samples (Table 1 and Figures 1 - 3). On the other hand, the control group, in which the specimens were not bleached, no enamel chemical changes were observed, as expected. Alterations in mineral content of enamel promoted by peroxides have also been detected by other studies using FT-Raman10,15,22. In this study,
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the number of applications did not decrease the integrated area of Raman peaks, indicating that, up to 3 HP applications, the effects of peroxides were similar. However, Bistey et al. reported that changes in the enamel surface were directly proportional to the HP concentration. In addition, these changes in the area of the peaks were more obvious when the duration of bleaching was 30 and 60 min. The 30-min time used in this study may not have been able to promote more alterations in enamel surface after first, second or third applications of bleaching agent regardless of the HP concentration. A previous has shown similar results on human enamel after using 35% HP, testing three commercially available, bleaching agents applied for 30 min.

The µEDXRF analysis provides information about tooth mineral content and could be used as an analytical technique to study chemical changes on enamel. However, in the conducted study it was not possible to identify any changes in mineral phase using this method, even after three 50% HP applications. Smidt et al. found no significant changes in the enamel Ca/P ratio after bleaching. Conversely, some authors reported a reduction in the Ca/P ratio, even after three 50% HP treatments.

Reis et al. investigated the effect of 24 h non-stop 3%, 10% or 30% HP exposure time (24 h versus 30 min used in the present work).

The Ca bonded weakly to the hydroxyapatite and it was lost mostly after the first and second peroxide applications, according to the AAS method. Thus, after the third application of peroxide, the Ca concentration decreased significantly in the rinsing solution (HP and water). According to Wang et al. the release of Ca from the enamel apatite may occur via atomic diffusion through the apatite channels along the crystallographic c-axis and the inter-crystallites and inter-rod special voids with openings on the surface. Another reason for bleaching having enhanced Ca leaching could be the degradation of the enamel organic component, which removes related mineral elements. Calcium removed by the HP application is the one present in the hydroxyapatite crystal, the building block of dental hard tissues. Probably polishing of the enamel surface, made in the present study in order to remove irregularities from the bovine enamel surface, produced a more reactive surface and in the first and second applications of HP, independent of its concentration, a greater amount of Ca was removed from the crystal.

Bovine teeth were used in this study because their chemical properties and the prismatic morphology are very similar to human enamel as reported by Yassen et al. and Reis et al., respectively. Studied have used bovine teeth as substitute for human teeth also in cosmetic dentistry and dental bleaching. However, the comparison of these findings with studies using human teeth must be careful because of the variations in bovine tooth age and the different methodologies used to prepare the samples.

This study did not test commercial whitening products; the used bleaching gels were a mixture of HP and a thicker. The commercially available bleaching agents have in their composition some additives like fluoride ions, potassium nitrate, carpolol, propylene glycol, sodium fluoride, calcium and others. These additives may minimize the mineral loss and produce different results from those obtained in this study. More studies are required to investigate the role of each component in the enamel mineral loss. An important factor that must be considered is that in this study the amount of bleaching agent used was standardized (2 mm thick on the enamel surface), since many studies do not mention the amount of bleaching agent used. There are no studies in the literature correlating the amount of bleaching agent and its effects on tooth structure.

Different methods were used in the present study to assess the mineral content of enamel after the bleaching treatment. While µEDXRF did not identify any changes in mineral content after bleaching, FT-Raman and AAS detected alterations in mineral components and Ca loss, respectively. The analysis of mineral content of enamel after bleaching...
requires the knowledge of the accuracy and limitations of each method used. The effect of dental bleaching on enamel mineral content has been frequently evaluated using EDXRF, FT-Raman and AAS, and the findings of the present study showed that these methods are accurate for this purpose. When used separately, different results were found for each method and this should be considered when comparing studies with different methodologies. According to the FT-Raman and AAS methods of detection, HP bleaching causes a reduction in the mineral content of enamel. Increasing the HP concentration did not increase the mineral loss.

Acknowledgments

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References