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ORIGINAL ARTICLE

In vitro effects of hydrogen peroxide combined with different activators for the in-office bleaching technique on enamel

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Abstract

Objective. The aim of this study was to evaluate the alteration of human enamel bleached with high concentrations of hydrogen peroxide associated with different activators. ***Materials and methods.*** Fifty enamel/dentin blocks (4 × 4 mm) were obtained from human third molars and randomized divided according to the bleaching procedure (n = 10): G1 = 35% hydrogen peroxide (HP – Whiteness HP Maxx); G2 = HP + Halogen lamp (HL); G3 = HP + 7% sodium bicarbonate (SB); G4 = HP + 20% sodium hydroxide (SH); and G5 = 38% hydrogen peroxide (OXB – Opalescence Xtra Boost). The bleaching treatments were performed in three sessions with a 7-day interval between them. The enamel content, before (baseline) and after bleaching, was determined using an FT-Raman spectrometer and was based on the concentration of phosphate, carbonate, and organic matrix. Statistical analysis was performed using two-way ANOVA for repeated measures and Tukey's test. ***Results.*** The results showed no significant differences between time of analysis ($p = 0.5175$) for most treatments and peak areas analyzed; and among bleaching treatments (p = 0.4184). The comparisons during and after bleaching revealed a significant difference in the HP group for the peak areas of carbonate and organic matrix, and for the organic matrix in OXB and HP+SH groups. Tukey's analysis determined that the difference, peak areas, and the interaction among treatment, time and peak was statistically significant ($p < 0.05$). ***Conclusion.*** The association of activators with hydrogen peroxide was effective in the alteration of enamel, mainly with regards to the organic matrix.

Key Words: *Bleaching, hydrogen peroxide, enamel, FT-Raman*

Introduction

Bleaching treatments are extensively used to treat discolored teeth, as they are considered effective, safe and a conservative procedure [1,2]. The whitening mechanism uses hydrogen peroxide, in different concentrations, and consists of an oxidation reaction with the release of free radicals. During bleaching, these oxygen free radicals penetrate the enamel and react with colored organic materials found within tooth structures [3–5].

The whitening agent may be hydrogen peroxide or products that break into hydrogen peroxide, such as sodium perborate and carbamide peroxide [2]. The bleaching treatment may be at-home bleaching, performed by the patient using custom trays and supervised by a dentist; or in-office bleaching, performed in the office by professionals. The in-office bleaching procedure has been used to achieve an optimal whitening effect in a reduced treatment time [6]. For this purpose, hydrogen peroxide has been used in high

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concentrations associated with or without light or chemical activators [3–5]. In this way, the use of chemical or physical catalysts in the bleaching procedure may be indicated to become the oxi-reduction reaction faster and to increase the liberation of free-oxygen radicals [7,8].

It has been reported that higher concentrations of peroxide lead to a lower pH of the bleaching product (more acidic) [9,10]. Hydroxyapatite is the main component of enamel; and it has been reported that its demineralization occurs when pH falls below 5.2 [5]. The use of bleaching agents with a pH below five may cause damage to the enamel, even in patients with appropriate salivary flow and with high concentrations of calcium and phosphate [11,12]. Overall, it has been determined that dental bleaching may produce some surface alterations in enamel, including increased roughness, decreased microhardness, reduced enamel strength and other modifications [13–15]. Another alteration that has been found is changes to the composition of enamel after treatment [16–22].

The alterations in mineral content of bleached enamel have been studied using different methodologies [23]. The Raman spectrum analysis of teeth can provide information about their composition and structures at a molecular level [24]. As it is a non-destructive method, FT-Raman allows for the evaluation of enamel before and after bleaching procedures [25]. This technique allows vibrational spectra of minerals to be obtained by analyzing scattered light caused by visible or near-visible monochromatic laser excitation [24]. The Raman spectrum of dental tissues can provide information regarding the relative concentration of the phosphate and carbonate groups associated with the hydroxyapatite molecule, while any change in the tooth will affect the vibrational mode of the molecules and could be analyzed by Raman spectroscopy [17].

Although the efficacy of bleaching agents has been widely proven [3–5], questions are raised in the literature about the effect of hydrogen peroxide on the molecular structure of teeth. Therefore, the aim of the present study was to evaluate the alteration of human enamel, in relation to structural phosphate and carbonate and organic content, after performing in-office bleaching with higher concentrations of hydrogen peroxide associated with different activators, such as a halogen lamp, sodium bicarbonate, sodium hydroxide and potassium hydroxide, using FT-Raman spectroscopy.

Materials and methods

Specimens preparation

Twenty-five sound, human, third molars were used. The research was approved be the Ethical Committee (Protocol no. 026/2006). The teeth were stored in thymol for 7 days. After cleaning, the teeth were examined under a light microscope (×4) to exclude those with staining, cracks and caries lesions. The specimens remained immersed in artificial saliva during the entire experiment. This solution was changed every day. The artificial saliva used in this study contained calcium and phosphate with a known concentration (50 mmol/l KCl, 1.5 mmol/l Ca, 0.9 mmol/l PO4, 20 mmol/l trihydroxymethyl-amino-methane, pH = 7.0) [13].

Fifty enamel/dentin blocks were obtained from the buccal/lingual surfaces of the teeth. The crown of each tooth was set in an acrylic plaque, which was fixed to a precision slow speed water-cooled diamond saw (Imptech PC10 - Equilam Lab Equip., Diadema, SP, Brazil), with two parallel disks set at a distance of 4 mm from each other and perpendicular to the buccal/lingual surface of the tooth. Each tooth was cut in the incisal–gingival and mesial–distal directions, resulting in 3 mm thick blocks with an area of 16 mm^2. The enamel surface was planed and flattened using silicon carbide papers of decreasing granulation (#300, #600 and #1200), felts and diamond paste of 1, 1/2 and 1/4 μm granulation; greased with a specific oil (Arotec, Cotia; SP, Brazil); and coupled to a circular polishing machine, under water cooling (Aropol E, Arotec, Cotia; São Paulo, SP, Brazil). Between the polishing steps and after the final polishing, all slabs were sonicated (Marconi, Piracicaba, SP, Brazil) with distilled water for 15 min to remove debris.

Table I. Groups of study.

Group	Bleaching agent	Activator
G1	35% hydrogen peroxide (HP) (Whiteness HP Maxx - FGM Products)	—
G2	HP (Whiteness HP Maxx)	Halogen Lamp (HL - Optilux 501C, Dec, Kerr)
G3	HP (Whiteness HP Maxx)	7% Sodium Bicarbonate (SB)
G4	HP (Whiteness HP Maxx)	20% Sodium Hydroxide (SH)
G5	38% hydrogen peroxide (OXB) (Opalescence Xtra Boost – Ultradent Products)	Potassium Hydroxide

Table II. In-office bleaching agents used in this study.

Bleaching agents	Main composition	pH
Whiteness HP Max	35% hydrogen peroxide, thickening agent, gel	5.8
Opalescence Boost	38% hydrogen peroxide, fluoride, potassium nitrate, potassium hydroxide (activator), thickening agent, gel	7.52

Groups

The specimens were randomly divided into five groups (n = 10) according to the bleaching agent and activator used treatment (Table I).

Bleaching procedure

The information about the two in-office bleaching products is shown in Table II. An ~ 1 mm thick layer of gel was applied to the enamel surface. For all groups, three gel applications of 15 min were made at each session, with three bleaching sessions performed. The intervals between the sessions were set at 7 days. All the bleaching treatments were performed in a controlled room temperature (23.0 ± 1°C). The bleaching protocols were as follows:

- HP: The gel remained on the enamel surface for 15 min.
- HP + physical activator (HL): Application of the gel on the enamel surface and light activation was performed after waiting 2 min. The gel was photoactivated using a halogen light (Power density = 470 mW) for two application periods of 30 s, with a 1 min interval. The light guide tip of the HL was positioned 2 mm from the specimen surface. The gel was removed 11 min after source activation. The gel remained on the enamel surface for a total of 15 min.
- HP + chemical activator (SB and SH): Before application on the enamel surface, 10 L of sodium hydroxide or sodium bicarbonate, according to the experimental group, was mixed with one portion of gel that consisted of 90 L hydrogen peroxide. The volume of activator used was defined in a pilot study. The gel remained on the enamel surface for 15 min.
- OXB: The Opalescence Xtra Boost was mixed with the chemical activator before use, in accordance with the manufacturer's instructions. The gel remained on the enamel surface for 15 min.

FT-Raman analysis

Before and after each bleaching treatment, the specimens were analyzed using FT-Raman spectroscopy (RFS 100/S, Bruker Inc., Karlsruhe, Germany). The analyses before treatment were used as a control (baseline). At the end of the bleaching treatment (after the three sections), there was a waiting period of 24 h for the specimens to re-hydrate before readings were taken. To excite the spectra, a focused = 1064.1 nm beam of an air-cooled Nd:YAG laser source was used. Maximum incident laser power on the sample surface was ~ 100 mW and spectrum resolution was 4 cm^1. To perform the analysis, the specimens were cleaned in an ultrasonic bath of distilled water for 5 min to remove impurities. The specimens were positioned in a glass sample holder and an IR354 lens collected radiation scattered over 180° of the exposed surface. The FT-Raman spectra were obtained using 100 scans and one spectrum was collected for each sample, using a central point on the enamel surface. The lower limit of laser penetration depth was ~ 500 μm [19]. Frequency of spectra ranged from 400–3000 cm^1 in the region of interest, thereby allowing a characterization of both mineral (hydroxyapatite) and organic constituents. The peaks analyzed were in the following Raman vibrational stretching modes: 431 cm^1 and 583 cm^1—phosphate, 1070 cm^1—carbonate and 2940 cm^1—C-H bonds of the organic matrix.

For the Raman analysis, the specimens were positioned over a glass slide in the sample holder compartment, and an IR354 lens collected radiation over 90° on the enamel surface. For each sample, one spectrum was collected at the central point of the enamel surface. To obtain a good signal-to-noise ratio, 100 scans were co-added for each spectra [17,25]. The obtained spectra, in the readings before and after treatment, were analyzed using analytical software (Microcal Origin 5.0 Software, Inc., Northampton, MA, USA). The luminescent background was removed using baseline correction for each spectrum collected before performing relative comparison studies of organic and inorganic content. The band fitting of characteristic peaks was performed using a combined Gaussian= Lorentzian function to determine the exact position, peak intensities and areas, as previously reported [17].

Statistical analysis

The results were submitted to two-way ANOVA for repeated measures, followed by the Tukey's test, considering treatment and evaluation time (baseline and after treatments) as factors. The SAS software system (SAS Institute Inc., Cary, NC) was used and the significance level was set at 5%.

Results

The mean values and standard deviations of the integrated areas of the vibrational stretching modes in

Table III. Mean values and standard deviations of the relative areas of the 431 cm^1 peak (phosphate) before and after bleaching.

	Bleaching	
Treatment	Before	After
HP	3.63 (0.5)^{Aa}	3.51 (0.4)^{Aa}
HP + HL	3.36 (0.5)^{Ba}	3.57 (0.6)^{Aa}
HP + SB	3.45 (0.4)^{Aa}	3.59 (0.4)^{Aa}
HP + SH	3.62 (0.5)^{Aa}	3.63 (0.4)^{Aa}
OXB	3.42 (0.3)^{Aa}	3.24 (0.4)^{Aa}

Mean values followed by different letters differ statistically for the Tukey test ($p < 0.05$). Capital letters are to be read horizontally and lower cases vertically.

Table V. Mean values and standard deviations of the relative areas of the 1070 cm^{-1} peak (carbonate) before and after bleaching.

	Bleaching	
Treatment	Before	After
HP	3.45 (0.4)^{Aa}	3.16 (0,31)^{Ba}
HP + HL	3.24 (0.6)^{Aa}	3.26 (0.6)^{Aa}
HP + SB	3.35 (0.4)^{Aa}	3.35 (0.4)^{Aa}
HP + SH	3.42 (0.5)^{Aa}	3.55 (0.3)^{Aa}
OXB	3.10 (0.3)^{Aa}	3.16 (0.4)^{Aa}

Mean values followed by different letters differ statistically for the Tukey test ($p < 0.05$). Capital letters are to be read horizontally and lower cases vertically.

Raman peaks for enamel before (baseline) and after bleaching are presented in Tables III, IV, V,VI. In the statistical analysis, the peak areas of the phosphate (431 cm^1, Table I and 583 cm^1, Table II), carbonate (1070 cm^1, Table III) and C-H bonds of organic matrix (2940 cm^1, Table IV) were compared.

ANOVA revealed no significant differences between time of analysis (before and after bleaching) ($p = 0.5175$) for most of treatments and peak areas analyzed; and among the bleaching treatments ($p = 0.4184$). The Tukey's test revealed significant differences among peak areas ($p < 0.0001$). In relation to the comparison before and after bleaching, a statistical difference in the HP group was found for the peak areas of carbonate and organic matrix and for the peak area of the organic matrix in the OXB and HP + SH group.

Discussion

In this current study, the peaks identified in the vibrational modes using FT-Raman spectroscopy were previously named as v_2 (430–450 cm^1) and v_4 (583–612 cm^1), both relating to phosphate (PO_4^3) in different modes and v_3 (1070 cm^1) attributed to carbonate (CO_3^2) [14,24,25]. The peaks ranging from 1247–2942 cm^1 are associated with dental organics, while the peak at 2940 found in this present study is related to C-H stretching vibrations [26]. To describe changes of tooth enamel, several peaks of phosphate groups are identified once the concentration of this component within enamel is a good indicator of the degree of mineralization [27]. Moreover, the intensity of PO_4^3 in Raman spectroscopy is linearly proportional to phosphate group concentration within hydroxyapatite molecule [28].

Table IV. Mean values and standard deviations of the relative areas of the 583 cm^1 peak (phosphate) before and after bleaching.

	Bleaching	
Treatment	Before	After
HP	4.76 (0.40)^{Aa}	4.64 (0.38)^{Aa}
HP + HL	4.69 (0.45)^{Aa}	4.65 (0.46)^{Aa}
HP + SB	4.66 (0.37)^{Aa}	4.80 (0.27)^{Aa}
HP + SH	4.85 (0.31)^{Aa}	4.77 (0.26)^{Aa}
OXB	4.56 (0.34)^{Aa}	4.40 (0.39)^{Aa}

Mean values followed by different letters differ statistically for the Tukey test ($p < 0.05$). Capital letters are to be read horizontally and lower cases vertically.

FT-Raman spectroscopy data showed no significant changes in the inorganic enamel components for tested groups in most peak areas analyzed. The carbonate and phosphate peak areas were not significantly changed after bleaching. The phosphate and carbonate content may indicate the degree of mineralization of enamel [29]. Thus, hydrogen peroxide used in high concentrations associated with activators did not demineralize enamel in the present study [17,30]. Similarly, several studies [16,17,26,30] did not find significant changes in enamel morphology after bleaching with high concentrations of hydrogen peroxide. Controversially, Bistey et al. [31] and Berger et al. [25] found morphological alterations in human enamel after different in-office bleaching protocols. According to those authors, the severity of enamel alteration was dependent on the treatment time, peroxide concentration and treatment protocol. The difference between the present study and the previously mentioned studies is in relation to storage of the samples. In this present study, the specimens were stored in artificial saliva at all times, with daily changes, simulating clinical conditions. Artificial saliva contains a high level of calcium and phosphate. These components create an optimum environment for the mineral recovery of an existing enamel lesion [13,32]. This is an important detail, since the effect of saliva on modulating the demineralization and remineralization process of the mineral content has been documented [33,34].

In relation to the carbonate peak analysis (1070 cm^1), treatment with HP reduced the content of this mineral, with a statistically significant

Table VI. Mean values and standard deviations of the relative areas of the peaks related to the C-H bonds of the organic matrix (2940 cm^1) before and after bleaching.

	Bleaching	
Treatment	Before	After
HP	5.98 (2.0)^{Aa}	4.84 (1.9)^{Ba}
HP + HL	5.37 (2.3)^{Aa}	6.08 (2.8)^{Aa}
HP + SB	5.96 (1.7)^{Aa}	6.29 (2.2)^{Aa}
HP + SH	6.08 (2.2)^{Ba}	7.61 (1.4)^{Aa}
OXB	4.11 (1.6)^{Ba}	5.24 (2.3)^{Aa}

Mean values followed by different letters differ statistically for the Tukey test ($p < 0.05$). Capital letters are to be read horizontally and lower cases vertically.

difference, in relation to the FT-Raman analysis at baseline. This alteration was not found in the treatment with HP and a physical or chemical activator. In relation to the physical activator, the reduction of carbonate might not occur due to the fact that, according to the manufacturer of HP, this product has a large light absorption spectrum, which accelerates the bleaching process under light irradiation [25]. In relation to the chemical activator, it has been noted that they do not improve the whitening effectiveness of high concentration bleaching agents [7,8].

The peak at 2940 cm^1, which corresponds to the organic phase (specifically C-H stretching vibrations), reduced after the application of hydrogen peroxide only (Table VI) as related by Eimar et al. [22]. However, when hydrogen peroxide was combined with an activator, the relative peak areas remained the same (HP+HL and HP+SB) or increased (HP +SH and OXB). Probably, this result is related to the acidity of this product, which presents lower pH than the other bleaching agent tested (OXB). This is in agreement with the study of Sun et al. [28], which concluded that bleaching agent with neutral pH should be recommended to tooth bleaching for the purpose of reducing deleterious effects on enamel. When chemical activators were used, the pH of the gel was elevated to an alkaline level and may have protected the organic structure. In addition, hydrogen peroxide in combination with a light source may have generated a type of free radical that did not damage the organic structure [35].

Considering the results of this present study, the in-office bleaching with high concentrations of hydrogen peroxide may be indicated when this compound is associated with chemical activators. These current and previous results [7] verify that this association is efficient in protecting the organic content of enamel, without reducing the clinical efficacy of the treatment. Since only the treatment with HP significantly reduced the peak corresponding to the organic content, the association of activators may be considered important for the bleaching technique. This was evident mainly for the 20% sodium hydroxide (in association with HP) and for that used with OXB (Opalescence Xtra Boost – Ultradent Products Inc., South Jordan, UT, USA), which is not disclosed by the manufacturer, as the results for the peak areas increased after treatment. Both activators are mixed with the hydrogen peroxide immediately prior to the bleaching treatment and, as previously discussed, this is an important factor to be considered to perform a safe clinical procedure.

The Raman spectroscopy presents as a limitation that some fluorescence of the sample, in this case tooth enamel, may interfere in the Raman signal of interest. However, with introduction of the Fourier transformation (FT-Raman) coupled with the use of infrared lasers, the effect of fluorescence was reduced [36]. This advance contributed to a more specific and accurate methodology, which justifies the use of FT-Raman in this study. However, further research should be performed to confirm if these results are consistent in more reliable clinical conditions, either by *in situ* or *in vivo* designs. However, under the conditions of this *in vitro* study, FT-Raman analysis showed that the association of hydrogen peroxide with a halogen lamp, as physical or chemical activators (sodium hydroxide, sodium bicarbonate or potassium hydroxide) were efficient in reducing damage on the enamel structure in relation to its molecular composition.

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