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Penetration of Hydrogen Peroxide and Degradation Rate of Different Bleaching Products

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Clinical Relevance

During in office bleaching techniques, the reapplication of products is not necessary.

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SUMMARY

This study's aim was to evaluate the degradation rate of hydrogen peroxide (H_2O_2) and to quantify its penetration in tooth structure, considering the residence time of bleaching products on the dental enamel. For this study, bovine teeth were randomly divided according to the bleaching product received: Opalescence Xtra Boost 38%, White Gold Office 35%, Whiteness HP Blue 35%, Whiteness HP Maxx 35%, and Lase Peroxide Sensy 35%. To analyze the degradation of H_2O_2, the titration of bleaching agents with potassium permanganate was used, while the penetration of H_2O_2 was measured via spectrophotometric analysis of the acetate buffer solution, collected from the artificial pulp chamber. The analyses were performed immediately as well as 15 minutes, 30 minutes, and 45 minutes after product application. The data of degradation rate of H_2O_2 were submitted to analysis of variance (ANOVA) and Tukey tests, while ANOVA and Fisher tests were used for the quantification of H_2O_2, at the 5% level. The results showed that all products significantly reduced the concentration of H_2O_2 activates at the end of 45 minutes. It was also verified that the penetra-

tion of H_2O_2 was enhanced by increasing the residence time of the product on the tooth surface. It was concluded that the bleaching gels retained substantial concentrations of H_2O_2 after 45 minutes of application, and penetration of H_2O_2 in the dental structure is time-dependent.

INTRODUCTION

The smile transmits most of a person's characteristics and expressions, thus influencing self-esteem and social relationships. In recent years, the search for cosmetic procedures has increased significantly, especially those that involve tooth whitening. Thus, tooth whitening has become a main demand for patients in dental clinics and has been incorporated as an essential procedure in most treatment plans that involve the esthetics of a smile.[1,2]

However, although bleaching vital teeth is considered to be a conservative treatment and is biologically safe, concerns still arise regarding the use of highly concentrated products, as their uses are related to structural changes in dental tissues and postoperative sensitivity.[3-7] Thus, a good professional should know the indications of the different bleaching agents, the advantages of each technique, and the potential effects on teeth.

Currently, the most common product used in the in-office bleaching technique is based on 35% and 40% hydrogen peroxide and is applied under the supervision of dental professionals. Although variations exist in the method of application for different products, this treatment requires multiple clinical sessions in order for an esthetic result to be obtained.[8,9] Although few studies assess the real need for reapplication during the same session, the professional usually replaces the whitening gel multiple times, continually renewing the product that is in contact with the tooth structure.

Despite the fact that the success of bleaching is directly related to the diffusion capacity of the peroxides, it is believed that successful bleaching is not related to continuous reapplication because color change is clinically observed even when reapplication is not performed.[10]

Studies that evaluate the concentration of peroxide in bleaching gels over time show continuous liberation of peroxide even after 15 minutes of application,[11-14] which indicates the real possibility of a bleaching product to maintain its bleaching capacity, even without reapplication.

Additionally, exposure to excessive and unnecessary bleaching products increases the levels of hydrogen peroxide in the pulp tissue, which could cause lipid peroxidation, cell membrane injury, and even cell death.[15,16] Given these facts, it is necessary to measure the concentration and quantify the penetration of hydrogen peroxide through the dental tissue during its contact with the enamel.

It is believed that this knowledge can contribute to the refinement of bleaching therapy, as clinicians are employing significant resources in order to offer the best whitening treatments for their patients, though they may be providing unnecessary exposure to peroxides.[3-6,17]

Therefore, this present study evaluated the degradation rate of hydrogen peroxide and quantified its penetration into the tooth structure when considering different application times.

METHODS AND MATERIALS

Factors Under Study

1. Bleaching products based on hydrogen peroxide at five levels: Opalescence Xtra Boost 38% (Ultradent, South Jordan, Utah, USA), White Gold Office 35% (Dentsply Ind Com Ltda, Petrópolis, RJ, Brazil), Whiteness HP Blue 35% (FGM Dental Products, Joinville, SC, Brazil), Whiteness HP Maxx 35% (FGM Dental Products), and Lase Peroxide Sensy 35% (DMC Equipment, São Carlos, SP, Brazil).
2. Time after placement at four levels: immediately, 15 minutes, 30 minutes, and 45 minutes after placement.

The response variables included the concentration of hydrogen peroxide in bleaching products as well as the transenamel and transdentinal penetration of hydrogen peroxide. The three basic principles of experimentation (repetition, randomization, and blocking) were respected.

Solution Preparation and Analysis of the Degradation Rate of Hydrogen Peroxide

Five syringes of each bleaching product were used to verify the decomposition of hydrogen peroxide at different times (Table 1). The method used is based on titration with potassium permanganate.[18,19] This method describes the amount of hydrogen peroxide in a bleaching product. This method is based on a reduction-oxidation reaction, according to the following formula: $2KMnO_4 + 5H_2O_2 + 4H_2SO_4 = 2KHSO_4 + 2MnSO_4 + 5O_2 + 8H_2O$.

Table 1: *Characteristics of the Materials Studied*

Group	Trademark	%[a]	Manufacturer/Lot	Manipulation
G1	Opalescence Xtra Boost	38%	Ultradent/B6BSV	Two coupled syringes, one containing the peroxide and the other with the activator
G2	White Gold Office	35%	Dentsply/675123E	Two coupled syringes, one containing the peroxide and the other with the activator
G3	Whiteness HP Blue	35%	FGM/240712	Two coupled syringes, one containing the peroxide and the other with the activator
G4	Whiteness HP Maxx	35%	FGM/290812	Phase I mixed with phase II at a ratio of a measure of powder to a liquid droplet
G5	Lase Peroxide Sensy	35%	DMC/30403	Phase I mixed with phase II at a ratio of a measure of powder to a liquid droplet

[a] Hydrogen peroxide concentration given by the manufacturer.

The solution of potassium permanganate (solution 1) was prepared by mixing 0.2 g of sodium oxalate, 250 mL of distilled water, and 15 mL of sulfuric acid at 80°C for 30 minutes. Then, the solution was kept in an amber glass that was protected from light for 24 hours.

The bleaching agent was mixed in accordance with each manufacturer's instructions and evaluated immediately and at 15 minutes, 30 minutes, and 45 minutes after mixing. During this period, the bleaching products were kept on a glass plate. In order to obtain the volume of hydrogen peroxide in a sample, the bleaching agents were analytically weighted, and a sample of approximately 2 mg of each bleaching product was collected and diluted in 10 mL of distilled water (solution 2). Then, solution 1 was added to solution 2 at a rate of 0.1 mL/sec until a violet color was observed. This color change indicated the equivalence point, ie, the moment when all of the H_2O_2 had been consumed. The volume of solution 1 required to change the color of the solution was applied to the following formula: $C = V \times Cf \times 1.701 \times 100m$, where

- C = hydrogen peroxide concentration (w/w);
- V = volume of solution 1 in milliliters added during titration;
- Cf = correction factor for the solution of 0.1 N potassium permanganate;
- m = mass of the bleaching product sample in milligrams.

Preparation and Standardization of Specimens

Seventy-five experimental specimens were obtained from bovine incisors taken from animals aged between 24 and 30 months. Analysis of enamel specimens was performed using stereomicroscopy (Stemi SV11, Carl Zeiss Microscopy, Thornwood, NY, USA) at 45×. Those specimens presenting with morphologic changes and/or the presence of cracks in the enamel were excluded.

The selected teeth were mechanically cleaned with periodontal curettes and received prophylaxis with pumice and water. Subsequently, the specimens were fixed on a device attached to a drill platform (FGC-16 model, Ferrari, São Paulo, SP, Brazil). Cylinders were obtained from the middle third of the buccal surface (5.7 mm in diameter) using a cylindrical diamond-cutting instrument designed to cut glass (diamond tip, 8 mm in diameter, Dinser Diamond Tools Ltda, Sacomã, SP, Brazil) under constant irrigation.

The dentin surface was smoothed using manual rotation with 600-grit aluminum oxide sandpaper (T469-SF-Noton, Saint-Gobam Abrasives Ltda, Jundiai, SP, Brazil) until the specimens presented a thickness of 3.5 mm (approximately 1.3 mm and 2.2 mm of enamel and dentin, respectively), as measured with a digital caliper (500 to 144 B, Mitutoyo South America Ltda, Suzano, SP, Brazil). The smear layer formed during grinding was removed by applying EDTA for 1 minute. The specimens were rinsed with deionized water.

Division of the Groups

After the manufacture of the specimens, they were divided into five groups (n=15) according to Table 1.

Preparation of Artificial Pulp Chamber

Each enamel/dentin disc was individually adapted to an artificial pulp chamber (APC), developed at the Laboratory of Experimental Pathology and Biomaterials of Araraquara School of Dentistry – Uni Estadual Paulista (UNESP).[17] Each APC was formed by two compartments: the upper portion with an 8-mm diameter opening and the lower

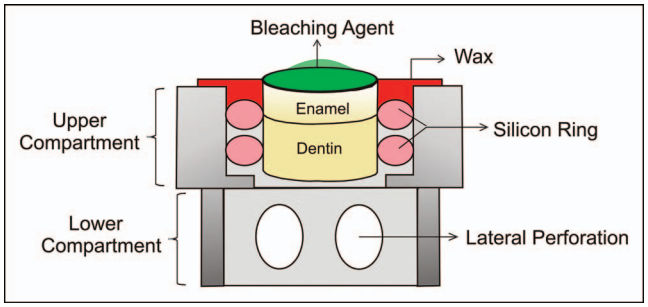

Figure 1. *Representative design of an enamel/dentin disc placed in an APC.*

portion with a 6-mm diameter opening, allowing the appropriate positioning and lateral sealing of the specimen. The lower portion presenting lateral perforations to circulate the acetate buffer solution was used to quantify the peroxide penetrating the specimen.

The specimens were positioned in the APCs between two silicone rings (5.60-mm inner diameter, 1.78-mm thick, Ref. OR 008, Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) and sealed with pink wax, restricting the lateral penetration of the bleaching agent (Figure 1).

Whitening Procedure

Three in-office bleaching sessions were performed at weekly intervals, using 0.04 mL of each bleaching product (Table 1). All procedures were performed in accordance with each manufacturer's instructions.

Preparation of Solutions and Transenamel and Transdentinal Penetration of H_2O_2

In order to quantify the H_2O_2 that penetrated the enamel/dentin discs, the artificial pulp chambers were individually placed in the wells of acrylic plates for cell culturing. Each well was filled with 1 mL of acetate buffer solution and subsequently received APCs that already contained dental fragments. Thus, the dentin surface remained in contact with the acetate solution during all bleaching procedures and the diffused hydrogen peroxide became part of the acetate buffer solution.

After the whitening procedures, 25 μL of acetate buffer solution was removed and mixed with 2750 μL distilled water, 100 mL leucocrystal violet (0.5 mg/mL, Sigma Chemical Co, St Louis, MO, USA) and 50 μL peroxidase (1 mg/mL, Sigma Chemical Co). The resultant solution was diluted to a final volume of 3 mL using distilled water.

Quantification of H_2O_2 Penetration

The quantification of hydrogen peroxide was carried out 15, 30, and 45 minutes after the placement of the bleaching product.

This method is based on the reaction of hydrogen peroxide with leucocrystal violet, as catalyzed by the peroxidase enzyme.[20] The coloring of the mixture varies in intensity according to the amount of peroxide. Thus, as the color value is proportional to the absorbance of the peroxide concentration, it is possible to indirectly assess the amount of peroxide that penetrated the tooth surface and the solution contained in the wells.

Readings were performed using Ultraviolet Visible reflectance spectrophotometric equipment (UV-2450, Shimadzu, Kyoto, Japan), 30 minutes after each bleaching session.

To obtain the calibration factor (CF) equivalent to the ratio of the concentration of the standard solution of hydrogen peroxide to its absorbance, the following equation was used: $CF = [Sample\ Solution]/Absorbance$.

The average calibration factor was used to calculate the hydrogen peroxide concentration contained in each specimen. Data were tabulated and the normality and homogeneity of variance assumptions were verified. Parametric tests were performed using analysis of variance (ANOVA) and Fisher test with the statistical program Stat View software at a significance level of 0.05.

RESULTS

The application of ANOVA and post-hoc Tukey test showed that all products significantly reduced the active concentration of H_2O_2 over time. Furthermore, the comparison among the products showed that Whiteness HP Maxx gel had the lowest concentration of hydrogen peroxide when compared to the other products, independent of the time of analysis. On the other hand, Opalescence Xtra Boost had the highest active concentration of the product. The mean values of the degradation rates over time are summarized in Table 2.

The application of ANOVA and Fisher test for the analysis of the data related to the transenamel and transdentinal penetration of hydrogen peroxide indicated that, in general, the penetration of H_2O_2 was intensified by increasing the dwell time of the product on the tooth surface ($p<0.0001$) (Figure 2).

Little variation was found in the penetration of hydrogen peroxide among the different products.

Table 2: *Mean Peroxide Concentrations (%) of the Degradation of the Bleaching Gel Over Time**

Products	0 Minutes	15 Minutes	30 Minutes	45 Minutes
Opalescence Xtra Boost	35.94 (0.62)Aa	35.49 (0.88)Aa	34.73 (0.17)Aab	33.80 (0.82)Ab
White Gold Office	35.21 (0.42)Aa	33.56 (1.20)ABb	32.15 (0.87)Bbc	30.79 (0.35)Bc
Whiteness HP Blue	34.67 (0.76)Aa	33.27 (0.93)Bb	32.10 (0.33)Bbc	31.11 (0.45)Bc
Whiteness HP Maxx	32.45 (0.71)Ba	30.97 (1.73)Cab	29.99 (0.89)Cbc	28.84 (0.60)Cc
Lase Peroxide Sensy	34.48 (1.37)Aa	33.36 (0.46)Bab	32.11 (0.75)Bb	29.94 (0.87)BCc

** Statistical significance is denoted by letters: upper-case letters in the columns and lower-case letters in the rows indicate significant differences between the products and time, respectively ($p<0.05$).*

However, Lase Peroxide Sensy 35%, Opalescence Xtra Boost 38%, and White Gold Office 35% penetrated more deeply when compared to the other products during the first 30 minutes. When evaluating the time of 45 minutes, Whiteness HP Blue 35% and Lase Peroxide Sensy 35% presented the greatest penetration, while Whiteness HP Maxx presented the lowest results (Figure 2; Table 3).

DISCUSSION

The use of bovine teeth allowed us to obtain experimental specimens with suitable dimensions to establish artificial pulp chambers in order to create an experimental model similar to the clinical application of bleaching agents on dental enamel during a session of in-office whitening. It is important to highlight that bovine teeth also present a uniform composition and have low variations in experimental responses.[21-25] However, human teeth are morphologically and histologically similar to bovine incisors.[26-28]

The use of peroxide in high concentrations (35%-38%) allows for noticeable bleaching results after the first bleaching session. This has been the main marketing appeal of in-office bleaching, which has increased the popularity of this technique in recent years.[29] However, reports of many and varied side effects of this treatment are common.[23,30,31] Thus, several authors have directed their research to the study of effective doses that would not cause postoperative sensitivity or other harmful effects on dental tissues.[32-35]

In this current study, the hydrogen peroxide was quantified from different products using a standard formula. This methodology has been used for a long time[11,13,19,29] and is widely used in the pharmaceutical industry (United States Pharmacopeia)[18] and allows for the estimation of the concentration of active products present in different bleaching gels.

White Gold Office and Whiteness HP Blue were the only products that presented an initial active concentration of H_2O_2 ($\pm 0.5\%$) that was indicated by the manufacturer. Moreover, all of the products revealed a significant reduction of hydrogen peroxide at the end of the 45-minute application. Lase Peroxide Sensy had the highest reduction in active

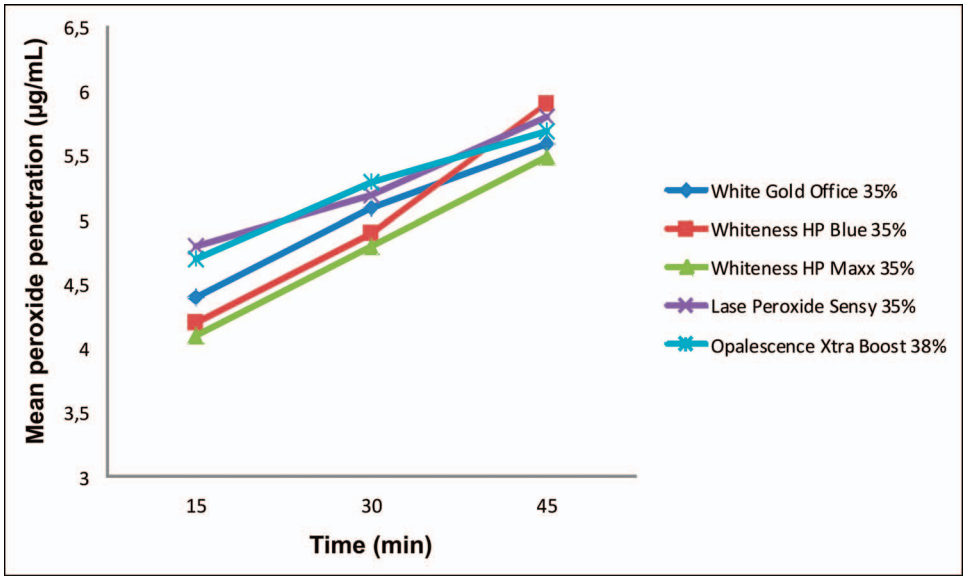

Figure 2. *Mean (µg/mL) of transenamel and transdentinal penetration of hydrogen peroxide obtained from artificial pulp chamber after bleaching, according to the different application times and products.*

Table 3: *Mean Peroxide Concentrations (μg/mL) Recovered From Artificial Pulp Chambers After Tooth Whitening, According to the Different Application Time and Products**

Time, min	White Gold Office 35%	Whiteness HP Blue 35%	Whiteness HP Maxx 35%	Lase Peroxide Sensy 35%	Opalescence Xtra Boost 38%
15	4.4 (0.5)Cabc	4.2 (0.6)Cbc	4.1 (0.7)Cc	4.8 (0.6)Ca	4.7 (0.4)Ca
30	5.1 (0.5)Bac	4.9 (0.3)Bbc	4.8 (0.3)Bb	5.2 (0.5)Ba	5.3 (0.3)Ba
45	5.6 (0.2)Abc	5.9 (0.3)Aa	5.5 (0.5)Ab	5.8 (0.3)Aac	5.7 (0.2)Abc

** Statistical significance is denoted by letters: upper-case letters in the columns and lower-case letters in the rows indicate significant differences between time and the products, respectively ($p < 0.05$).*

concentration of H_2O_2 when considering the initial concentration. However, this reduction may be considered small as more than 86% of the initial concentration of the product remained. These data suggest that the bleaching gels in the current study retain their whitening ability and maybe did not need to be reapplied during the sessions, thus contradicting many bleaching procedures. The present results are in opposition to those obtained by Al-Qunaian and others,[11] Matis and others,[13] and Wattanapayungkul and others,[36] where *in vivo* analysis verified that some bleaching gels used in at-home bleaching techniques exhibited intense degradation after a few hours of their applications. However, it should be emphasized that higher temperatures are found in the *in vivo* condition, and the constant contact of the saliva with the bleaching products could intensify the degradation of peroxides. This increased temperature did not occur in the experimental conditions of this study.

The results of the transenamel and transdentinal penetration of the H_2O_2 released by bleaching agents showed that all products presented rapid diffusion through the enamel and dentin, as also reported in other studies.[21,37-39] It was also verified that penetration of hydrogen peroxide is time-dependent. In other words, longer contact times of the bleaching gel with the enamel structure provides greater penetration of the peroxide through dental tissues. These results corroborate the findings of Soares and others,[40] who evaluated the effectiveness of bleaching gels on enamel after different application periods. Similar responses were also obtained by Camargo and others[38] and Bowles and Ugwuneri,[3] with both studies concluding that the diffusion of hydrogen peroxide through enamel and dentin is related to the contact time of the bleaching agent with the enamel.

When making a general analysis of the results obtained in the current study and considering that the bleaching gels substantially retained their initial concentrations of hydrogen peroxide, it was expected that the penetration quantified after a 45-minute application would be three times higher than that obtained after a 15-minute application. However, the current results showed that the penetration does not increase as a function of time. Similar results were obtained by Soares and others[40] when measuring H_2O_2 following different application periods on the dental enamel.

Thus, a single application of the bleaching product can produce results that are similar to those protocols already preestablished and evaluated with regard to the availability of H_2O_2 in a bleaching product.[41] In this context, a single application would involve a reduction in the time required to complete the clinical procedure, reduce the cost, and lessen the risk of accidents that involve the soft tissues.

However, other factors that are related to the safety of the bleaching procedures should be considered and studied. The pH variation of the product during its application can cause concern if it reaches values that are considered to be critical, where it may cause significant histomorphologic changes of the enamel surface.[42,43] On the other side, it has been reported that products with neutral pH do not alter enamel surface roughness, even after multiple applications.[44]

These results cannot be extrapolated directly to a clinical situation, since the current study is an *in vitro* study and did not include the presence of intrapulpal pressure, the presence of saliva, or cytoplasmic processes of odontoblasts that may decrease the peroxide diffusion.[4,45-48] Even so, the results of the current study strongly indicate the adoption and improvement of in-office bleaching protocols to include single applications of the bleaching product.

CONCLUSIONS

Under the conditions of the current study, it can be concluded that:

- Bleaching gels maintain more than 86% of their initial concentration of hydrogen peroxide after 45 minutes.

- The penetration of hydrogen peroxide in the tooth structure is time-dependent.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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