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Effect of Hydrogen Peroxide Concentration on Enamel Color and Microhardness

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Clinical Relevance

This study showed that 35% hydrogen peroxide gel exhibited higher whitening potential compared with 20% gel, without intensifying the adverse effects on enamel surface microhardness.

SUMMARY

Objectives: The aim of this study was to investigate the effect of hydrogen peroxide gels with different concentrations (20%, 25%, 30%, and 35%) on enamel Knoop microhardness (KNH) as well as on changes in dental color (C).

Methods: Cylindrical specimens of enamel/dentin (3-mm diameter and 2-mm thickness) were obtained from bovine incisors and randomly divided into six groups (n=20), according to the concentration of the whitening gel (20%, 25%, 30%, 35%, control, thickener). After polishing, initial values of KNH_0 and color measurement, assessed by spectrophotometry using the CIE L*a*b* system, were taken from the enamel surface. The gels were applied on the enamel surface for 30 minutes, and immediate values of KNH_i were taken. After seven days of being stored in artificial saliva, new measures of KNH_7 and color (L_7* a_7* b_7*, for calculating ΔE, ΔL, and Δb) were made. Data were submitted to statistical analysis of variance, followed by Tukey test ($p<0.05$).

Results: Differences in gel concentration and time did not influence the microhardness ($p=0.54$ and $p=0.29$, respectively). In relation

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to color changes, ΔE data showed that the 35% gel presented a higher color alteration than the 20% gel did (p=0.006).

Conclusion: Bleaching with 35% hydrogen peroxide gel was more effective than with the 20% gel, without promoting significant adverse effects on enamel surface microhardness.

INTRODUCTION

The pursuit of an esthetic smile has stimulated the search for effective treatments and alternatives to increase its attractiveness. Tooth whitening is a highly desirable esthetic treatment, since it is conservative and can lead to satisfactory results for changing dental color.[1] Hydrogen peroxide is an important agent used in dental bleaching that is capable of penetrating tooth structures, releasing free radicals, and oxidizing chromophore molecules, by means of redox processes.[2] The penetration of these oxidative agents in dental structures breaks these chromophore molecules into less complex molecules, giving a brighter aspect to the tooth.[3]

Dental bleaching procedures can be performed in a dental office, with total control of the dentist, or by the patient at home, with professional supervision. Although both techniques are shown to be effective,[4] in the in-office technique, higher concentrations of the hydrogen peroxide gel are usually used to reduce the clinical treatment time to 30 to 60 minutes.[5] The concentration of hydrogen peroxide in the whitening gel has an inverse correlation with the application time needed for achieving satisfactory outcomes;[6] thus, for faster results, with fewer applications, higher concentrations of hydrogen peroxide are required.[7]

However, there are some concerns regarding potential adverse effects that can happen to dental tissues after dental whitening. The results are controversial, but some authors claim that alterations in enamel surface morphology can happen,[8-10] as well as significant changes in microhardness values after bleaching.[8,11,12] In addition, Bistey and others[13] observed that significant structural alterations, with important loss of phosphate ions, occurred in the enamel surface when high concentrations of hydrogen peroxide (greater than 20%) were used. Furthermore, slightly erosive effects in bleached enamel were also described as promoted by the whitening agent.[10]

Therefore, investigations concerning bleaching efficacy of different hydrogen peroxide concentrations and the possible adverse effects on enamel are important to determine ideal protocols for better outcomes and less damage to tooth structure using the in-office technique. The aim of this study was to evaluate the color and microhardness of the enamel submitted to whitening treatments with hydrogen peroxide gels in different concentrations (20%, 25%, 30%, and 35%), immediately after application and after seven days. The null hypothesis tested was that higher concentrations of hydrogen peroxide do not improve the whitening effect and do not change enamel microhardness.

METHODS AND MATERIALS

Sample Preparation

Freshly extracted, undamaged, and intact bovine incisors were selected and stored in 0.1% thymol solution until required. One hundred enamel-dentin specimens 3 mm in diameter and 2 mm in height (1 mm of enamel and 1 mm of dentin) were prepared from the buccal surface of the tooth using a diamond trephine mill (Dentoflex, São Paulo, SP, Brazil).[14] Enamel and dentin thickness were standardized, ground flat, and polished with sequential water-cooled silicon carbide paper discs (1200-, 2400-, and 4000-grit; Fepa-P, Struers, Ballerup, Denmark). The enamel surfaces were verified with a stereomicroscope (Carl Zeiss, Stemi 2000-20×), and the surfaces presenting cracks and imperfections were discarded. The specimens were immersed in deionized water, placed in an ultrasonic bath for 10 minutes (Ultrasonic Cleaner, Odontobras, Ribeirão Preto, Brazil), and then stored in distilled water for rehydration. Specimens were randomly divided into six groups (n=20), according to the concentration of the hydrogen peroxide whitening gel: control (distilled water), thickener (gel without peroxide), 20%, 25%, 30%, and 35%.

Color Measurement

Prior to each bleaching treatment, the initial color of all specimens was taken. The baseline color coordinates were assessed in standard conditions using a reflectance spectrophotometer (CM-2600d Konica Minolta, Osaka, Japan).[5] The device was adjusted to use the D65 light source with 100% ultraviolet and specular reflection included. The observer angle was set at 2°, and the device was adjusted to a small reading area (SAV) with a total area of 3 mm^2. The spectrophotometer was adjusted for three consecutive measures, which were later averaged. The results of the color measurement were quantified in terms of the L*, a*, b* coordinate values established by the Commission Internatio-

Table 1: *Microhardness Mean Values (± SD) Obtained for Tested Groups*

Group	Initial	After Bleaching	After Seven Days
Control	347.41 (±23.16)	342.51 (±22.49)	348.03 (±15.73)
Thickener	351.91 (±21.68)	345.29 (±20.86)	341.08 (±16.43)
20%	352.75 (±25.39)	348.09 (±37.35)	330.38 (±42.18)
25%	353.17 (±20.68)	356.46 (±30.56)	344.81 (±27.18)
30%	353.68 (±22.53)	351.48 (±33.30)	352.62 (±40.00)
35%	353.11 (±22.99)	346.93 (±33.82)	355.64 (±45.38)

nale de l'Eclariage (CIE), in which the L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a* plane represents the degree of green/red color, and the b* plane represents the degree of blue/yellow color.[5,15]

The measurement of color change after the bleaching procedures was made by calculating the variation of L* (ΔL), a* (Δa), and b* (Δb). The total color change (ΔE) was calculated according to the following formula[15]:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

Microhardness Measurement

The initial surface microhardness (KNH_0) of all specimens was obtained before the bleaching procedures using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan), with a Knoop indenter, under 25 g load for 10 seconds. Three indentations were made in each sample, 100 μm apart, and the average was calculated for KNH_0.

Bleaching Procedures

The whitening gels used in this study were experimental and manipulated in our laboratory, following the protocol described previously,[6] resulting from the mixture of two parts: the first was a solution of 50% hydrogen peroxide containing an acrylic thickener, which in an acidic environment is a white solution (solution A). The second part consists of an aqueous solution containing an alkaline substance (solution B). They were manually mixed immediately before application in a 3:1 proportion by volume of peroxide base gel and thickener, respectively. Immediately after mixing, the pH of all gels was calculated using a pH meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, Brazil), with an electrode (Digimed DME-CV8) calibrated with buffer solutions of pH 4.00 and 6.86. The pH of thickener gel and 20%, 25%, 30%, and 35% gels was, respectively, 6.07, 5.66, 5.71, 5.70, and 5.36.

The products were applied over controlled conditions of temperature (25°C) and humidity (50%). A 1 mm layer of whitening gel was applied over the enamel surface of each specimen for 10 minutes and repeated three times, totaling 30 minutes of application. This protocol of application was chosen to simulate the clinical application defined for many gels available for clinical use. An aspiration cannula was used to remove the gel in between each application, simulating clinical bleaching treatment. After application, the specimens were washed with deionized water and submitted to an immediate measurement of microhardness (KNH_1) following the same specifications previously described for initial measurements. The specimens were then stored in artificial saliva, manipulated according to Gohring and others,[16] during seven days, with daily changes. After the storage period, new measures of microhardness (KNH_7) and color (L_7* a_7* b_7*, for calculation of ΔE, ΔL and Δb) were performed.

Statistical Analysis

After analyzing for normal distribution, data were submitted to analysis of variance (ANOVA), where the microhardness (KNH) was analyzed by repeated-measures ANOVA (with time the repeated variable) and color data (ΔE)—initial and after seven days of bleaching—submitted to one-way ANOVA (with gel pH the variable) and a post-hoc Tukey test ($p<0.05$) to compare the differences among groups.

RESULTS

Microhardness mean values are shown in Table 1. The repeated-measures ANOVA showed no significant difference among groups ($p=0.60$), or for the time factor ($p=0.15$), or the interaction groups × time ($p=0.45$). For the color data (Table 2), the one-way ANOVA test showed significant differences among groups for the values of ΔL ($p=0.0001$), Δb ($p=0.0001$), and ΔE ($p=0.0001$).

Table 2: *Mean (±SD) Color Change Parameters for Each Group*

Group[a]	ΔL	Δb	ΔE
Control	0.48 (±1.96)a	0.18 (±0.82)a	2.00 (±0.83)a
Thickener	−0.64 (2.04±)ab	0.42 (±0.81)a	2.09 (±0.98)ab
20%	1.55 (±1.07)bc	−2.19 (±1.73)b	3.15 (±1.25)bc
25%	2.25 (±1.03)c	−2.93 (±1.70)bc	4.03 (±1.36)cd
30%	2.21 (±0.88)c	−3.42 (±0.81)c	4.16 (±1.00)cd
35%	2.69 (±1.17)c	−3.59 (±1.28)c	4.61 (±1.52)d

[a] Different letters indicated significant differences among groups ($p<0.05$).

DISCUSSION

The null hypothesis tested was denied for color alteration, since a higher concentration of hydrogen peroxide in the bleaching gel proved to be more efficient in tooth whitening.

The gel concentration did not cause significant changes in the enamel surface microhardness, either immediately after application or after seven days. Indeed, previous data showed that the use of hydrogen peroxide does not alter enamel histomorphology or microhardness.[17] While some studies have observed demineralization of enamel submitted to bleaching procedures,[8,18,19] these structural modifications have been assigned mostly to the gel pH,[19-21] usually at less than 5.2. In fact, Sulieman and others[17] affirmed that studies reporting adverse effects on bleached enamel and/or dentin do not reflect the bleach itself; instead, they reflect the pH of the formulation used. Thus, neutral gels are recommended for tooth bleaching with the purpose of reducing deleterious effects on tooth enamel.[21] The gels tested in the present study have a pH between 5.3 and 5.7, and showed no significant changes for the time in contact with the enamel, which may have been insufficient to promote enough mineral modification to impact the enamel microhardness. Furthermore, Bistey and others[13] reported that, besides hydrogen peroxide concentration, structural changes of the enamel surface are also time dependent, with considerable changes happening at greater than 60 minutes of exposure to peroxide. The time of exposure used in this study was 30 minutes, as recommended by many bleaching gels, which might be insufficient to promote enamel demineralization. Also, previous *in situ* bleaching studies reported a decrease in enamel microhardness on placebo groups treated with thickener agents, such as carbopol and poloxamer.[22,23] The action mechanism by which these agents cause this reduction is still unknown, but it is speculated that they have an acidic nature. In the present study, we included a group using a gel (pH 6.07) with the same basic composition as the tested gels but without the peroxide, so we could verify that the thickener was unable to promote mineral dissolution by itself.

Almost all morphological studies evaluating the side effects of bleaching in dental tissues have been conducted *in vitro*, because it is difficult to perform microhardness, roughness and microscopic analysis *in vivo*. Amaral and others[24] evaluated the calcium and phosphorus concentration in human enamel *in vivo* and found no differences between in-office (35% and 38%) and home use (10% and 20%) bleaching gels. In addition, Metz and others[25] found no differences in enamel microhardness *in vivo* using teeth extracted for orthodontic reasons.

Regarding the color changes, the strength of the carbon bonds present in the chromophore molecules is inversely proportional to the dental color, meaning that molecules presenting carbonic rings in their structure absorb more light than linear chains with unsaturated double-bond molecules, and these, on the other hand, absorb more light than saturated linear chains without double bonds. Therefore, the higher the light absorption by complex molecules, the lower the reflection, giving the sensation of a darker tooth, requiring a higher acting time of the whitening gel or a higher concentration of the hydrogen peroxide.[26] Thus, according to Kawamoto and Tsujimoto,[27] higher concentrations of hydrogen peroxide solutions present larger amounts of free radicals, increasing the whitening potential. Indeed, in this study, the higher concentration of hydrogen peroxide (35%) promoted a better whitening effect on the enamel after seven days of the procedure, compared with the 20% concentration. Since the hydrogen peroxide that penetrates enamel prisms can be active for several days until being completely neutralized,[28] the color measurement was made after this period to allow enamel rehydration and color stability.[29]

The color change analysis is a complex issue, since the most important parameter of color reading is controversial. Color differences evidenced by the spectrophotometer might not necessarily be clinically relevant; however, the use of a spectrophotometer in this study is justified by the improvement in the standardization of shade assessment, allowing accuracy and reproducible results for color measurements, compared with the human eye, which presents differences in the color perception.[30,31] According with Dietschi and others,[15] when analyzing the three color dimensions of the CIE L*a*b* system separately, the L* values are important as they determine the lightness by quantifying the black-white color, while a* and b* values describe chroma and are less useful. However, Gerlach and others[28] evaluated the subjective response of individuals submitted to whitening procedures and concluded that the change from yellow to blue (decrease of b* value) is of primary importance in the perception of patients submitted to bleaching procedures, and their satisfaction is associated more strongly with variations in the b* coordinate than with a* and L*. In addition, ΔE describes the global color change, including all three color dimensions of the CIE L*a*b* system, and can also be used to compare the efficacy of different bleaching agents.[15] However, clinical color alteration impression is not well established in the literature. The threshold of color alteration perception has been reported as ΔE=1, but the threshold of color alteration acceptability has been stated as ΔE=3.7.[32]

Karpinia and others[33] observed in an *in vitro* study that bleached tooth presented a significant decrease of the Δb (reduction in the yellow color) and an increase of ΔL (brightness). These parameters were also verified in this study, and specimens bleached with higher-concentrated hydrogen peroxide gel (35%) showed greater values of ΔL and ΔE and lower values of Δb, indicating a better whitening performance of this gel. This better whitening performance of higher hydrogen peroxide concentrated gels was also verified in *in vitro*[5] and *in vivo*[7] studies, which showed that bleaching gel with 35% hydrogen peroxide promoted a better whitening effect compared with 10% carbamide peroxide gel and 20% hydrogen peroxide gel, with similar treatment times, respectively. Based on these findings, it can be suggested that the use of whitening gels with 35% hydrogen peroxide can be indicated for patients who desire a faster whitening effect with minor influence on enamel surface properties.

Nevertheless, as it was conducted *in vitro*, this study presents some limitations, especially regarding the absence of pulp tissue, making it impossible to predict the side effects of high-concentration gels on tooth sensitivity and pulp cells,[34] as well as the absence of pulp pressure, which can interfere in the penetration of the gel in vital teeth.[35]

CONCLUSION

Enamel microhardness was not influenced by different concentrations of hydrogen peroxide gels. The 35% hydrogen peroxide gel exhibited higher whitening potential than the 20% gel, without intensifying the side effects on the enamel surface property tested.

Conflict of Interest

The authors 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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