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Effect of Calcium and Fluoride Addition to Hydrogen Peroxide Bleaching Gel On Tooth Diffusion, Color, and Microhardness

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Clinical Relevance

The addition of calcium and fluoride in high-concentration hydrogen peroxide gel might be a viable alternative to decrease the negative effects of hydrogen peroxide on enamel microhardness and peroxide diffusion without impairing bleaching efficacy.

SUMMARY

Objectives: The aim of this study was to evaluate the effect of calcium and fluoride addition to a 35% hydrogen peroxide (HP) bleaching gel with regard to its diffusion through the tooth structure, enamel microhardness, and bleaching efficacy.

Methods and Materials: Eighty specimens (6 mm in diameter and 2 mm in height; 1 mm/enamel and 1 mm/dentin) were obtained from bovine incisors that were polished and divided into four groups (n=20) according to the remineralizing agent added to the gel: Ca = 0.5% calcium gluconate; F = 0.2% sodium fluoride; Ca+F = 0.5% calcium gluconate and 0.2% sodium fluoride; and control = no agent. Initial microhardness and color were assessed. The samples were positioned over simulated pulpal chambers filled with acetate buffer solution to capture the HP. Gels were applied over enamel for 30 minutes, and HP diffusion was assessed by spectrophotometry two hours after bleaching. Microhardness was measured immediately after bleaching and then the specimens were immersed into artificial saliva for seven days for final color assessment. Data were analyzed by one-way analysis of variance followed by Tukey test.

Results: Bleaching reduced microhardness for all groups (p=0.0001), but the Ca+F and F

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groups showed lower reductions after bleaching. The addition of Ca, F, and Ca+F decreased the peroxide penetration through the tooth structure (p=0.0001), but there were no differences in color change for ΔL (p=0.357), Δa (p=0.061), Δb (p=0.823), and ΔE (p=0.581).

Conclusion: The addition of calcium and fluoride in the gel did not affect bleaching efficacy, but it was able to reduce both the peroxide diffusion and the bleached enamel microhardness loss.

INTRODUCTION

Tooth bleaching is a highly desirable esthetic treatment as it is conservative and can lead to satisfactory results in terms of changing the color of vital and nonvital teeth.[1] Typically the gels used for bleaching utilize hydrogen peroxide as the active agent, with a wide range of concentrations (of up to 40%) being available. Hydrogen peroxide can diffuse through dental enamel and dentin and release free radicals, causing the oxidation of molecules' chromophores,[2,3] electron-rich unsaturated groups that absorb particular wavelengths of visible light, which are attacked by the free radicals, splitting the double bonds and rendering the chromophore colorless, displaying a lighter aspect of the tooth.[4,5]

Although the bleaching gels are generally safe for clinical use, some side effects have been reported, such as tooth sensitivity.[6] Mainly this is caused by the peroxide diffusion through enamel and dentin, which reaches the pulpal chamber, causing inflammation. Chemical and morphological alterations in enamel structure have also been reported.[7-9] Additionally, reduction of enamel microhardness due to a demineralization effect promoted by the bleaching gel is often reported, but controversial data have been published,[4,10-16] and this effect remains uncertain. In an attempt to reduce these potential adverse effects, studies have been conducted with the addition of remineralizing ions in the bleaching gel formulations.[2,10,11,17-20]

The addition of calcium, for example, can increase the bleaching gel saturation and decrease mineral loss, overcoming the nondesirable effects of the treatment.[17] Different forms of calcium have been investigated, with beneficial effects on bleached enamel microhardness recovery and wear reported.[10,21,22] Fluoride is also used in an attempt to create deposits of calcium fluoride (CaF_2) on the enamel surface, increasing the incorporation of these ions into enamel crystals during remineralization periods.[8] However, it has been speculated[8,18] that the presence of these ions could interfere with the hydrogen peroxide diffusion through enamel and dentin structures, thereby reducing the whitening effect.

The aim of this study was to evaluate the effect of calcium and fluoride addition to a 35% hydrogen peroxide gel in terms of its diffusion through the tooth structure and effects on enamel microhardness and bleaching potential. The null hypothesis tested was that the addition of calcium and/or fluoride to 35% hydrogen peroxide bleaching gel would not produce any significant effect on enamel microhardness, color change, and peroxide diffusion.

METHODS AND MATERIALS

Sample Size Calculation

Sample size was calculated based on mean and standard deviation from a pilot study used to calculate the effect size (f). The software G Power 3.1 statistical analysis software (Heinrich-Heine-Universität, Dusseldorf, Germany) was used, with the significance level set at 0.05 and the power test at 0.80. The calculation retrieved 16 specimens per group, but taking into account the potential losses during the experiment, 20 specimens per group were prepared.

Sample Preparation

Freshly extracted, nondamaged and intact bovine incisors were selected, cleaned, and stored in 0.1% thymol solution at 4°C until required. Teeth without incisal wear were selected to avoid the possibility of tubular sclerosis. Eighty circular specimens were obtained from the buccal surface of the crowns using a diamond trephine mill with 6-mm internal diameter, resulting in enamel/dentin specimens.

To standardize the height of the samples, a metallic holder was used to ground flat the cylindrical specimens until 1 mm of enamel and 1 mm of dentin remained. The enamel surface was polished with sequential water-cooled silicon carbide paper discs (P1200, P2400, and P4000) (Struers, Ballerup, Denmark). The enamel surfaces were verified with a stereomicroscope (20×, Carl Zeiss Stemi 2000, Oberkochen, Germany), and the ones presenting cracks and imperfections were discarded. Finally, the specimens were immersed in ultrapure water in an ultrasonic bath (Ultrasonic Cleaner, Odontobras, Ribeirao Preto, Brazil) for 10 minutes and then stored in ultrapure water for rehydration.

Microhardness Measurement

Baseline enamel surface microhardness (KHN_b) was determined before bleaching using a Knoop indenter with 50*g* load and 10-second dwell time. Three measurements with 100 μm of distance between them were performed in each specimen; these measurements were later averaged to determine the microhardness of the specimens.

Color Measurement

The baseline color of all specimens was measured under standard conditions using a colorimetric reflectance spectrophotometer (CM-2600d Konica Minolta, Osaka, Japan). The observer angle was set at 2° using D65 illuminant with 100% ultraviolet and with the specular component included.[4,23] The color measurements were quantified using the color coordinate values established by the Commission Internationale de l'Eclariage (CIE) using the L*a*b* system.[4] The spectrophotometer was adjusted for three consecutive measures of L*, a*, and b*, which were later averaged.

A ceramic white block (CERAM, Queens Road, Penkhull, UK) was positioned under the samples to standardize the background.[23] In addition, a 2-mm holder made of flexible white rubber was used to standardize the specimen position during the color reading.

Group Division

Specimens were stratified into four groups (n=20) using the baseline microhardness means, according to the presence of calcium and fluoride in the 35% hydrogen peroxide bleaching gel, as follows: Ca = addition of 0.5% calcium gluconate (Purac, Campo de Goitacazes, RJ, Brazil); F = addition of 0.2% sodium fluoride (Labsynth, Diadema, SP, Brazil); Ca+F = addition of 0.5% calcium gluconate and 0.2% sodium fluoride; and control = bleaching gel without addition of any substance. The bleaching gel used in this study was experimental and was manufactured in our laboratory, following the protocol described previously.[4,24] It results from the mixture of two parts: the first one was a solution of 50% hydrogen peroxide containing an acrylic thickener (solution A, pH 1.5). The second part consists of an aqueous solution containing an alkaline substance (solution B, pH 11.3). The gel was prepared mixing the two solutions in a 3:1 ratio by volume of solutions A and B using automatic micropipettes, resulting in a 35% hydrogen peroxide gel.[4] The final pH of all gels was as follows: Ca = 6.57; F = 6.51; Ca+F = 6.59; and control = 6.50.

Bleaching Procedures

Specimens were positioned in artificial pulpal chambers. Each chamber had an internal cavity, which was filled with 20 μL of 2 M acetate buffer (pH 4.5) to simulate the pulpal fluid and to collect the peroxide that penetrates the enamel/dentin structures. Acetate buffer was chosen for its ability to stabilize the peroxide that passed through the sample until its measurement. The specimen's dentin side was placed in contact with the acetate buffer, and the margins of the chamber's entrance were sealed with a rubber O-ring and a lid to avoid leakage of the bleaching gel by other means than the diffusion through tooth structures. This lid had a central hole measuring 4 mm, into which the bleaching gel was applied. A schematic drawing of the lid and the sample device have been provided previously.[25]

After the specimen positioning, a 2-mm layer of the respective bleaching gel from each group was applied on the enamel surface. Bubbles were eliminated with a disposable applicator to avoid interference between the gel and the enamel. After 10 minutes, the gel was removed by suction, simulating the clinical procedure, and these steps were repeated two more times, completing a total of three applications. After the third application, the gel was removed, and the surface was washed with air/water spray. After that, 20 μL of artificial saliva[26] was applied over each specimen and kept for two hours in a closed container with 100% relative humidity to allow the hydrogen peroxide inside the tooth structure to reach the pulpal chamber. It had previously been shown[25] that 35% hydrogen peroxide takes at least 90 minutes to reach the simulated pulp chamber in a measurable amount.

After two hours, the specimens were removed from the artificial pulpal chamber and the final microhardness (KHN_f) was measured, using the same parameters described previously. The percentage of microhardness change (%KHN) was determined in relation to the baseline value (KHN_b), which was considered the maximum hardness (100%). For the calculation of the microhardness change the following formula was used: $\%KHN = [(KHN_f - KHN_b)/KHN_b] \times 100$.

Specimens were kept in Eppendorf tubes with 2 mL of artificial saliva for seven days, with daily changes, for hydration and color stability.[4] Then, final color measurements were taken using the same parameters described previously. The color change after the bleaching procedures was calculated based on the changes in L* (ΔL), a* (Δa), and b* (Δb)

Table 1: *Mean of Microhardness and Percentage of Reduction for All Groups Tested*

Group[a]	KHN_b Mean (±SD)	KHN_f Mean (±SD)	%KHN, %	*t*-Test, *p*
Control	311.50 (±27.44) Aa	185.84 (±47.24) Ab	−40.09	0.0001**
Calcium	311.02 (±27.43) Aa	190.40 (±38.26) Ab	−39.04	0.0001**
Calcium+fluoride	310.44 (±27.38) Aa	225.85 (±33.71) Bb	−27.10	0.0001**
Fluoride	309.34 (±27.85) Aa	239.16 (±38.99) Bb	−22.33	0.0001**
ANOVA	$p = 0.994$	$p = 0.0001$**		

Abbreviations: ANOVA, analysis of variance; KHN_b, baseline enamel surface microhardness; KHN_f, final microhardness; %KHN, percentage of microhardness change; SD, standard deviation.
[a] Different uppercase letters in a column reflect differences among groups in each moment of evaluation. Different lowercase letters in a row reflect differences between the readings for each group.
*** Significant differences.*

coordinates. The total color change was calculated by the ΔE, according to the following formula: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.[27]

Quantification of Peroxide Diffusion

The quantification of the peroxide diffusion through enamel and dentin was made using the spectrophotometric analysis described by Hannig and others.[28] An aliquot of the acetate buffer was mixed with the enzymatic reagent, which changes the color depending on the hydrogen peroxide concentration. In this method, the reaction between 4-aminoantipirine and phenol with hydrogen peroxide, catalyzed by peroxidase, releases oxygen and changes the color of the solution from transparent to pink.[24]

The enzymatic reagent is composed of 4-aminoantipyrin (4 mmol/L), phenol (24 mmol/L), and peroxidase (0.4 U/mL), diluted in phosphate buffer (0.1 M; pH 7.0). A standard curve was prepared using a standard hydrogen peroxide solution with known concentrations, allowing us to determine the relationship between the absorbance and the concentration in mg/L. A 5-μL volume of acetate buffer solution was taken from inside the simulated pulpal chamber with a micropipette and transferred to an acrylic cuvette with 1000 μL of the enzymatic reagent. The amount of peroxide diffused through the specimens was determined by the absorbance of the solution in the spectrophotometer using a wavelength of 510 nm.[24]

Statistical Analysis

All data were checked for the normality assumption using the Kolmogorov-Smirnov test. For microhardness and color, normal distribution was observed and one-way analysis of variance (ANOVA) and Tukey tests were applied. For microhardness, the differences among the groups were analyzed separately for each evaluation (baseline and final). For each group, the baseline and final microhardness were compared using a paired *t*-test. For peroxide penetration, a normal distribution was not observed, and the results among the groups were compared using Kruskal-Wallis and Dunn tests. All analyses were performed using a significance level of 5%.

RESULTS

Results for one-way ANOVA and Tukey test for each moment of evaluation, the percentage of microhardness reduction, and the results of the *t*-test are shown in Table 1.

For the baseline evaluation, there were no significant differences among groups. The *t*-test showed a significant reduction of microhardness for all groups after the bleaching. However, after bleaching, groups containing fluoride and association between calcium and fluoride showed higher microhardness means than did the control and calcium groups. The percentage of reduction compared to baseline values for the groups containing fluoride was about half the value observed in the control group.

The results of color evaluation and hydrogen peroxide penetration are shown in Table 2. Nonsignificant differences were observed for color coordinates evaluated and for total color change. Significant differences were observed for hydrogen peroxide penetration. The addition of calcium and fluoride reduced the penetration in relation to the control group.

DISCUSSION

New formulations of bleaching gels with the addition of calcium and fluoride have been developed in an attempt to minimize side effects, such as reduction in enamel microhardness promoted by the hydrogen peroxide, mainly when it is used in high concentrations.[10,21,22] The null hypothesis tested was denied only for microhardness and peroxide diffusion, as the

Table 2: *Means (±SD) of Color Change (ΔL, Δa, Δb, and ΔE) and Hydrogen Peroxide Penetration (μg/mL)*

Group	ΔL	Δa	Δb	ΔE	Penetration[a]
Control	1.14 (±0.74)	0.67 (±0.38)	−3.91 (±0.83)	4.22 (±0.70)	0.75 (±0.66) a
Calcium	1.39 (±0.70)	0.47 (±0.38)	−3.97 (±0.69)	4.30 (±0.71)	0.49 (±0.67) b
Calcium+ fluoride	1.39 (±0.83)	0.56 (±0.27)	−3.91 (±0.82)	4.30 (±0.65)	0.52 (±0.47) b
Fluoride	1.05 (±0.72)	0.74 (±0.29)	−3.75 (±0.70)	4.04 (±0.67)	0.40 (±0.35) b
ANOVA/Kruskal-Wallis	$p = 0.357$	$p = 0.061$	$p = 0.823$	$p = 0.581$	$p = 0.001$

Abbreviation: ANOVA, analysis of variance.
[a] *Groups followed by different letters in a column represent significant differences.*

addition of calcium and fluoride decreased the demineralization effect, as measured by microhardness (Table 1), and decreased peroxide diffusion through enamel/dentin (Table 2).

Microhardness is a suitable, common, and well-accepted test by which to assess small alterations, such as initial demineralization, in the enamel surface.[12] Although ISO 28399,[29] which refers to bleaching procedures, states that the acceptable reduction of microhardness promoted by bleaching gels shall not be higher than 10%, the effects of high-concentration hydrogen peroxide gels on enamel microhardness remain uncertain in the literature. Previous studies[7,12,30,31] demonstrated that highly concentrated and acidic bleaching gels cause change in enamel chemical composition, calcium loss, demineralization, and microhardness reduction. Other studies, however, show that gels with higher pH are associated with increased bleaching effect[3] without causing alterations in the enamel surface.[4,32,33] The results found in this study showed that although the pH of the gels tested was around 6.5, and therefore nearly neutral, there was a significant decrease in microhardness for all groups, being higher than 20%, and therefore, higher than that recommended as acceptable by ISO 28399.[29]

It has been discussed that tooth bleaching agents can produce significant enamel microhardness loss regardless of the peroxide concentration, pH, or time of exposure,[16,34] and this loss also depends on the oxidative effect of the free radicals[12,35] and the composition of the gels with regard to the presence or absence of ions such as calcium and fluoride.[12,22] The unstable and nonspecific oxygen free radicals released by hydrogen peroxide gels act not only over the chromophore groups of the molecules but also over the organic structure of enamel, oxidizing the proteins in its composition (amelogenins and enamelins).[36,37] This induces structural changes in the protein matrix located between the enamel crystallites, interfering with the crystal structure and consequently making it more susceptible to mechanical impact such as reductions in microhardness and fracture toughness.[7,36]

As the minerals that form enamel and dentin are based on calcium and phosphate, when in contact with undersaturated solutions or gels in relation to these ions, the dissolution of hydroxyapatite may occur. However, when in contact with supersaturated solutions, mineral precipitation or remineralization can happen.[38] Therefore, the calcium supplementation of bleaching products aims to create a supersaturated gel in terms of Ca^{2+} ions, avoiding its dissolution from hydroxyapatite. The same principle has been tested for dental erosion prevention associated with the consumption of acidic beverages, reducing the mineral loss.[39] Often calcium gluconate is used to supplement bleaching gels as a result of its high solubility in terms of the conditions required for the gel formulation.[21] It has been reported that addition of 2% calcium gluconate can reduce enamel demineralization during bleaching.[37] However, literature is lacking in terms of information about the ideal concentration of the calcium ions during bleaching procedures. The results from this study showed that the addition of 0.5% calcium gluconate to the 35% hydrogen peroxide gel was not able to avoid enamel demineralization (Table 1). The low concentration of the salt used might have been insufficient to promote the supersaturation of calcium ions in relation to enamel crystals. This lower concentration was used based on previous research which found that 0.5% calcium gluconate maintained adequate viscosity and stability of the bleaching gel.[21,37] Additional studies aimed at determining the most adequate calcium salt for this purpose as well the ideal concentrations are needed.

When sodium fluoride was added to the bleaching gel formulation (Ca+F and F groups) there was a lower microhardness reduction in comparison to that observed with the control. This corroborates with the findings of previous studies which found that the addition of fluoride was beneficial, promoting remineralization and avoiding demineralization of the

enamel surface submitted to bleaching.[10,22] The favorable performance of fluoride groups is attributed to fluoride's ability to enhance crystal growth and slow enamel mineral dissolution,[40] and also for its contribution to enamel microstructural defect repair through the adsorption and precipitation of calcium.[20,22] Moreover, previous studies reported that the addition of fluoride into the bleaching gel renders the gel "supersaturated" in relation to this ion, allowing its incorporation into the enamel structure as fluoridated apatite.[20,40] In addition, enamel porosity might increase after bleaching, resulting in a higher number of retention sites and a better diffusion of the fluoride, and consequently increasing fluoride deposition.[40,41] It should be noted that despite the favorable result found for the fluoride-containing gel (F group), the association of this ion with calcium did not promote a better effect (Ca+F group was similar to F group). There may occur a premature interaction of both ions inside the bottle, decreasing the efficacy of the ions in protecting the enamel.[37] Therefore, bleaching gels containing both salts should ideally be presented in different bottles to avoid, or to minimize, this reaction before the application on the tooth structure.

The diffusion of hydrogen peroxide through enamel and dentin is necessary for the bleaching effect, but it has been speculated that the presence of ions, such as calcium and fluoride, might cause deposition of crystals over the enamel surface and reduce its permeability to hydrogen peroxide, thus reducing the whitening effect.[8,18] Although the penetration was really reduced with the addition of those ions, the results from this study showed that enough of the peroxide was able to diffuse through enamel and dentin, promoting a similar bleaching effect in relation to that observed for the control group (Table 2).

Despite the fact that peroxide diffusion through enamel and dentin is required to promote bleaching, a high rate of its diffusion through them can cause pulpal reactions, from minor histological changes such as degeneration sites, to inflammatory reactions, leading to tooth sensitivity.[42] Even though the concentration of hydrogen peroxide is directly proportional to its diffusion,[43,44] clinical findings suggest that the gel constituents, such as salts, have a greater effect on tooth sensitivity than does the peroxide concentration, with calcium-containing gels being less susceptible to tooth sensitivity.[45] Calcium-containing bleaching gels present a lower rate of peroxide diffusion,[42] and these results corroborate with the results from this study, which showed that the supplementation of calcium and fluoride in the bleaching gel decreased the peroxide diffusion through the tooth structure (Table 2). A previous study showed that even a small amount of peroxide diffusion (from 0.13 μl/mL to 1.69 μl/mL) was sufficient to cause cell alterations and death, proportional to the degree of penetration.[44] Table 2 shows that the peroxide diffusion means measured in this study were around 0.4 to 0.75 μl/mL when using the gel for 30 minutes, and, therefore, the treatment was able to potentially produce pulpal alterations. In addition, it has been reported that immediately after bleaching, the volume of peroxide diffusion is higher and the deleterious effect to the pulp cells is increased, but after three days it is possible to note their recovery, with an increase of metabolism and number of mitoses.[44] It is important to highlight that peroxide diffusion is dependent on the tooth thickness,[46] and the 2-mm enamel/dentin specimens in this study were intended to mimic the thickness of enamel/dentin from a maxillary central incisor. For mandibular incisors, for example, the peroxide diffusion might be much more deleterious to cell viability than it was for the teeth presenting a larger thickness of enamel and dentin.[46]

Color analysis is very subjective, and the human eye is not sensitive enough to detect slight color changes. The use of a spectrophotometer permits better accuracy and the ability to control variables, allowing data reproducibility, since the clinical perceptibility threshold can be higher than the differences detected by this equipment.[4] It is estimated[17,47] that a decrease in b* value (change from yellow to blue) and an increase in L* value are related to the perception of the whitening effect promoted by bleaching procedures and that this perception increases personal satisfaction. The threshold of color alteration acceptability has been stated as $\Delta E = 2.7$,[48] so values higher than this, associated with a reduction of b* values and increase of L* values, could indicate good whitening performance. In dental bleaching studies, generally the Δa values are of minor importance.[47] In this study, all gels were able to produce a bleaching effect, since ΔE values higher than 4.0 were observed, with negative Δb and positive ΔL values (Table 2). However, similar behavior was observed among the groups. Therefore, the addition of fluoride and/or calcium in the 35% hydrogen peroxide gel was not able to change its efficacy, allowing enough diffusion through enamel and dentin. Therefore, the color results from this study corroborate those of previous research in which similar addition of calcium and/or

fluoride to the bleaching agent did not affect the whitening efficacy,[8,18,49] so the supplementation of these compounds in bleaching gels can be viable as potentially remineralizing agents.

CONCLUSIONS

It can be concluded that the addition of fluoride, associated or not with calcium, to a 35% hydrogen peroxide gel reduced the peroxide diffusion through the tooth structure and the negative effects over the microhardness without interfering with bleaching efficacy.

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Regulatory Statement

This article was reviewed by the ICT-UNESP animal ethical committee, which dismissed the requirement for ethical analysis, regarding the use of bovine samples that are commercially available in Brazil (Arouca Law, 11794, 8/10/2008).

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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