

Influence of Bleaching Gel Peroxide Concentration on Color and Penetration through the Tooth Structure

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ABSTRACT

Aim and objective: The purpose of this study was to assess the effect of hydrogen peroxide concentration on the bleaching efficacy and penetration through the tooth structure.

Materials and methods: One hundred enamel/dentin specimens with cylindrical shape were obtained from bovine incisors. The surfaces were polished and the size standardized. They were divided into five groups ($n = 20$), following the concentration of hydrogen peroxide in the bleaching gels: 20, 25, 30, 35, and 40% (w/w). The specimens were placed over artificial pulpal chambers containing acetate buffer solution and bleached for 30 minutes (three applications of 10 minutes each). Aliquots of the acetate solution were collected, and the peroxide concentration was measured by an analytic spectrophotometer. The color of the samples was analyzed using a colorimetric spectrophotometer at the baseline and 7 days after the bleaching procedure. The color difference was calculated using the ΔE_{ab} formula. The data were analyzed by one-way ANOVA and Tukey's test ($p < 0.05$).

Results: The peroxide concentrations of 20–30% showed smaller bleaching effect than the higher concentrations ($p = 0.001$). The peroxide penetration was significantly higher ($p = 0.001$) for the more concentrated gels (35 and 40%).

Conclusion: The higher peroxide concentrations enhance the bleaching efficacy, but also increased the peroxide penetration through the tooth structure.

Clinical significance: In-office bleaching gels with higher concentrations of hydrogen peroxide (35 and 40%) present superior whitening efficacy. Nevertheless, they might also intensify the negative biological effects on the pulpal tissue, since they exhibit increased penetration potential.

Keywords: Bleaching, Color, Concentration, Peroxide.

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INTRODUCTION

Dental bleaching is a highly effective technique for the treatment of discolored teeth.¹ The whitening effect is promoted by hydrogen peroxide, which is a small molecule with a strong oxidizing effect, capable of forming reactive free radicals that oxidize chromophore molecules inside the dental hard tissues by means of a redox processes.^{2,3}

Different from the at-home technique, the in-office procedure uses bleaching gels with high hydrogen peroxide concentrations, generally around 35% (w/w), in order to promote a faster whitening outcome with reduced number of applications.⁴ In the highly competitive dental materials market, new bleaching gel formulations are often launched by manufacturers, and they use different approaches to attract the dentist attention to their brands. The first one is to increase peroxide concentrations, claiming to intensify the bleaching result and reduce the time required for the treatment. The second is to reduce the concentration, claiming to diminish the level of sensitivity during and after the treatment, as well the negative effects on the pulp. In both cases, the marketing intention is to make their products more attractive to the dentists, even sometimes without a clear scientific evidence supporting those claims.

The hydrogen peroxide concentration of mostly the currently available products varies between 20 and 40%. However, it has not yet completely demonstrated whether the higher concentrated gels are really more effective. This is due to the fact that other ingredients in the formulation of the bleaching gel can modulate the whitening outcome,^{5,6} besides the peroxide concentration per

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se, such as the pH,⁷ viscosity,⁸ kind of thickener,⁹ humectant and pH adjuster¹⁰ or presence of surfactants.¹¹ Therefore, a study testing the variation of peroxide concentration in the same bleaching gel formulation, in a dose–response analysis, could help to confirm or not this hypothesis.

Previous studies showed that the use of highly concentrated hydrogen peroxide gels produces whitening effect with minimal or no negative effect over the physical properties of the enamel surface.^{4,12–14} However, due to its low molecular weight, the

peroxide can diffuse through the intercrystalline spaces of enamel and inside the dentin tubules, reaching the pulpal chamber and starting an inflammatory process, which increases tooth sensitivity potential.^{2,15,16} When the amount of peroxide reaching the pulp chamber is excessive, the cells release large amounts of several inflammatory mediators into the tissue and consequently induce an inflammatory response.^{17,18} This response is nonspecific and may be intense, with vascular dilation and exudation of cells, such as macrophages, which are responsible for the degradation of the extracellular matrix.¹⁹ A direct correlation between the peroxide concentration in the bleaching gel and its diffusion through the enamel and dentin is expected,²⁰ although this was not observed in all studies.⁶ Since a higher quantity of molecules reaching the pulpal tissue can exceed the antioxidant capacity of the pulp cells and consequently lead to their damage, this issue is clinically relevant and deserves further investigation. Due to the effect of the various components in the formulation, a previous study showed that even gels with similar peroxide content can show different penetration rates, impairing the understanding of the real correlation between peroxide concentration and the pulpal penetration.²¹

Thus, this study aimed to evaluate the influence of hydrogen peroxide concentration on the bleaching efficacy and peroxide penetration through enamel and dentin. The null hypotheses tested were that peroxide concentration does not influence the bleaching effect or the penetration.

MATERIALS AND METHODS

Sample Preparation

This *in vitro* study was conducted in the Institute of Science and Technology of São José dos Campos (Sao Paulo State University—UNESP, Brazil). One hundred bovine incisors, freshly extracted and intact, were kept in 0.1% thymol solution at 5°C until use. A diamond trephine mill was used to cut specimen with 6 mm of diameter, from the central area of the buccal surface of the crowns, including enamel and dentin tissues, as previously described.^{16,20}

The thickness of the specimens was standardized in 2 mm (1 mm enamel and 1 mm dentin) using a P1200 silicon carbide (SiC) abrasive paper disk (FEPA-P, Struers, Ballerup, Denmark) and a dedicated specimen holder. For that, the specimen was attached to the holder and the desired enamel thickness adjusted based on DEJ. The excess was removed, and the surface was ground flat, using a polishing machine (DP-10, Panambra, São Paulo, SP, Brazil). Then, the specimen was reversed inside the holder and the dentin surface was flattened until reaching 1 mm thickness. The enamel surface was polished with a sequence of water-cooled SiC abrasive disks (P2400 and P4000 grit), applied for 20 seconds each. The enamel surfaces were analyzed with a stereomicroscope to verify the absence of cracks or defects. The smear layer on the dentin side was removed by the application of 37% phosphoric acid gel for 15 seconds, followed by copious washing with water. The specimens were immersed in 2 mL of distilled water inside Eppendorf tubes until required.

Baseline Color Assessment

The baseline color of all specimens was assessed with a spectrophotometer for colorimetric analysis by reflectance (CM-2600d, Konica Minolta, Osaka, Japan). The baseline light reflectance was assessed in standard conditions, with the device adjusted to small area view (SAV) of 3 mm, specular component

included, observer angle of 2°, standard D65 illuminant, and 100% UV included. A standard ceramic white background (CERAM, Lucideon, Staffordshire, UK) was placed under the specimen during the color measurement.¹¹ Between the specimen and the background, an optical contact was provided using polyethylene glycol 400 (Labsynth, Sao Paulo, SP, Brasil).¹ Three consecutive measures were taken from each specimen, and the values were later averaged. The color measurements were quantified in terms of the L*, a*, b* (CIELAB) coordinates.²

Dental Bleaching

After the initial color measurements, the enamel/dentin specimens were distributed into five groups ($n = 20$), according to the concentration of hydrogen peroxide present in the bleaching gels: 20, 25, 30, 35, and 40% (w/w). These concentrations were chosen for being the most commonly available in commercial products, allowing a dose–response analysis.

The bleaching gels tested were prepared in our laboratory, immediately before starting the application, as previously described by Torres et al.¹⁶ It was produced by the mixture of two solutions. One contained 50% hydrogen peroxide (w/w) associated with an acrylic thickener in acidic pH, while the other was an alkaline solution responsible for the gel formation. The solutions were mixed at 3:1 ratio by volume, using a 1000 μ L automatic micropipettes (Labmate Soft, HTL, Warsaw, Poland). The final pH of all tested gels was 6.7, checked by a benchtop pH meter (DM-22, Digimed, São Paulo, Brazil), previously calibrated with buffer solutions 6.86 and 4.01. The peroxide concentration on each gel was checked by titration using sodium permanganate.²

Hydrogen Peroxide Penetration

After initial color measurements, the specimens were placed inside an artificial pulpal chamber device to measure the diffusion of hydrogen peroxide, as described in previous works.^{2,16,20} A constant volume (20 μ L) of acetate buffer (pH 4.5) was placed inside the chamber, aiming to collect and stabilize the hydrogen peroxide that penetrated the tooth structure.¹⁶ The specimens were positioned inside the device, with the dentin touching the liquid simulating the pulpal fluid, while the surface was tightly sealed using a perforated lid and a rubber O-ring, allowing the penetration of peroxide only by diffusion through enamel and dentin.¹⁶

The gel was applied over the enamel with 2-mm-thick layer for 10 minutes and stirred every 2 minutes to displace the oxygen bubbles produced. After this time, the gel was aspirated with a vacuum cannula and reapplied two more times, with a total application time of 30 minutes. To allow the peroxide penetration through enamel/dentin, the specimens were kept inside a sealed box with 100% relative humidity for 2 hours.^{16,20}

After this time, the chambers were opened and 5 μ L of acetate buffer was collected from each one. The hydrogen peroxide concentration was quantified by the spectrophotometric method proposed by Bauminger²² and modified by Hannig et al.,²³ as fully described in previous studies.^{2,16}

Final Color Assessment

After the bleaching procedure, the specimens were kept in artificial saliva for 7 days to obtain color stability and to allow hydration.² The final color was evaluated the same way used for baseline. The color change was calculated using the total color difference formula: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$.²

Table 1: Mean and standard deviation for hydrogen peroxide penetration ($\mu\text{g/mL}$) and color change (ΔE) for all groups tested

Groups	Hydrogen peroxide penetration			Color change (ΔE)		
	Mean	SD	Homogeneous sets	Mean	SD	Homogeneous sets
BG _{20%}	4.32	1.93	A	4.30	1.63	A
BG _{25%}	4.33	1.75	A	4.40	1.21	A
BG _{30%}	6.42	2.64	AB	4.88	1.31	A
BG _{35%}	8.44	3.03	B	6.41	1.58	B
BG _{40%}	8.64	3.09	B	6.42	1.94	B

*Capital letter shows differences in columns, for hydrogen peroxide penetration and color change separately

Statistical Analysis

Color and penetration data were checked for normality assumption using the Kolmogorov–Smirnov test. Then, one-way ANOVA and Tukey's test were performed, with a significance level of 5%.

RESULTS

The Kolmogorov–Smirnov test showed normal distribution for color and peroxide penetration ($d > 0.05$). ANOVA showed significant differences among the groups regarding the peroxide penetration ($p = 0.0001$), and Tukey's test revealed that the 35 and 40% concentration promoted higher peroxide penetration than 20 and 25% (Table 1). Regarding color change (ΔE), ANOVA also showed significant differences among the groups ($p = 0.0001$). Tukey's test showed that the more concentrated gels (35 and 40%) presented higher ΔE values compared to the other concentrations tested (Table 1). In relation to the color coordinates, the L^* values increased, the b^* values reduced, and the a^* values showed small and nonsignificant changes, indicating that the bleaching treatment turned the teeth more luminous and less yellow for all groups.

DISCUSSION

The possibility of obtaining quicker bleaching is the main marketing appeal and explains the increasing popularity of the in-office bleaching technique.^{5,21} However, to reach the desired results, high concentrations of hydrogen peroxide gels are frequently used, which is correlated with the higher levels of sensitivity in relation to the at-home technique.²⁴

It is well known that the free radicals are responsible for the oxidation of chromophores on the organic components of the tooth and color change.²⁵ A study showed that the amount of free radicals in a peroxide solution is proportional to its concentration.²⁵ It is therefore expected that the higher concentrated gels would produce higher bleaching effect. In the current study, the results showed that peroxide concentration is directly proportional to the bleaching effect (Table 1), allowing to reject the first null hypothesis. Similar results were observed in *in vitro* studies.^{1,4,25-29} Borges et al. showed that 20% hydrogen peroxide gel produced a significantly smaller color change than a 35% hydrogen peroxide gel, but without differences on the effect over the enamel surface microhardness.⁴ Sulieman et al. analyzed the number of applications necessary for previously stained teeth to the shade C4 reach the shade B1. The number of applications increased

exponentially as the peroxide concentration decreased, requiring one application for 35%, 2 for 25%, 4 for 15%, 7 for 10%, and 12 for 5%. However, some *in vitro* studies failed to provide correlation between the concentration and the bleaching effect.³⁰⁻³² A clinical trial has also shown a correlation between the bleaching effect and the peroxide concentration,³³ although another did not confirm this effect.³⁴ Reis et al. compared similar gel formulations containing different peroxide concentrations (20 and 35% w/w). After two bleaching sessions, the more concentrated gel produced higher bleaching, although both products resulted in similar levels of tooth sensitivity.³³

Despite the effectiveness of the in-office dental bleaching procedure, around 70% of the patients that undergo this treatment reported dental sensitivity, from mild to severe.^{5,24,35,36} This can be explained by the rapid peroxide penetration into the pupal chamber, leading to an oxidative stress surpassing the antioxidant inherent capacity of the tissue,³⁷ which can result in inflammation,³⁸ small histological changes³⁹ or, in extreme situations, to necrosis of the pulp.⁴⁰ The penetration occurs due to the low molecular weight of the hydrogen peroxide molecule and the porosity of the enamel and dentin tissues. Although the penetration is essential to produce the desired bleaching effect, it is also responsible for the irritative effects on the pulp, and a balance between the two aspects must be reached. Some manufacturers created less concentrated gel in an empiric attempt to reduce the negative effects of the highly concentrated products, wishing to keep the effectiveness.^{5,6} According to the results of the current study, it is expected that peroxide reduction in the gel formulations will also reduce the effectiveness of the treatment.

Many *in vitro* studies showed that the amount and speed of pulpal penetration are directly proportional to the hydrogen peroxide concentration in the bleaching gel,^{20,26-28,31,32,39,41-43} which is in agreement with the results of the current study (Table 1), allowing to reject the second null hypothesis. The researches also showed the increase in cytotoxicity,²⁶ lymphocyte-like cell activation and expression of interleukin,⁴⁴ enzymatic inhibition,⁴⁵ formation of tertiary dentin,^{32,39,46} and dental permeability,⁴⁷ and that the peroxide concentration of the bleaching gel is higher. The higher peroxide penetration observed in the current study can explain the higher levels of sensitivity observed on some clinical trials for the more concentrated gels.^{34,48}

After applying the peroxide gel over the enamel surface, a certain amount will penetrate the tooth, while the rest will remain stable or suffer decomposition. A study showed that after the bleaching procedure, a small reduction of peroxide concentration occurs inside the gel layer applied over the tooth, indicating minor degradation and penetration rates.²¹ A study showed that the diffusion phenomena during dental bleaching are determined by the chemical affinity of the peroxide molecule with the organic components of the tissues.⁴⁹ The peroxide can easily and quickly penetrate the enamel due to its low organic content (2% by volume). From those peroxide molecules that actually entered the enamel, around 63% accumulate at the DEJ, due to its high organic content, reacting and attaching to the organic components of the dentin (38% by volume). Only, the remaining 37% is able to diffuse through the dentin tubules and to reach the pulpal chamber. That creates a concentration gradient of peroxide inside the tooth during its diffusion dynamics.⁴⁹ Therefore, not all peroxide molecules inside the bleaching gel actually penetrate the tooth, and from those that penetrated, just a small part really reaches the pulp. That explains the very low peroxide concentrations into the pulpal chamber

observed in this study as well in others,^{6,21,42} below 10 µg/L, while a 35% hydrogen peroxide gel (w/w) has a concentration of 388500 µg/L.

The level of peroxide penetration and the severity of the damage depend on the thickness of enamel and dentin, which varies among the groups of teeth.³⁹ A study showed that in-office bleaching with 38% hydrogen peroxide can cause irreversible pulpal damage in lower incisors but not in premolars.⁴⁰ In addition, the presence of cracks and a tooth abrasion can also increase the peroxide penetration.⁵⁰ However, a clinical trial did not find correlation between the thickness of enamel/dentin layer and the tooth sensitivity.⁴⁸

However, the levels of penetration and the tooth sensitivity are affected by other aspects of bleaching formulation besides the peroxide concentration. A clinical trial showed that the addition of calcium to the 35% hydrogen peroxide bleaching gel can significantly reduce absolute risk of tooth sensitivity, without affecting the bleaching effect.⁵¹ *In vitro* studies showed that the pulpal peroxide penetration was significantly reduced with the addition of calcium to the hydrogen peroxide gel.^{6,16} Some studies analyzed bleaching gels with different pHs and concluded that the smaller is the pH, the higher is the peroxide penetration into the pulpal chamber.^{52,53} Another study showed that chemical activation of bleaching gels can also reduce the pulpal penetration.²

The penetration and cytotoxicity are also increased by exposure to thermal sources⁴¹ or light, the existence of restorations,⁵⁴ and the contact time.^{21,26} Special protocols were suggested to minimize the negative effects on the pulp, changing the application time and peroxide concentration, aiming to increase the safety and comfort to the patients.^{27,28,32} Some studies showed that although the penetration of peroxide rises with the contact time with the surface, this increase is not proportional.^{21,26} The increase in time from 15 to 45 minutes raised the penetration of a 38% hydrogen peroxide gel from 4.7 to 5.7 µg/mL.²¹

The results of this study cannot be directly transferred to the clinical conditions since the penetration *in vivo* is affected by the positive pulpal pressure. Although a previous study showed that pulpal pressure does not influence the bleaching outcome,¹⁵ the amount of peroxide reaching the pulpal tissue will be certainly affected *in vivo*.²⁹ Besides the pulpal pressure, the presence of the odontoblastic processes can also interfere with the penetration, partially filling the tubule entrance. It is the first and more affected alive structure during the peroxide penetration.^{40,45} In addition, the enzymatic breakdown of the hydrogen peroxide by catalase and peroxidase, as part of the defense mechanism of the pulp, will reduce the peroxide concentration in the tissue.⁵⁵

CONCLUSION

It was concluded that the higher peroxide concentrations lead to the increase in tooth bleaching effect, but also increase the hydrogen penetration through the dental structure.

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