

Effects of the Concentration and Composition of In-office Bleaching Gels on Hydrogen Peroxide Penetration into the Pulp Chamber

AP Mena-Serrano • SO Parreiras • EMS do Nascimento
CPF Borges • SB Berger • AD Loguercio
A Reis

Clinical Relevance

The amount of hydrogen peroxide that reaches the pulp chamber of premolars after in-office bleaching depends on the bleaching protocol and the composition of the product.

SUMMARY

In tooth whitening, the hydrogen peroxide (HP) diffuses in the enamel and dentin, reaching the pulp. This *in vitro* study aimed to quantify the penetration of HP in the pulp chamber in teeth submitted to bleaching agents of different concentrations of HP without calcium (HP 20% [20CF], HP 35% [35CF])

Alexandra Patricia Mena-Serrano, DDS, MS, PhD, professor, Department of Restorative Dentistry, Universidad de las Americas, Quito, Pichincha, Ecuador

*Sibelli Olivieri Parreiras, DDS, MS, PhD student, Department of Restorative Dentistry, Ponta Grossa State University, Ponta Grossa, Paraná, Brazil

Emanuelly Messias Stanislawczuk do Nascimento, MS, Chemistry Department, Ponta Grossa State University, Ponta Grossa, Paraná, Brazil

Christiane Philippini Ferreira, PhD, associate professor, Chemistry Department, Ponta Grossa State University, Ponta Grossa, Paraná, Brazil

Sandrine B Berger, DDS, PhD, associate professor, Department of Restorative Dentistry, University of North Parana, Londrina, Paraná, Brazil

and with calcium (HP 20% [20CC], HP 35% [35CC]).

Method: Fifty human premolars were sectioned 3 mm from the cemento-enamel junction and the pulp tissue was removed. The teeth were divided into five groups according to treatment and with a control group (n=10). An acetate buffer solution was placed in the pulp chamber of all teeth. The control group was exposed only to distilled water, while the other groups were treated with a bleaching procedure, according to the manufacturer's recommendations. After treatment, the acetate

Alessandro D Loguercio, DDS, MS, PhD, adjunct professor, Department of Restorative Dentistry, Ponta Grossa State University, Ponta Grossa, Paraná, Brazil

Alessandra Reis, DDS, PhD, adjunct professor, Department of Restorative Dentistry, Ponta Grossa State University, Ponta Grossa, Paraná, Brazil

*Corresponding author: Rua Carlos Cavalcanti, 4748 – Bloco M, Sala 64A - Uvaranas, Ponta Grossa, Brazil, 84030-900; e-mail: sibelliolivieri@hotmail.com

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buffer solution was transferred to a glass tube in which leuco-crystal violet and peroxidase solutions were added, resulting in a blue solution. The optical density of this blue solution was determined spectrophotometrically and converted into micrograms equivalent to the HP. Data were analyzed using analysis of variance and Tukey tests ($\alpha=0.05$).

Results: The HP concentration did not affect the HP inside the pulp chamber, but the presence of calcium significantly reduced it ($p<0.0001$).

Conclusion: The amount of HP that reaches the pulp chamber depends on the bleaching protocol and the product employed, and it seems to be less affected by HP concentration.

INTRODUCTION

Vital tooth whitening is a noninvasive treatment commonly used in dental practice to achieve a harmonic smile in terms of shade.¹ Among the techniques used for vital tooth bleaching, the in-office protocol offers quicker whitening results than the at-home procedure, with fewer applications.^{1,2}

In both at-home and in-office bleaching techniques, hydrogen peroxide (HP) is the active molecule. It forms free radicals, reactive oxygen molecules, and HP anions,³ which oxidize the organic dentin matrix,^{4,5} leading to a whitening effect. Two clinical sessions of in-office bleaching allow a whitening effect of approximately five to eight classical shade guide units,^{6,7} and the bleaching has been shown to be stable after periods ranging from nine to 24 months.^{7,8}

However, the HP does not only whiten teeth. As a result of its low molecular weight, HP penetrates into the dental structure to the pulp chamber,⁹⁻¹¹ causing pulp reactions. This is reflected in minor histological changes,¹²⁻¹⁴ including pulp degeneration at some sites.¹⁵

High concentrations of HP and its by-products exceed the antioxidant capacity of the pulp tissue, causing oxidative stress^{16,17} and pulp inflammation,¹⁸ which trigger the most prevalent bleaching-induced side effect, tooth sensitivity.^{7,19}

Previous studies reported that the intensity of bleaching-induced tooth sensitivity varies from mild^{20,21} to severe.²² In some cases, the tooth sensitivity is so painful that it leads the patient to abandon the treatment.²³ In an attempt to reduce the bleaching-induced discomfort, some manufactur-

ers have released in-office bleaching gels with lower HP concentrations. This was based on the assumption that the amount of HP that reaches the pulp is proportional to its original concentration in the bleaching agent.^{11,24}

However, the results of a recent study²⁵ challenge the concept that tooth sensitivity is directly correlated to the initial HP concentration. This study compared the tooth sensitivity of two 35% HP gels. The alkaline calcium-containing gel presented lower absolute risk of tooth sensitivity than did the slightly acid calcium-free product.²⁵ The amount of HP that reaches the pulp chamber may be different as a result of the presence of other additives. To the extent of the authors' knowledge, this issue has not yet been investigated.

Therefore, the aim of the present study was to compare the amount of HP that reaches the pulp chamber using a calcium-free and a calcium-containing bleaching agent with different HP concentrations.

METHODS AND MATERIALS

We selected 50 extracted sound premolar teeth with only one root for this study. The roots of all teeth were cut approximately 3 mm apical to the cemento-enamel junction, and the pulp tissue was removed and washed with distilled water. The entrance to the pulp cavities was widened carefully with a round bur (#1014; KG Sorensen, Barueri, SP, Brazil) to allow the introduction of a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) inside the pulp chamber.

With the aim of measuring the thickness of the dental structure on the buccal surface of premolars, X-ray radiographs (Timex 70C, Gnatus, Ribeirão Preto, SP, Brazil) were taken with an exposure time of 0.5 seconds and a 30-cm focus-object distance (70 kVp and 7 mA). The central X-ray beam focused at a 90° angle to the buccal surface of the teeth. The images were digitalized, and we measured the buccal tooth thickness with the UTHSCSA ImageTool 3.0 software (University of Texas Health Science Center, San Antonio, TX, USA).

Four different bleaching gels were evaluated (Table 1) according to the combination of the main factors of HP concentration (20% to 35%) and composition (calcium-free and calcium-containing products). An additional control group, in which no bleaching treatment was performed, was added to the experimental design. Ten teeth were used in each group.

Table 1: Batch Number, Composition, pH, and Mode of Application of the Bleaching Products			
Bleaching Agent ^a	Composition ^b /Batch No.	pH ^c	Mode of Application ^b
35% Whiteness HP Maxx (35CF)	35% HP, thickeners, dye mixture, glycol, inorganic load, and deionized water/191111	6.5	3 Applications of 15 min each
35% Whiteness HP BLUE (35CC)	35% HP, thickeners, inert violet pigment, neutralizing agent, calcium gluconate, glycol, and deionized water/250712	9.0	A single 40-min application
20% Whiteness HP Maxx (20CF)	20% HP, thickeners, dye mixture, glycol, inorganic load, and deionized water	6.6	3 Applications of 15 min
20% Whiteness HP BLUE (20CC)	20% HP, thickeners, inert blue pigment, neutralizing agent, calcium gluconate, glycol, and deionized water/270511	9.2	A single 50-min application
<p>Abbreviation: HP, hydrogen peroxide.</p> <p>^a All products are manufactured by FGM Dental Products (Joinville, Santa Catarina, Brazil).</p> <p>^b According to the manufacturer's recommendations.</p> <p>^c Measured with a pH meter (pHmetro Nova Técnica NT-PHM, Piracicaba, São Paulo, Brazil) in triplicate.</p>			

Throughout this study, analytical-grade chemicals without previous purification were used; they were prepared with deionized water from a Millipore Milli-Q system (MS2000, Gehaka, São Paulo, SP, Brazil). HP was purchased from LABSYNTH (34%-36%, Diadema, SP, Brazil). A 5000-µg/mL stock solution was prepared in acetate buffer solution (pH 4) and standardized by conventional methods. The solution was titrated with potassium permanganate standard solution.²⁶ Aliquots of the stock solution of HP were diluted volumetrically to obtain working standard solutions of 0.032-0.397 µg/mL (Table 2; Figure 1).

All teeth were fixed vertically to a wax plaque, and the labial surface of each tooth was isolated by applying a light-cured resin dam (Top Dam, FGM Dental Products, Joinville, SC, Brazil). A 25-µL aliquot of acetate buffer (pH 4.5) was placed into the pulp chamber of each tooth to absorb and stabilize any peroxide that might penetrate into the pulp chamber.

The bleaching gels were applied over the enamel surface, as recommended by the manufacturer (Table 1). After the exposure period, the acetate buffer solutions in the pulp chamber of each tooth were removed by means of a mechanical micropipette (LABMATE Soft; HTL Lab Solutions) and transferred to a glass tube. The pulp chamber of each tooth was rinsed four times with 25 µL of acetate buffer, and this solution was removed from the pulp chamber and placed into the same glass tube. Next, more deionized water (2.725 µL) was added to the glass tube along with 100 µL of 0.5 mg/mL of leuco-crystal violet (Aldrich; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 50 µL of 1 mg/mL enzyme horseradish peroxidase (Peroxidase Type VI-A; Sigma Chemical Co, St Louis, MO, USA). When the absorbance value of this sample was higher than 1500 µL, the solution was diluted even further with 3000 µL of deionized water and measured again. This procedure was repeated separately for each tooth.

Table 2: Data Points for the Calibration Curve						
H ₂ O ₂ Data for Each Point		Solutions Required to Obtain 3000 µL for Each Point for the Calibration Line				
H ₂ O ₂ Weight, µg	H ₂ O ₂ Concentration, µg/mL	Acetate Buffer Solution, µL	47.67 µg/mL H ₂ O ₂ Solution, µL	Peroxidase, µL	Leuco-crystal Violet, µL	Deionized Water, µL
1.192	0.397	75	25	50	100	2750
0.953	0.318	80	20	50	100	2750
0.715	0.238	85	15	50	100	2750
0.477	0.159	90	10	50	100	2750
0.381	0.127	92	8	50	100	2750
0.191	0.064	96	4	50	100	2750
0.095	0.032	98	2	50	100	2750
0.000	0.000	100	0	50	100	2750

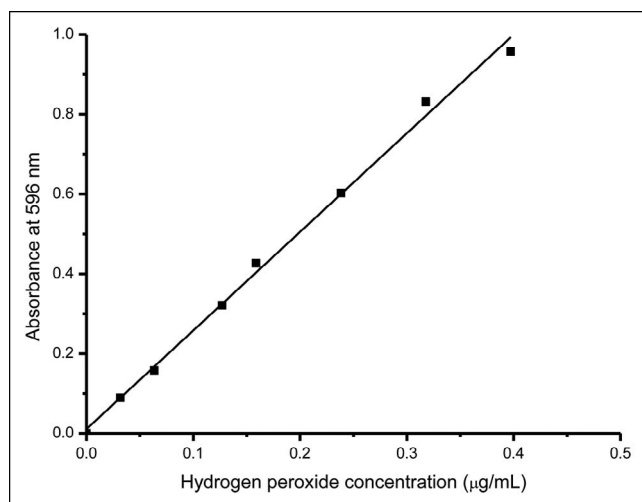


Figure 1. Spectrophotometric calibration curve used in this study. $R = 0.99524$.

The absorbance at 596 nm of the resultant violet color in the tubes was measured in a Cary 50 UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA). According to Beer's Law, absorbance is directly proportional to the concentration. Therefore, the concentration of HP ($\mu\text{g/mL}$) was determined by comparing it to the calibration curve previously obtained (Figure 1).²⁷ By knowing the concentration ($\mu\text{g/mL}$) and volume of the solution, the HP mass (μg) was calculated by the following equation: $m = C \times MM \times V$, where m represents mass, C is the concentration, MM is the HP molar mass (34,158), and V is the volume (3×10^{-3} L).

The data related to HP concentration and mass were subjected to one-way analysis of variance (ANOVA) and Tukey tests for pairwise comparisons ($\alpha=0.05$).

RESULTS

The mean buccal tooth thickness of the teeth employed in this study was 2.5 ± 0.5 mm. One-way ANOVA revealed statistically significant differences among groups ($p=0.001$ and $p=0.00001$ for HP concentration and HP mass, respectively).

As can be seen in Table 3, an insignificant amount of HP was detected in the pulp chamber of the control groups ($p<0.05$). When the bleaching gels were compared, a significantly lower amount of HP was found in the pulp chamber after application of the calcium-containing gel, regardless of the HP concentration.

Table 3: Means and Standard Deviations of the H_2O_2 Concentration ($\mu\text{g/mL}$) and the H_2O_2 Weight (μg) Detected Inside the Pulp Chamber for the Treatment Groups^a

Groups	HP Concentration, $\mu\text{g/mL}$	HP Weight, μg
Control	0.004 ± 0.002 C	0.012 ± 0.005 c
35% Calcium-free	1.156 ± 0.338 A	3.469 ± 1.014 a
35% Calcium-containing	0.201 ± 0.185 B	0.640 ± 0.554 b
20% Calcium-free	0.943 ± 0.487 A	3.251 ± 1.179 a
20% Calcium-containing	0.115 ± 0.082 B	0.664 ± 0.982 b

Abbreviation: HP, hydrogen peroxide.

^a Identical uppercase and lowercase letters within a column indicate statistically similar means (one-way analysis of variance and Tukey test, $\alpha=0.05$).

DISCUSSION

The results of the present study confirm the ability of the HP to penetrate the tooth structure and to reach the pulp chamber immediately after an in-office bleaching session. This finding had already been demonstrated by several researchers.⁹⁻¹¹

However, the present study showed that the amount of HP that reached the pulp chamber was not proportional to the HP concentration of the bleaching gel applied on the enamel surface. This is contrary to the findings of other published studies.^{9,11} For instance, Gokay and others⁹ demonstrated that the amount of HP detected in the pulp chamber was three times higher for a 30% HP than for a 35% carbamide peroxide product, which delivers approximately one-third less HP. In the same study, an even greater difference was reported when the 30% HP product was compared to 10% and 15% carbamide peroxide products.⁹ It is worth mentioning that none of these studies compared in-office bleaching gels: high HP concentration was compared to carbamide peroxide gels, which release one-third less HP.

Additionally, carbamide peroxide usually takes longer to deliver the HP¹ than do HP-based gels.²⁸ This may explain why a huge difference in the amount of HP in the pulp is detected when a high concentration of HP gel is compared with carbamide peroxide products.

The 15% difference in the concentration of HP between the in-office bleaching gels employed in this study does not yield a significant difference in the amount of HP that reaches the pulp, at least when measured immediately after bleaching. The findings of a recent study²⁹ strengthen this hypothesis. The

authors did not detect differences in the tooth sensitivity prevalence using in-office bleaching gels with 20% and 35% HP. Further studies comparing different concentrations of in-office bleaching gels should be encouraged to increase the generalizability of the findings of this study to other products available in the market.

However, the most surprising finding was the fact that the amount of HP that reached the pulp chamber was statistically lower for the calcium-containing agent than for the calcium-free product, regardless of the initial HP concentration. This finding correlates well with the finding of a recent clinical trial²⁵ that reported that the prevalence of tooth sensitivity with a calcium-containing agent was lower than that associated with the equivalent calcium-free product.

The fact that HP is detected within the pulp chamber means that not all HP molecules decompose into free radicals within the dental structure (ie, there is an exceedingly high amount of HP within dentin independent of the composition and concentration of the product). For the calcium-containing gel, this HP surplus may react with calcium gluconate present in its composition, leading to the formation of calcium hydroxide, reducing even further the surplus of HP that travels to the pulp chamber.

Additionally, the calcium-containing agent is delivered in an alkaline pH, which is different from the situation with the calcium-free product. In an alkaline media, the dissociation of HP into free radicals is the greatest, as the dissociation constant (pKa) of the HP is around 11.5. It has already been reported³⁰ that HP in a pH of 9 dissociated 2.7 times more than it did in a pH of 4.4. Thus, if more HP dissociates into free radicals within dental structure, less surplus of HP is available to travel within dentin and reach the pulp chamber. This may explain the lower amount of diffused HP for the high-pH, calcium-containing agent.

The pH of the media affects not only the decomposition kinetics but also the type of by-products produced. While in an acidic solution, free oxygen radicals and hydroxyl anions are produced, in an alkaline media there is a higher concentration of perhydroxyl ions.³¹ Although these variations in the bleaching gel composition did not produce differences in the degree of clinical whitening,²⁵ little is known about the deleterious effects of these different oxidizing agents on the dental-

pulp complex, a subject that deserves further evaluation.

This study design did not allow us to measure the decomposition by-products of the HP in the pulp chamber. Therefore, the fact that low HP was detected in the pulp chamber of the calcium-containing products does not mean that the HP by-products are in a reduced concentration in the pulp chamber. The use of electron spin resonance could offer a method with which to evaluate the presence of active oxygen or free radicals produced by the bleaching products in the pulp chamber.³²

We cannot rule out the role that the difference in bleaching protocols between the calcium-free and calcium-containing gels may have played with regard to the results presented herein. The calcium-containing gel was applied in a single 40-50-minute application (Table 1), per the manufacturer's directions, while the calcium-free gel was applied in three consecutive 15-minute applications. Considering that it took some minutes to remove and reapply the calcium-free gel, this protocol usually took 46 to 47 minutes to complete, meaning that the 35% calcium-free HP gel remained in contact with the buccal surfaces for approximately six to seven minutes longer than did the calcium-containing product with the same concentration. Previous studies³³⁻³⁵ have already demonstrated that the application time influences the amount of HP that reaches the pulp: the longer the period the HP is in contact with the buccal surface of the teeth, the higher the amount of HP detected in the pulp chamber.

Additionally, the calcium-free gel was replenished three times, while the calcium-containing product was only applied once. This means that we might have delivered more HP in the case of the calcium-free product. While the standardization of the bleaching protocols would allow us to eliminate these protocol variables, it would reduce the clinical significance of the study, as clinicians usually apply the material according to the manufacturer's instructions.

Finally, it is worth mentioning that besides the concentration, composition, and pH of the in-office products evaluated in this study, the literature reports that there are other important factors that can influence HP penetration into the pulp chamber, such as the presence of restorations,¹¹ enamel craze lines,³⁶ association with light sources,¹⁰ thickness and type of tooth,^{15,37} and chemical activation.³⁸ All of these factors might explain the high variability within the data when measuring the amount of HP

in the pulp chamber using the UV-VIS spectrophotometer.

CONCLUSIONS

The amount of HP that reached the pulp chamber after in-office bleaching was dependent on the bleaching protocol and gel composition, regardless of the product concentration (20% or 35%).

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Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies approved by the local Ethics Committee of the State University of Ponta Grossa. The approval code for this study was 11005/11. This study was conducted at the State University of Ponta Grossa.

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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