

Effects of pH and Application Technique of In-office Bleaching Gels on Hydrogen Peroxide Penetration into the Pulp Chamber

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Clinical Relevance

In-office bleaching gel with a neutral/alkaline pH resulted in less hydrogen peroxide penetration into the pulp chamber and could be safely applied using different techniques.

SUMMARY

Objective: This *in vitro* study aimed to quantify the penetration of hydrogen peroxide (HP) into the pulp chamber in teeth submitted to in-office bleaching with varied pH and application techniques. The color change and pH of the in-office bleaching product during application was also evaluated.

Methods and Materials: Ninety-six human premolars were used and randomly divided into

10 groups (n=9) according to the following combination of factors: pH of in-office bleaching agents (two neutral/alkaline pH: Opalescence Boost 38% and Whiteness HP Blue 35% and three acidic pH: Whiteness HP Maxx 35%, Lase Peroxide Sensy 35%, and Total Blanc Office 35%) and application modes (for 3 × 15 minutes [3×15] and 1 × 45 minutes [1×45]). An additional group of non-bleached teeth (control; n=6) was added. First, all teeth were sectioned 3 mm from the cemento-enamel junction and the pulp tissue was removed. An acetate buffer was placed in the pulp chamber of all teeth. After bleaching, this solution was

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transferred to a glass tube in which HP was allowed to react with other components, resulting in a pink solution. The optical density of this pink solution was measured using ultraviolet-visible spectroscopy and converted into amount of HP. Color change before and 1 week after bleaching was evaluated using a digital spectrophotometer. A pH meter with a 6-mm circular and flat surface was used in contact with the enamel surface to quantify the pH of the bleaching gels during application. Data were analyzed using two-way analysis of variance and Tukey tests ($\alpha=0.05$).

Results: Overall, lower mean HP penetration values were observed for Opalescence Boost 38% and Whiteness HP Blue 35% compared with other bleaching gels ($p<0.05$). Opalescence Boost 38% and Whiteness HP Blue 35% were not influenced by the application technique ($p>0.05$). However, lower mean HP penetration values were observed for Whiteness HP Maxx 35%, Total Blanc Office 35%, and Lase Peroxide Sensy 35% when using the 3×15 application technique compared with the 1×45 technique ($p<0.05$). Significant whitening was detected and no significant difference of color change was observed between groups ($p>0.54$). The pH did not change during the 3×15 application technique; however, all acidic bleaching gels significantly decreased in pH when applied for 1×45 ($p<0.01$).

Conclusions: The amount of HP that reaches the pulp chamber was lower when neutral/alkaline pH gels were used, independently of the application technique. When considering acidic pH gels, it is preferable to use the 3×15 application technique, mainly because longer application time (1×45) results in lower pH. No difference was observed between groups with regards to color change.

INTRODUCTION

Carbamide peroxide applied at home is the technique most employed by clinicians for bleaching vital teeth,^{1,2} and it provides a high satisfaction rate among patients.³ However, some patients have indicated that they could not adapt to the use of whitening trays or disliked waiting 2 to 3 weeks for the bleaching results.^{4,5} In these cases, an in-office bleaching technique is indicated because it is possible to obtain faster bleaching results compared with the at-home treatment,^{4,6} with the advantage of

exclusive in-office application and full control of the whole procedure, which prevents the intake of product.^{4,7,8}

Unfortunately, due to the higher concentration of hydrogen Peroxide (HP) used, in-office bleaching is related to a higher percentage of patients with tooth sensitivity (TS).⁴⁻⁸ Bleaching-induced TS is associated with an inflammatory response from the pulp cells due to the diffusion of HP,⁹ which may alter the dental pulp.^{10,11} This is the main reason for several changes to the in-office bleaching gels recently launched by manufacturers.

In the past, in-office bleaching gels were delivered with a low pH (around 2.0)^{12,13} to increase the product's shelf life.¹⁴ However, this leads to several modifications in the chemical composition, morphology, and mechanical properties of the tooth structure,^{15,16} which can increase the passage of HP and, consequently, increase TS.¹⁷⁻²⁰ These enamel alterations are more aggressive if the bleaching gels are in contact with the enamel surface for a long period of time. This occurs due to the prolonged contact of enamel with low pH in-office gels^{21,22} and is one of the reasons manufacturers have indicated replenishing the gel during in-office bleaching. The HP is typically applied and left undisturbed on tooth surfaces for 10-20 minutes during in-office bleaching, and this procedure is repeated two to five times at each clinical appointment, depending on the bleaching gel brand.^{23,24}

Recently, in-office bleaching gels began presenting with neutral to alkaline pH, which are less aggressive to the tooth structure.^{21,22,25,26} Some clinical studies have shown that neutral/alkaline pH bleaching gels reduced TS compared with acidic pH bleaching gels.^{17,19,20} Due to this, manufacturers have suggested that these new brands could be applied without replenishing the gel during in-office bleaching. This simplifies the clinical procedure, reduces the risk of occasional soft tissue burns, and lowers the cost because less material is used per patient.

Unfortunately, the pH of different available commercial products is not clear to clinicians, nor is the best application mode made known to clinicians. Therefore, the aim of the present study was to compare the amount of HP that reaches the pulp chamber using in-office bleaching gels with different pH applied using two application modes. Also, the pH of these in-office bleaching gels was evaluated during application, and color change was evaluated immediately and 7 days after bleaching.

Table 1: Material, Batch Number, Composition, pH, and Manipulation for In-office Bleaching Gels

Material/Batch Number	Composition	pH ^a	Manipulation ^a
Opalescence Boost PF 38% (190250)	38% Hydrogen peroxide, 20% water and desensitizing agents (3% potassium nitrate and 1.1% fluoride)	7.0	Attach both syringes before mixing. Press the plunger of the red syringe in, pushing all the contents into the clear syringe. Forcefully press the small clear stem completely into the larger clear stem. Then press the clear plunger completely into the red syringe. To activate, press the chemical from the red syringe into the clear syringe with thumbs. Reverse action, and mix a minimum of 25 times on each side
Whiteness HP Blue 35% (161012)	35% Hydrogen peroxide, deionized water, thickener, violet colorant, glycol, neutralized agents and desensitizing agents (3% calcium gluconate)	8-9	Prepare the gel: mix the two phases with the syringes connected, pushing the plungers four times on each side (total of eight times), then push all the mixed content into one of the syringes, and it is ready for use. Make sure both syringes are well attached; Place a tip on the syringe that still has the gel and apply a layer to the entire vestibular surface of the teeth to be whitened (including the interproximals) and extend a little onto the incisal and occlusal faces. The layer of gel should be between 0.5 and 1 mm thick.
Whiteness HP Maxx 35% (200814)	35% Hydrogen peroxide, thickener, colorants, glycol, filler, deionized water	6.5	Using the mixture plaque provided in the kit, mix the peroxide phase (phase 1) with the thickener phase (phase 2) in the following proportion: three drops of peroxide to one drop of thickener. Shake vigorously the thickener bottle before using it. This mix quantity is sufficient for one tooth application. With the help of a spatula, mix both phases for 10 seconds.
Lase Peroxide Sensy 35% (11002)	35% Hydrogen peroxide, thickener, water, vegetables extracts, sequestrating agents, amide, colorants and glycol	6.5	Remove the caps of the syringes with peroxide and thickener, and attach the syringes tightly. Make sure they are well connected to avoid material leakage. Poke the plungers alternately until the product reaches a homogeneous yellow coloring, indicating that it is active. Then, transfer all the mixture to the orange plunger syringe (hydrogen peroxide). Couple the applicator tip in the syringe and apply the bleaching gel over the teeth labial surface. The mixture content is sufficient for simultaneous application on the two arcades.
Total Blanc Office 35% (13040589)	35% Hydrogen peroxide, thickener, water, vegetable extracts, sequestrating agents, amide, colorants and glycol	6.5	Remove the caps of the syringes with peroxide and thickener and attach the syringes tightly. Make sure they are well connected to avoid material leakage. Poke the plungers alternately until the product reaches a homogeneous yellow coloring, indicating that it is active. Then, transfer all the mixture to the orange plunger syringe (hydrogen peroxide). Couple the applicator tip in the syringe and apply the bleaching gel over the teeth labial surface. The mixture content is sufficient for simultaneous application on the two arcades.

^a According to the manufacturer's indications.

The null hypotheses tested were that the pH and application mode of in-office bleaching gels does not affect (1) the amount of HP that reaches the pulp chamber and (2) color change after bleaching.

METHODS AND MATERIALS

This study was reviewed and approved by the Ethics Committee of the local university, under protocol No. 1355037. A total of 96 sound premolar human teeth, free of fracture lines, enamel defects, fissures, or any pathologic lesions, were divided according to a

combination of the following main factors: 1) bleaching agents (Opalescence Boost PF 38% [Ultradent, South Jordan, UT, USA]; Whiteness HP Blue 35% [FGM, Joinville, SC, Brazil]; Whiteness HP Maxx 35% [FGM]; Lase Peroxide Sensy 35% [DMC, São Carlos, SP, Brazil]; or Total Blanc Office 35% [Nova DFL, Estrada do Guerengué, RJ, Brazil]) (Table 1); and 2) application technique (three 15-minute applications [3×15] or a single 45-minute [1×45] application). An additional, unbleached control group was added to the experimental design.

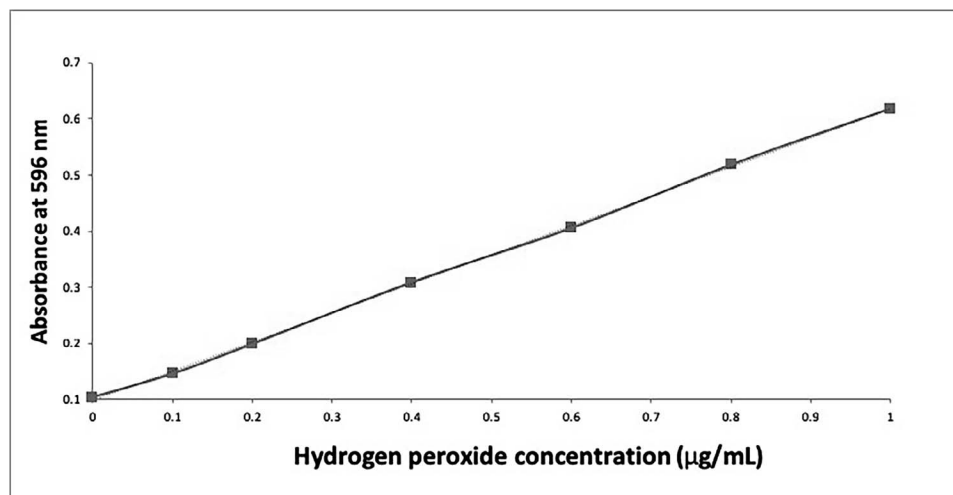


Figure 1. Spectrophotometric calibration curve used in this study ($R=0.999524$).

Sample-Size Calculation

The primary outcome of this study was the amount of HP penetration inside the pulp chamber. According to previous literature, the amount of HP penetration for the control group was determined to be $0.6 \pm 0.2 \mu\text{g/mL}$.^{27,28} Using an α of 0.05, a power of 80%, and a two-sided test, the minimal sample size was six teeth in each group in order to detect a difference of $0.3 \mu\text{g/mL}$ among the tested groups.

Quantification of HP Penetration

The roots of all teeth ($n=6$ for each group) were sectioned using a diamond disc (KG Sorensen, Barueri, SP, Brazil, double-sided segmented, No. 7011) under constant water irrigation 3 mm apical to the cemento-enamel junction. The pulp tissue was then carefully removed, and the pulp chamber washed with distilled water. The pulp chamber was carefully widened with a round bur (#1014, KG Sorensen). The entrance to the pulp chamber was widened using a round bur (No. 1014, KG Sorensen) to accommodate a micropipette (LABMATE Soft, 50 μL of capacity, HTL Lab Solutions, Warsaw, Poland), while avoiding touching the pulp chamber walls.

All teeth were vertically fixed to a wax plaque. Adhesive tape (Missner, Missner & Missner Ltda, Blumenau, SC, Brazil) was placed before nail polish application to limit the exposure area to 6 mm (radius) (Colorama, L'Oreal Brasil, Rio de Janeiro, RJ, Brazil), standardizing the area for bleaching gel application and color measurement.

Before applying the bleaching gels, an analytic curve was prepared with a standard HP solution at 30% concentration (Labsynth, Diadema, SP, Brazil)

to obtain the relation between the light absorbance and the HP concentration. For this purpose, an acetate buffer solution (pH 4.5) was used to attract and stabilize the HP that might penetrate the pulp chamber. The solution was titrated with a potassium permanganate standard solution (Figure 1).²⁹ Next, a 25- μL aliquot of acetate buffer (pH 4.5) was placed into the pulp chamber of each tooth to absorb and stabilize any HP that might penetrate the pulp chamber.

All bleaching gels were manipulated according to the description in Table 1 and placed over the enamel surface. In the 1 \times 45 group, the product was placed on the enamel surface and remained untouched for the entire time (45 minutes). In contrast, the product on the tooth surface in the 3 \times 15 group was removed using an aspirating tip, and the product reapplied two additional times, until 45 minutes had elapsed (Table 1).

After the treatment period, a micropipette was used to transfer the acetate buffer solution from the pulp chamber of each tooth to a glass tube. The pulp chamber of each tooth was then rinsed four times with 25 μL of acetate buffer, which was placed into the same glass tube. Then, deionized water (2.725 μL) was added to the glass tube along with 4 mmol/L of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolyl-5, 24 mol/L of phenol, 0.4 U/mL of peroxidase dissolved in phosphate buffer, 0.1 M pH = 7.0 (Glucose pp, Gold Análise Diagnóstica Ltda, Belo Horizonte, MG, Brazil). When stored at 4°C, the peroxidase catalyzes the degradation of HP in the presence of amino-phenazone 4-aminoantipyrine with phenol. This reaction releases oxygen that oxidizes the chromogenic hydrogen donor, making the originally transparent solution turn to a shade of pink.

The amount of HP in the solution was then measured by evaluating the color absorbance using an ultraviolet-Vis spectrophotometer (Shimadzu UV 1601, Kyoto, Japan) at a wavelength of 510 nm to obtain the optical density.³⁰ 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 obtained previously (Figure 1).^{29,30} Once the concentration ($\mu\text{g/mL}$) and volume of the solution were known, the HP mass (l g) was calculated using the following equation: $m = C * MM * V$, 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).³⁰ This procedure was repeated separately for each tooth.

Color Change Evaluation

The same teeth used in the "Quantification of HP Penetration" section were used for color change measurement. The color change was evaluated using the spectrophotometer, VITA Easyshade (VITA Zahnfabrik, Bad Säckingen, Germany). The tip of the device was put in contact with the previously delimited area and the L^* , a^* , and b^* parameters of color were obtained from the spectrophotometer. The L^* value represents the luminosity (value from 0 [black] to 100 [white]), the a^* value represents the measurement along the red-green axis, and the b^* value represents the measurement along the yellow-blue axis. The color change (ΔE) before (baseline) and 1 week after the bleaching procedure was given by differences between the two colors measured with the spectrophotometer, which was calculated using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. All teeth were immersed in distilled water at 37°C from the completion of the bleaching procedures until the final color was measured.

Quantification of pH in Contact With Enamel Surface

Thirty sound premolar human teeth were used in this part of the study ($n=3$ for each group). All teeth were fixed vertically to a wax plaque, and adhesive tape (Missner, Missner & Missner Ltda) was placed before nail polish application to limit the exposure area to 5 mm² (Colorama, L'Oreal Brasil) to standardize the area of bleaching gel application. All bleaching gels were manipulated according to the description in Table 1 and placed over the enamel surface as described previously.

A pH meter with a 6-mm circular and flat surface pH electrode (Extech pH100: ExStik pH Meter;

Table 2: Means and Standard Deviations of the Hydrogen Peroxide (HP) Concentration ($\mu\text{g/mL}$) Detected Inside the Pulp Chamber for the Treatment Groups and the Statistical Comparison

Bleaching Gel	Application Technique ^a	
	3×15 min	1×45 min
Opalescence Boost PF 38%	0.13 ± 0.06 A	0.19 ± 0.04 A,B
Whiteness HP Blue 35%	0.12 ± 0.02 A	0.19 ± 0.08 A,B
Whiteness HP Maxx 35%	0.14 ± 0.08 A	0.32 ± 0.07 C
Lase Peroxide Sensy 35%	0.19 ± 0.08 A,B	0.28 ± 0.04 C
Total Blanc Office 35%	0.21 ± 0.06 A,B	0.30 ± 0.10 C
Control	0.03 ± 0.002*	

^a Different letters indicate that groups are statistically different (two-way analysis of variance, Tukey test, $p < 0.05$).

* All groups showed higher and statistically significant values compared with the control group (Dunnett test, $p = 0.00001$).

Extech instruments, Nashua, NH, USA) was positioned directly onto the delimited area and held in position until the pH was stabilized.²⁰ Because the pH electrode is very sensitive, it was possible to make three measurements for each tooth. For both application protocols (3×15 and 1×45 applications), pH was registered at the baseline and every 15 minutes until 45 minutes had elapsed.

Statistical Analysis

The data from the HP concentrations and ΔE were subjected to a two-way analysis of variance (ANCOVA) (bleaching agent and application technique) and Tukey test for pairwise comparisons. Also, all groups from the HP concentrations were compared against the control group using the Dunnett test. The data from the pH values were compared using a two-way repeated measures ANOVA (bleaching agent and application technique). All tests were carried out using the Sigma Plot 11 software (Systat Software Inc, Chicago, IL, USA) with a preset level of significance of 5%.

RESULTS

Quantification of HP Penetration

The HP concentration that reached the pulp chamber in each group is depicted in Table 2. The two-way ANOVA revealed a statistically significant effect for the cross-product interaction (Table 2; $p = 0.001$). The amount of HP was lower in the pulp chamber of the control group than the bleached groups (Table 2; $p < 0.05$). In general, there was no difference between the in-office bleaching agents tested when they were used in the 3×15 technique (Table 2; $p > 0.41$). However, there was a significant difference when the gels were applied using the 1×45 technique

Table 3: Means and Standard Deviations of the ΔE for the Treatment Groups and the Statistical Comparison

Bleaching Gel	Application Techniques ^a	
	3×15 min	1×45 min
Opalescence Boost PF 38%	13.2 ± 3.3	14.2 ± 3.4
Whiteness HP Blue 35%	12.7 ± 2.9	13.7 ± 3.2
Whiteness HP Maxx 35%	12.3 ± 2.9	12.7 ± 3.9
Lase Peroxide Sensy 35%	11.7 ± 4.5	12.5 ± 4.1
Total Blanc Office 35%	12.2 ± 3.2	12.1 ± 3.9

^a No significant differences were observed between different groups (two-way analysis of variance, Tukey test, $p>0.54$).

(Table 2; $p=0.001$). In the 1×45 technique, the neutral/alkaline in-office bleaching gels Opalescence Boost PF 38% and Whiteness HP Blue 35% showed lower mean HP penetration than the acidic bleaching gels (Table 2; $p=0.001$).

Color Change Evaluation

The ΔE in each group is shown in Table 3. Two-way ANOVA revealed no statistically significant effect for the cross-product interaction and the main factors (Table 3; $p>0.54$). These results indicate that no significant difference in color change was observed between groups. However, a significant whitening effect was detected by all groups, regardless of the pH and application mode of the evaluated in-office bleaching gels (Table 3).

Quantification of pH in Contact With Enamel Surface

The quantification of pH in contact with the enamel surface in each group is shown in Table 4. The two-way ANOVA revealed a statistically significant effect for the cross-product interaction (Table 4; $p=0.01$). The baseline results confirmed that Opalescence Boost PF 38% and Whiteness HP Blue 35% are neutral/alkaline in-office bleaching gels. On the other hand, Whiteness HP Maxx 35%, Lase Peroxide

Sensy 35%, and Total Blanc Office 35% were confirmed to be acidic in-office bleaching gels (Table 4). The pH remained stable during the 3×15 application technique for all bleaching gels evaluated (Table 4; $p>0.05$). However, when applied in the 1×45 technique, there was a significant difference between the in-office bleaching agents tested (Table 4; $p=0.01$). When the neutral/alkaline in-office bleaching gels were applied, no significant change in pH was observed during the 1×45 application (Table 4; $p>0.61$). On the other hand, all acidic bleaching gels significantly decreased in pH during the 1×45 technique, mainly after 30 minutes (Table 4; $p<0.01$).

DISCUSSION

The results of the present study showed that when the different application techniques were evaluated, a significant difference was observed for the acidic pH in-office gels compared with the other gels, leading us to reject the first null hypothesis. A higher amount of HP was usually found inside the pulp chamber for the 1×45 technique. According to the pH measurement, three products (Whiteness HP Maxx 35%, Lase Peroxide Sensy 35% and Total Blanc Office 35%) were considered acidic pH in-office gels in agreement with the measurements of several authors.^{21,22,26} This is expected because acidic gel could cause more microhardness loss and morphologic change of enamel due to the enamel surface demineralization compared with the neutral/alkaline gels.^{15,16}

The results of the present study also showed a significant decrease in the pH of the acidic in-office gels with prolonged contact with enamel.^{21,22} This may lead to further damage to the surface of the teeth, resulting in an increase in the superficial porosities of the enamel^{15,16,31} and, consequently, greater passage from HP to the pulp chamber, in agreement with the results of HP penetration in the present study. This is the main reason the replen-

Table 4: Means and Standard Deviations of pH at the Different Assessment Points for the Treatment Groups and the Statistical Comparison

Bleaching Gel	3×15 Application Technique ^a				1×45 application technique ^a			
	Baseline	15 min	30 min	45 min	Baseline	15 min	30 min	45 min
Opalescence Boost PF 38%	7.5 ± 0.3 A	7.5 ± 0.6 A	7.6 ± 0.3 A	7.8 ± 0.3 A	7.4 ± 0.3 A	7.5 ± 0.6 A	7.7 ± 0.4 A	7.7 ± 0.4 A
Whiteness HP Blue 35%	7.7 ± 0.4 A	7.6 ± 0.2 A	7.9 ± 0.2 A	8.0 ± 0.2 A	7.8 ± 0.3 A	7.5 ± 0.3 A	7.7 ± 0.3 A	7.4 ± 0.3 A
Whiteness HP Maxx 35%	6.5 ± 0.3 B	6.4 ± 0.3 B	6.6 ± 0.2 B	6.6 ± 0.4 B	6.5 ± 0.4 B	6.2 ± 0.4 B	5.7 ± 0.2 B,C	5.3 ± 0.3 C
Lase Peroxide Sensy 35%	6.1 ± 0.2 B	6.1 ± 0.3 B	6.2 ± 0.4 B	6.3 ± 0.2 B	6.1 ± 0.3 B	5.7 ± 0.2 B,C	5.5 ± 0.3 B,C	5.0 ± 0.3 C
Total Blanc Office 35%	6.4 ± 0.5 B	6.3 ± 0.4 B	6.2 ± 0.3 B	6.4 ± 0.3 B	6.3 ± 0.2 B	5.8 ± 0.3 B	5.6 ± 0.3 B,C	5.2 ± 0.4 C

^a Different letters indicate that groups are statistically different (two-way repeated measures analysis of variance, $p=0.01$).

ishing technique is indicated for the majority of in-office bleaching gels available on the market.^{7,32} When the acidic gel is removed after 15-20 minutes of application, it prevents further changes on the enamel surface and, consequently, decreases the passage of HP, as observed in the results of the present study.

On the other hand, the results of the present study showed no significant difference for Opalescence Boost PF 38% and Whiteness HP Blue 35% with regards to the application technique. According to the pH measurement, these gels are considered neutral/alkaline pH gels. It has been described that a higher pH in bleaching gels leads to more dissociation of HP into free radicals. For instance, HP with a pH of 9 dissociated 2.7 times more than HP with a pH of 4.4.³³ Thus, if more HP dissociates into free radicals within the dental structure, less surplus of HP is available to travel within the dentin and reach the pulp chamber. This may explain the lower amount of diffused HP for the Opalescence Boost PF 38% and Whiteness HP Blue 35%.

When the 1×45 technique was evaluated, the neutral/alkaline gels typically showed a lower amount of HP inside the pulp chamber compared with the acidic gels, which was previously shown by Mena-Serrano and others.²⁷ This is in agreement with recent published clinical studies that showed a lower percentage of patients who reported TS when neutral/alkaline pH bleaching gels were compared with acidic pH bleaching gels.^{17,19,20}

The results of the present study and the study of Mena-Serrano and others²⁷ are not in consensus with the current literature. For instance, Pignoly and others³⁴ and Marson and others³⁵ showed that the amounts of HP found in the pulp chamber were similar, regardless of the pH of the gel used. However, methodologic differences may explain this controversy. While some studies used a small part of a bovine tooth, turning it into a simulated pulp chamber, the present study used human teeth without any preparation, which better simulates the clinical situation.^{34,35} Also, Kwon,³⁶ who was the first to introduce the protocol of not replenishing the gel during in-office bleaching more than 10 years ago, showed that the amount of HP inside the pulp chamber was significantly lower when one prolonged application was evaluated. However, the application of the bleaching gel was evaluated using a different technique (sealed technique), so a clear comparison cannot be made between the results of the present study and Kwon's results.³⁶

It is worth mentioning that the results of the present study showed that these neutral/alkaline pH gels maintain their pH for the entire application duration, which was also previously observed by Trentino and others.²¹ This could be explained by the fact that these gels have a pH that is almost neutral; few ion exchange reactions occur with the surface of the enamel,^{37,38} which enables pH to be maintained. This helps to explain why the application technique did not significantly change the amount of HP inside the pulp chamber when neutral/alkaline gels were applied.

Unfortunately, the products with the lower concentration of HP in the pulp chamber (alkaline/neutral gels) contain desensitizing agents. Opalescence Boost PF 38% contains potassium nitrate and sodium fluoride, and Whiteness HP Blue 35% contains calcium digluconate. These agents act in different ways; for instance, potassium nitrate reduces sensitivity by decreasing the capacity of the nerve fibers to propagate nerve impulses. On the other hand, sodium fluoride and calcium digluconate act by blocking the dentinal tubules, leading to a reduced flow of fluid into the pulp chamber.³⁹ A closer view of clinical trials that evaluated the TS of in-office bleaching gels containing desensitizer agents have shown controversial results.^{17,19,20,40} Future clinical studies need to be done to evaluate the effect of neutral/alkaline gels with and without desensitizer on HP penetration.

It is also worth mentioning that it is not only the pH and the addition of desensitizing agents that could influence the amount of HP inside the pulp chamber. Other factors may affect the amount of HP that enters the pulp cavity, such as HP concentration and the viscosity of the bleaching gels.^{27,41} Future studies need to be done to evaluate how these factors interact with the pH of different bleaching gels.

Finally, independent of the pH and application technique applied, a significant whitening was detected and no significant difference in color change was observed between groups, leading us to accept the second null hypothesis. This is in agreement with several clinical studies that have shown that all in-office bleaching materials evaluated in the present study are effective in terms of whitening.^{17-19,20,40} However, according to the results of the present study, the pH and application technique of in-office bleaching gels could impair the amount of HP reaching the pulp chamber.

CONCLUSION

The amount of HP that reaches the pulp chamber was lower when neutral/alkaline pH gels were used, independent of the application technique. For acidic pH gels, it is preferable to use the 3×15 application technique, mainly because the 1×45 technique results in lower pH.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of State University of Ponta Grossa. The approval code for this study is 1355037.

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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REFERENCES

- Leonard RH Jr, Bentley C, Eagle JC, Garland GE, Knight MC, & Phillips C (2001). Nightguard vital bleaching: a long-term study on efficacy, shade retention, side effects, and patients' perceptions *Journal of Esthetic and Restorative Dentistry* **13**(6) 357-369.
- Haywood VB, Leonard RH, Nelson CF, & Brunson WD (1994) Effectiveness, side effects and long-term status of nightguard vital bleaching *Journal of the American Dental Association* **125**(9) 1219-1226.
- Perdigao J, Baratieri LN, & Arcari GM (2004) Contemporary trends and techniques in tooth whitening: a review *Practical Procedure in Aesthetic Dentistry* **16**(3)185-192.
- Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC, & Baratieri LN (2010) Clinical performance of vital bleaching techniques *Operative Dentistry* **35**(1) 3-10.
- Reis A, Kossatz S, Martins GC, & Loguercio AD (2013) Efficacy of and effect on tooth sensitivity of in-office bleaching gel concentrations: a randomized clinical trial *Operative Dentistry* **38**(4) 386-393.
- Kose C, Calixto AL, Bauer JR, Reis A, & Loguercio AD (2016) Comparison of the effects of in-office bleaching times on whitening and tooth sensitivity: A single blind, randomized clinical trial *Operative Dentistry* **41**(2) 138-145.
- de Geus JL, Wambier LM, Kossatz S, Loguercio AD, & Reis A (2016) At-home vs in-office bleaching: A systematic review and meta-analysis *Operative Dentistry* **41**(4) 341-356.
- Tay LY, Kose C, Herrera DR, Reis A, & Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: A 2-year double-blind randomized clinical trial *American Journal of Dentistry* **25**(4) 199-204.
- Markowitz K (2009) The original desensitizers: strontium and potassium salts *Journal of Clinical Dentistry* **20**(5) 145-151.
- Soares DG, Basso FG, Hebling J, & de Souza Costa CA (2014) Concentrations of and application protocols for hydrogen peroxide bleaching gels: Effects on pulp cell viability and whitening efficacy *Journal of Dentistry* **42**(2) 185-198.
- Soares DG, Basso FG, Pontes EC, Garcia Lda F, Hebling J, & de Souza Costa CA (2014). Effective tooth-bleaching protocols capable of reducing H₂O₂ diffusion through enamel and dentine *Journal of Dentistry* **42**(3) 351-358.
- Price RB, Sedarous M, & Hiltz GS (2000) The pH of tooth-whitening products *Journal of the Canadian Dental Association* **66**(8) 421-426.
- Freire A, Archegas LR, de Souza EM, & Vieira S (2009) Effect of storage temperature on pH of in-office and at-home dental bleaching agents *Acta Odontologica Latinoamericana* **22**(1) 27-31
- Torres CR, Crastechini E, Feitosa FA, Pucci CR, & Borges AB (2014) Influence of pH on the effectiveness of hydrogen peroxide whitening *Operative Dentistry* **39**(6) E261-E268.
- Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, & Wang Y (2011) Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide *Journal of Dentistry* **39**(10) 686-692.
- Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, & Wang Y (2013) Effects of two in-office bleaching agents with different pH on the structure of human enamel: an in situ and in vitro study *Operative Dentistry* **38**(1) 100-110.
- Kossatz S, Martins G, Loguercio AD, & Reis A (2012) Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel. *Journal of the American Dental Association* **143**(12) e81-e87.
- Reis A, Tay LY, Herrera DR, Kossatz S, & Loguercio AD (2011) Clinical effects of prolonged application time of an in-office bleaching gel *Operative Dentistry* **36**(6) 590-596.
- Loguercio AD, Servat F, Stanislawczuk R, Mena-Serrano A, Rezende M, Prieto MV, Cereño V, Rojas MF, Ortega K, Fernandez E, & Reis A (2017) Effect of acidity of in-office bleaching gels on tooth sensitivity and whitening: A two-center double-blind randomized clinical trial *Clinical Oral Investigation* **21**(9) 2811-2818.
- Martins IEB, Onofre S, Franco N, Martins L, Montenegro-Arana A, Arana-Gordillo LA, Reis A, Loguercio AD, & Silva LM (2018) Effectiveness of in-office hydrogen peroxide with two different protocols: A two-center randomized clinical trial *Operative Dentistry* **43**(4) 353-361.

21. Trentino AC, Soares AF, Duarte MA, Ishikiriama SK, & Mondelli RF (2015) Evaluation of pH levels and surface roughness after bleaching and abrasion tests of eight commercial products *Photomedical Laser in Surgery* **33(7)** 372-377.
22. Soares AF, Bombonatti JF, Alencar MS, Consolmagno EC, Honório HM, & Mondelli RF (2016) Influence of pH, bleaching agents, and acid etching on surface wear of bovine enamel *Journal of Applied Oral Science* **24(1)** 24-30.
23. Al Shethri S, Matis BA, Cochran MA, Zekonis R, & Stropes M (2003) A clinical evaluation of two in-office bleaching products *Operative Dentistry* **28(5)** 488-495.
24. Sulieman M (2005) An overview of bleaching techniques: 3. In-surgery or power bleaching *Dental Update* **32(2)** 101-104, 107-108.
25. Majeed A, Grobler SR, & Moola MH (2011) The pH of various tooth whitening products on the South African market *South African Dental Journal* **66(6)** 278-281.
26. Jadad EBRM, Arana G, Palo RM, Gomes OMM, & Arana-Gordillo LA (2014) In vitro evaluation of pH values of whitening agents in relationship to time *Revista APCD de Estética* **2(2)** 520-526.
27. Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD, & Reis A (2015) Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber *Operative Dentistry* **40(2)** E76-E82.
28. Parreiras S, Mena-Serrano A, Moreira CG, Otuki M, Loguercio D, & Reis A (2014) Penetration and cytotoxicity of a bleaching gel activated by LED/laser in restored teeth. *American Journal of Dentistry* **27(6)** 301-306.
29. Mendham J & Afonso JC (2002). *Vogel: Quantitative Chemical Analysis* [in Portuguese] Livros Técnicos e Científicos Editora SA, Rio de Janeiro.
30. Hannig C, Weinhold HC, Becker K, & Attin T (2011) Diffusion of peroxides through dentine in vitro with and without prior use of a desensitizing varnish *Clinical Oral Investigation* **15(6)** 863-868.
31. Pinto CF, Oliveira RD, Cavalli V, & Giannini M (2004) Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology *Brazilian Oral Research* **18(4)** 306-311.
32. He LB, Shao MY, Tan K, Xu X, & Li JY (2012) The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: A systematic review and meta-analysis *Journal of Dentistry* **40(8)** 644-653.
33. Frysh H, Bowles WH, Baker F, Rivera-Hidalgo F, & Guillen G (1995) Effect of pH on hydrogen peroxide bleaching agents *Journal of Esthetic Dentistry* **7(3)** 130-133.
34. Pignoly C, Camps L, Susini G, About I, & Camps J (2012) Influence of in-office whitening gel pH on hydrogen peroxide diffusion through enamel and color changes in bovine teeth *American Journal of Dentistry* **25(2)** 91-96.
35. Marson FC, Goncalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, & Briso AL (2015) Penetration of hydrogen peroxide and degradation rate of different bleaching products *Operative Dentistry* **40(1)** 72-79.
36. Kwon S (2007) The sealed bleaching technique *Aesthetic Dentistry Today* **1(3)** 14-18.
37. Cadenaro M, Breschi L, Nucci C, Antonioli F, Visintini E, Praci C, Matis BA, & Di Lenarda R (2008) Effect of two in-office whitening agents on the enamel surface in vivo: A morphological and non-contact profilometric study *Operative Dentistry* **33(2)** 127-134.
38. Alexandrino L, Gomes Y, Alves E, Costi H, Rogez H, & Silva C (2014) Effects of a bleaching agent with calcium on bovine enamel *European Journal of Dentistry* **8(3)** 320-325.
39. Wang Y, Gao J, Jiang T, Liang S, Zhou Y, & Matis BA (2015) Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment—A systematic review and meta-analysis *Journal of Dentistry* **43(8)** 913-923.
40. Basting RT, Amaral FL, Franca FM, & Florio FM (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents *Operative Dentistry* **37(5)** 464-473.
41. Kwon SR, Pallavi F, Shi Y, Oyoyo U, Mohraz A, & Li Y (2018) Effect of bleaching gel viscosity on tooth whitening efficacy and pulp chamber penetration: An in vitro study. *Operative Dentistry* **43(3)** 326-334.