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RESEARCH

Can all highly concentrated in-office bleaching gels be used as a single-application?

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Abstract

Objective This in vitro study aims to evaluate of hydrogen peroxide (HP) diffusion into the pulp chamber, bleaching efficacy (BE), and pH stability (pH) of single-application high concentrated in-office bleaching gels.

Materials and methods Eighty-eight healthy premolars were randomly into eleven groups ($n = 8$) according to the in-office dental bleaching: DSP White Clinic 35% calcium (DW), Nano White 35% (NW), Opalescence XTra Boost 40% (OB), Pola Office + 37.5% (PO), Potenza Bianco Pro SS 38% (PB), Total Blanc 35% (TB), Total Blanc One-Step 35% (TO), Whiteness Automixx 35% (WA), Whiteness Automixx Plus 35% (WP), and Whiteness HP Blue 35% (WB). A group not exposed to bleaching agents was the control group (CG). All bleaching agents were applied in one session with a single application. After the bleaching procedure, the concentration of HP diffusion (µg/mL) into the pulp chamber was assessed using UV-Vis spectrophotometry. The BE (ΔE_{ab} and ΔE_{00}) was evaluated before and 1 week after the bleaching procedure using a digital spectrophotometer. The pH of each bleaching gel was evaluated by digital pHmeter. The one-way ANOVA and Tukey's was used for a statistical analysis ($\alpha = 0.05$).

Results The concentration of HP diffusion into the pulp chamber was higher in all in-office bleaching gels when compared to CG ($p < 0.0000001$). However, there are a significant difference between them ($p = 0.0001$). A significant BE was observed in all in-office bleaching gels ($p < 0.0001$ for ΔE_{ab} and ΔE_{00}), with a significant difference between them ($p < 0.0001$). PO, OB, TB, WP, and WB showed a higher BE when compared to DW, PB, and WA ($p < 0.0001$). Most bleaching gels were slightly acidic or alkaline during the total application time, while DW, PB, TB, and WA showed a high acidic behavior after 30 min of application.

Conclusions A single application was able to produce a bleaching efficacy. However, usually, gels with slightly acidic or alkaline pH during the application time reduces the HP diffusion into the pulp chamber.

Clinical relevance The single-application of bleaching gels with slightly acidic or alkaline and stable pH decreased the penetration of hydrogen peroxide into the pulp chamber in in-office bleaching and maintained the bleaching efficacy.

Keywords Hydrogen peroxide · Tooth bleaching · Dental enamel permeability

Introduction

Dental bleaching is one of the most conservative and popular aesthetic treatment choices to reduce patients' dissatisfaction with dental discoloration [1, 2]. Among the techniques used for dental bleaching, the in-office procedure promotes faster results than at-home bleaching [3, 4] and does not requires patient collaboration [5].

In-office bleaching usually requires the use of bleaching gels containing high concentrations of hydrogen peroxide (HP) [3, 6]. For this purpose, there are numerous commercial bleaching gels available on the market. These gels vary

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in their mode of use and can be applied in 2 or 3 applications at each bleaching session. Application mode for these gels varies according to characteristics of viscosity, proper pH, and pH stability of each material for in-office bleaching [7–9].

Actually, the number of applications is related to the need for product exchange due to falling pH during the bleaching procedure [10–12] and because the vast majority of in-office bleaching gels are still supplied with a higher acidic pH to increase the product's shelf life [13–15].

Therefore, the use of bleaching gels with low or unstable pH can be clinically harmful. One of the main consequences of the low pH of bleaching gels is the higher index of bleaching-induced tooth sensitivity [11, 16–19]. As demonstrated in several studies [7, 20, 21], the use of lower pH bleaching gels is responsible for increased diffusion of hydrogen peroxide into the pulp, leading to the high rates of tooth sensitivity [16–18], because once HP diffuses into the pulp, it is able to trigger an inflammatory process [22] with the release of several inflammatory chemical mediators [23, 24], which modify the local microcirculation, generating pressure on peripheral nerve fibers and activating nociceptors [25] related to pain sensation. In addition, the fact that use low pH bleaching gels can promote changes in the morphology and mechanical properties of the tooth structure [21, 26–29], contributing to increased PH diffusion, reinforces the need for less acidic or alkaline pH bleaching gels.

Another reason for replacing the available bleaching gel would be to increase the effectiveness of bleaching. However, some clinical studies have shown that replacing the bleaching gel is not necessary [30–32]. However, this replacement of bleaching gel increases the hydrogen peroxide penetration in the pulp chamber [33], and consequently, bleaching-induced tooth sensitivity [30, 34].

Thus, recently, the manufacturers of bleaching gels have introduced single-application gels that not only simplify the technique by avoiding product replacement, but also promise a stable pH throughout the application [35]. In addition, some of these new gels feature desensitizing and remineralizing agents that attempt to not produce deleterious effects on the enamel surface [21, 28] and decrease hydrogen peroxide penetration into the pulp chamber [7, 20, 21]. In addition to being unnecessary, changing the bleaching gel during the procedure can lead to a greater expense of material, besides leading to a longer, more complex procedure due to the multiple replacement of materials.

However, a few commercial brands indicate that their gels can be applied in a single application. Taking into account that several characteristics of the bleaching gel—such as viscosity, initial pH, and stability of pH—could impair the penetration of HP inside the pulp chamber and bleaching efficacy when in-office gels are used in a single application, the aim of this study is to evaluate the penetration into the pulp chamber and bleaching efficacy, as well as the initial pH and pH during the application of in-office bleaching gels containing high concentrations of HP applied in a single application. The null hypotheses tested were that the pH and single-application of the different in-office bleaching gels does not affect (1) the amount of HP that reaches the pulp chamber and (2) bleaching efficacy.

Material and methods

Ethical approval

This study was approved (34232-22) by the Research Ethics Committee of the State University of Ponta Grossa, Ponta Grossa, PR, Brazil.

Teeth selection

To carry out the study, 88 healthy premolars of similar size were obtained from the Human Teeth Local Bank at the University. The teeth were evaluated under a microscope at 10× magnification (Lambda LEB-3, ATTO instruments, Hong Kong, China) to standardize the selection. We excluded teeth with enamel cracks or morphological changes, and teeth lighter than A2, measured by a digital spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany). Teeth were analyzed by radiography (Timex 70C, Gnatus, Ribeirão Preto, SP, Brazil), and if these teeth showed with a thickness from vestibular until the pulp chamber with less than 2.5 mm and greater than 3.5 mm, they were excluded.

Experimental design

The selected teeth were randomly distributed into eleven groups ($n = 8$) according to the in-office bleaching gel used: DSP White Clinic 35% Calcium (DW) [DSP Biomedical Group, Campo Largo, PR, Brazil], Nano White 35% (NW) [DMC, São Paulo, SP, Brazil], Opalescence XTra Boost 40% (OB) [Ultradent, Salt Lake, UT, USA], Potenza Bianco Pro SS 38% (PB) [PHS Group, Joinville, SC, Brazil], Pola Office + 37.5% (PO) [SDI, Bayswater, Australia], Total Blanc 35% (TB) [DFL, Rio de Janeiro, RJ, Brazil], Total Blanc One-Step 35% (TS) [DFL, Rio de Janeiro, RJ, Brazil], Whiteness Automixx 35% (WA) [FGM, Joinville, SC, Brazil], Whiteness Automixx Plus 35% (WP) [FGM, Joinville, SC, Brazil], and Whiteness HP Blue 35% (WB) [FGM, Joinville, SC, Brazil]. A group not exposed to bleaching agents was the control group (CG). More details regarding the bleaching agents used are provided in Table 1.

Table 1 Commercial bleaching gel used in each group (batch number, initial pH, and composition)

Product (batch number)	Composition
DSP White Clinic 35% Calcium (229220221)	35% hydrogen peroxide, thickeners, neutralizers, coloring mix, glycols, deionized water, calcium.
Nano White 35% (14608)	35% hydrogen peroxide, thickeners, neutralizers, glycols, deionized water.
Opalescence XTra Boost 40% (D09XR. D0BIK)	40% hydrogen peroxide, thickeners, pH regulators, 1.1% sodium fluoride, 3% potassium nitrate.
Potenza Bianco Pro SS 38 (16052022-30279)	38% hydrogen peroxide, thickeners, neutralizers, colorant and deionized water.
Pola Office+ 37.5% (P2102431)	37.5% hydrogen peroxide and potassium nitrate
Total Blanc 35% (21030252. 21060627)	35% Hydrogen peroxide, thickener, water, vegetable extracts, sequestrating agents, amide, colorants and glycol
Total Blanc One-Step 35% (160322LP)	35% Hydrogen peroxide, thickener, water, vegetable extracts, sequestrating agents, amide, colorants and glycol
Whiteness Automixx 35% (201021)	35% hydrogen peroxide, deionized water, thickeners, neutralized agents.
Whiteness Automixx Plus 35% (250422)	35% hydrogen peroxide, deionized water, thickeners, neutralized agents.
Whiteness HP Blue 35% (140121)	35% hydrogen peroxide, deionized water, thickeners, violet colorant, glycol, neutralized agents and desensitizing agents, 3% calcium gluconate.

Sample size calculation

The primary results of this study involved the quantification of HP into the pulp chamber. Based on previous study [36], an average of 0.511 ± 0.156 µg/mL of hydrogen peroxide was quantified into the pulp chamber of teeth submitted to in-office bleaching with high-concentration of HP applied for around 30 min. Using a bilateral test with an alpha of 0.05 and a power of 80%, six teeth were needed in each group to detect a difference of 0.250 µg/mL; two extra teeth were assigned to each group due to possible sample losses during the experiment.

Sample preparation

With the help of a low-speed diamond disk (Isomet 1000, Buehler Ltd., Lake Bluff, USA), the roots of the teeth were removed approximately 3 mm from the cementum-enamel junction, and the pulp tissue was removed and rinsed with deionized water. With care taken not to touch the internal occlusal region of the pulp chamber, the access to the pulp chamber was expanded using a #1014 spherical bur (KG Sorensen, SP, Brazil) and to 25 µL for solution could be introduced into the pulp chamber using a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland) [8, 37]. After sample preparation, a new radiography and initial color evaluation were taken. Each radiography was taken with an exposure time of 0.5 s and a 30-cm focus-object distance (70 kVp-7 mA). The central X-ray beam was focused at a 90° angle to the tooth's buccal surface. After exposure, the images were digitally obtained and the corresponding buccal tooth thickness (external point of the enamel to the innermost point of dentin, corresponding to the pulp horn) was measured with New IDA software (Dabi Atlante, Ribeirão Preto, SP, Brazil) [36, 37].

Obtaining the analytical curve

The study used analytical products without prior purification, and all solutions were prepared using deionized water. Initially, a standard analytical curve was drawn from a 5000 µg/mL stock solution prepared from a concentrated solution (34–36% hydrogen peroxide, LABSYNTH, Diadema, SP, Brazil). This solution was diluted in an acetate buffer solution (pH = 4.5) and titrated using traditional methods. The solution was titrated with a potassium permanganate solution to determine the analytical grade and the actual concentration of the solution [36, 37]. Based on this initial concentration, serial volumetric dilutions of 0.000–0.409 µg/mL were performed to draw the analytical curve. The known concentrations of hydrogen peroxide were obtained using a Cary UV-Vis 50 spectrophotometer (Varian, Palo Alto, CA, USA). This procedure yielded a standard reference line for the extrapolation of the study samples' results ($R = 0.998$); these data are not shown.

Hydrogen peroxide diffusion into the pulp chamber

A single experienced and calibrated operator was responsible for performing all bleaching protocols. The specimens were placed with the occlusal face in contact with a wax plate allowing access to the pulp chamber. All teeth had their buccal area isolated by the application of a light-cure resin barrier (Top Dam, FGM, Joinville, SC, Brazil) to standardize an area of 6 mm^2. The delimitation of 6 mm^2 was guided by a dry-ended compass with the standardized measurement

on the instrument. A 25-µL aliquot of the acetate buffer (pH = 4) was inserted into the pulp chamber of each tooth to absorb and preserve any hydrogen peroxide that may diffusion into the pulp chamber during bleaching procedures [36, 37].

All the bleaching gels were manipulated and applied to the vestibular enamel area according to the in-office bleaching gel used for 30 min. The bleaching gel was applied until it completely covered the area of the teeth to be whitened. Only a session was performed, followed by removal of the bleaching gel with gauze and washing with deionized water only on the vestibular surface. A control group was used and maintained without contact with bleaching agents.

Following the bleaching procedure, the acetate buffer solution in the pulp chamber of each sample was removed using a micropipette and transferred to a glass tube. To remove the hydrogen peroxide completely, this procedure was repeated with the cleaning of the pulp cavity of each tooth four times with 25 µL of the acetate buffer. This solution was transferred to the same glass tube. Sequentially, 100 µL of 0.5 mg/mL (Leucocrystal Violet, Sigma Chemical Co., St Louis, MO, USA) and 50 µL of 1 mg/mL of horseradish peroxidase enzyme (Peroxidase Type VI-A, Sigma Chemical Co., St. Louis, MO, USA) were added to the glass tube, along with deionized water (2.725 µL). This sequence was repeated separately for each of the teeth. The resulting solution was measured using a Cary 100 UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA) [8, 36, 37], and the absorbance used was the highest absorption peak of the resulting reaction between hydrogen peroxide and Leucocrystal Violet (Crystal Violet - 596 nm). According to Beer's Law, absorbance directly corresponds to the concentration. Therefore, the concentration of hydrogen peroxide (µg/mL) was determined by comparison with the calibration curve already obtained.

Color change evaluation

The color evaluation was measured before the bleaching procedure and 1 week after the treatment protocol. The color evaluation in this study was performed in triplicate, and an average value was obtained in each time cut. It was performed for all groups, using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik, Bad Säckingen, Germany). The specimens were immersed in artificial saliva (Pharmacy Eficácia, Ponta Grossa, PR, Brazil) pH = 7.0, composed of carboxymethylcellulose, sodium chloride, potassium chloride, magnesium chloride, dibasic calcium phosphate, glycerin, xylitol, and distilled water between evaluation periods. Daily changes of artificial saliva were carried out at a controlled temperature of 37 °C. To measure the color of the specimens, guides were made with dense condensation silicone to standardize the position of the spectrophotometer (Coltoflax and Cub Kit Profile, Vigodent, Rio de Janeiro, RJ, Brazil) through a 6-mm-diameter window with a metal device in the middle third of the vestibular surface for each specimen, where the tip of the spectrophotometer was inserted [8, 36, 37].

The tip of the spectrophotometer was inserted in the silicone guide, and the color parameters were measured (L*, a*, and b*). The value L* represented the luminosity (values varying from 0 (black) to 100 (white), the value a* represented the color along the red-green axis, and the value b* represented the color along the yellow-blue axis. The color change before (baseline) and after treatment (1 week after bleaching) was given by the difference between the colors measured with the spectrophotometer using the CIELab formula: $\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [38]. In addition, the color change was also calculated using the CIEDE00 formula: $\Delta E_{00} = [(\Delta L/kLSL)^2 + (\Delta C/kCSC)^2 + (\Delta H/kHSH)^2 + RT\,(\Delta C^*\Delta H/SC^*SH)]^{1/2}$ [39]. Perceptual changes will be accepted when the differences in the initial and after bleaching colors present $\Delta E_{ab} > 2.7$ and $\Delta E_{00} > 1.2$ [40, 41].

pH stability measurements of bleaching gels

During the bleaching procedure, the pH of each bleaching gel by each application time (immediately and every 10 min of application, up to 30 min) was measured. For this purpose, a 6-mm circular pH meter and a flat surface pH electrode (Extech pH100: ExStik pH Meter; Extech Instruments, Nashua, NH, USA) were positioned in the areas delimited for the application of the bleaching gel and held in position until stabilization. Three measurements were carried out on each tooth [36]. The pH of bleaching gels can be classified into acidic pH between 0 to 6, neutral pH 7, and alkaline pH between 8 and 14.

Statistical analysis

The data were analyzed using the Kolmogorov–Smirnov test to assess whether they were normally distributed and using the Barlett test to verify the assumption of equality of variances (not shown data). The data of the amount of the HP concentration (µg/mL) detected inside the pulp cavity, as well as the bleaching efficacy (ΔE_{ab} and ΔE_{00}) were subjected to two statistical evaluations: (1) one-way ANOVA and Dunnet's post hoc test to compare the values obtained in different bleaching techniques with those of the control group and (2) one-way ANOVA and Tukey's post hoc test to compare different bleaching materials. The statistical significance was set at $\alpha = 0.05$. The pH was only qualitatively evaluated.

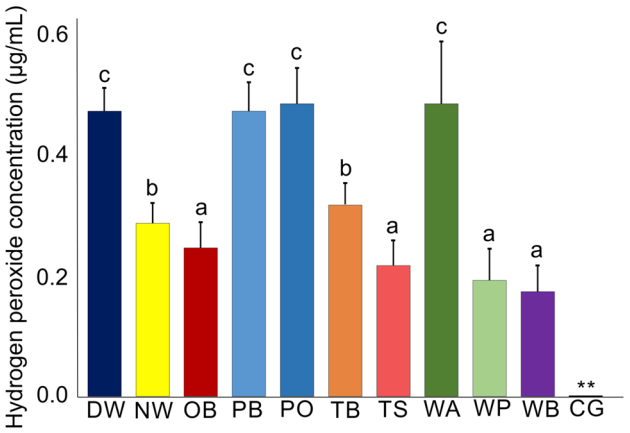

Fig. 1 Means (± standard deviations) of the hydrogen peroxide concentration (µg/mL) detected into the pulp chamber in different experimental groups

Results

Hydrogen peroxide concentration diffusion into the pulp chamber

The specimens of all groups showed standardized thicknesses with values varying between 3.1 and 3.5 mm ($p = 0.52$). Figure 1 shows the amount of HP inside the pulp chamber. All in-office bleaching materials showed a significant and higher amount of HP inside the pulp chamber when compared to CG (Fig. 1; $p < 0.0000001$; Dunnet's post hoc test). When different in-office bleaching materials were compared, a significant difference was observed (Fig. 1, $p = 0.0001$). The in-office bleaching materials can be divided in three groups. In the first group, four in-office bleaching materials (OB, TS, WP, and WB) showed a lower amount of HP inside the pulp chamber when compared to four other in-office bleaching materials (DW, PB, PO, and WA) that showed a higher amount of HP inside the pulp chamber (Fig. 1, $p = 0.0001$). Two in-office bleaching materials (NW and TB) showed intermediary results in relation to the other groups.

Evaluation of bleaching efficacy

Table 2 shows the bleaching efficacy measured by ΔE_{ab} and ΔE_{00}. All in-office bleaching materials showed a significant and higher bleaching efficacy when compared to CG (Table 2; $p < 0.0001$ for ΔE_{ab} and $p < 0.00001$ for ΔE_{00}.; Dunnet's post-hoc test). When different in-office bleaching materials were compared, a significant difference was observed (Table 2, $p < 0.0001$). Five in-office bleaching materials (OB, PO, TS, WP, and WB) showed higher bleaching efficacy when compared to three in-office bleaching materials (DW, TB, and WA) (Table 2, $p < 0.0001$) that showed lower bleaching efficacy (Table 2, $p = 0.0001$). NW and TB showed intermediary bleaching results.

Table 2 Means (± standard deviations) of the color change in different objective assessments (ΔE_{ab} and ΔE_{00}) in different experimental groups

Experimental groups*	ΔE_{ab}	ΔE_{00}
DSP White Clinic 35% Calcium (DW)	4.4 ± 1.3 c	2.8 ± 0.8 [c]
Nano White 35% (NW)	6.1 ± 1.8 b	3.6 ± 0.9 [b]
Opalescence XTra Boost 40% (OB)	7.0 ± 1.2 a	4.6 ± 0.9 [a]
Potenza Bianco Pro SS 38 (PB)	5.0 ± 2.1 c	3.1 ± 0.9 [b,c]
Pola Office+ 37.5% (PO)	7.2 ± 2.2 a	4.4 ± 1.7 [a]
Total Blanc 35% (TB)	5.9 ± 0.9 b	4.4 ± 1.3 [b]
Total Blanc One-Step 35% (TS)	6.6 ± 1.8 a	4.1 ± 1.1 [a]
Whiteness Automixx 35% (WA)	5.5 ± 1.1 b,c	3.1 ± 0.9 [b,c]
Whiteness Automixx Plus 35% (WP)	7.1 ± 0.7 a	4.6 ± 0.8 [a]
Whiteness HP Blue 35% (WB)	6.9 ± 1.2 a	4.7 ± 1.1[a]
CG (**)	1.3 ± 0.7	0.8 ± 0.5

*Same capital, lowercase, or superscript letters in each column indicate statistically similar means among groups (Tukey's post hoc test, $p < 0.05$)

**All experimental groups were statistical different when compared with control group (Dunnett's post hoc test, $p < 0.05$)

Measurements of pH stability

Figure 2 shows the pH behavior of all in-office bleaching materials evaluated. The in-office bleaching gels NW, OB, TS, WB, and WP showed alkaline pH, while DW, PO, TB, and WA showed a less acidic behavior (pH higher than 6). Only one in-office bleaching gel showed a high acidic behavior (PB). The in-office bleaching gels usually showed a stable pH with values ranging between 6.2 and 8.2. However, at the end of 30 min, DW, PB, TB, and WA showed a high acidic behavior (pH lower than 6).

Discussion

One of the main objectives of the present study was to evaluate whether all highly concentrated in-office bleaching gels evaluated when applied in a single application promoted a whitening effect without generating a huge amount of HP inside the pulp chamber. For this purpose, one of the greatest challenges encountered in relation to the in-office bleaching technique is to produce a less acidic gel if possible, and at the same time, to maintain the pH during the entire application time.

We observe that all bleaching gels showed a significant and higher amount of HP within the pulp chamber when

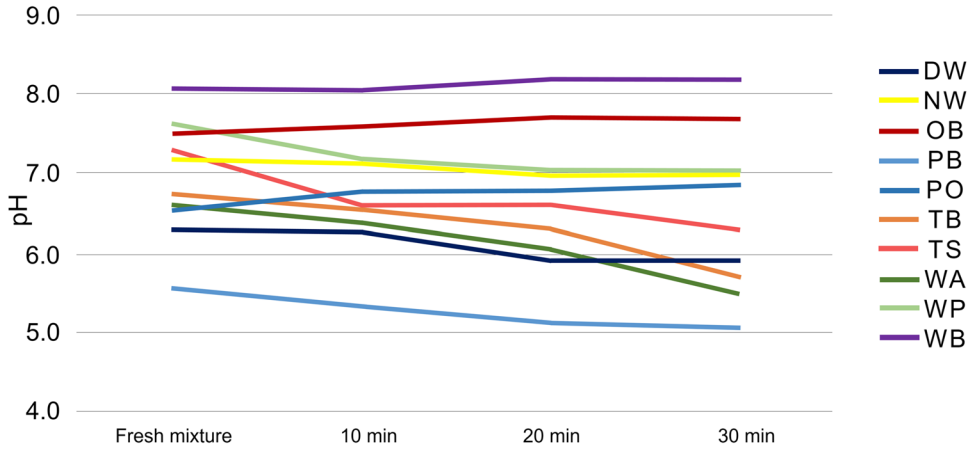

Fig. 2 pH stability of each bleaching gel applied during a time of 30 min

compared to the control group. This was already expected, since in the control group no product containing the active principle of bleaching agents, which is HP, was applied. However, a significant difference was observed when all materials were compared among them. We observe that the majority of the in-office bleaching gels contain the same amount of available HP (35%), with the exception of PO (37.5%), PB (38%), and OB (40%), which contain a higher concentration of HP. However, one of these three (OB) showed a lower amount of HP inside the pulp chamber. Therefore, characteristics other than the amount of available HP need to be taken into account.

A closer view showed that four of the in-office bleaching gels tested (OB, TS, WP, and WB) showed the lowest amount of HP in the pulp chamber. One of the characteristics that these gels have in common is a pH range of less acidic (around pH 6) to alkaline. This pH was maintained during the application period. As previously observed, less acidic or alkaline materials showed lower amounts of HP in the pulp chamber [7, 20, 21].

On the other side, the in-office bleaching gels that demonstrated a more acidic (less than pH 6) and/or unstable pH after 30 min (DW, PO, TB, PB, and WA) [10–12] were the ones with the highest penetration of HP into the pulp chamber, in agreement with some in vitro studies [7, 20, 21]. This demonstrates once again the effect of pH on HP penetration into the pulp chamber.

The main explanation for the difference in penetration in relation to pH is that more acidic gels have the ability to alter the roughness of dental enamel, thus producing deleterious effects on its surface [21, 27], and providing greater diffusion of HP into the pulp [7, 20, 21]. This can be explained due to the reactions that can happen during the bleaching process [42], which can be influenced by several factors, such as pH. When more acidic gels are applied to the enamel surface, there is a tendency for reactions to form H^+ and HO_2 radicals [42]. The presence of H^+ causes the medium to become more acidic and leads to negative instability in the gel, making it more acidic over time and not allowing other molecules to dissociate. Ideally, in-office bleaching gels with a pH range from less acidic (pH 6) to alkaline are more interesting as they are closer to the dissociation constant of HP (pH where the drug is 50% dissociated and 50% non-dissociated), which is pKa = 11.5 [42]. This can be directly related to the lower amount of HP in the pulp, as the molecules have already been broken down during dental bleaching reactions.

However, there is not a consensus in the literature on the association between pH and the amount of HP inside the pulp chamber, as other studies demonstrate that the amounts of HP found in the pulp chamber were similar, regardless of the pH of the gel used [43, 44]. These differences can be explained due to methodological differences among studies. For instance, in some studies, human teeth without preparation were used, which is the best way to simulate the clinical situation [7] in a manner similar to that realized in the present study. On the other hand, other studies used only a small part of a bovine tooth to simulate a pulp chamber [43, 44].

Also, we need to mention that a difference in HP diffusion could have occurred in some groups due to the presence of remineralizing or desensitizing agents in the bleaching gels used. Although the use of these agents for reducing tooth sensitivity during dental bleaching is a controversial issue, some studies have shown that the addition of these compounds to bleaching gels could help reduce damage to the enamel surface preventing mineral loss [21, 28, 45, 46] by reducing HP diffusion chamber [7, 20, 21]. However, other studies have shown that this reduction occurred only after prior application of these agents [47–49]. When incorporated into the bleaching gel, the calcium compounds, despite remaining longer in contact with the tooth structure, are delivered simultaneously to the HP, unlike what occurs with previously applied desensitizing agents [50]. Therefore, although in the previous application a tendency for HP diffusion reduction occurs, in our study, such agents were not previously applied, this influence did not occur. Furthermore,

with regard to remineralizing or desensitizing agents, the main influence may have occurred only in relation to pH maintenance, since calcium compounds keep the pH stable throughout the entire bleaching process [18].

Interestingly, the same effect was observed when the bleaching efficacy of different in-office bleaching gels was compared. In other words, in-office bleaching gels with higher and/or more stable pH during application (PO, TS, OB, WP, and WB) were those that produced the highest values of ΔE_{ab} and ΔE_{00}. On the other hand, the bleaching gels that were more acidic or with an unstable pH showed the lower values of ΔE_{ab} and ΔE_{00}. As mentioned earlier, the dissociation of HP into free radicals is greater at alkaline pH, as the HP pKa is approximately 11.5 [42]. The more alkaline the bleaching agent, the greater the number of free radicals that will be formed to oxidize the organic content of the tooth [51], improving the whitening efficacy [16–18].

However, although a significant difference was found among the different gels evaluated, the values of ΔE_{ab} and ΔE_{00} indicate values higher than the acceptability threshold of 50:50 for all bleaching gels ($\Delta E_{ab} = 2.7$ and $\Delta E_{00} = 1.2$) [40, 41], which may indicate that a single application of 30 min is capable of providing satisfactory bleaching. This seems to be in accordance with clinical trials that showed that the ability of whitening is independent of the pH of in-office bleaching gels [16–18].

It is worth mentioning that two in-office bleaching materials, NW and TB, exhibited intermediate behavior. Despite NW having an alkaline and stable pH compared to TB, the amount of HP inside the pulp chamber was comparable to that of TB. On the other hand, the pH profile of TB was similar to DW and PB, but the amount of bleaching gel in the pulp chamber was significantly lower for the former. The authors of this study could not provide an explanation for the behavior of these two bleaching gels. However, it is important that future studies evaluate more physical-chemical properties to clarify these conflicting results.

The results found in this study again reinforce the idea that it is not necessary to use acid products to obtain better results in dental bleaching. Based on the results of the present study, the use of bleaching gels with a less acidic and stable pH decreased the penetration of HP into the pulp chamber, as PO, TS, OB, WP, and WB should therefore be used as the first choice in clinical practice. However, not all products cited should be used as a single application. For instance, PO became completely fluid after 10 min of application (not shown data); therefore, due to the danger of running down to the gingival tissue and causing some burning, this application should not be considered for this material.

It is important to note that all the gels used in this study were only applied for 30 min. It is recognized that some gels are typically applied for up to 40 or 50 min, which could result in lower pH values than those observed in this study, as several authors have previously noted [7, 20, 21]. However, the use of a shorter application time such as 30 min demonstrated a decrease in the amount of HP penetration [33, 43, 52]. For this reason, the fact that all the bleaching gels were only applied for 30 min may have been a limiting factor for this study. Future studies should be conducted in order to determine if use of these gels for a shorter time would be able to produce lower HP diffusion by pulp. Also, the use of these gels for a longer time than normally recommended by the manufacturers could be studied.

This shorter application period together with the use of a less acidic or alkaline gel is able to provide a reduction in HP penetration, which could provide clinical benefits regarding tooth sensitivity. However, this did not happen for all the bleaching agents tested, so clinical studies that evaluate the effectiveness of bleaching using the single application of bleaching agents are needed to better elucidate this research question.

Conclusions

It is possible to use several in-office bleaching gels in a single application. However, these bleaching gels should have a less acidic and stable pH during application to guarantee a reduced penetration of hydrogen peroxide into the pulp chamber. All the bleaching gels employed were effective in achieving satisfactory teeth whitening results after a single 30-min application.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Karine Letícia da Silva, Michael Willian Favoreto, Gabrielle Gomes Centenaro, Laís Giacomini Bernardi, Christiane Philippini Ferreira Borges, Alessandra Reis, and Alessandro D. Loguercio. The first draft of the manuscript was written by Karine Letícia da Silva and Michael Willian Favoreto, and all authors commented on previous versions of the manuscript. The authors read and approved the final manuscript.

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Declarations

Ethical approval The clinical investigation was approved (34232-22) by the scientific review committee and by the committee for the protection of human participants of the State University of Ponta Grossa.

Informed consent Not applicable.

Conflict of interest Karine Letícia da Silva declares that she has no conflict of interest. Michael Willian Favoreto declares that he has no conflict of interest. Gabrielle Gomes Centenaro declares that she has

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