



Use of an applicator brush with high concentration bleaching gels

Laís G. Bernardi¹ · Michael W. Favoreto¹ · Taynara S. Carneiro¹ · Alexandra Mena-Serrano² · Christiane P. F. Borges³ · Alessandra Reis¹ · Alessandro D. Loguercio¹

Received: 23 February 2022 / Accepted: 20 June 2022 / Published online: 1 July 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Objectives To evaluate in vitro two high concentration self-mix bleaching gels (35% or 37.5%) with different application tips (with or without an applicator brush) during in-office bleaching.

Materials and methods Healthy premolars were randomly assigned to five groups ($n = 8$): no treatment; 35% HP without applicator brush, 35% HP with applicator brush, 37.5% HP without applicator brush, and 37.5% HP with applicator brush. After the procedures, the concentration of HP transferred into the pulp chamber was evaluated using UV–Vis. The amount of gel used in each group was measured on a precision analytical balance. Color change (ΔE_{ab} , ΔE_{00} , and ΔWI_D) was evaluated with a digital spectrophotometer. Initial concentration was measured by titration with potassium permanganate. The pH was evaluated using a digital pH meter. The data from each test were submitted to nonparametric tests ($\alpha = 0.05$).

Results Using a tip with an applicator brush expended less gel and left a lower amount of HP inside the pulp chamber compared to the tip without a brush for both bleaching gels ($p < 0.0003$), although no significant difference in color change was observed ($p < 0.05$). The 37.5% HP showed a more stable and less acidic pH and a lower amount of HP in the pulp chamber than the 35% HP ($p < 0.00001$).

Conclusion The HP penetration into the pulp chamber was lower when using an applicator with a brush tip than when using one with a conventional tip. As for the color, both tips were considered to lighten teeth.

Clinical significance For the application of a self-mixing high concentration in-office bleaching gel, a brush tip should be recommended because its use diminishes the penetration of HP into the pulp chamber and wastes less bleaching gel.

Keywords Tooth bleaching · Tooth permeability · Hydrogen peroxide · Dental enamel permeability

Introduction

Tooth bleaching has been carried out for over 100 years and is considered a safe and effective procedure [1]. As the images and color quality of television, media, and films have improved, people's self-awareness of white teeth has

increased, further expanding the demand for esthetic procedures, such as tooth bleaching [2].

The most common substance used in bleaching procedures is hydrogen peroxide (HP), and depending on its concentration, the HP is applied in-office (high concentrations of HP) or at-home (low concentrations of HP) [3]. In-office bleaching promotes faster results than at-home bleaching and does not depend on patient collaboration [4]. However, due to the high concentrations of the active ingredient, it demands greater control by the dentist, mainly through the use of a mouth opener and gingival barrier to protect the soft and gingival tissues [4]. However, higher levels of tooth sensitivity (TS) are reported with in-office bleaching [5, 6]; the main explanation for this discomfort is the ability of HP to penetrate enamel and dentin and enter the pulp chamber after application of the gel [7–9]. HP and its by-products can cause oxidative stress in pulp cells, promoting the release of inflammatory mediators [10–12].

✉ Alessandro D. Loguercio
aloguercio@hotmail.com

¹ Department of Restorative Dentistry, Dental Post-Graduate Program, State University of Ponta Grossa, Rua Carlos Cavalcanti, Bloco M - Uvaranas, Ponta Grossa, Parana 4748, Brazil

² Department of Dentistry, Universidad de Las Américas, Quito, Ecuador

³ Department of Chemistry, State University of Ponta Grossa, Parana, Brazil

Although the higher concentration of HP can be considered the most important factor in causing TS [13], it is not the only factor. For instance, the amount of bleaching gel applied to the surface during the procedure could influence the amount of HP seeping into the pulp chamber [14]. It seems obvious that a greater amount may increase the likelihood of generating TS [15]. However, the diffusion coefficient of HP could be dependent on the amount of PH available [14] and the time spent on application [16]. According to Fick's second law, the greater the volume of product applied, the greater the diffusion [17]. Therefore, a lower amount of HP would be expected to penetrate into the pulp chamber if a lower amount of bleaching gel were applied to the tooth surface. For this reason, most manufacturers' instructions recommend applying only a thin layer of bleaching gel [18–20].

Recent randomized clinical trial showed that the greater the amount of bleaching agent applied, the greater the sensitivity observed [15]. However, only one acidic in-office bleaching gel was available [21]. More recently, some in-office bleaching agents have become available in a self-mixing form, where the two phases combine during the application process. These bleaching gels have also been available for application with or without a brush tip. According to manufacturers, brush tips have the ability to spread a thin and homogeneous layer of gel over the entire tooth surface, compared to tips without brushes, saving a considerable amount of whitening gel. However, to the extent of the authors' knowledge, no studies have evaluated the effects of different applicators/tips on TS and the whitening effect of highly concentrated in-office bleaching gels.

Therefore, the aim of the present *in vitro* study was to evaluate whether the application method (applicator with or without a brush tip) of self-mixing in-office bleaching gels in high concentrations influences the penetration of HP into the pulp chamber, amount of bleaching gel used, and color change. The null hypotheses tested were that (1) there will be no difference in the concentration of HP within the pulp chamber, (2) there will be no difference in the amount of gel used, and (3) there will be no difference in the bleaching effectiveness when higher concentrations of in-office bleaching gels are applied with and without a brush tip.

Material and methods

Selection of teeth and inclusion and exclusion criteria

Forty healthy 1st and 2nd superior premolars, without caries and enamel cracks, darker than A_2 , were obtained from the tooth bank of State University of Ponta Grossa and used in the present study. To standardize the selected

teeth, all teeth were analyzed under a microscope at $10\times$ magnification (Lambda LEB-3, ATTO instruments, Hong Kong, China). Teeth with morphological changes or enamel cracks were excluded. The teeth were previously analyzed with a digital spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany); teeth lighter than A_2 were also excluded. To standardize the vestibular thickness of the specimens and to prevent this thickness from influencing the penetration of HP, radiographs were taken (Timex 70C; Gnatus, Ribeirão Preto, SP, Brazil). Each radiograph was made with an exposure time of 0.5 s and a focus-object distance of 30 cm (70 kVp, 7 mA). The central X-ray beam was focused at a 90° angle on the buccal surface of the tooth. After exposure, the images were digitally obtained, and the buccal dental thickness was measured with the New IDA software (Dabi Atlante, Ribeirão Preto, SP, Brazil). Based on previous studies [22], teeth with thicknesses between 2.5 and 3.5 mm were selected for the present study.

Experimental design

After the selected teeth were randomly distributed into five groups ($n = 8$) according to the self-mix bleaching gel used (Whiteness HP Automixx 35% (FGM, Joinville, SC, Brazil) and Pola office + Plus 37.5% (SDI, Victoria, Australia)) and according to the applicator used without or with a brush tip (Sulzer, Haag, Switzerland, Fig. 1). A group not exposed to bleaching agents was the negative control. More details regarding the two bleaching agents used are provided in Table 1.

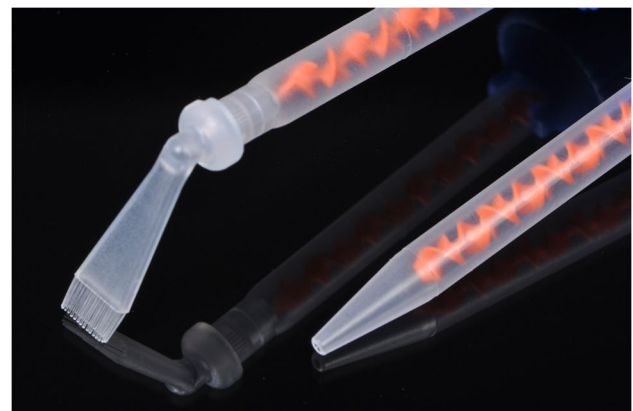


Fig. 1 Self-mixing tips used in the present study. Tip without brush, also like the conventional tip and routine use in dentistry. Tip with brush, coupled to the conventional system where the gel is distributed between the bristles improving the application and spreading of the product

Table 1 Commercial bleaching gel used in the study (manufacturer, composition, and application method)

Product (manufacturer)	Composition	Application method
Whiteness HP AutoMixx 35% (FGM, Jonville, Brazil) (6.5)*	35% Hydrogen peroxide (final concentration of HP 33.0%***) and digluconate calcium	<ol style="list-style-type: none"> 1. With the self-mixing pointer (without applicator brush or with applicator brush) duly coupled to the double-body syringe that contains the bleaching gel; 2. Press the piston until the phases (peroxide and thickener) are slowly mixed; 3. A small quantity of gel was dispensed into a container prior to applying the product to the tooth surface, to assure that the applied product is properly homogenized; 4. The bleaching gel was applied until it completely covered the area of the teeth that were bleached, with a gel layer as thin as possible, using the self-mixing without applicator brush or with applicator brush, according to the experimental group; 5. After applied, the gel left in contact with the tooth for 50 min 6. At the end, the gel was removed with a suction cannula and washing with deionized water only on the vestibular surface.
Pola office + 37% (SDI, Bayswater, Australia) (6.5)*	37.5% hydrogen peroxide (final concentration of HP 28.2%***) and potassium nitrate	<ol style="list-style-type: none"> 1. With the self-mixing pointer (without applicator brush or with applicator brush) duly coupled to the double-body syringe that contains the bleaching gel; 2. Press the piston until the phases (peroxide and thickener) are slowly mixed; 3. A small quantity of gel was dispensed into a container prior to applying the product to the tooth surface, to assure that the applied product is properly homogenized; 4. The bleaching gel was applied until it completely covered the area of the teeth that were bleached, with a gel layer as thin as possible, using the self-mixing without applicator brush or with applicator brush, according to the experimental group; 5. After applied, the gel left in contact with the tooth for 8 min; 6. Four applications of 8 min were performed, totaling 32 min; 7. At the end, the gel was removed with a suction cannula and washing with deionized water only on the vestibular surface.

(*) Measured pH assessed in triplicate ($n=3$)

(**) Measured HP amount assessed in triplicate ($n=3$)

Sample size calculation

The primary results of this study involved the quantification of HP within the pulp chamber. Based on previous studies [22, 23], an average of 0.399 ± 0.119 $\mu\text{g/mL}$ of HP was quantified within the pulp chamber of teeth submitted to in-office bleaching with 35% HP. 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 0.200 $\mu\text{g/}$

mL; two extra teeth were assigned to each group due to possible sample losses during the experiment.

Sample preparation

The roots of all forty premolars were removed approximately 3 mm from the cementum-enamel junction, using a low-speed diamond disk (Buehler Ltd., Lake Bluff, USA). The pulp tissue was removed and rinsed with deionized water.

Access to the pulp chamber was expanded using a #1014 spherical bur (KG Sorensen, SP, Brazil), with care taken not to touch the internal occlusal region of the pulp chamber. This was done so that 25 μL of solution could be introduced into the pulp chamber using a micropipette (HTL Lab Solutions, Warsaw, Poland).

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 5.000 $\mu\text{g}/\text{mL}$ stock solution prepared from a concentrated solution (Eficácia Pharmacy, Ponta Grossa, PR, Brazil). This solution was diluted in an acetate buffer solution ($\text{pH}=4$) 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. Based on this initial concentration, serial volumetric dilutions of 0.000–0.464 $\mu\text{g}/\text{mL}$ were performed to draw the analytical curve. The known concentrations of HP were obtained using a Cary UV–Vis 50 spectrophotometer (Palo Alto, CA, USA). This procedure yielded a standard reference line for the extrapolation of the study samples' results ($R=0.996$; these data are not shown).

Penetration of HP into the pulp chamber

The all forty premolars were placed with the occlusal surface in contact with a wax plate, allowing access to the pulp chamber. The vestibular area of each tooth was isolated with the application of a light-cure resin barrier enclosing an area of 6 mm^2 . A 25- μL aliquot of the acetate buffer ($\text{pH}=4$) was inserted into the pulp chamber of each tooth to absorb and preserve any HP that could penetrate the pulp chamber during the bleaching procedures.

The materials were manipulated and applied to the vestibular enamel area according to the self-mix bleaching gel used (Table 1). The self-mixing was done using an applicator without or with a brush tip. The bleaching gel was applied until it completely covered the area of the teeth to be whitened. One 50-min session for 35% HP and four 8-min sessions for 37.5% HP were performed, per the manufacturer's recommendations (Table 1); then, the bleaching gel was removed with gauze, and only the vestibular surface was washed with deionized water. The control group was maintained without contact with the 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 HP completely, this procedure was repeated, with the pulp chamber of each tooth cleaned 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 tooth. The resulting solution was measured using a Cary 50 UV–Vis spectrophotometer (Palo Alto, CA, USA). According to Beer's law, the absorbance directly corresponds to the concentration. Therefore, the concentration of HP ($\mu\text{g}/\text{mL}$) was determined by comparison with the calibration curve already obtained.

Evaluation of color change

The same forty premolars used in the previous test were used in the present test. The color change was measured before and 1 week after bleaching, using a digital spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany). To measure the initial color of the specimens, guides were made with dense condensation silicone to standardize the position of the spectrophotometer through a window with an area of 6 mm^2 diameter with a metal device in the middle third of the vestibular surface for each specimen, and the tip of the spectrophotometer was inserted [9]. During this period, the specimens were immersed in artificial saliva (Eficácia Pharmacy, Ponta Grossa, PR, Brazil). Daily changes of the artificial saliva were performed at a controlled temperature of 37 °C. The color parameters (L^* , a^* , and b^*) were recorded through the tip of the device inserted in the silicone guide. The L^* value represented the lightness (the values ranged from 0 for black to 100 for white), the a^* represented the color along the green–red coordinate ($-a^*$ green, $+a^*$ red), and the b^* value represented the color along the blue–yellow coordinate ($-b^*$ blue, $+b^*$ yellow). The color change was calculated as the difference in the sample before (baseline) and 1 week after bleaching for each group using CIELab's 1976 formula [24]: $\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. In addition, the color change (ΔE_{00}) was calculated using CIEDE's 2000 formula [25]: $\Delta E_{00} = [(\Delta L/kLSL)^2 + (\Delta C/kCSC)^2 + (\Delta H/kHSH)^2 + RT(\Delta C * \Delta H/SC * SH)]^{1/2}$ and the whiteness index [26]: $\Delta WI_D = (0.511L^*) - (2.3424a^*) - (1.100b^*)$.

Evaluation of the amount of gel used for bleaching

The bleaching gel syringes and tips were weighed on a precision analytical digital scale (AUX220, Shimadzu, Kyoto, Japan) before and immediately after the application of the bleaching agents, as described in the literature [14].

Initial concentrations of bleaching agents

The bleaching gels used in the study were titrated with a standardized potassium permanganate solution before the bleaching procedure, as described in the literature [9, 27]. This was done to determine the initial concentrations inside the bleaching gel tube and compare it with the information provided by the manufacturer.

pH measurements of bleaching agents

The pH of each bleaching agent was measured with a pH meter (Extech pH100, Extech Instruments, Nashua, NH, USA) placed directly in contact with the bleaching gel on a tooth [9, 16]; measurements were made at different times, beginning right after application and continuing every minute to evaluate the protocol indicated by Pola office + Plus 37.5% (SDI, Victoria, Australia) and every 10 min to evaluate the protocol indicated by Whiteness HP Automixx 35% (FGM, Joinville, SC, Brazil).

Statistical analysis

The Kolmogorov–Smirnov test was used to assess whether the data were normally distributed, and the Bartlett test for equality of variance was used to verify the assumption of an equality of variances (these data are not shown). As the data failed to show normality, the data on the amount of gel used (g), the penetration of HP ($\mu\text{g}/\text{mL}$) into the pulp chamber, and the color change (ΔE_{ab} , ΔE_{00} , and ΔWI_D) were subjected to the nonparametric Kruskal–Wallis test and the Mann–Whitney test for pairwise comparisons ($\alpha = 0.05$). The initial concentrations and pH data were evaluated by *t*-test for independent samples. The Pearson correlation was calculated between the concentration of the penetration of HP and the amount of gel used in each product according to the tip used.

Results

HP quantification in the pulp chamber

The amounts of HP in the pulp chambers are shown in Table 2. A lower amount of HP was found in the pulp chambers of the control group (Table 2; $p < 0.000001$). Independent of the bleaching gel used, the applicator without a brush tip resulted in a significant and higher amount of HP penetrating into the pulp chamber compared to the applicator with a brush tip (Table 2; $p < 0.000001$). Regardless of bleaching agents, 35% HP applied without a brush showed a significant and greater amount of HP penetrating into the pulp chamber compared to 37.5% HP applied without a brush (Table 2; $p < 0.000001$).

Evaluation of the amount of bleaching gel used

The amounts of HP expended during the bleaching procedure are shown in Table 2. Independent of the bleaching gel used, the applicator without a brush tip resulted in a significant and higher amount of gel being expended compared to the applicator with a brush tip (Table 2; $p < 0.0003$). However, when application with the same brush tip was compared, the 35% HP expended significant and less bleaching gel than the 37.5% HP (Table 2; $p < 0.0003$).

Correlation HP quantification in the pulp chamber vs. the amount of bleaching gel used

A strong and positive correlation ($r = 0.85$; $p = 0.006$) was found between the amount of HP measured inside the pulp chamber and the amount of gel used for HP 35% without applicator brush (Table 2). However, a weak and non-significant correlation was observed between the amount of HP measured inside the pulp chamber and the amount of gel used for HP 35% with applicator brush ($r = 0.40$; $p = 0.32$), for HP 37.5% with an applicator brush ($r = 0.65$; $p = 0.07$), and for HP 37.5% with an applicator brush ($r = -0.02$; $p = 0.96$).

Table 2 Means (\pm standard deviations) of the hydrogen peroxide concentration ($\mu\text{g}/\text{mL}$) detected into the pulp chamber and amount of gel used for bleaching (g) in different experimental groups (*)

Experimental groups	HP concentration ($\mu\text{g}/\text{mL}$)	Gel used for bleaching (g)
Control	0.003 \pm 0.003 A	0.000 \pm 0.000 a
HP 35%	With applicator brush	0.338 \pm 0.118 B
	Without applicator brush	0.927 \pm 0.328 D
HP 37.5%	With applicator brush	0.156 \pm 0.051 c
	Without applicator brush	0.324 \pm 0.098 d

(*) Identical capital or lowercase letters in each column indicate statistically similar means ($p < 0.05$)

Evaluation of color change

The color change measurements are shown in Table 3. Significant differences were found when the control group was compared with all experimental groups ($p=0.009$ for ΔE_{ab} , $p=0.001$ for ΔE_{00} , and $p=0.006$ for ΔWI_D ; Table 3). Regarding the bleaching gels or the tips used, no significant differences in terms of color change were observed when all color measurements evaluated were compared (ΔE_{ab} , ΔE_{00} , and ΔWI_D ; Table 3; $p>0.32$).

Initial concentrations of HP in the bleaching agents

The initial concentration of HP in the 35% HP was 33.0 ± 1.1 and in the HP 37.5% was 28.2 ± 5.4 with a significant difference between them ($p<0.0001$). While the concentration of the former is closer than indicated by the manufacturer, the concentration of the latter was below that indicated by the manufacturer.

pH measurements of bleaching agents

Initially, after mixing, the 35% HP had an initial pH of 6.5, reaching a pH of 5.6 at the end of treatment. For the 37.5% HP, the pH measured after mixing was 6.5, and it remained stable throughout the application time (Fig. 2). When both bleaching gels were compared, a significant difference was observed in the pH at the end of treatment. At this time,

37.5% HP showed a significant and higher pH when compared with 35% HP ($p<0.001$).

Discussion

Despite manufacturers' recommendations for application of in-office bleaching indicates the amount of bleaching gel to be applied need to be homogeneous and, in enough amount, to guarantee bleaching efficacy. Usually, it is difficult to control these factors. Therefore, the use of different applicator brushes, as evaluated in the present study, seems to be an interesting alternative. According to manufacturers, it will be expected, when a brush tip will be used, a homogeneous and uniform film thickness of bleaching gel will be applied on the dental surface, reducing the amount of bleaching agents spent during application. Indirectly, the use of brush tip will reduce the penetration of hydrogen peroxide into the pulp, because less amount of bleaching gel will be used.

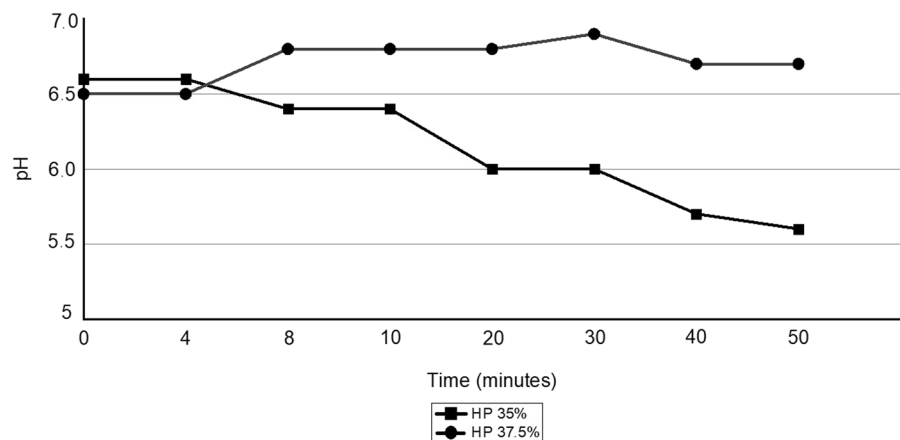
These statements seem to be proved in the present study, since it showed a significantly lower penetration of HP into the pulp chamber for groups in which the application was done using an applicator with a brush tip, regardless of the concentration used, leading to partial rejection of the first null hypothesis. Also, the application of HP using an applicator with a brush tip decreased the amount of HP in the pulp chamber by 58–64%, compared to the use of an applicator without a brush tip. This seems to be related to

Table 3 Means (\pm standard deviations) of the color change in different objective assessments (ΔE_{ab} , ΔE_{00} , and ΔWI_D) in different experimental groups (*)

Experimental groups		ΔE_{ab}	ΔE_{00}	ΔWI_D
Control		1.3 ± 0.5 A	1.1 ± 0.5 a	1.6 ± 0.9 A
HP 35%	With applicator brush	3.6 ± 1.8 B	2.0 ± 0.9 b	5.6 ± 1.8 B
	Without applicator brush	4.2 ± 1.6 B	2.6 ± 0.9 b	6.7 ± 3.5 B
HP 37.5%	With applicator brush	4.6 ± 2.2 B	2.8 ± 0.9 b	6.9 ± 1.8 B
	Without applicator brush	5.7 ± 2.4 B	3.5 ± 1.2 b	7.1 ± 2.0 B

(*) Identical capital, lowercase, or superscript letters in each column indicate statistically similar means

Fig. 2 Evaluation of pH stability during the application time of 50 min for both in-office bleaching gels evaluated. Initially both gels have a slightly acidic and similar pH. However, during the application time, HP 35% becomes more acidic, while HP 37.5% showed pH stability



the amount of HP expended. When the applicator with a brush tip was used, the amount of expended gel showed a decrease of 63.5% on average compared to the use of an applicator without a brush tip, regardless of the concentration used. This led to the partial rejection of the second null hypothesis.

Some studies have shown that as the amount of HP increased, so too did the damage to the pulp tissue in animals [28]. The application of a 35% HP bleaching gel caused more damage to the pulp tissue in animals than the application of a 20% HP gel [29]. Recently, Esteves et al. [15] indicated a directly proportional relationship between the volume of the bleaching product applied on the tooth surface and the number of patients with TS.

However, as far as the authors of this paper are aware, only Carneiro et al. [14] evaluated the amount of HP penetrating into the pulp chamber and the amount of bleaching gel expended when different tips were used. Their results showed that a lower amount was spent with the use of a brush tip, and this significantly decreased the amount of HP in the pulp chamber when compared to application without brush tip, as was observed in the present study.

On the other hand, a significant difference was observed when both in-office bleaching gels were evaluated after application without a brush tip. A higher amount of HP in the pulp chamber was observed for the use of 35% HP compared to 37.5% HP. Several factors could help explain these findings. If one takes into consideration that the HP concentration described on the label is similar for both in-office bleaching gels available, no significant difference will be expected. However, while the concentration of the 35% HP gel was closer than the manufacturer described—33% according to the present measurement—the 37.5% HP gel had a concentration 9.3% lower than that stated on the label, with a real concentration of 28.2%. It is well known that when the amount of HP available is lower, the amount of HP that penetrates into the pulp chamber is also lower [27]. In fact, many commercial brands have less HP in the syringe than is described on the label, as shown by Matis et al. [30].

Another factor is the application mode, while 37.5% HP requires product reapplications in 4 times of 8 min, and 35% HP only indicates a single application of 50 min. Therefore, the gel expense for 37.5% HP was considerably higher than for 35% HP. In a pilot study, the 37.5% HP was applied for 50 min. However, a higher increase of flowability of the in-office gel after 10–12 min of application occurred, preventing the evaluation in the proposed time (not showed data). Several *in vitro* studies showed that an increase in the amount of HP inside the pulp chamber according to the increase of the number of changes occurred [16, 21, 31]. This increase showed

significant impact in the viability of pulp cells [31]. In a clinical scenario, Kose et al. [32] demonstrated that the number of patients with TS was higher when the 35% HP in-office bleaching procedure was performed in two or three 15-min applications instead of only one application.

A third factor to be considered is the pH. While the 35% HP bleaching gel showed an acidic and unstable pH during the time it remained on the tooth, the 37.5% HP bleaching gel showed the opposite characteristics, less acidity and stability. This confirms the previous results observed by de Mendonça et al. [21]. Actually, most in-office bleaching gels are supplied with a low pH to increase the product's useful life [33–35]. The disadvantage of a low pH is that it can promote enamel demineralization [8, 36]; changes in the chemical composition, morphology, and mechanical properties of the tooth structure [37–39]; and increased diffusion of HP to the pulp [8, 16, 22].

In vitro studies have shown that less acidic products do not alter the enamel's roughness and thus do not have deleterious effects on the enamel surface [8, 37]. Therefore, a decreased diffusion of HP into the pulp will be expected [8, 16, 22]. These results seem to be confirmed by some clinical trials that showed less acidic in-office bleaching agents generate fewer patients with TS compared to more acidic bleaching gels [18, 19].

Regarding color change, although the attached brush tip deposited a smaller amount of bleaching gel on the tooth surface compared to a tip without a brush, this seems not to impact the whitening effect observed. In addition, despite a difference in the amount of HP available and the pH observed for both bleaching gels, no significant difference in color change was observed when both in-office bleaching gels were compared, as has been previously observed in different studies [8, 16, 22]. This led to the partial rejection of the third null hypothesis.

Finally, the current study showed promising results regarding the use of an application tip with a brush attached for self-mixing bleaching materials, since the penetration of HP into the pulp was reduced and the bleaching's effectiveness was maintained. Therefore, future studies need to evaluate whether the use of a brush tip with in-office bleaching gels can affect TS and the whitening effect of self-mixing bleaching materials. Although two different commercial products were evaluated, and at the margin of the authors' knowledge, unique in self-mix form among bleaching gels available commercially, the results should not be extrapolated to all commercial brands, since the tip to be coupled is not universal, making it impossible to use for all commercial brands. This should be considered a limitation of the present study. Therefore, future studies evaluating different commercial brands should be conducted.

Conclusion

Regarding the different tips evaluated, the HP penetration into the pulp chamber as well as the amount of gel used was lower when using an applicator with a brush tip than when using one with a conventional tip for both in-office gels used. Regarding the in-office bleaching gels evaluated, the HP penetration into the pulp chamber was lower for HP 37.5% than HP 35% when used without tip. Despite more gel was expended when HP 37.5% was mixed in comparison with HP 35%, the HP 37.5% maintained their pH (6.5) along the application time, while HP 35% showed a significant decrease (6.5 to 5.5). Independently of the tips or in-office gels evaluated, all groups showed a higher whitening effect.

Acknowledgements The authors would like to thank FGM Dental Group (Brazil), SDI (Australia), and Sulzer (Switzerland) for the generous donation of the bleaching and tips products employed in this study.

Funding This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 303332/2017–4 and 305588/2014–1 as well as the Coordination of Improvement of Higher-Level Personnel (CAPES; no. 001).

Declarations

Ethical approval This study was submitted for approval by the Research Ethics Committee of the State University of Ponta Grossa (#4.647.755) and was carried out at the same university.

Informed consent Not applicable.

Conflict of interest The authors declare no competing interests.

References

- Haywood VB (1992) History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. *Quintessence Int* 23:471–488
- Carey CM (2014) Tooth whitening: what we now know. *J Evid Based Dent Pract* 14(Suppl):70–76. <https://doi.org/10.1016/j.jebdp.2014.02.006>
- de Geus JL, Wambier LM, Kossatz S, Loguercio AD, Reis A (2016) At-home vs in-office bleaching: a systematic review and meta-analysis. *Oper Dent* 41:341–356. <https://doi.org/10.2341/15-287-lit>
- Reis A, Mendonça da Silva L, Martins L, Loguercio A (2018) In-office tooth whitening. *Clin Dent Rev* 2:10. <https://doi.org/10.1007/s41894-018-0021-9>
- de Sá JL, Silva JS, Herkrath FJ, Favoreto MW, Reis A, Silva LM, Loguercio AD, Martins LM (2021) In-office bleaching with complete cervical third protection protocol: a split-mouth, double-blind, randomized clinical trial. *Am J Dent* 34:281–285
- Meireles SS, Santos ME, Lustosa ÍMC, Leite ELL (2021) Effects of a reduced in-office bleaching protocol with 37.5% hydrogen peroxide on effectiveness and tooth sensitivity: a double-blind randomized clinical trial. *J Esthet Restor Dent* 33:824–831. <https://doi.org/10.1111/jerd.12744>
- Torres CR, Zanatta RF, Godoy MM, Borges AB (2021) Influence of bleaching gel peroxide concentration on color and penetration through the tooth structure. *J Contemp Dent Pract* 22:479–483
- Acuña ED, Parreiras SO, Favoreto MW, Cruz GP, Gomes A, Borges CPF, Loguercio AD, Reis A (2019) In-office bleaching with a commercial 40% hydrogen peroxide gel modified to have different pHs: color change, surface morphology, and penetration of hydrogen peroxide into the pulp chamber. *J Esthet Restor Dent* 34:322–327. <https://doi.org/10.1111/jerd.12453>
- Favoreto MW, Madureira MP, Hass V, Maran BM, Parreiras SO, Borges CPF, Reis A, Loguercio AD (2021) A novel carbamide peroxide polymeric nanoparticle bleaching gel: color change and hydrogen peroxide penetration inside the pulp cavity. *J Esthet Restor Dent* 33:277–283. <https://doi.org/10.1111/jerd.12652>
- Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J (2010) Human pulp responses to in-office tooth bleaching. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109:e59–64. <https://doi.org/10.1016/j.tripleo.2009.12.002>
- Vaz MM, Lopes LG, Cardoso PC, Souza JB, Batista AC, Costa NL, Torres EM, Estrela C (2016) Inflammatory response of human dental pulp to at-home and in-office tooth bleaching. *J Appl Oral Sci* 24:509–517. <https://doi.org/10.1590/1678-7752/0160137>
- Roderjan DA, Stanislawczuk R, Hebling J, Costa CA, Reis A, Loguercio AD (2015) Response of human pulps to different in-office bleaching techniques: preliminary findings. *Braz Dent J* 26:242–248. <https://doi.org/10.1590/0103-6440201302282>
- Maran BM, Matos TP, de Castro ADS, Vochikovski L, Amadori AL, Loguercio AD, Reis A, Berger SB (2020) In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: a systematic review and meta-analysis. *J Dent* 103:103499. <https://doi.org/10.1016/j.jdent.2020.103499>
- Carneiro TS, Favoreto MW, Bernardi LG, Bandeca MC, Borges CPF, Reis A, Loguercio A (2022, in press) Application tip and concentration of a self-mixing bleach: hydrogen peroxide inside the Pulp chamber, color change and amount of bleaching used. *Oper Dent*.
- Esteves LMB, Dos Santos PH, Fagundes TC, de Oliveira GM, de Mello Antonaccio GB, Cintra L, Briso ALF (2021) Effect of bleaching gel volume on color change and postoperative sensitivity: a randomized clinical study. *Clin Oral Investig* 26:2527–2536. <https://doi.org/10.1007/s00784-021-04220-z>
- Balladares L, Alegría-Acevedo LF, Montenegro-Arana A, Arana-Gordillo LA, Pulido C, Salazar-Gracez MT, Reis A, Loguercio AD (2019) Effects of pH and application technique of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. *Oper Dent* 44:659–667. <https://doi.org/10.2341/18-148-1>
- Crank J (1979) *The mathematics of diffusion*. Oxford University Press
- 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. *J Am Dent Assoc* 143:e81–7. <https://doi.org/10.14219/jada.archive.2012.0075>
- 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. *Clin Oral Investig* 21:2811–2818. <https://doi.org/10.1007/s00784-017-2083-5>
- Martins I, Onofre S, Franco N, Martins LM, Montenegro A, Arana-Gordillo LA, Reis A, Loguercio AD, da Silva LM (2018) Effectiveness of in-office hydrogen peroxide with two different protocols: a two-center randomized clinical trial. *Oper Dent* 43:353–361. <https://doi.org/10.2341/17-128-c>

21. de Mendonça RP, Baliza JR, Burey A, Cavalcante LM, Loguercio AD, Calazans FS, Barcelheiro MO (2021) In vitro analysis of the pH stability of dental bleaching gels during in-office procedures. *J Clin Exp Dent* 13:e22–e29. <https://doi.org/10.4317/jced.57367>
22. Mena-Serrano AP, Parreiras SO, do Nascimento Borges Berger-Loguercio Reis AMCP SBADA (2015) Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. *Oper Dent* 40:E76–82. <https://doi.org/10.2341/13-352-1>
23. 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. *Am J Dent* 27:301–306
24. De L'Eclairage CI (1978) CIE recommendations on uniform color spaces, color-difference equations, and metric color terms. *Color Res Appl Paris, France*. <https://doi.org/10.1002/j.1520-6378.1977.tb00102.x>
25. Luo MR, Cui G, Rigg B (2001) The development of the CIE 2000 colour-difference formula: CIEDE2000. *Color Res Appl* 26:340–350. <https://doi.org/10.1002/col.1049>
26. Pérez Mdel M, Ghinea R, Rivas MJ, Yebra A, Ionescu AM, Paravina RD, Herrera LJ (2016) Development of a customized whiteness index for dentistry based on CIELAB color space. *Dent Mater* 32:461–267. <https://doi.org/10.1016/j.dental.2015.12.008>
27. Parreiras SO, Favoreto MW, Cruz GP, Gomes A, Borges CPF, Loguercio AD, Reis A (2020) Initial and pulp chamber concentration of hydrogen peroxide using different bleaching products. *Braz Dent Sci* 23:1–8. <https://doi.org/10.14295/bds.2020.v23i2.1942>
28. Llena C, Collado-González M, García-Bernal D, Oñate-Sánchez RE, Martínez CM, Moraleda JM, Rodríguez-Lozano FJ, Forner L (2019) Comparison of diffusion, cytotoxicity and tissue inflammatory reactions of four commercial bleaching products against human dental pulp stem cells. *Sci Rep* 9:1–11. <https://doi.org/10.1038/s41598-019-44223-1>
29. Cintra LT, Benetti F, Ferreira LL, Gomes-Filho JE, Ervolino E, Gallinari Mde O, Rahal V, Briso AL (2016) Penetration capacity, color alteration and biological response of two in-office bleaching protocols. *Braz Dent J* 27:169–175. <https://doi.org/10.1590/0103-6440201600329>
30. Matis BA, Matis JJ, Wang Y, Monteiro S, Al-Qunaian TA, Millard R (2013) Labeled vs actual concentration of bleaching agents. *Oper Dent* 38:334–343. <https://doi.org/10.2341/11-367-1>
31. 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. *J Dent* 42:185–198. <https://doi.org/10.1016/j.jdent.2013.10.021>
32. 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. *Oper Dent* 41:138–145. <https://doi.org/10.2341/15-085-c>
33. 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 Odontol Latinoam* 22:27–31
34. Price RB, Sedarous M, Hiltz GS (2000) The pH of tooth-whitening products. *J Can Dent Assoc* 66:421–426
35. Chen JH, Xu JW, Shing CX (1993) Decomposition rate of hydrogen peroxide bleaching agents under various chemical and physical conditions. *J Prosthet Dent* 69:46–48. [https://doi.org/10.1016/0022-3913\(93\)90239-k](https://doi.org/10.1016/0022-3913(93)90239-k)
36. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A (2009) Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. *Dent Mater* 25:143–157. <https://doi.org/10.1016/j.dental.2008.05.010>
37. Azrak B, Callaway A, Kurth P, Willershausen B (2010) Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. *J Esthet Rest Dent* 22:391–399. <https://doi.org/10.1111/j.1708-8240.2010.00372.x>
38. 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. *Oper Dent* 38:100–110. <https://doi.org/10.2341/11-173-1>
39. Xu B, Li Q, Wang Y (2011) Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. *Oper Dent* 36:554–562. <https://doi.org/10.2341/11-045-1>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.