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RESEARCH

Low and high hydrogen peroxide concentrations of in-office dental bleaching associated with violet light: an in vitro study

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Abstract

Objectives This study aims to assess hydrogen peroxide (HP) penetration within the pulp chamber, color change (CC), physical–chemical properties, and temperature using in-office different concentration bleaching gels with or without violet light.

Materials and methods Fifty teeth were divided into five groups ($n = 10$) based on the HP concentration bleaching gels used (6% and 35%) and the used violet light (with or without). HP penetration within the pulp chamber was measured using UV–Vis. The CC was evaluated with a digital spectrophotometer. Initial and final concentration, and pH were measured through titration, and a Digital pHmeter, respectively. Temperature analyses were measured through a thermocouple. Statistical analysis included two-way ANOVA, Tukey's, and Dunnett's test ($\alpha = 0.05$).

Results The presence of violet light did not affect the amount of HP within the pulp chamber, or the CC ($p > 0.05$). Greater penetration of HP was observed within the pulp chamber, as well as CC when using 35% HP ($p < 0.05$). The final concentration of both gels was lower than the initial concentration, regardless of the use of violet light ($p < 0.05$). The initial and final pH levels remained neutral and stable ($p > 0.05$). The pulp temperature increased when the gels were used in conjunction with violet light ($p < 0.05$).

Conclusions Using violet light in conjunction with 6% or 35% HP does not alter the physical properties of the bleaching agents, the penetration of HP or enhance color change. However, an increase in temperature was observed when violet light was applied associated with bleaching gels.

Clinical relevance While the simultaneous use of violet light with hydrogen peroxide 6% or 35% does not alter the material's properties, it also does not bring benefits in reducing hydrogen peroxide penetration and improving color change. Furthermore, the use of violet light increases pulp temperature.

Keywords In-office dental bleaching · Lower concentration and violet LED

Introduction

In-office dental bleaching has gained popularity due to its ability to produce noticeable color changes in just one treatment session [1]. This technique has undergone various advancements, allowing for customization to suit each patient's needs, making the treatment individualized. Typically, the procedure involves safeguarding the soft tissues using buccal retractors and light-cured gingival barriers, followed by the application of the active bleaching agent into the enamel surface [1]. Nowadays, it is possible to apply hydrogen peroxide (HP) for in-office-bleaching in concentrations ranging from 6 to 40% [2, 3].

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The mechanism of action is associated with the penetration of HP into the tooth structure and the subsequent breakdown to free radicals [4]. These free radicals then oxidize the organic components within the tooth structure, resulting in a bleaching effect [4]. However, it's important to note that the active agent is not limited to the hard tissues and can reach the pulp [4–8], leading to inflammatory processes [9, 10] and causing tooth sensitivity following the bleaching procedure [11, 12].

While HP remains the most effective agent for dental bleaching [13], certain manufacturers have suggested the incorporation of light activation protocols to enhance the bleaching process. Nevertheless, recent systematic reviews on this matter have indicated that the combination of light emitting diode (LEDs), quartz tungsten halogen lights, plasma arc, and lasers with in-office bleaching gels did not yield any discernible clinical benefits [11–15] and even with the increase in temperature, tooth sensitivity does not seem to be more severe [11, 12].

However, as the association of different lights with bleaching is always a topic under constant discussion, same manufactures introduced a new wavelength of visible spectrum light (violet light wavelength: 405–410 nm) to be used, either in isolation or in conjunction with bleaching gels [16, 17]. There appears to be a consensus that the use of the violet light alone did not achieve the same bleaching effect as 35–37% HP used by itself [18–20]. This can be explained by the fact that around 1 mm of enamel thickness is sufficient to attenuate the penetration of violet light into hard tissues, reaffirming that its action is superficial [18]. Studies combining violet light with high-concentrated HP gels have yielded inconsistent results regarding their effectiveness [19–22]. However, some research using this combination observed a significant increase in pulpal temperature [19, 23–25], suggesting a potential risk of pulpal injury.

The use of high (> 30%) or medium (20% to 30%) concentrations of HP seems to be enough to produce an excellent efficacy without the addition of light. In an attempt to maintain the use of violet light, and at the same time decrease potential pulpal damage, different studies had been indicated their use associated with lower (< 20%) concentrated HP [5, 20, 24–29]. The reason is that using low concentrations requires more sessions than usual to achieve the expected result and patients seek in-office bleaching as it produces faster results.

However, until now, much research has focused on evaluate the bleaching efficacy of different concentration of HP with or without violet light [5–8, 27, 28]. But to extent of authors' knowledge, no previous studies have compared the use of violet light associated with concentrations of HP for in-office bleaching can affect the penetration of HP into the pulp chamber. The aim of this study was to assess HP penetration within the pulp chamber, color change, physical–chemical properties (initial and final concentration and pH), and temperature in human teeth treated with low-concentration (6% HP) and a high-concentration (35% HP) bleaching agents, both with and without the use of a violet light source.

Materials and methods

Ethical approval

The State University of Ponta Grossa (PR/Brazil) Ethics Committee approved this study under protocol number 5.731.728.

Selection of teeth and inclusion and exclusion criteria

A total of 50 caries-free maxillary first premolars (Fig. 1a) were obtained from the tooth bank of the State University of Ponta Grossa (PR/Brazil). They were cleaned, disinfected in 0.5% chloramine for one day, and stored in distilled water until use. The selection of teeth was carried out using a 10× magnification microscope (Lambda LEB-3, ATTO instruments, Hong Kong, China). Teeth with fractures, enamel defects, and colors greater than > 20 according to the Whiteness Index for Dentistry (WI_D) [30] (VITA Easyshade Advance 4.0, VITA Zahnfabrik, Bad Säckingen, Germany) were excluded. Additionally, teeth with a buccal thickness less than 2.5 mm and greater than 3.5 mm, as determined through previously performed radiography (Timex 70C, Gnatus, Ribeirão Preto, SP, Brazil), as described in the specimen preparation section, were also excluded [31].

The selected teeth were randomly distributed into five groups ($n = 10$) according to the following variables: (1) bleaching gel concentration: low-concentration ([6% HP]; Whiteness HP Automixx 6%; FGM Dental group, Joinville, SC, Brazil), and high-concentration ([35% HP]; Whiteness HP Automixx Plus 35%; FGM Dental group); and (2) association of not with a violet light source (Bright Max Whitening, MMOptics, São Carlos, SP, Brazil). A group treated with ultra-purified water served as the negative control.

Sample size calculation

The primary purpose of this study is the quantification of the HP within the pulp chamber. Considering the values of HP penetration in a previous study [32], teeth submitted to treatment with high concentrated HP presented 0.92 ± 0.32 µg/mL of HP within the pulp chamber. Using a two-sided test with an alpha of 0.05 and 80% power, it should be selected at least 8 teeth in each group to detect a difference of 0.46 µg/mL. Two extra teeth were assigned to each group to compensate for possible losses. Therefore, 10 teeth were used in each experimental group.

Specimen preparation

A low-speed diamond disk (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) was utilized to remove the tooth root approximately 3 mm apically to the cementum-enamel

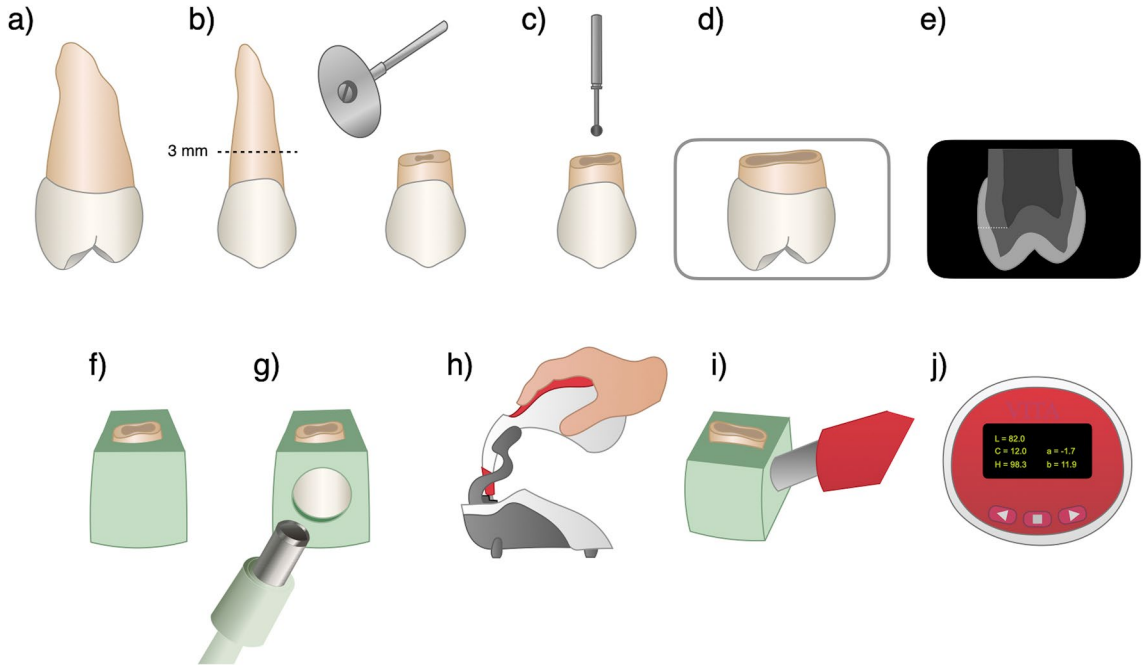

Fig. 1 **a)** Maxillary first premolar teeth were used in the experiment; **b)** Approximately 3 mm of the tooth root were removed apically to the cementum-enamel junction using a diamond disk; **c)** A spherical bur was used to enlarge access to the pulp chamber; **d)** The mesial or distal face of the specimen was placed in contact with the X-ray film; **e)** The thickness of the buccal tooth was measured; **f)** Individual impressions were made using a green dense silicone paste; **g)** A 6 mm diameter window was created on the buccal surface within the middle third of the specimens using a circular punch scalpel; **h)** The digital spectrophotometer was calibrated.; **i)** The spectrophotometer was positioned within the prepared window, and; **j)** L*, a*, and b* coordinates were obtained from the device display

junction (Fig. 1b). Pulp tissue was carefully removed and flushed with deionized water [31]. A spherical bur (#1014, KG Sorensen, SP, Brazil) was then employed to expand access to the pulp chamber (Fig. 1c), creating an access point capable of accommodating up to 25 µL of solution using a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland).

Next, X-ray radiographs were taken using the Timex 70C X-ray machine (Gnatus). For this purpose, the mesial face of the specimen was placed in contact with the X-ray film (Fig. 1d). Each radiograph was captured 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 distal surface. After exposure, the images were digitally obtained, and the corresponding buccal tooth thickness was measured using New IDA software (Dabi Atlante, Ribeirão Preto, SP, Brazil) [31] (Fig. 1e).

Initial color change

To standardize the position of the spectrophotometer, individual impressions were made using a green dense silicone paste (Coltoflax and Perfil Cub Kit, Vigodent, Rio de Janeiro, RJ, Brazil) (Fig. 1f). Using a circular punch scalpel (Fig. 1g), a 6 mm diameter window was created on the buccal surface within the middle third of the specimens. (Fig. 1g) [33].

The initial color coordinates (L*, a*, and b*) were measured using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik). Before each measurement, the digital spectrophotometer was calibrated (Fig. 1h) and subsequently positioned within the pre-established window (Fig. 1i). The L* value represents lightness, with values ranging from 0 for black to 100 for white. The a* value represents the color along the red-green axis, and the b* value represents the color along the yellow-blue axis. These values appear on the device display (Fig. 1j). We calculated the change in WI_D baseline color using the formula [30]: $WI_D = 0.551 \times L - 2.324 \times a - 1.1 \times b$.

Obtaining the study calibration curve

The analytical products used in this study were not pre-purified, and all solutions were prepared with deionized water. Initially, a typical reference line was plotted using a 5.000 µg/mL stock solution prepared from a concentrated solution (35% HP, Pharmacy Eficácia, Ponta Grossa, PR, Brazil). Subsequently, this solution was diluted in an acetate buffer solution (pH = 4) and calibrated using conventional methods. To determine the analytical grade and actual

concentration of the solution, a potassium permanganate solution was used for titration [31].

Based on the verified initial concentration, serial volumetric dilutions ranging from 0.000 to 0.414 µg/mL were performed to construct the calibration curve. Known HP concentrations were added to glass tubes and placed in a Cary 100 UV–Vis spectrophotometer (Varian, Palo Alto, CA, USA). This procedure resulted in a standard reference line used for extrapolating the results of the study samples (R = 0.99, data not reported) [31].

Treatment protocols and HP penetration within the pulp chamber

For all groups, the specimens were vertically fixed to a wax plate with the occlusal surface facing the plate (Fig. 2a). Prior to the application of the bleaching agent, the buccal surface of each specimen was isolated by applying a light-cured resin barrier, enclosing an area of 6 mm × 6 mm (Top-dam, FGM Dental Products) (Fig. 2b). To retain any HP that entered the pulp chamber during the bleaching procedures, a 25-µL aliquot of acetate buffer (pH = 4) was inserted into the pulp chamber of each specimens (Fig. 2c).

A single and experienced operator was responsible for the materials application. The bleaching agent was applied in the buccal enamel area according to the different experimental groups (Fig. 2d). Both bleaching gels were applied until the buccal area of the teeth to be bleached was completely covered. For the groups that received violet light irradiation (Bright Max Whitening, MMOptics), the gel was applied first, followed by a 1-min irradiation and 30 s of rest, repeating this process for a total of 20 applications, with a distance of 2 cm from the specimens. The device used had four LED diodes that emitted light in the spectral range of 405–410 nm, with each LED having an output power of 350 mW, resulting in a total optical power of 1.2 W and an irradiance of 140.2 mW/cm^2. The negative control group was kept out of contact with bleaching agents or violet light. After 50 min in each session the bleaching gel was removed with gauze and carefully washed with deionized water (Fig. 2e).

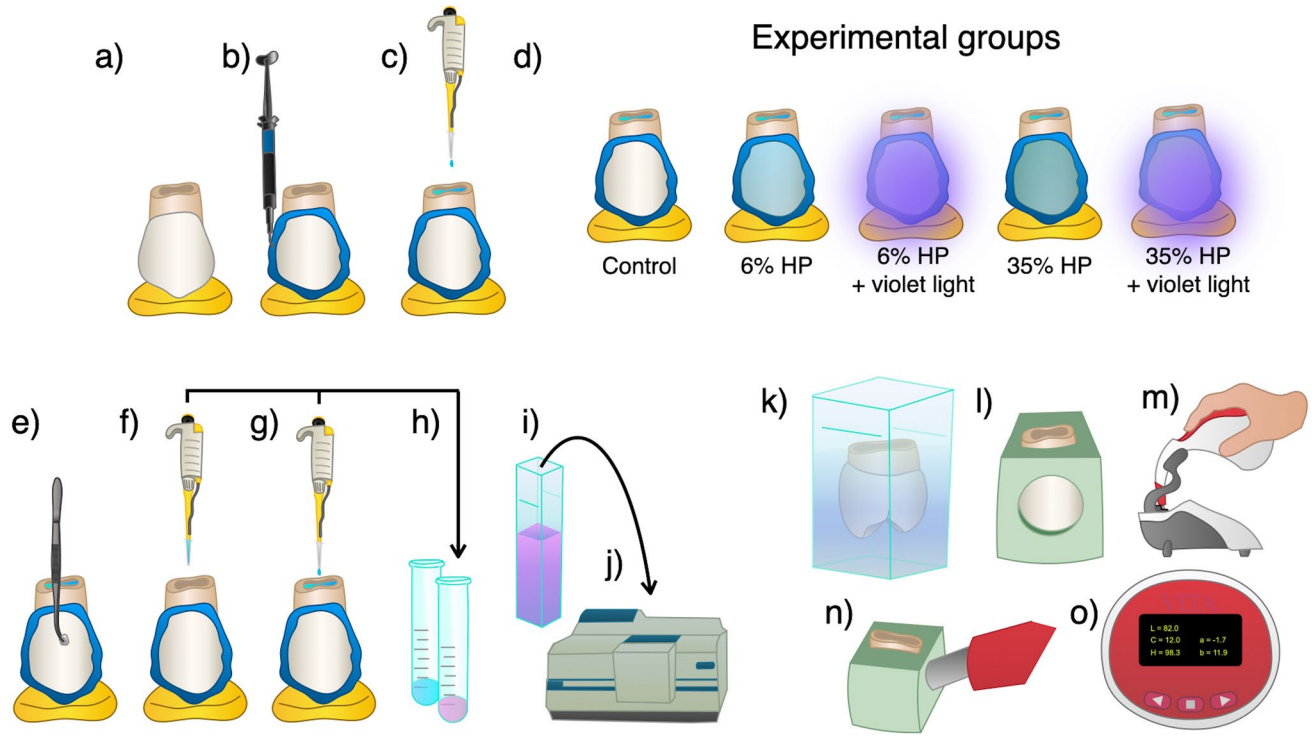

Fig. 2 **a)** Specimens were vertically fixed to a wax plate; **b)** Each specimen was isolated with a light-cured resin barrier, enclosing a 6 × 6 mm area; **c)** 25-µL of acetate buffer was placed in each pulp chamber of each specimens; **d)** The bleaching agent was applied to the buccal enamel area as per the experimental groups; **e)** After each 50-min session, the bleaching gel was removed with gauze and carefully washed with deionized water; **f)** The acetate buffer solution from each pulp chamber was removed and transferred to a glass tube; **g)** Each pulp chamber was rinsed four times with 25 µL of acetate buffer, and the rinse was then transferred to a glass tube; **h)** Distilled water, Leucocrystal Violet (a colorimetric indicator), horseradish peroxidase enzyme, and acetic acid were added to the glass tube; **i)** The resulting solution displayed a violet color; **j)** The solution's absorbance was measured using a UV–Vis spectrophotometer; **k)** Specimens were immersed in artificial saliva for one week; **l)** Specimens were positioned in their dense silicone guide; **m)** The digital spectrophotometer was calibrated; **n)** The spectrophotometer was positioned within the pre-established window; **o)** L*, a*, and b* coordinates were obtained from the device display

After that, using a mechanical micropipette, the acetate buffer solution within the pulp chamber of each tooth was removed and transferred to a glass tube (Fig. 2f). This procedure was performed by rinsing the pulp chamber of each tooth four times with 25 µL of acetate buffer and transferring this solution to the same glass tube (Fig. 2g). Thereafter, more distilled water (2.725 µL) was added to the glass tube along with 100 µL of 0.5 mg/mL (Leucocrystal Violet, Sigma Chemical Co., St Louis, MO, USA) and 50 µL of 1 mg/mL horseradish peroxidase enzyme (Peroxidase Type VIA, Sigma Chemical Co.) (Fig. 2h). This procedure was repeated separately for each specimen. The resulting solution had a violet color (Fig. 2i) with a maximum absorbance peak at 591 nm, which was measured using a Cary 100 UV–Vis spectrophotometer (Varian) (Fig. 2j), and the absorbance used was the highest absorption peak of the resulting reaction between HP and Leucocrystal Violet (Crystal Violet—591 nm). According to Beer's Law, absorbance corresponds directly to concentration. Therefore, HP concentration (µg/mL) was determined by comparing it with the calibration curve already obtained [31].

Final color change evaluation

Throughout this period, the specimens were immersed in artificial saliva (Pharmacy Eficácia) (Fig. 2k) composed of carboxymethylcellulose, sodium chloride, potassium chloride, magnesium chloride, dibasic calcium phosphate, glycerin, xylitol and distilled water, with daily changes of artificial water maintained at a controlled temperature of 37 °C. Afterward, using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik), the final color coordinates (L*, a*, and b*) were measured. Before each measurement, the specimens were positioned in their dense silicone guide (Fig. 2l). The digital spectrophotometer was calibrated (Fig. 2m) and subsequently positioned within the pre-established window (Fig. 2n). These values were displayed on the device (Fig. 2o).

The color change before (baseline) and after one week of bleaching was determined by calculating the difference between the measurements with the spectrophotometer. This calculation was performed using the CIELab formula (ΔE_{ab}) [34], CIEDE 2000 formula (ΔE_{00}) [35], and Whiteness Index for Dentistry (WI_D) [30]. Furthermore, changes in WI_D caused by each step were calculated by subtracting the values observed at each assessment time from those calculated in the previous step (ΔWI_D) [30]. Perceptual changes were considered significant when the differences in the initial and post-bleaching colors presented $\Delta E_{ab} > 2.7$ and $\Delta E_{00} > 1.8$ [36] and $\Delta WI_D > 2.6$ [37]. To ensure optimal patient outcomes and minimize perceivable color shifts within a patient's smile, we employ a stringent 50:50% acceptability threshold for color difference measured as ΔE [36, 37]. This conservative approach prioritizes natural-looking results, deeming any change below this threshold as clinically insignificant [36, 37].

Initial and final concentrations of bleaching agents

The bleaching gels used in the study were titrated with a standardized potassium permanganate solution before the bleaching procedure and at the end of the bleaching procedure, following the procedure described in the literature [33, 38]. This titration was performed to determine the initial and final concentrations within the bleaching gel. The analyses were performed in triplicate to ensure accuracy and consistency. Acceptable limits of variation of up to -30% and + 10% of the original concentration stated by the manufacturer were considered [39].

pH measurements of bleaching agents

The pH of each bleaching agent was measured using a pH meter (Extech pH100, Extech Instruments, Nashua, NH, USA) placed directly in contact with the bleaching gel on a tooth [32, 40]. Measurements were taken at various time points, starting immediately after application and then at 10-min intervals. The analyses were performed in triplicate at each time point to ensure accuracy and reliability.

Temperature analysis

In this analysis, the same samples used in previous experiments were introduced with a 0.011″ diameter ultra-fast response T-type thermocouples (IT-23 Physitemp Instruments, Clifton, NJ, USA) into the buccal pulp horn (dentin), as previous described [19, 23–25]. Then, the temperature variation was analyzed throughout the second bleaching session. For better heat conduction, thermal paste (Implastec, Votorantim Ind. Brasileira, São Paulo, SP, Brazil) and wax (Lysanda, São Paulo, SP, Brazil) were used to ensure that the tip of the thermocouple was in contact with the horn sample mouthpiece without moving.

Statistical analysis

The data were subjected to statistical analysis using the Kolmogorov–Smirnov test to assess normality and the Barlett test for equality of variances to examine the assumption of equal variances (unreported data). As the data exhibited normal distribution, two-way ANOVA (concentration of HP vs. violet light) was performed to analyze the buccal teeth thickness, the color alteration in different parameters

(ΔE_{ab}, ΔE_{00}, and ΔWI_D), the concentration of HP within the pulp chamber (μg/mL) and the variation of temperature (°C). Tukey's post-hoc test was employed to compare different bleaching techniques. Additionally, a one-way ANOVA was conducted, followed by Dunnett's post-hoc test to compare the values obtained from different bleaching techniques with those of the control group ($\alpha = 0.05$).

Results

Buccal teeth thickness

Table 1 showed the buccal teeth thickness of all teeth evaluated. No significant difference was observed when all groups were compared to control group ($p > 0.05$; Dunnet's post-hoc test). Also, no significant difference among experimental groups were observed when different experimental groups were evaluated ($p > 0.05$; Tukey's post-hoc test).

Table 1 Means (± standard deviations) of buccal thickness (mm) and the HP concentration (μg/mL) detected within the pulp chamber in different experimental groups (*)

Experimental groups	Buccal thickness (mm)	HP concentration (μg/mL)
Control (**)	3.3 ± 0.2 =	0.001 ± 0.002 ≠
6% HP	3.4 ± 0.2 A	0.027 ± 0.023 a
6% HP + violet light	3.3 ± 0.2 A	0.009 ± 0.007 a
35% HP	3.3 ± 0.2 A	0.202 ± 0.073 b
35% HP + violet light	3.4 ± 0.2 A	0.165 ± 0.073 b

(*) Identical capital or lower-case letters indicate statistically similar means for each column (Tukey's test; $p > 0.05$)

(**) Symbol "=" means that control group was not significant different when compared to all experimental groups (Dunnet's test; $p > 0.05$) and symbol "≠" means that control group was significant different when compared to all experimental groups (Dunnet's test; $p < 0.05$)

HP concentration within the pulp chamber

The average values of the amount of HP within the pulp chamber are showed in the Table 1. All experimental groups showed a significant and higher amount of HP within the pulp chamber when compared to control ($p < 0.00000001$; Dunnet's post hoc test). When only experimental groups were compared, the cross-products interaction, as well as main factor 'violet light' was not significant different ($p > 0.10$). However, the main factor 'concentration of HP' was statistically significant with less amount of HP inside the pulp chamber for 6% HP when compared to 35% HP ($p < 0.000000001$; Tukey's post-hoc test).

Color change

Table 2 showed the baseline WI_D color, as well as the color alteration measured by ΔE_{ab}, ΔE_{00} and ΔWI_D for all experimental groups. Regarding the WI_D baseline color evaluation, no significant difference among experimental groups were observed ($p > 0.35$; Tukey's post-hoc test). All groups showed a significant and higher color alteration when compared to control group ($p < 0.00001$ for ΔE_{ab}; $p < 0.000001$ for ΔE_{00}; and $p < 0.000001$ for ΔWI_D; Dunnet's post-hoc test).

The cross-product interaction was not statistically significant for all measurement performed ($p = 0.32$ for ΔE_{ab}; $p = 0.48$ for ΔE_{00}; and $p = 0.71$ for ΔWI_D), as well as for the main factor 'violet light' ($p = 0.82$ for ΔE_{ab}; $p = 0.76$ for ΔE_{00}; and $p = 0.37$ for ΔWI_D). However, only the main factor 'concentration of HP' was statistically significant ($p < 0.05$). The 35% HP showed better bleaching efficacy when compared to 6% HP ($p < 0.00000001$ for ΔE_{ab}, ΔE_{00}, and ΔWI_D). The application of violet light did not improve the effectiveness of whitening when compared to the group without violet light ($p = 0.25$ for ΔE_{ab}; $p = 0.32$ for ΔE_{00}; and $p = 0.48$ for ΔWI_D).

Table 2 Means (± standard deviations) of the baseline color (WI_D baseline), as well as color change in different objective assessments (ΔE_{ab}, ΔE_{00} and ΔWI_D) in different experimental groups (*)

Experimental groups	WI_D baseline (**)	ΔE_{ab}	ΔE_{00}	ΔWI_D
Control	19.0 ± 3.8 =	1.8 ± 0.6 ≠	1.7 ± 0.6 ≠	0.2 ± 1.5 ≠
6% HP	19.0 ± 2.2 A	5.0 ± 1.8 a	4.7 ± 1.8 ^{A}	3.9 ± 2.7 ^{a}
6% HP + violet light	19.1 ± 2.1 A	6.8 ± 2.6 a	6.3 ± 2.5 ^{A}	6.7 ± 3.6 ^{a}
35% HP	19.9 ± 3.2 A	9.7 ± 4.4 b	9.4 ± 4.4 ^{B}	11.1 ± 3.5 ^{b}
35% HP + violet light	19.4 ± 4.2 A	10.6 ± 4.3 b	10.1 ± 4.2 ^{B}	12.7 ± 3.3 ^{b}

(*) Identical capital or lowercase letter superscript or not indicates statistically similar means for each experimental groups (Tukey's test; $p > 0.05$)

(**) Symbol "=" means that control group was no significant different when compared to all experimental groups (Dunnet's test; $p > 0.05$) and symbol " ≠ " means that control group was significant different when compared to all experimental groups (Dunnet's test; $p < 0.05$)

Initial and final concentrations of bleaching agents and pH measurements of bleaching agents

Table 3 showed the initial and final concentrations of bleaching gels. The cross-product interaction was not statistically significant for initial and final concentrations (initial; $p = 0.82$ and final; $p = 0.92$), as well as for the main factor 'violet light' (initial; $p = 0.74$ and final; $p = 0.89$). However, only the main factor 'concentration of HP' was statistically significant ($p < 0.05$). The 35% HP showed higher amount of HP when compared to 6% HP ($p < 0.000001$ for initial and final). As it is possible to see in the Fig. 3, no significant change was observed in the pH of bleaching gels during application time, regardless of concentration of HP or use of violet light ($p > 0.45$).

Temperature analysis

Also, in the Table 3, it was possible to see the variation of temperature for different experimental groups. The cross-product interaction was not statistically significant ($p = 0.25$), as well as for the main factor 'concentration of HP' ($p = 0.56$). However, only the main factor 'violet light' was statistically significant ($p = 0.001$). The use of violet light showed higher increase of temperature when compared to without violet light ($p = 0.001$).

Table 3 Means (± standard deviations) of the initial and final concentration hydrogen peroxide, as well as temperature (ΔT, ºC) in different experimental groups (*)

Experimental groups	Concentration (%)		ΔT
	Initial	Final	
6% HP	5.7 ± 0.0 A	5.2 ± 0.0 a	0.3 ± 0.3 ^{A}
6% HP + violet light	5.7 ± 0.0 A	4.2 ± 0.0 a	7.9 ± 0.6 ^{B}
35% HP	34.3 ± 0.0 B	30.0 ± 0.0 b	0.7 ± 0.0 ^{A}
35% HP + violet light	34.3 ± 0.0 B	29.6 ± 0.0 b	7.5 ± 0.5 ^{B}

(*) Identical capital or lowercase letter superscript or not indicates statistically similar means for each experimental groups (Tukey's test; $p > 0.05$)

Discussion

The purpose of light use in in-office dental bleaching has never been focused on reducing tooth sensitivity resulting from the penetration of HP within the pulp chamber. The association between in-office bleaching with light has always been geared towards protocols aimed at enhancing the bleaching effectiveness. This may explain why there is limited exploration in studies of dental bleaching that assess pulp concentration when violet light are associated to in-office bleaching [19–22]. The results of this study revealed a higher amount of HP observed within the pulp for 35% HP when compared to 6% HP. This was expected because several studies have shown that the higher the concentration of HP, the greater the amount of HP within the pulp chamber [38, 41].

The excessive amount of HP reaching the pulp tissue when using 35% HP can trigger an intense inflammatory response [10]. For instance, clinical studies have demonstrated that 80% to 100% of patients undergoing bleaching reported tooth sensitivity during and immediately after an in-office session with 35% HP [42–45]. While this adverse effect is typically considered temporary and moderate, it can cause significant discomfort for the patient, and in some cases, substantial pulpal damage has been observed in histological studies [9].

Despite these differences regarding the HP concentration, the use of violet light did not have a significant influence on amount of HP penetration within pulp chamber. The authors

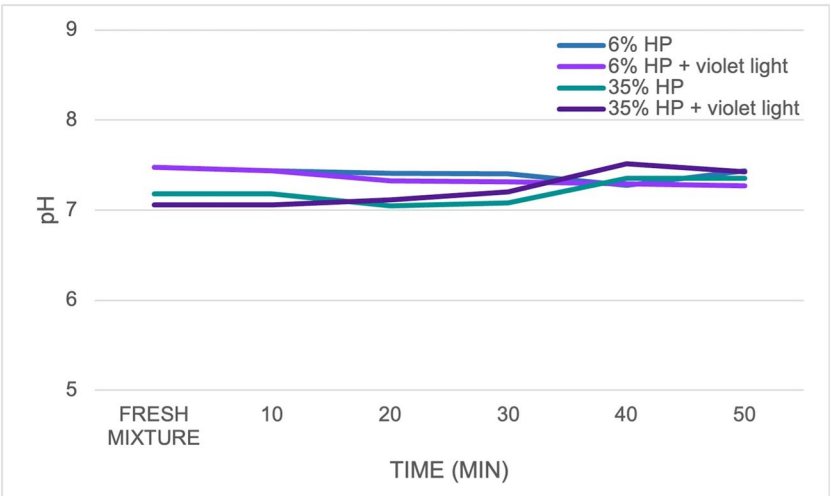

Fig. 3 Evaluation of pH stability during the application time of 50 min for both in-office bleaching gels evaluated

aimed to understand the mechanism of action of HP when used with violet light. Bleaching gels used in this study did not contain activators or dyes to accelerate the processes [20, 28, 46, 47]. The authors sought to comprehend the direct reaction of HP with violet light. Consequently, a constant permeability was expected because it is impossible to prevent the HP from permeating hard structures and reaching the pulp chamber. In fact, when it was examined the final concentrations of the bleaching gel after the procedure, they are very similar whether irradiated by violet light or not [48, 49].

The observed findings could be attributed, in part, to changes in surface morphology, as suggested by some authors [27, 46]. However, among all potential factors, pH emerges as the most significant. As you can see in Fig. 3, both bleaching gels maintained a stable and alkaline pH throughout the application period. This suggests that a significant increase in surface porosity is unlikely to be the primary driver of the observed effects.

An increase in the temperature observed when violet light was applied, in agreement with previous studies [19, 23–25]. However, even though an increase in temperature was observed, this increase was not able to improve the degradation of HP available to react. This implies that even with a slight degradation from 13% [that means only 4.5% of HP for HP 35%] and 8% [that means only 0.5% of HP for HP 6%], there was still an amount of active HP available to react with dental tissues and reach the pulp. Furthermore, as observed in the pH data, it was possible to see stability even in the presence of violet light irradiation [50]. This indicates that the violet light is not capable of modifying the inherent reactions of HP alone [50, 51]. Although there is speculation about a theoretical advantage of using violet light sources, it can be explained by the violet light source's ability to heat the HP-based bleaching gel [48], thereby increasing its decomposition rate to form free radicals capable of interacting with the dental structure, the physicochemical characteristics demonstrate the opposite.

As expected, lower amount of HP is available in the HP 6%, lesser bleaching effect was observed when compared to higher concentrated HP evaluated [41]. In fact, as the bleaching effect occurs due to structural oxidation by HP, the higher the concentration, the greater the bleaching effect [29, 52, 53]. It's worth to mention that the bleaching values observed in the present study are similar to previous studies [32, 40, 41, 54].

While all groups that underwent tooth bleaching showed color changes, their efficacies differed significantly. HP 6% both with and without violet light, exhibited very good efficacy [55]. Similarly, HP 35% groups, regardless of violet light use, demonstrated exceptional efficacy [55]. This classification is based on the number of times the 50:50 acceptability limits stipulated for ΔE_{ab} (2.7) and ΔE_{00} (1.8) are exceeded [55]. It is noteworthy to mention that although these formulas have been widely used, the ΔWI_D stands as the most current and accurate method for evaluating tooth bleaching [30, 37]. Nonetheless, the other parameters remain valuable for comparisons with previous studies.

The lower bleaching efficacy observed with 6% HP gels, even when using violet light, can be considered a disadvantage. In-office dental bleaching is often chosen for its ability to produce immediate results. However, an alternative approach could involve conducting more bleaching sessions using 6% HP. In fact, several in vitro studies have demonstrated that a simple way to enhance the bleaching efficacy when using bleaching gels (17–20%) is to increase the number of bleaching sessions [56]. However, it is not clear in the existing literature how many sessions are needed for 6% HP gels to achieve the same bleaching results as high-concentration in-office gels. Future studies are required to explore this hypothesis.

It's crucial to highlight that this in vitro study doesn't encompass certain clinical features, such as simulating pulp pressure during bleaching. This was not feasible because the acetate buffer solution in the pulp chamber scavenges HP before it reaches the pulp, hindering measurement. We therefore acknowledge this as a limitation of the present study. This is the main reason that an in vitro study, as the present one, it can't be extrapolated for any in vivo situation. Although two different commercial products were evaluated, the results should not be extrapolated to all commercial brands, and this should be considered a limitation of the present study. Therefore, future studies assessing different commercial brands should be conducted. These data also cannot be extrapolated to gels that are doped with substances where violet light can act and thus provide benefits in radical formation.

Conclusion

Using violet light in conjunction with 6% or 35% HP does not alter the physical properties of the bleaching agents, does not reduce the penetration of HP, and does not enhance color change. However, an increase in temperature was observed when violet light was applied associated to bleaching gels.

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Declarations

Ethical approval The clinical investigation was approved (5.731.728) by the scientific review committee and by the committee for the protection of human participants of the State University of Ponta Grossa (PR/Brazil).

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