Received: 20 June 2023 | Revised: 9 August 2023 | Accepted: 28 August 2023

DOI: 10.1111/jerd.13133

RESEARCH ARTICLE

WILEY

In vitro evaluation of the effect of different bleaching varnishes: Hydrogen peroxide penetration into the pulp chamber and color change

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Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Abstract

Objective: To evaluate the penetration of hydrogen peroxide (HP) into the pulp chamber and the color change of different bleaching varnishes in low concentrations used for at-home bleaching.

Materials and Methods: Ninety healthy premolars were used, randomly distributed into nine groups ($n = 10$) according to bleaching varnish (PL, PolaLuminate; VS, VivaStyle Paint On Plus; CA, Cavex Bite&White whitening pen and; AW Aligner-White) and time (10 and 30 min), and a control group (no bleaching). The penetration of HP was evaluated by UV–Vis spectroscopy. To evaluate the color change (ΔE_{ab}, ΔE_{00}, ΔWI_D) a digital spectrophotometer was used ($\alpha = 0.05$).

Results: The AW group in 10 min and the control group showed similar and lower HP penetration in the pulp chamber when compared to the other groups ($p = 0.003$). Increasing the application time to 30 minutes elevated the amount of HP inside the pulp chamber for all groups ($p = 0.003$), except for PL ($p > 0.05$). When applied for 30 min all bleaching varnishes showed higher color change (ΔWI_D) when compared to 10 min ($p = 0.04$).

Conclusions: For all bleaching varnishes evaluated, PolaLuminate applied for 30 min showed lower penetration into the pulp chamber and higher bleaching effects.

Clinical Significance: The use of bleaching varnishes seems promising for teeth bleaching, but it varies according to user product and protocol.

KEYWORDS
bleaching agents, dental enamel permeability, hydrogen peroxide, In vitro techniques, tooth bleaching

1 | INTRODUCTION

Cosmetic procedures, such as tooth bleaching, have increased patients' self-confidence and improved emotional well-being,[1] making the procedure increasingly popular.[2,3] In a recent study, it was observed that 86% of the participants underwent the bleaching technique on their initiative and not at the dentist's suggestion.[4]

Different tooth bleaching techniques on vital teeth performed or supervised by the dentist are described in the literature in search of color change.[5–8] Among them, the most established is the in-office

bleaching which uses higher concentrations of hydrogen peroxide (HP) (up to 40%),[9–13] the gel application being performed by the dentist,[14] and the at-home bleaching, which uses lower concentrations of HP (up to 10%), performed by the patient himself with the application of bleaching gel in custom trays.[15] However, both techniques showed adverse effects and tooth sensitivity induced by bleaching was among the most prevalent.[16]

Tooth sensitivity induced by bleaching can be explained by damage to pulp cells and oxidative stress caused by HP and/or by-products that manage to reach the pulp due to their low molecular weight.[17–26] Given this fact, there is a tendency to decrease the concentration of HP to be applied, and some studies demonstrate that concentrations of around 6% would be ideal because they produce lower aggression to the metabolism and cell morphology.[27,28] It has already been demonstrated that peroxide of hydrogen at high concentrations may have more cytotoxic effects on MDPC-23 cells[29] than at low concentrations.[30]

Thus, 6% HP has become the maximum allowable concentration in some places around the world,[31] and different forms of application for this concentration were developed by manufacturers, not only for the traditional at-home bleaching with trays[24] but also in-office bleaching[32] and at-home bleaching through varnishes.[33–40]

The use of bleaching varnishes has several advantages, such as disposing of the need to make a custom tray for bleaching or facilitating the day-to-day for both the clinician and the patient. Also, it is easy to use by the patient himself because all commercial brands include an applicator, similar to the brushes used to apply nail polish. It has already been demonstrated that when a brush applicator was used, a thin layer was achieved due to the increase in the contact surface of the HP with the tooth.[32,41] Recently, at least two studies showed that the thinner the bleaching layer, the less penetration of HP into the pulp,[32,41] and this makes the use of bleaching varnishes seemingly promising.[33]

As far as the authors are aware, and due to the recent launching of some commercial brands, in vitro studies evaluating several bleaching varnishes and the different application times indicated by the manufacturers have not yet been carried out. With this in mind, this in vitro study's objective was to evaluate HP's penetration into the pulp chamber and the color change through different bleaching varnishes with HP around 6% and application times. The null hypothesis to be tested is that: (1) there will be no difference in HP penetration, and (2) there will be no difference in color change using different bleaching varnishes at different application times.

2 | MATERIALS AND METHODS

The study was submitted to the Ethics Committee of the State University of Ponta Grossa, PR, Brazil, which approved it under number 5.778.595. Ninety healthy premolars with similar sizes were obtained from Human Teeth Bank of the State University of Ponta Grossa. A stereomicroscope (Lambda LEB-3; ATTO instrument, Hong Kong, China) was used to analyze the enamel surface at 10× magnification. Teeth with morphological changes or enamel cracks were excluded. The teeth were previously analyzed with a digital spectrophotometer (VITA Easyshade Advance 4.0; VITA Zahnfabrik, Germany), and teeth lighter than A_2 were also excluded. To standardize the buccal surface thickness of the specimens and to prevent this thickness from influencing the penetration of HP, radiographs were taken (Timex 70C; Gnatus, Ribeirão Preto, 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 surface thickness was measured with the New IDA software (Dabi Atlante, Ribeirão Preto, Brazil), teeth with a thickness of <2.5 and >3.5 mm were excluded.[42,43]

2.1 | Sample size calculation

The primary outcome of this study was the amount of HP within the pulp cavity. Based on a previous study where the penetration value of 6% of HP was 0.033 μg/mL with a deviation of 0.016 μg/mL,[18,32] for control group (30 min). Taking into consideration that in the experimental group, the bleaching materials were applied for 10 min, i.e., for only 35% of the application time previous evaluated (30 min), it was expected a decrease of the amount of HP inside the pulp chamber of 65% (0.012 μg/mL) in the experimental group. Using a bilateral test with an alpha of 5% and a study power of 80%, ten teeth were needed for each experimental group.

Nevertheless, the sample size was also determined based on color change (secondary outcome) as per the whiteness index for dentistry (ΔWI_D).[8] A previous study showed that the ΔWI_D value of 6% of HP was 5.7 with a deviation of 2.1.[32] Taking into consideration that the acceptability threshold (AT) for ΔWI_D is 2.6,[6] that means, it was occur a bleaching considered clinically acceptable and using a bilateral test with an alpha of 5% and a study power of 80%, 10 teeth were needed for each experimental group.

2.2 | Experimental groups

The selected teeth were randomly assigned into nine groups ($n = 10$). A group not exposed to bleaching agents was the control (GC). The other groups were divided according to the commercial brands and time of application, as follows: PL (Pola Luminate HP 6%; SDI); VS (VivaStyle Paint On Plus HP 6%; Ivoclar Vivadent, Schaan, Liechtenstein); CA (Cavex Bite&White whitening pen HP 6%; Cavex Holland BV, Netherlands) and AW (Aligner White HP 5%, EverSmile; Garden Grove, CA, USA). All products were applied for 10- or 30-min.

2.3 | Specimen preparation

The roots of the teeth were removed approximately three millimeters from the enamel-cement junction, using a low-speed diamond disk (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA). The pulp tissue was removed, and the pulp chamber was rinsed with deionized water. The access to the

pulp cavity was slightly expanded using a #1014 spherical drill (KG Sorensen, Serra, ES, Brazil), taking care not to touch the inner vestibular region of the pulp cavity. This was done with the purpose of introducing 25 µL of solution inside, using a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland). After specimen preparation, new radiographs (Timex 70C; Gnatus) were taken to verify the buccal surface thickness of the specimens by groups measured with the New IDA software (Dabi Atlante).[32,41] The premolars were procured from the Human Teeth Bank of the same university when all teeth were stored in distilled water. Only teeth extracted within the last 6 months were selected for use in the present study. After the preparation of the specimens, 1 week before the start of the study, all teeth were stored in artificial saliva.

2.4 | Obtaining the analytical curve and quality control measurements

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 µg/mL stock solution prepared from a concentrated solution (50% HP; Pharmacy Eficácia, Ponta Grossa, PR, Brazil). This solution was diluted in an acetate buffer solution (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 0.000–0.403 µg/mL were performed to draw the analytical curve. The known concentrations of HP 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 ($R = 0.992$; not shown data).[32,41]

For quality control measurements, the pH and initial concentration were measured. The pH of each bleaching agent was measured with a pH meter placed directly in contact with a bleaching gel (Extech pH 100: ExStik pH Meter; Extech Instruments, Nashia, NH, USA; Table 1).[44] To determine the initial concentrations of HP, the bleaching gels used in the study were titrated with a standardized solution of potassium permanganate before the bleaching procedure, as described in the literature[18] and compared with those provided by the manufacturers.

2.5 | Treatment bleaching protocols and HP quantification into the pulp chamber

A single experienced and calibrated operator, blinded to the assigned groups, was responsible for the application of the material. For all groups, the specimens were vertically fixed to a wax plate with the occlusal surface toward the plate. Before the bleaching agent was applied, the buccal surface of each specimen was isolated by applying a light-cured resin barrier enclosing an area of 6 mm^2 (Topdam, FGM Dental Group, Joinville, SC, Brazil). A 25 µL aliquot of the acetate buffer (pH = 4) was inserted into the pulp chamber of each tooth to preserve and absorb all the HP that entered the pulp chamber during bleaching procedures.

In the groups that received bleaching treatment, the varnishes were applied in the region of the vestibular enamel according to the different experimental groups. The bleaching varnishes were applied until they completely covered the buccal area of the teeth to be whitened. For all bleaching varnishes, a thin layer of bleaching varnish was applied for 10 or 30 min according to each experimental group. For all groups, the bleaching varnishes were applied for 14 days. Each product was removed with gauze and carefully washed with deionized water only on the vestibular surface. The control group was kept out of contact with bleaching agents.

After performing the first application of bleaching varnish, the acetate buffer solution in the pulp cavity of each sample was removed immediately after the bleaching session, according to the application time to be tested. The removed solutions were transferred to a glass tube. To remove the maximum amount of HP, 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

TABLE 1 Means (± standard deviations) of the buccal surface thickness, initial concentration, pH of varnishes, and hydrogen peroxide (HP) concentration detected inside the pulp chamber in different experimental groups.

Bleaching varnishes	Bleaching time (min)	Buccal thickness (mm)[a]	Initial concentration (%)	pH	HP (µg/mL)[a]
PL	10	3.0 ± 0.2 A	5.6	5.7 ± 0.1	0.034 ± 0.024 B
	30	3.3 ± 0.3 A			0.030 ± 0.030 B
VS	10	3.5 ± 0.6 A	6.8	5.0 ± 0.0	0.039 ± 0.040 B
	30	3.4 ± 0.3 A			0.077 ± 0.077 C
CA	10	3.3 ± 0.6 A	4.7	5.4 ± 0.2	0.023 ± 0.024 B
	30	3.3 ± 0.4 A			0.068 ± 0.045 C
AW	10	3.2 ± 0.5 A	4.6	4.9 ± 0.1	0.005 ± 0.004[b] A
	30	3.2 ± 0.3 A			0.040 ± 0.031 B
Control	–	3.2 ± 0.4 A	–	–	0.001 ± 0.001[b] A

[a]Identical capital letters in each column indicates statistically similar means (Two-way ANOVA and Tukey's test, $\alpha = 0.05$).
[b]The control group and Aligner White were statistically similar and statistically different when compared to all groups (Two-way ANOVA and Dunnet's test, $\alpha = 0.05$).

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 (Peroxidase Type VI-A, Sigma Chemical Co.) were added to the glass tube, along with deionized water (2.725 μL). This sequence was repeated separately for each tooth at different times. The resulting solution was measured using a Cary 50 UV–Vis spectrophotometer (Varian). Despite other available methods in the literature,[25] this is the most used to measure the amount of HP inside the pulp chamber.[9–11,18,19,22,23,27–29,32,33,41–43] According to the Beer Law, absorbance directly corresponds to the concentration. Therefore, the concentration of HP (μg/mL) was determined by comparing it with the calibration curve previously obtained.[32,41]

2.6 | Color change evaluation

The color change was measured before and after 14 days of the bleaching procedure. It was performed using a digital spectrophotometer (VITA Easyshade Advance 4.0; VITA Zahnfabrik). To measure the initial color of the specimens, guides were made with dense condensation silicone (Coltoflax and Cub Kit Profile; Vigodent, Rio de Janeiro, RJ, Brazil), and a 6 mm diameter window was made with a metal device in the middle third of the buccal surface of each specimen to standardize the position of the tip of the spectrophotometer. During this period, the specimens were immersed in artificial saliva. Daily changes of 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^* value represented the green-red coordinate ($-a^*$ green and $+a^*$ red), and the b^* value represented the blue-yellow coordinate ($-b^*$ blue and $+a^*$ yellow). The color change before (baseline) and after bleaching was given by the difference between the colors measured with the spectrophotometer using the following CIELab formulas: CiELab (Delta E_{ab}) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$,[45] and CIEDE 2000 (Delta E_{00}) = $[(\Delta L/kLSL)^2 + (\Delta C/kCSC)^2 + (\Delta H/kHSH)^2 + RT\ (\Delta C^*\Delta H/SC^*SH)]^{1/2}$.[7] Also, the Whiteness index for Dentistry was calculated according to the following formula: $WI_D = 0.551 \times L - 2.324 \times a - 1.1 \times b$. Moreover, changes in WI_D caused by each step were calculated by subtracting the values observed at each assessment time from those calculated in the prior step (ΔWI_D).[8]

2.7 | Statistical analysis

Data were analyzed using the Kolmogorov–Smirnov test to assess whether the data had a normal distribution and using the Barlett test for equality of variance to verify the hypothesis of equality of variances (data not shown). As the data showed normality and homoscedasticity of variances, the thickness of buccal surface (mm), amount of HP concentration (μg/mL) detected in the pulp chamber, as well as color change (ΔE_{ab}, ΔE_{00}, and ΔWI_D) were submitted to two statistical evaluations: (1) two-way ANOVA (bleaching varnish vs. bleaching time) and Tukey's post hoc test to compare different bleaching varnishes and; (2) two-way ANOVA (bleaching varnish vs. bleaching time) and Dunnet's post hoc test to compare the values obtained in different bleaching varnishes with those of the control group ($\alpha = 0.05$).

3 | RESULTS

3.1 | Buccal surface thickness and hydrogen peroxide quantification into the pulp chamber measurements

Regarding the buccal surface thickness, the cross-product interaction (bleaching varnishes vs. bleaching time), as well as the main factors were not statistically significant ($p < 0.24$; Table 1). The mean and standard deviation of the buccal surface thickness of the specimens, according to the radiographs taken for all groups are described in Table 1.

The analysis of the hydrogen peroxide (HP) content within the pulp chamber revealed a statistically significant cross-product interaction between bleaching varnishes and bleaching time ($p = 0.003$; Table 1). Regarding the factor "bleaching varnishes", the control group and the AW group at 10 min showed a smaller amount of HP in the pulp chamber compared to the other experimental groups. In contrast, the VS and CA groups displayed a notably higher and more significant HP content within the pulp chamber when subjected to a bleaching time of 30 min (Table 1). Regarding the factor "bleaching time", the PL groups exhibited no significant difference in the amount of HP within the pulp chamber. In contrast, the VS, CA, and AW groups demonstrated a considerable and significant increase in the amount of HP within the pulp chamber when subjected to a bleaching time of 30 min ($p = 0.003$; Table 1).

3.2 | Color change assessment

The color changes observed in the different experimental groups are presented in Table 2. Statistical analysis revealed a significant cross-product interaction between bleaching varnishes and bleaching time for both ΔE_{ab} and ΔE_{00} ($p = 0.03$ and $p = 0.01$, respectively; Table 2). Regarding the factor "bleaching varnishes", a smaller color change was evident in the control group, exhibiting lower ΔE_{ab} and ΔE_{00} values in comparison to all experimental groups (Table 2; $p = 0.03$ and $p = 0.01$ respectively). Notably higher and significant color changes (ΔE_{ab} and ΔE_{00}) were observed in the VS 30 min and CA 10- and 30-min groups when compared to the other groups. Irrespective of bleaching time, no significant differences were observed in terms of color change as measured by ΔE_{ab} and ΔE_{00} for PL, AW, and CA (Table 2).

Regarding ΔWI_D, the cross-product interaction (bleaching varnishes vs. bleaching time) was not statistically significant ($p = 0.95$), but both main factors displayed significance ($p = 0.002$ and 0.04,

TABLE 2 Means (± standard deviations) of the color change in different objective assessments (ΔE_{ab}, ΔE_{00}, and ΔWI_D) in different experimental groups.[a]

Bleaching varnishes	Bleaching time (min)	ΔE_{ab}	ΔE_{00}	ΔWI_D[c]
PL	10	3.4 ± 1.3 B	2.1 ± 0.8 b	−0.9 ± 4.4 ^{Aab}
	30	2.9 ± 1.6 B	1.9 ± 1.0 b	3.1 ± 5.7 ^{Bab}
VS	10	3.7 ± 1.8 B	2.3 ± 1.0 b	2.3 ± 3.1 ^{Aa}
	30	6.4 ± 2.1 A	4.0 ± 1.4 a	6.0 ± 1.7 ^{Ba}
CA	10	6.5 ± 1.9 A	3.9 ± 0.8 a	−2.0 ± 7.2 ^{Aab}
	30	6.1 ± 1.8 A	4.0 ± 1.2 a	1.9 ± 5.8 ^{Bab}
AW	10	3.5 ± 1.4 B	2.3 ± 1.2 b	−3.6 ± 6.1 ^{Ab}
	30	4.6 ± 2.0 B	3.0 ± 0.8 b	2.1 ± 7.2 ^{Bb}
Control[b]	–	1.3 ± 0.8	0.9 ± 0.5	−0.5 ± 1.2

[a]Identical letters indicate statistically similar values among the groups in each column. (Two-way ANOVA and Tukey's test, $\alpha = 0.05$).
[b]The control group was statistically different when compared to all groups in each column (Two-way ANOVA and Dunnet's test, $\alpha = 0.05$).
[c]For ΔWID, identical capital letters indicate statistically similar values for different bleaching time in each bleaching varnish and identical lower case letters indicate statistically similar values for different bleaching varnishes in each bleaching time.

respectively; Table 2). Among the bleaching varnishes, VS exhibited a significantly higher color change, showing a difference only when compared to AW ($p = 0.002$; Table 2). Furthermore, for all bleaching varnishes, significantly higher color changes were detected with a 30-minute application ($p = 0.04$; Table 2).

4 | DISCUSSION

Low-concentration bleaching varnishes seem promising since they are used quickly and do not require trays,[33] making day-to-day life easier for patients and clinicians. Different bleaching varnishes are available on the market, and few have been described in the literature.[35–37] Therefore, this is the first study that compares different bleaching varnishes in terms of penetration into the pulp chamber and color change. The present study evaluated the buccal surface thickness of all analyzed groups. This is important because it has already been described that the tooth's thickness influences HP's penetration.[21] According to the results, no significant difference in buccal surface thickness among groups was observed.

The results of the present study, in terms of HP penetration, showed that significant differences were observed when different bleaching varnishes and times were evaluated, leading to the no acceptance of the first null hypothesis. However, several differences between materials and protocols were observed. HP penetration may be related to tooth sensitivity, as previously described, and may depend on some variables, such as the concentration of the bleaching agent and the application time.[18,19,32]

Regarding the bleaching varnishes, note that the AW showed low penetration of HP inside the pulp chamber when applied for 10 min, like that of the control group. This may be related to the viscosity of AW since, from the clinical point of view, it is the most viscous varnish among those evaluated. When the material is very viscous, there is not enough release of HP. Consequently, the lower HP release of the material could be responsible for the lower HP inside the pulp chamber.[19,32] As pointed out previously, not only the viscosity can affect the amount of hydrogen peroxide released, but also solvent, matrix composition, and type of application could be considered as potential factors to influence the kinetics of release of hydrogen peroxide.[25] Unfortunately, only a few studies evaluated the viscosity of bleaching gels, which did not allow the authors give more explanations.[13,26] It is worth mentioning that, except for AW applied for 10 min, all the values of HP penetration observed in the present study are in accordance with the previous literature.[18,32,34]

When different application times were compared, an increase in the amount of HP inside the pulp chamber was registered for three of the four bleaching varnishes when applied for 30 min. The longer the bleaching agent remains in contact with the tooth surface, the greater penetration has been observed when peroxide is still available,[22,27] which can be explained by HP's diffusion dynamics in the dental tissues.[20]

The unique exception was PL, which showed no significant differences when comparing both application times. The pH can explain this. It is well known that the higher the pH of a bleaching material, the lower the penetration of HP inside the pulp chamber.[9,23] It should be noted that PL exhibited a higher pH (5.7) compared to the other evaluated bleaching varnishes. Typically, the pH of bleaching gels is highly acidic to enhance the product's shelf life.[24] However, more acidic gels can have detrimental effects on the enamel surface,[9,12] and enhance the diffusion of HP into the pulp, as previously observed for in-office bleaching gels.[10,23]

Regarding the color change, it was expected that the color change would be similar because the bleaching varnishes tested had similar concentrations of HP. However, according to the manufacturer's recommendation, the application time of the bleaching varnish is relatively short, therefore, it was not expected significant differences between 10- and 30-min. Despite that, the results of the present study showed that significant differences were observed for a color change when different bleaching varnishes were applied at various times on the tooth surface, leading to the no acceptance of the second null hypothesis.

Different formulae were used to evaluate color change in the present study. The ΔE_{ab} was used because it is the most widely used parameter in bleaching studies.[5] The ΔE_{00}, a formula improvement of ΔE_{ab}, is more faithful to color differences as the human eye can perceive them.[5,7] When the different experimental groups were compared, similar results were observed when ΔE_{ab} or ΔE_{00} were applied. However, although the ΔE_{ab} and ΔE_{00} can give the magnitude of color change, they do not provide information on the direction of the color change, whether whiter or darker. WI_D indicates the degree of whitening toward the lighter end of the scale.[6,8]

Despite some differences among bleaching varnishes and application time, when the color change was evaluated by ΔE_{ab} or ΔE_{00}, for all experimental groups, the color change was higher than the 50:50 acceptability (AT) threshold for ΔE_{ab} (AT = 2.7) or ΔE_{00} (AT = 1.8). The AT value represents an existing difference acceptable to most people. This means that a perceptible color change occurs, and the results are similar to those observed in the two studies.[27,34] Nevertheless, a closer view regarding the ΔWI_D showed that for all bleaching varnishes applied for 10 min and for CA and AW applied for 30 min, the values observed did not reach the AT threshold value (2.6) for ΔWI_D.[6] This means that, despite some bleaching occurring, it should not be considered clinically acceptable. However, when a longer application time (30 min) is performed for PL and VS, the bleaching values exceed the AT threshold value. This finding seems to be the most important for color change since the Whiteness Index for Dentistry is recently used specifically to evaluate how much the teeth became whiter.[8]

This does not imply that CA and AW should not be used. In fact, this suggests that it is necessary to prolong the application duration of these bleaching varnishes to achieve improved bleaching outcomes. For example, following the manufacturer's recommendations, CA should be applied twice daily, and AW should be used for a duration of 8 hours. Increasing the application time is likely to yield better results. Future studies need to be conducted to evaluate the bleaching effect of CA and AW when applied according to the manufacturers' recommendations.

Unfortunately, there is a high variation in manufacturer's instructions for the materials evaluated in the present study. Also, despite an increase in the availability of bleaching varnishes on the market, only a few studies were found and usually evaluated only one commercial material (VS).[38–40] Therefore, future studies need to be performed to assess these hypotheses.

The exception of higher values than ΔWI_D AT threshold was observed for PL and VS applied for 30 min. Despite that, a closer view of the ΔWI_D results showed that for all bleaching varnishes, a significant and higher color change occurred when 30 min was compared to 10 min. This indicates that the increase in time of application should improve the final bleaching results, as previously observed.[11] When different varnish bleaching was compared, significant differences were only observed between VS and AW. The former is more fluid than AW, leading to the fastest release of hydrogen peroxide.[38] Also, VS showed a higher initial concentration (6.8%) when compared to all varnishes evaluated.

Finally, it is essential to clarify that, despite a better whitening effect observed for PL and VS when applied for 30 min, only the manufacturer of the former indicates the application for 30 min. Also, VS showed twice as much HP in the pulp chamber as PL, mainly when evaluated for 30 min. Therefore, considering both properties, PL seems to be better than the other bleaching varnishes evaluated.

Although, to the authors' knowledge, this is the first study to evaluate different bleaching varnishes at different times, one of the study's limitations can be attributed to using only four commercial brands. Since the differences in the composition of bleaching varnishes from other brands may differ from the results presented in this study. In addition, there is huge variability in the application times of the varnishes, since only two times of use were tested. Furthermore, since the collection of the permeability solution was conducted immediately after the final application time, it is important to note that all interpretations of the results in the present study should be confined to the immediate time point. Therefore, further studies are needed to evaluate bleaching varnishes that may be launched. Given this, and based on these results, randomized clinical trials must be developed to verify the advantages of using different bleaching varnishes clinically.

5 | CONCLUSIONS

Despite the numerous differences observed among the various combinations of bleaching varnishes and application times assessed, the following conclusions can be drawn:

1. A lower amount of hydrogen peroxide within the pulp chamber was noted when all bleaching varnishes were applied for 10 min over a span of 14 days. However, none of these treatments achieved a clinically acceptable bleaching outcome.
2. Only two of the tested bleaching varnishes, namely Pola Luminate HP 6% and VivaStyle Paint On Plus HP 6%, exhibited a clinically acceptable level of bleaching when applied for 30 min over a period of 14 days. Nonetheless, Pola Luminate HP 6% demonstrated a lower hydrogen peroxide content compared to VivaStyle Paint On Plus HP 6% when both were applied for a duration of 30 min.

ACKNOWLEDGMENTS

This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 304817/2021-0 and 308286/2019-7 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any financial interest in the companies whose materials are included in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Carneiro TS, Favoreto MW, Mena-Serrano A, et al. In vitro evaluation of the effect of different bleaching varnishes: Hydrogen peroxide penetration into the pulp chamber and color change. *J Esthet Restor Dent*. 2023;1-8. doi:10.1111/jerd.13133