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Article

Evaluation of Internal Bleaching with 35% Hydrogen Peroxide in Dentin Conditioned with 37% Phosphoric Acid and 17% EDTA.

Evaluación del blanqueamiento interno con peróxido de hidrógeno al 35% en dentina acondicionada con ácido fosfórico al 37% y EDTA al 17%.

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Abstract: Introduction: This study aimed to evaluate the effect of dentin surface treatment with 37% phosphoric acid or 17% ethylenediaminetetraacetic acid (EDTA) before Internal Bleaching with 35% hydrogen peroxide using the walking bleach technique. **Material and Methods:** This experimental *in vitro* study used 66 human premolars extracted for orthodontic reasons, which were debrided, endodontically prepared, and pigmented with chromogens derived from blood decomposition. The samples were randomly divided into three groups (n=22). Group A: bleaching agent without dentin conditioning; group B: bleaching agent in dentin conditioned with phosphoric acid 37%; group C: bleaching agent in dentin conditioned with 17% EDTA. 4 applications of bleaching agent were used with a separation of 4 days between each session. The initial color (baseline) and after each application was determined by spectrophotometry, recording the CIE L*a*b* values and the total color variation between the initial parameters and the different evaluation times. **Results:** Data were statistically analyzed with the Wilcoxon test. This showed statistically significant differences for the total variation of the color between the study groups, with the control group in no case inferior to the rest. **Conclusion:** The application of 37% phosphoric acid increased the effectiveness of the bleaching agent when compared to 17% EDTA. However, these did not increase the effectiveness compared to the application of the bleaching agent without a previous dentin surface treatment.

Keywords: *Hydrogen Peroxide; EDTA; Dental Acid Etching, Teeth Bleaching, Spectrophotometry.*

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Resumen: Introducción: El objetivo de este estudio fue evaluar el efecto del tratamiento de superficie dentinaria con ácido fosfórico al 37% o EDTA al 17% previo al blanqueamiento Interno con peróxido de hidrógeno al 35% por medio de la técnica Walking Bleach. **Material y Métodos:** Para este estudio experimental *in vitro*, se utilizaron 66 premolares humanos extraídos por indicación ortodóncica, los cuales fueron desbridados, preparados endo-

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dónticamente, y pigmentados con cromógenos derivados de la descomposición sanguínea. Las muestras fueron divididas aleatoriamente en 3 grupos (n=22). Grupo A: agente blanqueador sin acondicionamiento dentinario, grupo B: agente blanqueador en dentina acondicionada con ácido fosfórico 37% y grupo C: agente blanqueador en dentina acondicionada con EDTA 17%. Se utilizaron 4 aplicaciones de agente blanqueador con una separación de 4 días entre cada sesión. El color inicial (baseline) y tras cada aplicación fue determinado mediante espectrofotometría, registrando los valores CIE L*a*b* y la variación total de color entre los parámetros iniciales y los diferentes tiempos de evaluación. **Resultados:** Los datos fueron analizados estadísticamente con la prueba de Wilcoxon, arrojando diferencias estadísticamente significativas para la variación total del color entre los grupos de estudio, siendo en ningún caso el grupo control inferior al resto. **Conclusión:** La aplicación de ácido fosfórico al 37% aumenta la eficacia del agente blanqueador al compararlo con el EDTA 17%, sin embargo, no aumentan la eficacia respecto a la aplicación del agente blanqueador sin un tratamiento de superficie dentinaria previo.

Palabra Clave: *Peróxido de Hidrógeno, EDTA, Grabado Ácido Dental, Blanqueamiento Dental, Espectrofotometría.*

INTRODUCTION.

Tooth color alteration is a frequent aesthetic problem, mainly attributed to dentoalveolar trauma or the permanence of endodontic filling materials in the pulp chamber for prolonged periods.[1] Color defects can be corrected by different procedures, with tooth bleaching the least invasive and conservative procedure to modify the color and appearance of both vital and devitalized teeth.[2,3]

In the case of devitalized teeth, Internal bleaching based on peroxides employing the walking bleach technique is an effective and conservative alternative. However, it requires an indeterminate number of clinical sessions, which is not currently standardized and depends on the clinical case or the manufacturer's recommendations.[4]

Peroxide-based bleaching agents must have a low molecular weight and the ability to denature proteins to penetrate dentin tissue and enamel.[5] Therefore, the success of whitening is directly related to the degree of transdentinal penetration of these agents.[6] The use of rotary instruments, necessary to generate the intrachamber space for the bleaching agent, produces smear layer. This consists of a mixture of dentin particles, remaining odontoblastic extensions, pulp tissue, and bacteria.[7] The permanence of this smear layer on the dentin surface reduces its permeability[8] and therefore could decrease the diffusion of bleaching agents, affecting the final bleaching result.

Ethylenediaminetetraacetic acid (EDTA) and phosphoric acid are effective agents in the removal of the smear layer,[9-11] which could favor the diffusion of bleaching agents by increasing tubular permeability.[11]

Given this, the objective of the present study was to evaluate the effect of dentin surface treatment with 37% phosphoric acid or 17% EDTA before Internal bleaching with 35% hydrogen peroxide using the walking bleach technique.

The null hypothesis was that surface treatment with 37% phosphoric acid and 17% EDTA before the application of 35% hydrogen peroxide through the walking bleach technique would not show differences compared to the application of the bleaching agent on an untreated dentin surface.

MATERIALS AND METHODS.

This study was submitted to the Scientific Ethics Committee of the Faculty of Dentistry of the Andrés Bello University, Viña del Mar, Chile. On September 25, 2018, the committee authorized its implementation and execution under resolution number 102018.

This experimental *in vitro* study was blinded in the measurement of the effect and the data analysis. The study used upper and lower premolars extracted for orthodontic reasons and immediately preserved in physiological saline, which were collected during 2018 in various health institutions of the Valparaiso region and the Metropolitan region (Chile).

Calculation of sample size

To determine the minimum sample size (n), the formula for comparison of means employed was $n=2 \times (Z\alpha + Z\beta)2 \times S2/d2$, where $Z\alpha=1.645$ in the one-sided case of 95%

confidence, and Ƶβ=0.842 for a statistical power of 80%. A standard deviation (S) of 3.8 was considered, taking as reference the results proposed in the meta-analysis and systematic review by Luque-Martínez *et al*.,[12] The maximum difference in color change (d) was considered equal to 5 units, based on the results obtained by Bizhang *et al*.,[13] who concluded that dental bleaching is effective when a total color variation (delta E [DE]) of at least 5 units occurs. These parameters resulted in a sample size of 11 units per group, including a loss ratio of 10%. Due to the successful sample collection, n=22 was finally used for each study group.

Standardization of samples

The samples were chemically disinfected with a gauze moistened with 2.25% sodium hypochlorite and subsequently mechanically debrided using ultrasonic periodontal tips and prophylaxis brushes with pumice stone, eliminating the remains of organic and inorganic material. After debridement, endodontic opening was performed with high speed using a 0.16 round bur (Microdont, São Paulo, Brazil) and an Endo-Z bur (Dentsply Maillefer, Ballaigues, Switzerland).

The biomechanical preparation was performed with Gates Glidden drills #1, #2, and #3 (Dentsply Maillefer) for the cervical and middle third of the canal, and the apical third was prepared with K files (Dentsply Maillefer) up to a #45 instrument. The biomechanical preparation used 10 milliliters (mL) of 2.25% sodium hypochlorite, the main irrigant recommended for the chemical disinfection of root canals.[14] Finally, the canals were dried with paper points (Dentsply Maillefer), and #45 gutta-percha cones with 2% taper (Dentsply Maillefer) were adjusted, cemented with AH Plus (Dentsply Sirona), and adjusted with the lateral compaction technique, using a #35 spreader and #30 accessory gutta-percha cones. The residual obturator material was cut 2 millimeters (mm) below the amelo-cemental limit, sealing the cervical area with Riva Light Cure glass ionomer cement (SDI, Victoria, Australia).

Staining and mounting of samples

The staining of the samples used the method proposed by Freccia *et al*.,[15] consisting of the use of fresh human blood voluntarily donated by the study participants. Once the blood was separated from the serum, the tubes were filled with the teeth, ensuring that they were completely immersed in blood. They were centrifuged at 3,200 revolutions per minute for 20 minutes at 37 degrees Celsius (°C), twice a day for 3 days, to accelerate hemolysis and allow blood breakdown products to penetrate the dentin tubules. The teeth remained inside the tubes with blood during the 3 days. Once the staining protocol was finished, cotton was placed in the operated pulp chamber and the cavities were superficially sealed with Fermin (Detax GmbH & Co., Ettlingen, Germany). The samples were stored in containers with distilled water at room temperature to prevent dehydration.

The samples were mounted up to the middle third of the root in Marché self-curing white acrylic (Félix Martín y Cía. Ltda, Santiago, Chile) using an Elite HD silicone matrix (Zhermack, Badia Polesine, Italy) to obtain a base in the shape of a cube. The clinical crowns were stamped with 3-mm-thick sheets of soft acetate, obtaining a matrix of each sample. These were perforated in the middle third of the vestibular region using a 6-mm diameter biopsy punch (Kai Medical, Seki, Japan), obtaining a standardized matrix that guaranteed the same measurement point in each of the samples (Figure 1A, Figure 1B and Figure 1C).

Study group conformation and color evaluation

The samples were randomly divided in an Excel spreadsheet (Microsoft Office 2016, Seattle, USA) into three groups (n=22).

Group A: 35% hydrogen peroxide, Opalescence Endo (Ultradent Products Inc., Utah, USA);

Group B: dentin conditioning with 37% phosphoric acid, Scotchbond (3M, Minnesota, USA) + 35% hydrogen peroxide, Opalescence Endo (Ultradent);

Group C: dentin conditioning with 17% EDTA (Química Hertz, Santiago, Chile) + 35% hydrogen peroxide, Opalescence Endo (Ultradent). Once the groups were obtained, the initial color (baseline) of all the samples was measured using an Easyshade V spectrophotometer (VITA, California, USA), recording the CIE L*, a*, and b* values.

For calibration, the researchers initially performed two theoretical–practical activities on the application of bleaching agents according to the instructions of each manufacturer and on the use of a spectrophotometer and color measurement. All samples were subjected to four whitening sessions with an interval of 4 days between each session. For group A, the bleaching agent was applied directly to the pulp chamber without prior surface conditioning. For group B, the intrachamber dentin was conditioned with 37% phosphoric acid (Scotchbond, 3M) for 15 seconds (Figure 2A), sufficient time to increase the permeability of the dentin tubules and promote the

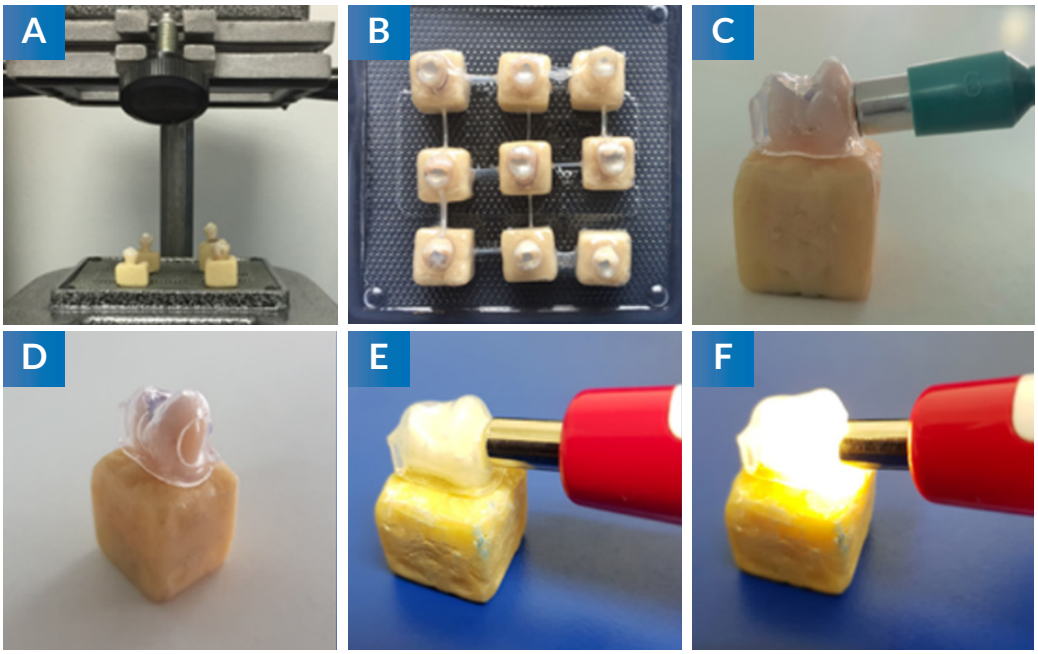
removal of traces of endodontic sealers.[16] It was washed thoroughly with distilled water, the chamber was dried, and the bleaching agent was applied. For group C, the intrachamber dentin was conditioned with 17% EDTA (Química Hertz) for 2 minutes (Figure 2B), a protocol that has shown results in the effective removal of detritus and smear layer.[17] It was washed profusely with distilled water, the chamber was dried, and the bleaching agent was applied. After the application of the whitening agent, it was covered with a small amount of cotton and temporarily sealed with Fermin (Detax GmbH & Co.). In the three subsequent sessions, all groups underwent the walking bleach technique in a conventional way and without dentin conditioning for groups B and C.

Between each of the sessions, the samples were conserved and stored in distilled water in a Culture incubator (Ivoclar Vivadent, Schaan, Liechtenstein) at 37°C until the last spectrophotometric measurement. The color evaluation at 4, 8, 12, and 16 days was carried out with the Easyshade V spectrophotometer (VITA). This objective measurement instrument is considered the gold standard method for determining tooth color,[18,19] since it takes into account all clinical factors that can significantly affect color perception, obtaining objective agreement in 93.3% of cases.[18] This is automatically calibrated and assesses the color of the teeth by measuring the amount and spectral composition of the total light reflected on the tooth surface in intervals of 1 to 25 nm along the visible spectrum. The results are expressed in CIE L*, a*, and b* values.[18] The color measurement was based on the middle third of the buccal aspect of the teeth. To ensure the reproducibility of the measurement point, a customized acetate positioner was made for each premolar (Figure 1D, Figure 1E and Figure 1F).

At each measurement, the temporary Filling material and the cotton covering the bleaching agent were removed, the pulp chamber was thoroughly washed with distilled water and dried, and then the spectrophotometer tip was positioned in the positioner. For each tooth and by the same operator, three color measurements were made in each session, thus following the manufacturer's instructions (VITA), taking the average of the three measurements as the final result. After registering the values, the color variation (DE) between the baseline and the different measurement times was calculated.

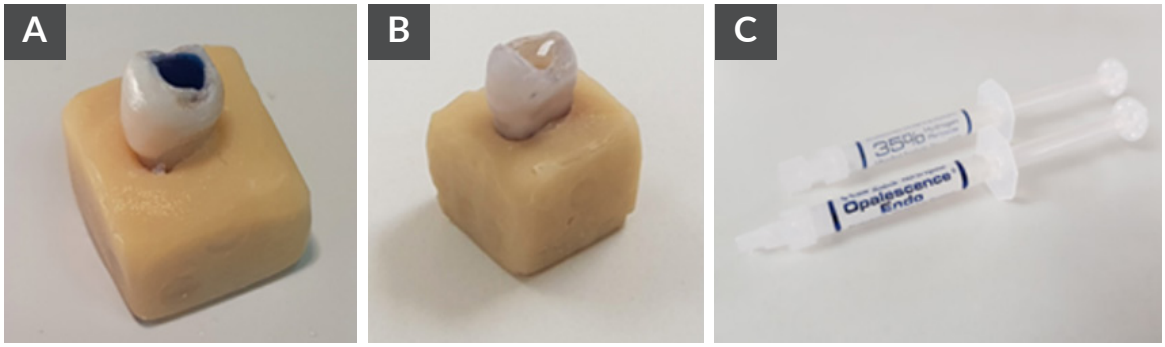
The normality of the data distribution according to the Shapiro–Wilks test was verified using the statistical analysis program STATA/SE 13.0. Based on this, a normal distribution of the sample was defined, for which the inferential analysis was performed using the ANOVA test. The comparison of the DE distribution was based on the Wilcoxon test for independent and paired samples.

Figure 1. Mounting in acrylic and making the positioner.



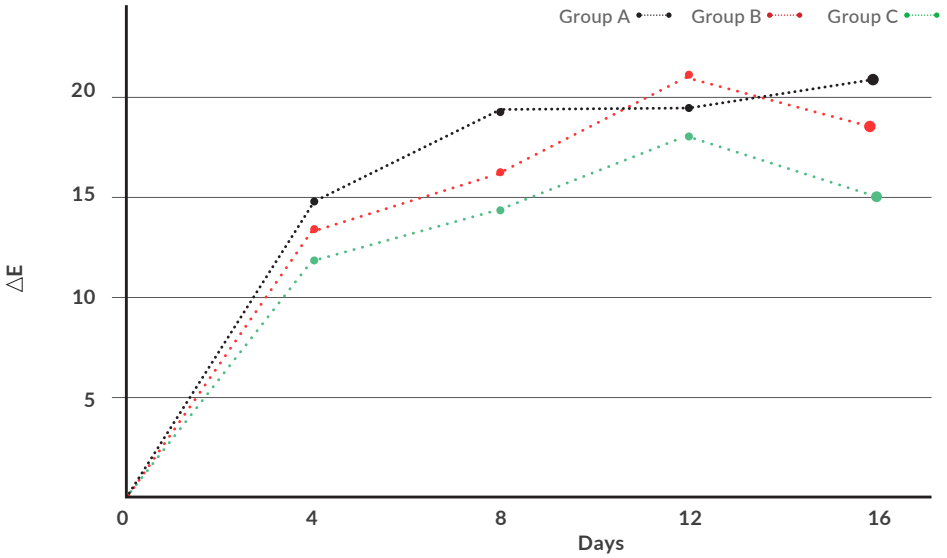
A. and B: Part of the sample in the assembly and stamping process. **C. and D:** Adjusted positioner, being drilled in the middle third of the vestibular region with a 6-mm diameter biopsy punch **E. and F:** The coincidence of diameters between the positioner and the tip of the spectrophotometer is observed

Figure 2. Dentin conditioning.



A. 37% phosphoric acid. **B.** 17% EDTA. **C.** Bleaching agent based on 35% hydrogen peroxide .

Figure 3. Average values of the total color variation according to time and study group. An increasing behavior was observed for all groups.



Group A. Without surface treatment. **Group B.** Treatment of the dentin surface with 37% phosphoric acid. **Group C.** Dentin surface treatment with 17% EDTA.

Tabla 1. Descriptive measures for color variation values by group and time.

Group	Time	Min.	Max.	Median	Standard Deviation
A	4	4.55	26.3	15.08	4.968
	8	10.2	30.5	19.25	4.681
	12	11	29.8	18.74	4.956
	16	9.71	66.8	19.75	11.437
B	4	3.11	27.5	12.99	5.216
	8	11.2	24.2	15.98	4.074
	12	15.8	37.6	19.06	5.260
	16	10	30.1	17.15	4.493
C	4	5.22	18.9	12.06	5.501
	8	3.39	25.4	15.33	5.980
	12	8.56	26.4	19.07	4.930
	16	7.43	23.6	15.59	4.166

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Tabla 2. Color variation between the study groups.

Day	Comparison by groups		*p*-value
4	A	B	0.278
	A	C	0.052
	B	C	0.395
8	A	B	0.039*
	A	C	0.013*
	B	C	0.395
12	A	B	0.492
	A	C	0.395
	B	C	0.179
16	A	B	0.701
	A	C	0.015*
	B	C	0.016*

*: Represents statistically significant differences.

Tabla 3. Variation of delta E between the different observation times. *represents statistically significant differences.

Group		Observation time			
A	**Bleaching agent application day**	**4**	**8**	**12**	**16**
	4	1	0.005*	0.000*	0.001*
	8	0.005*	1	1	0.848
	12	0.000*	1	1	1
	16	0.000*	0.848	1	1
B	**Bleaching agent application day**	**4**	**8**	**12**	**16**
	4	1	0.000*	0.000*	0.000*
	8	0.001*	1	0.000*	0.002*
	12	0.000*	0.000*	1	0.001*
	16	0.000*	0.002*	0.001*	1
C	**Bleaching agent application day**	**4**	**8**	**12**	**16**
	4	1	0.001*	0.000*	0.002*
	8	0.001*	1	0.000*	0.388
	12	0.000*	0.000*	1	0.000*
	16	0.003*	0.388	0.000*	1

RESULTS.

Analyzing the groups separately showed that group A presented an increasing behavior over time with respect to the median DE, unlike groups B and C, which showed this same pattern until day 12 and then presented a slight decrease (Table 1). The results showed an increasing behavior of the mean DE for all groups (Figure 3).

Statistically significant differences were found between groups in the color variation after the second application of the bleaching agent, where the group without dentin surface treatment was superior to that treated with 37% phosphoric acid and 17% EDTA.

After the fourth application, the group treated with 37% phosphoric acid was superior to that treated with 17% EDTA. However, both groups presented lower values than the group without surface treatment (Table 2 and Table 3).

DISCUSSION.

The evaluation of color and its variation throughout the study was carried out using the Easyshade V (VITA) spectrophotometer. Although this is not the most widely used method for evaluating color, due to its high cost and difficulty in operation, it is considered the most objective and reproducible.[18,19]

Since spectrophotometry allows it, it is interesting to assess what happens with tooth color components after internal bleaching. Several studies have used this method to evaluate color before and after treatment,[20,21] using the (DE) parameter, which is obtained with a formula that integrates all the components of the CIE system in a single numerical value. This parameter takes into account the differences between the measurements of the L*, a*, and b* coordinates, evaluating the accuracy and true acceptability of the color variation between two objects.[22]

The results show that in the three groups, an effective change in color was achieved from the first control, according to the parameters proposed by Bizhang *et al*.,[13] However, when statistically comparing the groups with each other, the estimated averages of the DE parameter were always lower in the group with 17% EDTA dentin conditioning, and the highest values were those of the group without dentin conditioning.

On the other hand, the group conditioned with 37% phosphoric acid obtained its peak in relation to day 12, but this did not present significant differences from the other groups.

This allows accepting the null hypothesis, that is, that the surface treatment with 37% phosphoric acid and 17% EDTA after applying 35% hydrogen peroxide using the walking bleach technique does not show differences compared to the application of the bleaching agent on a dentin surface without previous surface treatment.

Hydrogen peroxide and carbamide peroxide are the main bleaching agents used today. The quest to improve the speed and results of bleaching agents has led manufacturers to modify the chemical compositions and test different concentrations.[23] The results regarding the efficacy of the bleaching agent obtained in this study agree with those of Dufey *et al*.,[4] who observed an effective color change using 35% hydrogen peroxide in four sessions for 16 days. They also coincide with the systematic review by Luque-Martínez *et al*.,[12] who corroborated the efficacy of this agent.

The number of bleaching agent applications used in this study is based on Dufey *et al*.,[4] who found no differences in the effective color change when using 35% hydrogen peroxide, 37% carbamide peroxide, and 100% carbamide peroxide after the fourth application. Independent of the study group analyzed, applying 35% hydrogen peroxide using the walking bleach technique results in successful intrachamber bleaching for premolars stained with chromogens derived from the decomposition products of the blood.

Regarding dentin surface treatments before intrachamber bleaching, studies have shown that EDTA is one of the most effective calcium chelating agents to eliminate the inorganic components of the smear layer.[9,10] It allows direct contact of the agents with the surface and also influences its structure, which can cause erosion in peritubular and intertubular dentin.[24]

For its part, etching with 37% phosphoric acid not only acts on the inorganic part of the tissue, eliminating the mineral content, removing the dentin mud and therefore increasing the permeability of the tubules,[11] but in turn activates the degradation of the organic component of dentin. This promotes endogenous matrix metalloproteinases (MMPs) that activate the degradation of collagen[25] and decreases the amount of residue of endodontic sealers of the dentin surface.[16]

By increasing dentin permeability, greater diffusion of peroxide would be expected, increasing the effectiveness of bleaching.[6] Although this would justify surface treat-ment before bleaching to increase the diffusion of peroxides, internal bleaching agents based on peroxides by themselves have been shown to act chemically with the dentin components, modifying their morphology and structure, promoting changes in the organic composition through an increase in the degradation of collagen by activation of MMPs[25] and in the inorganic component reducing the mineral content,[26] acting similarly to EDTA and phosphoric acid, and increasing dentin permeability significantly.[6] Given this and according to the results of this study, the use of phosphoric acid or EDTA after applying the bleaching agent would not improve the results of intrachamber whitening. When comparing the surface treatments used in the present study, dentin conditioning with 37% phosphoric acid generated an increase in the efficacy of the bleaching agent when compared to 17% EDTA. This can be explained by the results obtained by Carrasco *et al*.,[27] who demonstrated that the use of 17% EDTA passively in the pulp chamber before Internal bleaching

does not generate a significant increase in dentin permeability—not so when it is ultrasonically activated. On the other hand, neither of the two surface treatments managed to increase the effectiveness of hydrogen peroxide by 35% compared to its application on unconditioned dentin.

This can be explained since the smear layer of the pulp chamber, generated by the rotating instruments, is not dense and impermeable and leaves open tubules that allow the bleaching agent to diffuse. In addition, the interconnections presented by the system of dentin tubules allow fluids to penetrate and diffuse through the dentin, regardless of whether some are blocked by the smear layer.[28]

In the results of this study, the groups where dentin surface treatments were performed before the application of the whitening agent did not obtain differences compared to the group without conditioning. Hydrogen peroxide generates large amounts of hydroxyl ions, which act on the inter- and peritubular dentin, degrading the collagen and hyaluronic acid of the dentin and causing it to increase its permeability.[29] Thus, this bleaching agent by itself is only a powerful oxidant capable of modifying the chemical and mechanical structure of dentin,[5] and the use of dentin surface treatments before its application only weakens the tooth structure even more without affecting the effectiveness of the procedure.

Damage to the dental structure after bleaching has been reported by De Oliveira Moreira *et al.*,[30] and Klaric *et al.*,[31] who observed lower microhardness values in teeth subjected to bleaching treatment. Free radicals produced by bleaching agents bind to hydroxyapatite, generating apatite peroxide, breaking the balance of calcium and phosphate and thus weakening the inorganic phase of dentin.[30,32] This loss of elements and the reduction of the percentage weight of the hydroxyapatite crystals is considered demineralization.[31,33]

The results of the present study show that the use of 37% phosphoric acid and 17% EDTA as a dentin conditioner before Internal bleaching does not increase the effectiveness of 35% hydrogen peroxide. Its use only generates a greater demineralization of the dental tissue, which could compromise the rehabilitation and therefore the function of the tooth. It should be mentioned that the staining method used in this study corresponds to a laboratory model in which dental stains are induced in a short period, which often does not resemble a clinical situation in which stains produced by hemorrhagic processes and blood breakdown products remain for long periods in the pulp chamber. More studies are required to support the clinical relevance of these results, especially aspects related to the removal of long-standing coronary stains.

CONCLUSION.

Dentin conditioning with 37% phosphoric acid increased the effectiveness of the bleaching agent when compared to 17% EDTA. However, these dentin surface treatments did not demonstrate a greater efficacy of 35% hydrogen peroxide compared to its application to unconditioned dentin during internal bleaching with the walking bleach technique.

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