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Effect of prior application or addition of desensitizers into hydrogen peroxide gels on bleached enamel

Efeito da aplicação prévia ou adição de dessensibilizantes em géis de peróxido de hidrogênio no esmalte clareado

Efecto de la aplicación previa o la adición de desensibilizantes a los geles de peróxido de hidrógeno sobre el esmalte dental blanqueado

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Abstract
This in vitro study evaluated the effect of 35% hydrogen peroxide (35HP) gels with different desensitizing agents on color, microhardness and roughness of bleached enamel. Forty enamel-dentin specimens (6x6x2 mm^2) were obtained from twenty human molars. After color measurement with a spectrophotometer, the specimens were randomized into four groups (n= 10): 35HPw- 35HP without desensitizing agent; 35HPCa- 35HP with calcium; 35HPK- 35HP with 0.5% potassium nitrate; 35HPTFa- 35HPw + topical application of 5% potassium nitrate and 2% sodium fluoride (TFa). Specimens were evaluated for color (ΔE_{ab*} and ΔE_{00}), Vickers microhardness (VHN) and, superficial and volumetric roughness (Ra and Sa, µm) using a 3D non-contact profilometer before and 1-week after bleaching. Data were analyzed by ANOVA and Tukey tests ($p< 0.05$). Both treatment groups promoted a significant whitening effect and there was no difference between them for any color parameters evaluated. All groups reduced significantly the VHN, but the 35HPK showed a reduction significantly major than the other groups. 35HPCa and 35HP+TFa did not avoid the of Ra and Sa increasing. It can be concluded that 35HP bleaching gels with different desensitizing agents did not affect the whitening efficacy. However, all treatments decreased the microhardness, the addition of calcium into 35HP gel and the application of TFa before bleaching did not revert the enamel roughness under in vitro conditions.
Keywords: Hydrogen peroxide; Tooth bleaching; Dental enamel.

Resumo
Este estudo *in vitro* avaliou o efeito de géis de peróxido de hidrogênio a 35% (35PH) com diferentes agentes dessensibilizantes na cor, microdureza e rugosidade do esmalte clareado. Quarenta espécimes de esmalte-dentina (6x6x2 mm^2) foram obtidos de vinte molares humanos. Após a aferição da cor com espectrofotômetro, os espécimes foram randomizados em quatro grupos (n= 10): 35PHw- 35PH sem agente dessensibilizante; 35PHCa- 35PH com cálcio; 35PHK- 35PH com nitrato de potássio a 0,5%; 35PHTFa- 35PHw + aplicação tópica de nitrato de potássio 5% e fluoreto de sódio 2% (TFa). Os espécimes foram avaliados quanto à cor (ΔE_{ab*} e ΔE_{00}), microdureza Vickers (VHN) e rugosidade superficial e volumétrica (Ra e Sa, µm) através de perfilômetro 3D sem contato antes e 1 semana após o clareamento. Os dados foram analisados pelos testes ANOVA e Tukey ($p <0,05$). Ambos os grupos de tratamento promoveram efeito clareador significativo e não houve diferença entre eles para nenhum dos parâmetros de cor avaliados. Todos os grupos reduziram significativamente o VHN, mas o 35PHK apresentou uma redução significativamente maior que os outros grupos. 35PHCa e 35PH+TFa não evitaram o aumento de Ra e Sa. Pôde-se concluir que os géis de 35HP com diferentes agentes dessensibilizantes não afetaram a eficácia do clareamento. No

entanto, todos os tratamentos diminuíram a microdureza, a adição de cálcio ao 35PH e a aplicação de TFa antes do clareamento não reverteram a rugosidade do esmalte nas condições *in vitro*.
Palavras-chave: Peróxido de hidrogênio; Clareamento dental; Esmalte dental.

Resumen
Este estudio *in vitro* evaluó el efecto de los geles de peróxido de hidrógeno al 35% (35PH) con diferentes agentes desensibilizantes sobre el color, la microdureza y la rugosidad del esmalte blanqueado. Se obtuvieron cuarenta muestras de esmalte-dentina (6x6x2 mm^2) de veinte molares humanos. Después de la medición del color con un espectrofotómetro, las muestras se distribuyeron al azar en cuatro grupos (n= 10): 35HPw-35HP sin agente desensibilizante; 35HPCa-35HP con calcio; 35HPK-35HP con 0,5% de nitrato de potasio; 35HPTFa- 35HPw + aplicación tópica de nitrato de potasio al 5% y fluoruro de sodio al 2% (TFa). Las muestras se evaluaron en cuanto al color (ΔE_{ab*} y ΔE_{00}), microdureza Vickers (VHN) y rugosidad superficial y volumétrica (Ra y Sa, µm) utilizando un perfilómetro 3D sin contacto antes y 1 semana después del blanqueamiento. Los datos fueron analizados por ANOVA y pruebas de Tukey ($p < 0.05$). Ambos grupos de tratamiento promovieron un efecto blanqueador significativo y no hubo diferencia entre ellos para ninguno de los parámetros de color evaluados. Todos los grupos redujeron significativamente el VHN, pero el 35PHK mostró una reducción significativamente mayor que los otros grupos. 35PHCa y 35PH+TFa no evitaron el aumento de Ra y Sa. Se concluyó que los geles de 35PH con diferentes agentes desensibilizantes no afectaron la eficacia blanqueadora. Sin embargo, todos los tratamientos disminuyeron la microdureza, la adición de calcio al gel de 35PH y la aplicación de TFa antes del blanqueamiento no revirtieron la rugosidad del esmalte en condiciones *in vitro*.
Palabras clave: Peróxido de hidrógeno; Blanqueamiento dental; Esmalte dental.

1. Introduction

Tooth bleaching is a conservative technique and minimally invasive for treating tooth discoloration. It can be performed at home, when the patient uses prefabricated trays loaded with low concentrations of carbamide peroxide (Kwon & Wertz, 2015), or in-office, when the dentist usually applies high concentrations (20% to 40%) of hydrogen peroxide (HP) on the enamel surface, with the 35% HP being the most commonly concentration used (Kwon & Wertz, 2015; Vieira et al., 2020). Individuals who want faster results or who refuse to use the trays indicated in the home technique often request in-office tooth bleaching (De Geus et al., 2016; Kutuk et al., 2018).

The bleaching agents act by a complex oxidation process with the release of reactive oxygen species, which penetrate through the enamel interprismatic spaces and reach the dentin, breaking organic molecules and producing lighter, smaller and lighter compounds (De Geus et al., 2016; Kutuk et al., 2018). Regardless of the technique and bleaching agent used, adverse effects have been reported, including: tooth sensitivity (Rezende et al., 2020; 2021), changes in the surface morphology (Torres et al., 2019; Parreiras et al., 2020) and changes in the physical-chemical properties of dental hard tissues (Jurema et al., 2018; Kutuk et al., 2018; Martini et al., 2020).

In an attempt to minimize the enamel demineralization and tooth sensitivity, an adverse effect often reported by patients during and after tooth whitening procedures, desensitizing agents, such as calcium gluconate, fluoride, potassium nitrate, bioactive glass and nanohydroxyapatite have been used before, after or incorporated into whitening gels (Publio et al., 2013; De Moraes et al., 2015; Dionysopoulos et al., 2017; Torres et al., 2019; Vieira et al., 2020). However, while previous studies reported that desensitizing agents incorporated into bleaching gels minimized the demineralization caused by high HP concentrations (Kemaloğlu, Tezel & Ergücü, 2014; Kutuk et al., 2018; Torres et al., 2019; Vieira et al., 2020), others showed that adding these components did not reverse the enamel damage (De Moraes et al., 2015; Cavalli et al., 2018).

Another important point to consider is the pH of the bleaching gels, since it can vary from acidic (pH around 2.0) to alkaline (pH around 9.0) (Xu, Li & Wang, 2011; Loguercio et al., 2017; Furlan et al., 2017; Jurema et al., 2018). The majority of in-office bleaching gels are supplied at a low pH to increase the product's shelf life (Torres et al., 2014; Acuna et al., 2019). However, the low pH can promote enamel demineralization (Jurema et al., 2018) and changes in the chemical composition,

morphology and mechanical properties of the dental structure (Xu, Li & Wang, 2011; Sa et al., 2013; Trentino et al., 2015; Jurema et al., 2018; Alkahtani et al., 2020).

As previously reported, the effect of adding desensitizing agents into bleaching gels on enamel microhardness and surface roughness is still controversial. Furthermore, to our knowledge, no study has evaluated if the application of desensitizing agent before in-office bleaching can protect the enamel dissolution. Therefore, this study aimed to evaluate the effect of 35% HP gels with different desensitizing agents on the color, microhardness and roughness of human enamel. The null hypotheses tested were that the addition of desensitizing agents into bleaching gels or its application before treatment: 1) would not affect the bleaching efficacy, and 2) would not protect the enamel against alterations in microhardness or surface roughness.

2. Methodology

2.1 Ethical considerations

This is a prospective, quantitative and experimental research (Pereira et al., 2018). This study was conducted in accordance with the Declaration of Helsinki (1964) and was approved by the local Ethics and Research Committee (CAAE: 99907618.9.0000.5188). Twenty human molars without cracks, fractures or enamel defects were donated by the Human Tooth Bank from Federal University of Paraiba. Teeth were stored in distilled water and kept at 4 °C until testing.

2.2 Specimen preparation

Teeth roots were removed using diamond discs, and the molar crowns were sectioned longitudinally in the mesiodistal direction (Labcut 1010, Extec Corp., Enfield, CT, USA) to obtain two enamel-dentin blocks (6×6×2 mm^2) from labial and lingual surfaces. The blocks' dimensions were measured using a digital caliper (500-144B, Mitutoyo Corp., São Paulo, SP, Brazil). The specimens were positioned inside a cylindric plastic holder with the enamel surface located in the bottom of the mold, and then the molds were filled with self-cure acrylic resin. The enamel surfaces were ground flat using 320-grit silicon carbide abrasive paper and polished with 600- and 1200-grit aluminum oxide papers under water-cooling for 60 seconds in a polishing machine (Politriz ERIOS-27000, São Paulo, SP, Brazil). At the end of the polishing procedures, the specimens were washed in an ultrasonic machine (Digital Ultrassonic Cleaner CD-4820, Kondentech, São Carlos, SP, Brazil) containing distilled water for 10 minutes and then stored in recipients with distilled water (Klimek, Hellwig & Ahrens, 1982) (pH = 7.0) at 37 °C until the beginning of and during the experimental phase.

2.3 Color assessment

Color evaluation was performed by an examiner not directly involved in whitening procedures. Specimens were positioned in a white background and color measurements were performed at baseline (T_0) and one week after treatment (T_1) with a spectrophotometer (Vita EasyShade® Advance, Vita Zahnfabrik, Bad Sackingen, Germany). A putty silicon paste (Zetaplus, Zhemarck Dental, São Paulo, SP, Brazil) mold was used to standardize the area of measurement. For each specimen, we produced a circular window (6 mm) on the buccal surface of the silicon guide. The active point of the spectrophotometer was positioned into the silicone guide and the readings were repeated three times at each time evaluation, and then color parameters averages were calculated.

Color measurements were determined according to CIEL*a*b* coordinates, where L* represents the value (lightness or darkness); the a* is a measure of redness (positive a*) or greenness (negative a*); and the b* is a measure of yellowness (positive b*) or blueness (negative b*) (Pecho et al., 2018). ΔL^*, Δa^* and Δb^* were calculated by the difference between T_1 and T_0. Subsequently, the color difference between T_1 and T_0 were calculated using both CIEL*a*b* (ΔE^*_{ab}) (Zlataric et al., 2019) and CIEDE2000 (ΔE_{00}) (Pecho et al., 2018) formulas.

2.4 Whitening procedures

Each bleaching agent was prepared according to the manufacturer instructions. The pH values of each bleaching gel, distilled water and desensitizing agent were measured using a pH meter with a micro pH electrode (Hanna Instruments, Romania, USA), which was initially calibrated using pH 4.0 and 7.0 standard buffer solutions (Dinâmica Química Contemporanea Ltda., Indaiatuba, SP, Brazil). Two samples of each bleaching/desensitizing agent were tested in triplicate to obtain the mean pH values. Each product was kept in contact with a pH electrode for the time the manufacturer recommended for each treatment at room temperature (24 ºC ± 1 ºC) until the pH value stabilized. Then, the electrode was thoroughly washed between samples using a stream of water and rinsed with distilled water and dried with absorbent paper. The pH meter was re-calibrated before analyzing each sample.

After tooth color measurement, specimens were randomized into four groups (n = 10) and bleaching gels were applied according to the manufacturer's instructions:

• 35HPw (control): 35% hydrogen peroxide without a desensitizing agent (Whiteness HP 35%, FGM Dental products, Joinville, SC, Brazil). The gel was applied twice for 15 minutes in each clinical session.

• 35HPCa: 35HP with calcium (Whiteness Automixx 35%, FGM Dental products). The gel was applied once for 50 minutes in each clinical session.

• 35HPK: 35HP with 0.5% potassium nitrate (Pola Office 35%, SDI, Southern Dental Industries, Bayswater, Victoria, Australia). The gel was applied three times for 8 minutes in each clinical session.

• 35HP+TFa: 35HPw + topical application of 5% potassium nitrate and 2% sodium fluoride (Whiteness HP 35% + Desensibilize KF 2%, FGM Dental Products). The desensitizing agent was applied for 10 minutes before beginning the treatment. Then, it was removed with sterilized gauze, washed with an air-water spray and the bleaching gel was applied twice for 15 minutes per clinical session.

During application, each bleaching gel was moved over the enamel surface with a disposable applicator four times to eliminate the bubbles and enhance the contact between the enamel and bleaching agent. After each application, the bleaching gel was removed with sterilized gauze, the specimens were washed with an air-water spray and the gel was renewed (except for the 35HPCa group). At the end of each clinical session, specimens were stored in distilled water at 37 °C, which was changed daily. Three sessions were performed with an interval of 7 days. The materials used in this study, their compositions, manufactures and pH values are described in Table 1.

Table 1. Composition and manufacturers of the products used.

Products	Abreviations	Composition	Manufacturers
Whiteness HP 35%	35HPw (control)	35% hydrogen peroxide, thickening, inert filler, red pigment, glycol, deionized water, pH= 5.7.	FGM Dental Products, Joinville, SC, Brazil
Whiteness Automixx 35%	35HPCa	35% hydrogen peroxide, thickening, neutralizing, calcium gluconate, glycol, dye, inorganic filler, deionized water, pH= 7.7.	FGM Dental Products, Joinville, SC, Brazil
Pola Office 35%	35HPK	Liquid: 35% hydrogen peroxide, 65% water. Powder: 73.26% thickener, 26.2% catalyst, 0.04% dye, 0.5% potassium nitrate, pH= 2.6.	SDI, Southern Dental Industries, Bayswater, Victoria, Australia
Desensibilize KF 2%	TFa	Active ingredients: 5% potassium nitrate and 2% sodium fluoride. Inactive ingredients: deionized water, glycerin, neutralizing agent, thickener, pH= 6.8.	FGM Dental Products, Joinville, SC, Brazil

Source: Authors (2021).

2.5 Analysis in noncontact 3D profilometry

Enamel surface topography was analyzed using a 3D non-contact profilometer (CCI MP-L, Taylor Hobson Ltd, Leicester, England). Measurements of linear (Ra) and volumetric (Sa) roughness were obtained using a 0.25 mm cut-off, scan speed of ×1 in xyz mode with an area of 340 × 340 µm.

Eight linear measurements (three horizontal, three vertical and two diagonal) with a distance of 0.85 µm between them were performed in each specimen. The average of these eight linear measurements was used to determine Ra (µm) values before (T_0) and one week after treatment (T_1). Additionally, two scan areas (340 × 340 µm) were obtained from each specimen. The average of the two areas was used to determine Sa (µm) at T_0 and T_1 evaluations.

2.6 Microhardness assessment

Enamel microhardness was determined using a microhardness tester (HMV-2000, Shimadzu, Tokyo, Japan) with a Vickers indenter (100 g/ 10 s) at T_0 (VHN_0) and T_1 (VHN_1) appointments. Three indentations were performed for each specimen with a distance of 100 µm between them and 200 µm from the margins. The VHN for each specimen was obtained from the average of these indentations. Additionally, we calculated the percentage of microhardness change (%VHN) in relation to the baseline using the following formula: $\%VHN = (VHN_{1-} VHN_0/ VHN_0) \times 100$.

2.7 Statistical analysis

All data were checked for normal distribution using the Shapiro-Wilk test of normality. Means and standard deviations of color coordinates (L*, a*, b*), color change between baseline and 1-week post-bleaching (ΔL*, Δa*, Δb*, ΔE^*_{ab} and ΔE_{00}), VHNs and Ra and Sa roughness were evaluated using ANOVA and Tukey´s test (between treatment groups) and Paired Student´s t-test (within the same group). For all statistical analyses, the significance level was 5%.

3. Results

All treatment groups resulted in significant whitening ($p < 0.001$) and there were no significant differences between then in color change coordinates (ΔL*, Δa*, Δb*) or color differences (ΔE^*_{ab} and ΔE_{00}). Both groups showed a ΔE^*_{ab} greater than 13.0 units and a ΔE_{00} over 7.8 units ($p > 0.3$). Means for ΔE^*_{ab} and ΔE_{00} between 1-week and baseline were above 50:50% perceptibility (PT) and acceptability (AT) thresholds for both treatment groups (Table 2).

Table 2. Means (standard deviation) of color coordinates at baseline (L*, a*, b*), color change coordinates (ΔL*, Δa*, Δb*) and color differences (ΔE^*_{ab} and ΔE_{00}) between baseline and 1-week postbleaching for different treatment groups.

Color evaluation		In-office bleaching			
		35HPw	**35HPCa**	**35HPK**	**35HP+TFa**
Color coordinates (baseline)	**L***	73.8 (7.3)^A	72.8 (4.1)^A	72.1 (5.5)^A	74.6 (2.3)^A
	a*	-1.9 (2.5)^A	-0.4 (2.5)^A	-1.0 (1.6)^A	-0.9 (2.2)^A
	b*	29.7 (7.5)^A	30.2 (8.6)^A	29.3 (4.0)^A	33.2 (5.6)^A
ΔL*		6.6 (2.9)^A	7.7 (3.3)^A	4.6 (4.0)^A	4.5 (4.0)^A
Δa*		-1.7 (1.5)^A	-3.3 (1.8)^A	-2.8 (1.5)^A	-2.9 (2.6)^A
Δb*		-10.5 (2.8)^A	-12.8 (3.2)^A	-12.7 (3.1)^A	-13.5 (7.2)^A
ΔE^*_{ab}		13.0 (2.0)^A	15.6 (3.7)^A	14.4 (3.0)^A	16.0 (5.2)^A
ΔE_{00}		7.8 (1.5)^A	9.3 (2.0)^A	8.5 (2.0)^A	8.6 (2.4)^A

* Different *capital* letters indicate significant differences between treatment groups (p< 0.05, One-way ANOVA and Tukey test).
Source: Authors (2021).

After treatments, all groups reduced significantly the enamel microhardness when compared to baseline ($p = 0.001$), and 35HPK showed the lowest microhardness values ($p < 0.01$). The percentage of microhardness reduction for 35HPK also was significantly higher than that of the other groups (Table 3).

Table 3. Means (standard deviation) of enamel microhardness at baseline and after treatment and percentage of microhardness change (%VHN) for different in-office bleaching groups.

In-office bleaching	Enamel microhardness (VHN)		%VHN	*p*
	Baseline	Final		
35HPw	312.7 (34.7)^{A}	206.9 (32.2)^{A}	33.24 (11.5)^{A}	0.001
35HPCa	312.4 (37.0)^{A}	224.1 (48.5)^{A}	28.0 (13.9)^{A}	0.001
35HPK	330.8 (14.1)^{A}	157.5 (20.8)^{B}	52.34 (6.4)^{B}	0.001
35HP +TFa	312.3 (17.6)^{A}	207.7 (25.3)^{A}	33.45 (8.2)^{A}	0.001

* Different *capital* letters indicate significant differences between treatment groups (p< 0.05, One-way ANOVA and Tukey test).
* Significant differences within the same treatment group and for different periods were evaluated using Paired Student´s t-test (p< 0.05).
Source: Authors (2021).

Regarding enamel surface roughness, the control ($p < 0.005$) and 35HPCa ($p < 0.008$) groups increased Ra and Sa parameters when compared to baseline. After treatment, Ra values for 35HP+TFa group did not differ from 35HPw ($p = 1.0$). There were no significant differences between the groups for Sa values after treatment (Table 4).

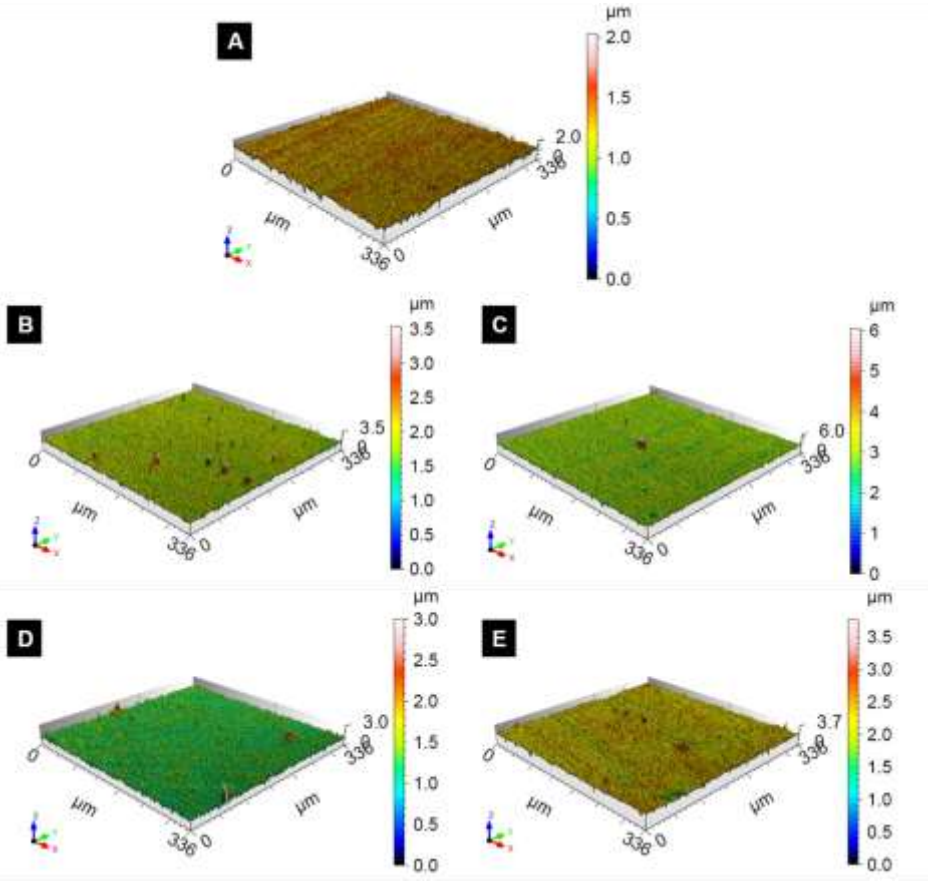
Table 4. Means (standard deviation) of linear (Ra) and volumetric (Sa) roughness at baseline and after treatment for different in-office bleaching groups.

Enamel roughness	In-office bleaching			
	35HPw	35HPCa	35HPK	35HP+TFa
Ra (µm)				
Baseline	0.10 (0.04)^{A}	0.10 (0.04)^{A}	0.10 (0.02)^{A}	0.10 (0.02)^{A}
Final	0.13 (0.03)^{AB}	0.14 (0.04)^{A}	0.10 (0.03)^{B}	0.12 (0.02)^{AB}
p	0.003	0.001	0.6	0.07
Sa (µm)				
Baseline	0.12 (0.04)^{A}	0.12 (0.04)^{A}	0.11 (0.02)^{A}	0.12 (0.03)^{A}
Final	0.14 (0.04)^{A}	0.15 (0.05)^{A}	0.12 (0.03)^{A}	0.13 (0.02)^{A}
p	0.005	0.008	0.3	0.2

* Different *capital* letters indicate significant differences between treatment groups (p< 0.05, One-way ANOVA and Tukey test).
* Significant differences within the same treatment group and for different periods were evaluated using Paired Student´s t-test (p< 0.05).
Source: Authors (2021).

Figure 1 shows the 3D profilometry images of the enamel surface before (A) and after (B-E) bleaching procedures. The loss of tooth structure was evident for all treatment groups. There was also an increase in the peaks' height in the enamel surfaces treated with 35HPw, 35HPCa and 35HP+TFa, and these alterations are in accordance with Ra and Sa data.

Figure 1. 3D profilometry images of enamel surface before (A) and 1-week after (B-E) bleaching procedures. (A) Specimen untreated, (B) 35HPw, (C) 35HPCa, (D) 35HPK, (E) 35HP+TFa.



Source: Authors (2021).

4. Discussion

New bleaching gels formulations containing desensitizing agents have been introduced in the market to minimize the adverse effects such as tooth sensitivity, changes in the microhardness or in the enamel surface morphology that whitening procedures cause (Xu, Li & Wang, 2011; Kutuk et al., 2018; Vieira et al., 2020). It has been suggested that the presence of calcium and fluoride ions could decrease the HP diffusion through the tooth structure by the deposition of the crystals on the enamel surface, but without decreasing the whitening effect (De Moraes et al., 2015; Pecho et al., 2018; Torres et al., 2019; Parreiras et al., 2020). Additionally, the degradation of HP into free radicals rises at neutral or alkaline pH, which could increase the bleaching efficacy (Xu, Li & Wang, 2011; Torres et al., 2014). However, other studies reported that the pH values were not interfered with in the whitening effectiveness (Loguercio et al., 2017; Jurema et al, 2018; Acuna et al., 2019). The findings of this study showed that the 35% HP gels promoted an effective tooth whitening, regardless of pH, time of application or type of desensitizing agent associated with the bleaching protocol. Color difference means for both treatment groups were higher than 13.0 (ΔE^*_{ab}) and 7.8 units (ΔE_{00}), which indicates a color variation clinically perceptible and higher than the acceptability thresholds accepted by ISO (ΔE^*_{ab} = 2.7 units and ΔE_{00} = 1.7 units) (ISO, 2016; Crastechini et al., 2019). Therefore, the first null hypothesis tested was accepted, since the addiction of desensitizing into 35% HP gels or its application before treatment did not interfere in the bleaching efficacy.

Studies have demonstrated that adding calcium into bleaching gels could minimize the negative effects on microhardness and increase the mineral content of the enamel surface after bleaching treatment (Chen et al., 2008; Borges et al., 2014; Kutuk et al., 2018; Torres et al., 2019; Vieira et al. 2020), but could not reverse the subsurface enamel

demineralization (Furlan et al., 2017; Cavalli et al., 2018; Jurema et al., 2018). The purpose of incorporating calcium gluconate into bleaching gel is to make it supersaturated in Ca^{2+} ions to prevent its dissolution from enamel hydroxyapatite during the whitening procedures (Borges et al., 2015; Torres et al., 2019). In this study, the 35% HP gel containing calcium gluconate in a concentration not informed by the manufacturer was not able to recover the microhardness decreasing. Probably, the concentration of this salt was insufficient in providing the supersaturation of Ca^{2+} ions in relation to the enamel. This corroborates with previous studies that found adding 0.5% calcium gluconate into 35% HP did not reduce the enamel demineralization (De Moraes et al., 2015; Furlan et al., 2017; Torres et al., 2019). Although this bleaching gel has a slightly alkaline pH, the pH increases can facilitate the releasing of free radicals, resulting from the hydrogen peroxide breakdown, which promotes the degradation of inorganic and organic enamel matrix (Dionysopoulos et al., 2017; Jurema et al., 2018). Another factor that may also explain the microhardness reduction in the 35HPCa group was the longest contact time (50 minutes per session) of this bleaching gel with tooth surface (Vilhena et al., 2019; De Carvalho et al., 2020).

It has been discussed that high concentrated bleaching gels with pH values varying from acidic to alkaline promoted alterations in enamel chemical composition, demineralization and microhardness reduction (De Moraes et al., 2015; Furlan et al., 2017; Pecho et al., 2018). These changes also have been attributed to the oxidative effect and chemical composition of bleaching agents (Sa et al., 2013; Kwon & Wertz, 2015; Alkahtani et al., 2020). In this study, one of the factors that may have contributed to the greater enamel demineralization in the group containing 0.5% potassium nitrate was the low pH (2.6) of the bleaching gel, which was lower than the pH values of other treatment groups and also of the critical pH of enamel dissolving (5.5). When the pH falls below the critical value (Sa et al., 2013), the concentration of calcium and phosphate ions in the enamel reduces, resulting in the loss of mineral content and the hardness decreasing (Furlan et al., 2017). Adding potassium nitrate in the bleaching gels aims to reduce the incidence and severity of tooth sensitivity by depolarizing the dentinal sensorial nerve fibers and blocking the conduction of pain signal transmission (Kwon & Wertz, 2015). The results from this study showed that adding potassium nitrate into 35% HP gel did not attenuate the enamel microhardness reduction. This corroborates with previous studies that found this desensitizing agent did not provide any beneficial effects on the enamel microhardness (Furlan et al., 2017; Kutuk et al., 2018).

The use of fluoridated agents before and/or after bleaching treatments has been recommended for both the protection and remineralization of the enamel surface (China et al., 2014; Kemaloğlu et al., 2014; Dionysopoulos et al., 2017; Kutuk et al., 2018). This procedure can promote the precipitation and deposition of calcium fluoride on the enamel surface, making it more resistant to dissolution (Pecho et al., 2018; Torres et al., 2019). In an attempt to protect the enamel during bleaching with 35% HP without the desensitizing agents (the same bleaching gel used in the control group), we applied the gel containing 5% nitrate potassium and 2% sodium fluoride (Desensibilize KF 2%) at 10 minutes before the bleaching procedure. However, using this desensitizer did not reverse the decrease in enamel microhardness. We hypothesized that an insufficient amount of fluoride was deposited on the enamel surface once the sound enamel was less porous than bleached enamel. This condition could have resulted in a smaller number of retention sites, which jeopardized the fluoride diffusion, and consequently decreased the protection of the bleached enamel.

Previous studies have reported that using HP-based products promotes slight alterations on the enamel surface such as porosities, erosion pits, cracks and grooves, which increase the enamel roughness (Xu et al., 2011; De Moraes et al., 2015; Trentino et al., 2015; Vieira et al., 2020; De Miranda et al., 2020). In this study, we also observed an increase of linear and volumetric roughness for 35HPCa, which was confirmed by the 3D images obtained in the profilometric analysis. A possible explanation for this may be related to the insufficient amount of calcium ions precipitated on the defects of the enamel surface, which did not allow the filling of these irregularities and as a consequence a smoother surface (De Moraes et al., 2015; Trentino et al., 2015; Vieira et al., 2020). Additionally, applying the desensitizing agent before bleaching did not prevent the

enamel roughness increasing. However, these alterations may be clinically irrelevant once that the specimens were stored in distilled water and the human saliva can restore the roughness of the bleached enamel over time (Sa et al., 2013; Grazioli et al., 2018; De Carvalho et al., 2020). Therefore, the second null hypothesis can be rejected, since the addition of desensitizing agents into 35HP gels or its application before bleaching did not protect against a decrease in the enamel microhardness and an increase in the roughness surface.

Saliva has a protective effect, which occurs by its buffer capacity and the supplementation of calcium and phosphate ions, reducing the mineral loss and promoting an equilibrium between the demineralization and remineralization phenomena (De Moraes et al., 2015; Kutuk et al., 2018). Furthermore, studies reported that the saliva can partially restore the microhardness of bleached enamel until two weeks after whitening procedures, to decrease the alterations in the enamel surface and to reduce the roughness of the bleached enamel (Borges et al., 2015; Kutuk et al., 2018; Acuna et al., 2019; De Carvalho et al., 2020). In this study, the specimens were stored in distilled water (pH = 7.0) to evaluate if only the desensitizing agents added in the 35% HP gels or used before bleaching would be able to minimize the bleached enamel's microhardness loss or the increase of roughness. Unfortunately, the use of distilled water as a storage medium may also have contributed to the great reduction in enamel microhardness for all treatment groups (greater than 28%), being higher than values recommended by ISO 28399 (ISO, 2020). This fact highlights the important role of saliva in minimizing the negative effects cause by bleaching procedures on the enamel surface (Zanolla et al., 2017; Grazioli et al., 2018; De Carvalho et al., 2020).

4. Conclusion

Within the limitations of this in vitro study, we concluded that adding calcium or potassium nitrate into 35% HP gels or applying desensitizer before treatment did not prevent the reduction of enamel microhardness or protect against increasing roughness and, not interfere in the bleaching effectiveness.

In addition, other studies are needed to evaluate the effects of desensitizing agents used before or added into high HP concentrations on the bleached enamel surface in order to suggest a safer protocol for patients subjected to this treatment modality.

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