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Assessment of a novel bleaching agent formula containing 35% hydrogen peroxide and titanium tetrafluoride: an *in vitro* study

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Abstract: This study developed experimental gels containing titanium tetrafluoride (TiF_4) combined with commercial 35% hydrogen peroxide (HP), and evaluated bleaching efficacy and pH of the gels, and mineral content and morphology of enamel submitted to these treatments. In phase-1, different stock gels mixed with TiF_4 were combined with HP. In phase-2, the selected gels were tested on enamel/dentin specimens (n=8): HP; HP and Natrosol+TiF_4 (HPnT); HP and Natrosol+Chemygel+TiF_4 (HPncT); HP and Aristoflex+TiF_4 (HPaT). Bleaching was performed in four sessions (3x15min-application/session). Color (CIEL*a*b*) and whiteness index (WI_D) were measured after each session, whereas whiteness index differences (ΔWI_D), color alteration (CIELab–ΔE, CIEDE2000-ΔE_{00}), enamel morphology and pH, at end of bleaching therapy. The change in Knoop microhardness (ΔKHN) was compared before and after bleaching. Data were analyzed by two-way repeated measures ANOVA and Bonferroni (CIEL*, a*, b*), one-way ANOVA and Tukey (ΔWI_D, ΔE, ΔE_{00}), and LSD (ΔKHN) tests (α=5%). SEM and pH measurements were submitted to descriptive analysis. No differences were observed in lightness (L*) or WI_D among the groups ($p > 0.05$), but HP exhibited lower b* values ($p<0.05$), higher ΔWI_D than HPnT, and the highest ΔE among the groups ($p < 0.05$). No differences in ΔE_{00} were observed between HP and HPncT ($p > 0.05$), and HPncT showed higher ΔKHN than HP ($p < 0.05$). HP presented pH values closer to neutral (6.9), whereas experimental agents showed acidic pH values (2.3–3.9). No morphological changes were observed in HP or HPncT groups. HPncT was able to bleach the enamel and maintain enamel microhardness and surface integrity, even at low pH.

Keywords: Bleaching Agents; Fluorides; Titanium; Tooth Bleaching.

Introduction

Minimally invasive operative dentistry focused on high esthetic criteria requires safe and effective techniques, procedures, and products. In this respect, dental bleaching is a common clinical procedure, because it is undoubtedly an effective technique,[1] regardless of the hydrogen peroxide (HP) concentration used.[2,3]

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However, safety has always been a concern, since literature reports damage to the dental structure promoted by HP agents, including a decrease in microhardness,[3] an increase in roughness, tooth sensitivity, morphological changes and enamel mineral loss.[4] These adverse effects may be transitory, considering the remineralization action played by saliva or topical fluoride application.[5] Therefore, using remineralizing agents like sodium fluoride (NaF),[6] or incorporating NaF into bleaching agents could be feasible alternatives for controlling or reversing enamel mineral loss.[7,8]

Although the ability of sodium fluoride to reduce the solubility of components of the mineralized structure has already been proven,[9] other fluoride agents, such as titanium tetrafluoride (TiF_4), have drawn the interest of researchers to their therapeutic properties, such as reversing mineral loss, and triggering remineralization of mineral structures.[10,11,12] According to previous studies, TiF_4 can prevent demineralization, because it forms a titanium-rich layer on top of the enamel.[11,13,14]

Solutions or varnishes with different concentrations of TiF_4 (mostly 4%) have been designed and indicated for application on erosion lesions, owing to their ability to inhibit mineral loss.[14] The effectiveness of 4% TiF_4 in remineralizing artificial white spot lesions has been found to be similar to 2% NaF.[11] TiF_4 has an extremely low pH (≈ 1.2)[11] that might favor remineralization,[15] and that is believed to form a new compound (hydrated hydrogen titanium phosphate) by a process of dissolution-precipitation.[16] During application, titanium ions interact with phosphate compounds, replacing the calcium ions on the hydroxyapatite dental surface.[15,17] Based on the findings for the remineralizing ability of TiF_4,[18] its incorporation into bleaching agents could benefit esthetic procedures by preventing structural/morphological changes in enamel, and the damage promoted by HP. So far, only one *in vitro* study has performed topical application of a TiF_4 solution after in-office bleaching treatment. According to the results, 4% TiF_4 was as effective as NaF in preventing bleached enamel mineral loss.[19] Based on the aforementioned, this *in vitro* study aimed to test an experimental gel containing TiF_4, with different stock gels, and evaluate the bleaching efficiency and pH of the gels, and the mineral content and morphology of the enamel submitted to a commercial 35% HP bleaching agent associated with the experimental gel formulations. TiF_4 powder was homogenized by producing experimental agents with different stock gels (Natrosol, Aristoflex and Chemygel), before combining them with a 35% HP commercial agent (HP).

Therefore, the null hypotheses were that the experimental TiF_4 gels, compared with a commercial HP agent: a) would not interfere in the bleaching effectiveness; b) would not change the enamel mineral content of the bleached enamel; and c) would not affect the surface morphology of the bleached enamel surface.

Methodology

Experimental design

This *in vitro* study evaluated the color alteration, the enamel mineral content and the surface morphology of enamel blocks submitted to a 35% HP agent (HP, Whiteness HP 35% FGM, Joinville, SC, Brazil) with or without different TiF_4-based formulations. The experimental TiF_4-based formulations were obtained and tested using a study divided in two phases: 1- preliminary manipulation of different stock gels combined with TiF_4, and 2- testing of the selected TiF_4 formulations combined with 35% HP.

In phase 1, different reagents and stock gels were combined with TiF_4 to obtain a gel-based TiF_4 that had a homogeneous appearance, and that could be applied to enamel, after which the gels obtained were analyzed visually. The most suitable combinations obtained in phase 1 were selected and tested in phase 2, in which enamel blocks were prepared, selected according to surface microhardness, and randomly divided into the following groups (n = 8) (independent variable at four levels): HP (control); HP combined with Natrosol + 4% TiF_4 (HPnT); HP combined with Natrosol + Chemygel + TiF_4 (HPncT); HP combined with Aristoflex + TiF_4 (HPaT). The response variables (dependent variables) were color evaluation (according to CIEL*, a*, b* parameters) and whiteness index for dentistry (WI_D), measured

at baseline and 24 h after each bleaching session. Following the bleaching process, whiteness index differences (ΔWI_D), color alteration (CIELab – ΔE and CIEDE2000 - ΔE_{00}), changes in Knoop microhardness (ΔKHN), and enamel morphology (scanning electron microscopy – SEM) were evaluated.

Phase 1. Preliminary manipulation of gel-based TiF_4

Different stock gels and diluents were tested (Tables 1 and 2) to produce the experimental gel able to dissolve TiF_4, and having adequate viscosity and homogeneity. In this phase, no analytical method was used, except for the visual analysis of the gel combination tested. The experimental gels were manipulated under controlled temperature (20°C). Based on scientific literature, 4% TiF_4 in powder form was defined as the standard concentration for all manipulations,[20] and the weight equivalence (g) of the others compounds was calculated and weighed on an analytical precision balance (Chyo JEX-200, YMC Co Ltda, Tokyo, Japan). First, the TiF_4 powder was crushed with a plastic stick into the plastic beaker, and mixed with the amount of stock gel required to

Table 1. Gel formulations tested and respective occurrences.

TiF_4	Stock gel	Diluent agent	Manipulation outcome / Occurrences	Visual analysis
0.813 g	4.5g Chemygel®	42 µl Propylene Glycol	presence of TiF_4 clusters	Inadequate
0.271 g	1.499 g Chemygel®	13 µl DMSO	presence of TiF_4 clusters	Inadequate
0.406 g	2.248 g Chemygel®	800 µl DMSO	homogeneous gel with high DMSO concentration	Inadequate
0.271 g	1.499 g Chemygel®	45 µl DMSO	less presence of TiF_4 clusters but high concentration of DMSO	Inadequate
0.2 g	5 g Carbopol®	90 µl DMSO	solution with low viscosity	Inadequate
0.2 g	5 g Aristoflex®	90 µl DMSO	presence of white agglomerates circled by a transparent solution	Inadequate
0.2 g	5 g Natrosol™	45 µl DMSO	homogeneous gel with high DMSO concentration	Inadequate
0.1g	2.4g Natrosol™	Not applicable	homogeneous gel with translucent aspect	Adequate
0.1g	1.2g Natrosol™ and 1.2g Chemygel®	Not applicable	homogeneous gel with whitish appearance	Adequate
0.1g	2.4g Aristoflex®	Not applicable	homogeneous gel with translucent aspect	Adequate

Table 2. Composition of the materials used, as reported by the manufacturer.

Material	Composition	Manufacturer
TiF_4	Titanium Tetrafluoride	Sigma-Aldrich® Brasil Ltda, Cotia,Brazil
Chemygel® gel	Mineral oil, paraffinum liquidum, liquid petrolatum, polyethylene based-gel	Chemyunion® Ltda, Manalapan, USA
Natrosol™ gel	Hydroxyethyl cellulose, disodium EDTA, water, triethanolamine, methylparaben, propylene glycol	Mapric®, Greentech Company, São Paulo, Brazil
Aristoflex® gel	2% Aristoflex AVC®, 0.6% Optiphen, 3% glycerin, 100g deionized water qsq	Drogal, Piracicaba, Brazil
Carbopol® gel	Carbopol 940, disodium EDTA, aqua, carbomer, phenoxyethanol, methylparaben, propylene glycol, ammonium lactate, glycerin, butylene glycol, polyethylene glycol 400, triethanolamine	Mapric, Greentech Company, São Paulo, Brazil
DMSO	Dimethyl sulfoxide	Sigma-Aldrich® Brasil, Cotia, Brazil
Propylene Glycol	1,2 Propanediol	Labsynth, São Paulo, Brazil

dissolve 4% TiF_4, with or without the diluent agent. Subsequently, the plastic beaker containing the TiF_4 stock gel was maintained on an ice plate to control heating, and was further homogenized using an Ultra-Turrax™ homogenizer (IKA® T10 basic, Staufen, Germany) for approximately 5 min.

According to the data reported in Tables 1 and 2, and based on visual analysis, the combinations selected for phase 2 were those capable of homogenizing TiF_4 without forming clusters or precipitation, and of maintaining gel viscosity (as opposed to liquid), compared with the commercial bleaching gel combinations. The experimental groups selected were:

a. 96% Natrosol and 4% TiF_4;
b. 48% Natrosol, 48% Chemygel and 4% TiF_4;
c. 96% Aristoflex and 4% TiF_4.

Phase 2. Testing of the selected gel-based TiF_4

Specimen preparation

Thirty-two enamel/dentin bovine blocks free of caries and defects were obtained from the buccal surface of sound bovine incisor crowns. The blocks with standard dimensions (5x5 mm) were polished with silicon carbide paper (#600, #1500 and #4000-grit) (Buehler®, Lake Bluff, USA), and cleaned ultrasonically for 10 min (Marconi®, Piracicaba, Brazil) at the intervals of the polishing steps.

The dentin surface was isolated with transparent acid resistance varnish (L'Apogée Alfaparf, Campo Grande, Brazil), and the blocks with the exposed enamel surface were immersed in black tea solution (Dr. Oetker, São Paulo, Brazil) for 24 h at room temperature (25°C). After staining, the specimens were stored in distilled water for one week, which was changed daily.[21,22]

The surface microhardness (Knoop Hardness Number – KHN, Shimadzu® Corporation, Kyoto, Japan) was determined, and enamel blocks with mean values of 292.9Kg/mm^2 +/- 29.01 were selected and randomly allocated into four treatment groups (n = 8), as described in Table 3.

Bleaching treatment protocol

The control group (HP) was submitted to the bleaching protocol, according to the manufacture's instruction: the solution was manipulated in the plastic container – it consisted of one drop of thickener and three drops of 35% hydrogen peroxide, both from the commercial product. Manipulation of the experimental agents was performed with 4% TiF_4 combined with the stock gels (Natrosol, Natrosol + Chemygel or Aristoflex), as described in phase 1. One drop (or 0.05g) of these experimental gel-based TiF_4 alternatives was incorporated into the commercial bleaching agent (HP), as descripted for the control group, and mixed manually with a plastic spatula immediately before applying the solution to the enamel surface. Bleaching treatment consisted of three applications of 15 min each, in four sessions at 72-h intervals.[23] Following the bleaching protocol, the specimens were washed with distilled water and stored in artificial saliva (1.5 mM CaCl, 0.9 mM NaH_2PO_4, 150 mmol/l KCl, pH 7.0) at 37°C,[24] changed every 24 h.

Color measurement

The color parameters were evaluated before bleaching (baseline), and 24 h after each of the four bleaching sessions. The evaluation was performed

Table 3. Treatment groups (phase 2) and composition.

Variable	Group	Abbreviation
35% Hydrogen Peroxide (control) (Whiteness HP 35% FGM, Joinville, Brazil)	HP (After mixture: 35% Hydrogen peroxide, stock gel, red dye, glycol, inorganic filler, deionized water)	HP
Experimental groups (phase 2)	HP + Natrosol + 4% TiF_4	HPnT
	HP + Natrosol + Chemygel + 4% TiF_4	HPncT
	HP + Aristoflex + 4% TiF_4	HPaT

TiF_4: Titanium tetrafluoride; HP: Hydrogen peroxide.

with the Vita Easyshade spectrophotometer (Vita-Zahnfabrik, Bad Säckingen, Germany), attached to a three-fingered laboratory clamp (Jack lift – Q219, Quimis), with standard height, and with the tip pointing downwards to the dental block. The specimen was placed over an opaque white ceramic background, and the color was measured under a controlled light environment. Three color measurements were taken on each specimen in different directions, by rotating the specimen and the ceramic underneath, without displacing the spectrophotometer, considering that variations may occur if the tip is placed at a distinct angle.[25] Mean values of the CIELab parameters (L*, a*, and b*) were obtained. Later, the whiteness index for dentistry (WI_D), whiteness index differences (ΔWI_D), color change (ΔE) and CIEDE2000 color difference (ΔE_{00}) were determined according to the following equations:[26,27,28]

$$WI_D = 0.511L^* - 2.324a^* - 1.100b^* \quad (1)$$

$$\Delta WI_D = WI_D\ 4^{th}\ \text{application} - WI_D\ \text{baseline} \quad (2)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

$$\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T*(\Delta C'/K_C S_C)*(\Delta H'/K_H S_H)]^{1/2} \quad (4)$$

Color changes were evaluated between the bleaching sessions: ΔE_0 (1st session – baseline), ΔE_1 (2nd session – baseline), ΔE_2 (3rd session – baseline) and ΔE_3 (4th session – baseline).

CIEDE2000 was performed according to the L*, a* and b* parameters, and to the c* and h* parameters calculated by a software program validated by Sharma et al.[29]

Microhardness analysis

Knoop microhardness measurements (KHN, Shimadzu, Kyoto, Japan) were performed on the enamel surface before and after 24 h of the last bleaching application (4th) to calculate the change in Knoop microhardness (ΔKHN). Five indentations were made 500 µm from the margin and 100 µm apart, under a 490.3mN load for 10 sec. Mean values were obtained for each specimen.[12]

pH measurement

Bleaching gels (control and experimental agents) were submitted to pH measurement (MS Tecnopon, Piracicaba, Brazil) using a small ion-electrode device (Sensoglass, São Paulo, Brazil).[3,30,31,32] The pH meter was calibrated with three standard solutions (pH 1.0, 4.0 and 7.0) (Dinâmica®, Diadema, Brazil) and 0.01 accuracy. The pH of each group was evaluated at four different times (baseline, 5, 10 and 15 min, corresponding to a single bleaching application of 15 min) in triplicate (three pH readings).

Scanning electron microscopy

Three specimens from each group were selected and observed under scanning electron microscopy (SEM) (JEOL-JSM, 6460LV, Tokyo, Japan). The specimens were left to dry in an incubator for 24 h, sputter-coated with gold (MED 010, Balzers, Liechtenstein), and submitted to evaluation using SEM (1,500x magnification), operating at 15 kV in vacuum mode (45 Pa).[12]

Statistical analysis

The normal distribution and homoscedasticity of the data were confirmed by the Shapiro-Wilk and Levene tests ($p > 0.05$), and parametric analyses were then performed. CIELab parameters (L*, a*, b*), and the whiteness index for dentistry (WI_D) were analyzed by two-way repeated-measures ANOVA and Bonferroni's *post-hoc* test. Whiteness index differences (ΔWI_D), color alteration (ΔE), and CIEDE2000 color difference (ΔE_{00}) were tested by one-way ANOVA and Tukey's *post-hoc* test. Changes in Knoop microhardness (ΔKHN) were analyzed by one-way ANOVA and LSD *post-hoc* test. The analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA), with a significance level set at 5%. SEM evaluation and pH measurement were submitted to descriptive analysis. The test power was determined by GPower 3.1 (GPower, ASA Group, Autenzell, Bayern, Germany), considering the main variables of the study (surface microhardness and the color parameters of CIELab, whiteness index for dentistry - WI_D, whiteness index differences - ΔWI_D, color change difference - ΔE and CIEDE2000 - ΔE_{oo}) ($\beta > 0.9$, $\alpha = 0.05$).

Results

Color measurement

The test power showed values higher than 0.9578 (95%) for all the methodologies performed, thus ensuring high sample value confidence.

The CIELab color parameters (L*, a* and b*) values for HP, and the experimental agents at baseline and after bleaching applications are shown in Table 4. The L* coordinate did not differ among the bleaching sessions or the groups ($p > 0.05$). At the end of bleaching, HP and HPncT groups showed no differences in a* mean values ($p > 0.05$), but were higher than groups HPaT and HPnT ($p < 0.028$). Furthermore, only HPaT showed a decrease in the a* value at the end of bleaching ($p < 0.01$). The yellow appearance of the teeth (b* mean values) decreased after HP ($p < 0.014$) and HPncT applications ($p < 0.03$), unlike the other groups that maintained similar b* values ($p > 0.05$). HP displayed lower b* values ($p = 0.038$) than HPnT after the bleaching treatment, denoting that values were closer to blue than yellow.

The whiteness index for dentistry (WI_D) and the color change (ΔE) values for all the treatment groups throughout the bleaching application period are shown in Table 5. No differences in WI_D were observed among the groups at any bleaching application, or throughout the bleaching sessions ($p > 0.05$), except for HP between baseline and the 2nd, 3rd and 4th applications. HP had higher ΔE values, based on the 2nd bleaching application, compared with HPnT at ΔE_1, and compared with all the experimental agents at ΔE_2 and ΔE_3 ($p < 0.004$). Moreover, no differences were found among the experimental groups at any color change evaluated (ΔE_0 - ΔE_3) ($p > 0.05$).

Table 4. Mean and standard deviation (SD) of whiteness index for dentistry (WI_D), and color changes (ΔE) results

	WI_D				
	Baseline	*1st application*	*2nd application*	*3rd application*	*4th application*
HP	14.43 (9.08) Ba	21.75 (3.53) ABa	24.98 (3.24) Aa	25.99 (3.38) Aa	26.63 (2.54) Aa
HPnT	20.35 (4.62) Aa	21.91 (3.50) Aa	22.28 (4.87) Aa	21.85 (4.12) Aa	22.58 (4.95) Aa
HPncT	17.06 (4.64) Aa	20.35 (3.73) Aa	22.56 (3.59) Aa	22.88 (4.08) Aa	23.90 (4.25) Aa
HPaT	17.76 (11.69) Aa	22.91 (7.70) Aa	21.98 (6.67) Aa	24.62 (5.88) Aa	25.67 (6.15) Aa
	ΔE				
	ΔE_0	ΔE_1	ΔE_2	ΔE_3	
HP	6.07 (3.36) a	8.66 (3.65) a	9.45 (3.95) a	10.16 (3.87) a	
HPnT	3.69 (0.61) a	4.14 (1.65) b	4.88 (1.72) b	5.15 (1.89) b	
HPncT	3.73 (2.05) a	5.10 (2.54) ab	4.75 (2.40) b	5.62 (2.91) b	
HPaT	4.28 (2.45) a	6.59 (2.66) ab	4.85 (3.23) b	5.52 (2.73) b	

Mean values followed by distinct letters differ statistically at 5%, according to two-way repeated measures ANOVA and Bonferroni *post-hoc* test (WI_D), or one-way ANOVA and Tukey *post-hoc* test (ΔE). Uppercase letters compare enamel color changes (WI_D) within bleaching agents (lines). Lowercase letters compare enamel color changes (WI_D, ΔE) at each time point (columns). n=8 specimens/group.

Table 5. Mean and standard deviation (SD) of the whiteness index difference (ΔWI_D), color change difference (ΔE) and CIEDE2000 color difference (ΔE_{00}) results

	ΔWI_D	ΔE	ΔE_{00}
HP	10.50 (4.54) a	10.16 (3.87) a	5.06 (1.72) a
HPnT	2.24 (4.16) b	5.15 (1.89) b	2.92 (1.55) b
HPncT	6.84 (5.21) ab	5.62 (2.91) b	3.22 (1.35) ab
HPaT	7.91 (6.97) ab	5.52 (2.73) b	3.03 (1.22) b

Mean values followed by distinct letters differ statistically at 5%, according to one-way ANOVA and Tukey *post-hoc* test (ΔWI_D, ΔE and ΔE_{00}). Lowercase letters compare enamel color changes (ΔWI_D, ΔE, and ΔE_{00}) at each time point (columns). n=8 specimens/group.

The whiteness index difference (ΔWI_D) and color change (CIELab - ΔE and CIEDE2000 - ΔE_{00}) values for all the treatment groups are shown in Table 6. HP had a higher whitening effect (ΔWI_D) than HPnT ($p = 0.002$), but it was no different from HPncT and HPaT ($p > 0.05$). HP exhibited higher color change values (ΔE and ΔE_{00}) than the experimental groups ($p < 0.047$), but no differences in color change (ΔE_{00}) were found between HP and HPncT ($p > 0.05$).

Surface microhardness

Figure 1 shows the results for the change in Knoop microhardness (ΔKHN, before and after bleaching treatment) among the bleaching formulations. HPncT increased the enamel microhardness, and had a higher ΔKHN than any other group ($p < 0.022$). HP and HPaT decreased enamel microhardness with no statistical difference between them ($p = 0.716$). HPnT caused the highest microhardness loss among the groups, after bleaching ($p < 0.006$).

pH-measurement

The control group (HP) presented the highest pH values (6.9) among the groups (Figure 2). On the other hand, HP showed pH values closer to neutral, and the HPnT, HPncT, HPaT experimental groups showed initial acid pH values of 2.27 / 3.58 / 2.89, and final values of 2.30 / 3.91 / 3.03, respectively.

Scanning electron microscopy

The representative enamel surface areas of the control and experimental groups are shown in Figure 3. The HP group showed a flat and relatively smooth surface, whereas the HPnT and HPaT groups presented similar surface topography, revealing rough enamel areas, and the presence of enamel cracks. In contrast, HPncT displayed a regular, flat surface, with no topographical enamel alterations.

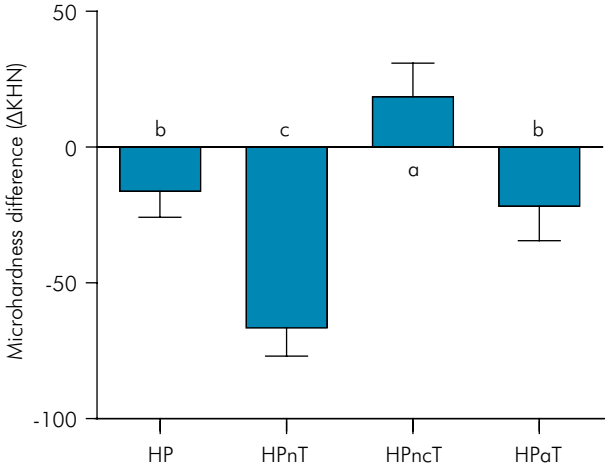
Table 6. Mean and standard deviation (SD) of the whiteness index difference (ΔWID), color change difference (ΔE) and CIEDE2000 color difference (ΔE00) results.

Variable	ΔWI_D	ΔE	ΔE_{00}
HP	10.50 (4.54) a	10.16 (3.87) a	5.06 (1.72) a
HPnT	2.24 (4.16) b	5.15 (1.89) b	2.92 (1.55) b
HPncT	6.84 (5.21) ab	5.62 (2.91) b	3.22 (1.35) ab
HPaT	7.91 (6.97) ab	5.52 (2.73) b	3.03 (1.22) b

Mean values followed by distinct letters differ statistically at 5%, according to one-way ANOVA and Tukey post-hoc test (ΔWID, ΔE and ΔE00). Lowercase letters compare enamel color changes (ΔWID, ΔE, and ΔE00) at each time point (columns). n = 8 specimens/group.

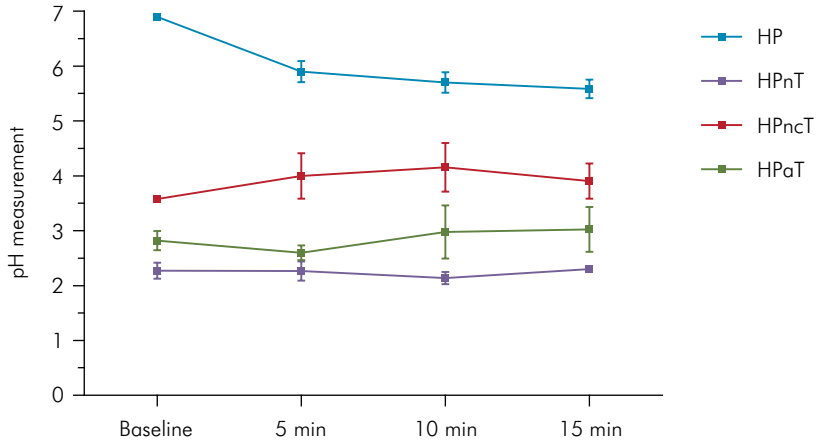
Discussion

The primary goal of this research was to determine if the experimental gels containing TiF_4 (a potential remineralizing agent) combined with 35% hydrogen peroxide would influence the bleaching efficacy of the oxidizing agent (HP). The bleaching ability was determined by measuring color according to three distinct parameters (L*, a*, and b*), which individually indicate variations of lightness (L* -black; +white) and chrome (a*-green; +red and b* -blue; +yellow).[33] For bleaching purposes, changes in the L* and b* parameters denote bleaching, since lightness increases (L* values), and the yellow appearance of the teeth (b* values) decreases.[33] According to the results, no increase in lightness (L* values) was observed



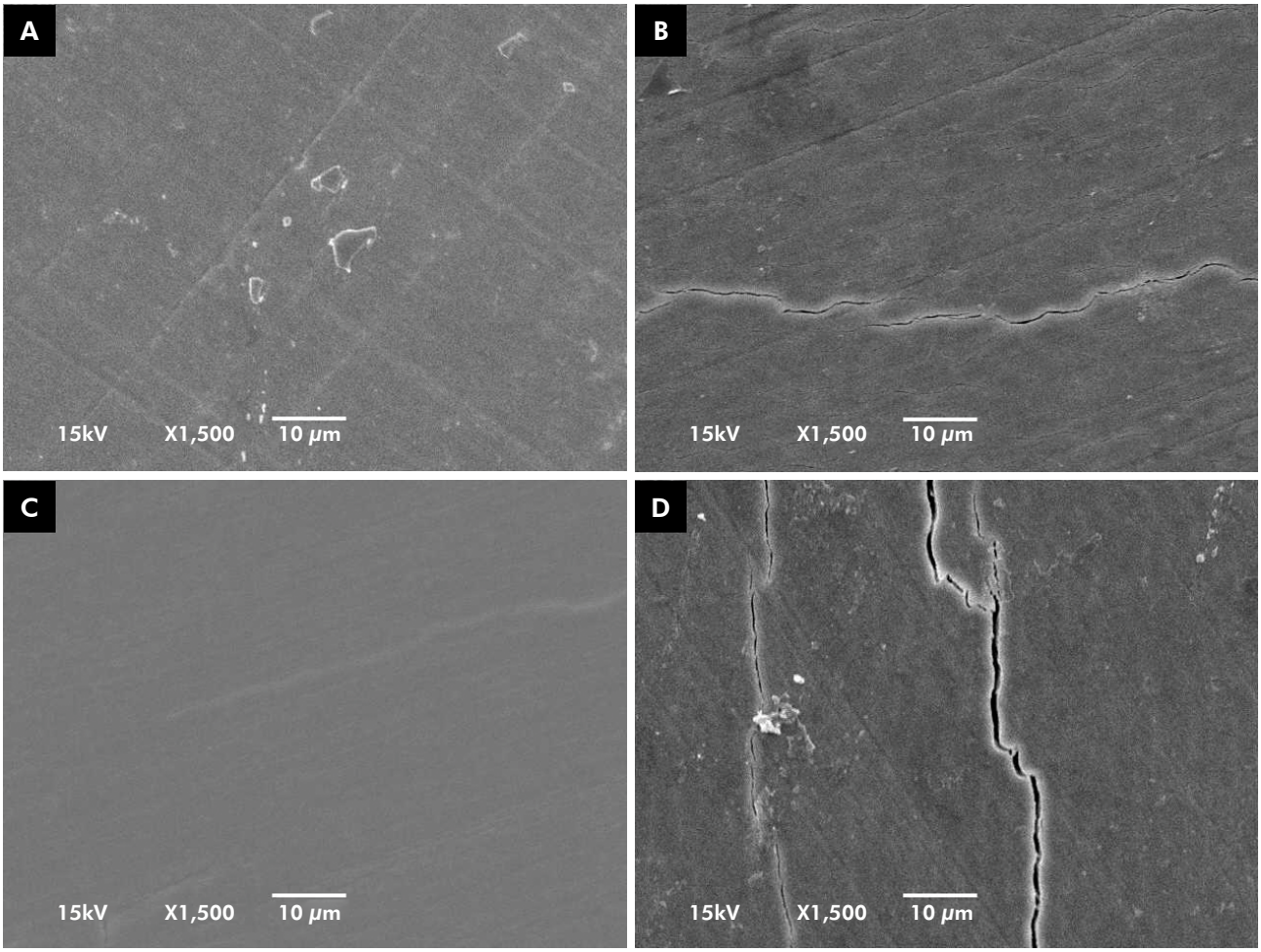
HP: hydrogen peroxide; HPnT: hydrogen peroxide with Natrosol and titanium tetrafluoride; HPncT: hydrogen peroxide with Natrosol, Chemygel and titanium tetrafluoride; HPaT: hydrogen peroxide with Aristoflex and titanium tetrafluoride. Mean followed by distinct letters differ statistically at 5%, according to one-way ANOVA and LSD *post-hoc* test. N = 8 specimens / group. Lowercase letters compare bleaching treatments.

Figure 1. Graphic representation of mean and standard deviation values of change in Knoop microhardness (ΔKHN) values after treatments.



HP: hydrogen peroxide; HPnT: hydrogen peroxide with Natrosol and titanium tetrafluoride; HPncT: hydrogen peroxide with Natrosol, Chemygel and titanium tetrafluoride; HPaT: hydrogen peroxide with Aristoflex and titanium tetrafluoride.

Figure 2. Graphic representation according to mean and standard deviation values of pH measurements at baseline, at 15 minutes.



A) HP - hydrogen peroxide: the enamel surface presented slight alterations in the control group; B) HP + Natrosol + 4% TiF_4: irregular enamel surface with cracks; C) HP + Natrosol + Chemygel + 4% TiF_4: without any enamel surface alteration; D) HP + Aristoflex + 4% TiF_4: irregular enamel surface with cracks.

Figure 3. Representative SEM images of enamel surface after bleaching protocols. Magnification was set at 1,500x.

after dental bleaching regardless of the bleaching gel tested, but the yellow appearance of the HP and HPncT treated groups decreased (b*). This result was expected for the HP group, but was a positive outcome for the HPncT group.

Variations in the a* parameter indicate whether color is changing to red (a* > 0) or green (a* < 0), and although this parameter could hardly apply to bleaching, specimens in this study were stained with black tea solution;[21] therefore, variations in the a* parameter could denote removal of the black tea stains. After bleaching, all the groups showed negative values (a* < 0), indicating the ability of all the agents to decompose the red stains promoted by the tea, but HPaT and HPnT showed the lowest a* values.

The whiteness index for dentistry (WI_D) indicates that higher values correspond to whiter teeth, and lower WI_D (including negative values) denotes darker teeth. The positive WI_D results for all the groups indicated the whitening ability of the agents,[27] and no differences were observed between the experimental groups and the control (HP) for any of the bleaching applications. Moreover, the ΔWI_D values for HPncT and HPaT were similar to HP. However, the commercial HP gel showed greater color changes (ΔE) than the experimental agents. Although ΔE is an important parameter for observing color changes based on the L*, -a*, -b* parameters, we should bear in mind that it should not be interpreted individually, but together with other variables, like WI_D and ΔWI_D, which correlate color with proximity to white.[27] In addition, all the groups showed ΔE higher than 2.7, which is the limit previously established for observing color changes clinically.[34]

Another formula used in dentistry to evaluate color change is CIEDE2000, which uses the CIEL*a*b* parameters, but provides adjustments in the way color difference is evaluated to make it acceptable for human accuracy ($\Delta E_{00} \geq 1.8$).[28,35] According to the results, no differences in ΔE, ΔWI_D, or ΔE_{00} were found among the HPaT, HPncT, and HPnT experimental groups; however, only HPncT exhibited two of these parameters (ΔWI_D and ΔE_{00}), similar to the control group (HP). Overall, the color results seem to suggest that hydrogen peroxide was able to diffuse through the enamel and dentin, and react with organic and bioorganic matter, or biological residual products of dental tissues, and achieve bleaching by breaking down the stain. The stain removal occurred even when HP was combined with the experimental TiF_4-based gels.[4,33,36] On the other hand, the different stock gels tested (Aristoflex, Natrosol or Natrosol with Chemygel) in combination with an acidic agent (TiF_4) may have interfered with the decomposition rate of HP.[37] Therefore, although we expected the experimental agents to perform comparably to HP, or preferably better in every color parameter, the first hypothesis was not accepted, since the TiF_4-based gels interfered with the bleaching efficacy of the commercial HP agent.

In the present study, the reason for using different stock gels was based on having to dilute TiF_4 before it could be incorporated into the bleaching agent (phase 1). TiF_4 is a highly unstable agent in water[17], and the first trials used Chemygel, a water-free petroleum polymer-based gel. Unexpectedly, Chemygel alone had unsatisfactory results, because it was unable to homogenize TiF_4, even after sonication and centrifugation. Subsequently, other stock gels were tested (Aristoflex, Natrosol and Natrosol combined with Chemygel), and these combinations were able to homogenize TiF_4.

Stock gels are used as vehicles for bleaching formulations, because they turn solid or liquid compounds into gel consistency, and thus prolong the decomposition rate of oxygen ions.[37,38] Aristoflex is a versatile polymer, and is frequently used as a vehicle in cosmetic products.[39] Natrosol is a cellulose-based polymer found in some existing bleaching products. It remains stable in acidic media (such as TiF_4 solution or gel) without damaging the enamel structure.[37,40]

Previous studies have observed that Natrosol promotes acceptable color change when combined with HP, and minor enamel surface alterations after bleaching, thus qualifying this agent as a promising substitute for Carbopol.[38] However, although TiF_4 is unstable, the results of this study indicate that the combination of Natrosol with Chemygel performs better in regard to color change, compared with the other experimental formulations. The fact that HPncT was able to change the enamel color to one that was closer to that obtained for the HP group

may indicate that the addition of TiF_4 with Natrosol and Chemygel did not completely hamper the ability of HP to release free radicals, and oxidize the stained molecules. Nevertheless, adjustments in the formulation made by adding catalyst substances could be of interest.

The control group (HP) – a stable commercial reference – decreased enamel microhardness, a result confirmed by a previous study.[3] In fact, this was expected, and was why TiF_4-based gels were combined with HP. However, not all the TiF_4-based gels performed appropriately. HPnT, for instance, had the lowest microhardness difference among the groups, and the smallest surface morphological alterations, as observed under SEM. In addition, although HPaT succeeded in bleaching, it also decreased the enamel microhardness and promoted morphological alterations in different parts of the enamel, ultimately compromising the performance of this gel.

As opposed to these results, the HPncT experimental agent increased microhardness after bleaching, and no morphological changes or surface defects were detected on the enamel, representing a very important finding. Thus, since experimental formulations decreased (HPnT) or increased (HPncT) the enamel microhardness difference, and promoted enamel surface morphology alterations (HPnT and HPaT) compared with the control group (HP), the 2nd and 3rd null hypotheses were rejected, respectively.

The pH value plays a major role in enamel mineral content balance.[41] It can influence the kinetics and stability of the stock gel,[38] and how TiF_4 interacts with enamel. For example, TiF_4 combined with Natrosol + Chemygel (HPncT) was able to maintain enamel microhardness and surface characteristics even in acidic pH (final pH = 3.91), unlike HPnT (final pH = 2.30), which included only Natrosol as a stock gel, and which had lower microhardness values. Yu et al.[15] observed that the TiF_4 solution performed better (remineralizing potential) in acidic media. This could explain the remineralizing action of HPncT, compared with that of the HP group. It should be pointed out that higher pH values interfere in the storage stability of HP, and increase the bleaching efficacy of peroxide-based gels,[41] which could explain why the commercial HP had superior color results for most color parameters, compared with the experimental acidic TiF_4 gels.

The TiF_4 mechanism is based on its ability to deposit titanium ions on the enamel surface, thus ensuring acid resistance and producing a layer with hydrated titanium phosphate, titanium oxide and calcium fluoride, able to prevent acid penetration.[10,17,42] This layer is formed on enamel treated with solutions or varnishes containing 4% TiF_4,[13,14,15,42] and is not homogeneous because of differences in the concentration of inorganic content (phosphate crystals and hydroxyapatite) on the enamel surface.[11]

One limitation of the current study was that microhardness assessment was limited to the surface, and no cross-sectional microhardness evaluation was performed to determine the integrity of the enamel subsurface. Another limitation was that no chemical analysis was performed on the enamel to confirm whether a layer with hydrated titanium phosphate or titanium oxide actually was formed. In addition, although the experimental gel containing 4% TiF_4, Natrosol and Chemygel combined with 35% HP showed promising results (since it increased enamel microhardness and showed no morphology changes), no post-bleaching microhardness evaluation was performed, and the ability of artificial saliva to control mineral loss over time[43] was not assessed. Possibly, the reduction in microhardness caused by the commercial HP gel and the experimental HPaT could be reversed even in *in vitro* conditions, and the microhardness results observed would not represent a major concern over time.

Since no reports in the literature describe the combination of stock gels/TiF_4/HP, further laboratory investigations are essential to evaluate the enamel color change of TiF_4 associated with different bleaching products that have distinct pH values. Moreover, the post-bleaching enamel mineral content, viscosity and cytotoxicity of this experimental gel must still be determined.

Conclusions

The different stock gels (Aristoflex, Natrosol or Natrosol with Chemygel®) evaluated were able to homogenize 4% titanium tetrafluoride (TiF_4). Furthermore, 35% hydrogen peroxide combined

with an experimental gel containing TiF_4, Natrosol and Chemygel (HPncT) was able to control enamel mineral loss and enamel morphological alterations, even at low pH values.

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