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RESEARCH ARTICLE

Evaluation of Enamel Surface Properties Submitted to Bleaching With 35% Hydrogen Peroxide Associated With Titanium Tetrafluoride (TiF_4)

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ABSTRACT

Objective: This study evaluated the color change, surface roughness, mineral content and morphology of enamel bleached with 35% hydrogen peroxide (HP) combined with an experimental gel containing 1% titanium tetrafluoride (TiF_4).

Materials and Methods: Bovine enamel blocks were treated with ($n = 12$): (TiF) experimental gel containing 1% TiF_4, (HP) 35% HP, (HPT) 35% HP + 1% TiF_4 and (CT) control. Bleaching with HP was performed in 3 sessions (3 × 15 min/applications). pH, colorimetric parameters, surface roughness, mineral content and enamel morphology were determined. The pH was evaluated for 45 min. The color parameters were determined before bleaching (T_0), and 14 days elapsed from the last bleaching session (T_4). Surface roughness was analyzed at T_0 and immediately after last bleaching session (T_3). Enamel mineral content and morphology were verified at T_4. Data were statistically analyzed by one-way, two-way ANOVA and Kruskal–Wallis ($\alpha = 0.05$).

Results: TiF increased surface roughness, and no differences between HP and HPT in terms of color and CO_3^{2-}–PO_4^{3-} mineral content. Ti was detected only on TiF, and slight surface morphology changes were observed in bleached enamel.

Conclusions: The combination of TiF_4 and 35% HP did not interfere with the enamel bleaching effect, controlled surface roughness, and kept mineral content but promoted a minor surface morphology alteration.

Clinical Significance: Due to the adverse effects of bleaching, titanium tetrafluoride (TiF_4) has gained attention for its therapeutic properties, including the ability to reverse mineral loss and neutralize remineralization of mineral structures. Therefore, TiF_4's remineralizing capacity may be a good alternative for incorporation into hydrogen peroxide bleaching agents.

1 | Introduction

Although the in-office bleaching technique using 35% HP effectively promotes color changes [1], a significant number of reports indicate that bleaching increases surface roughness, decreases enamel microhardness, and changes enamel surface morphology [2–4]. The overall enamel surface changes are related to the composition, concentration of the active compound (HP), pH values, and bleaching protocols [5, 6]. To reverse such changes on the bleached enamel surface, the incorporation of remineralizing agents into bleaching gels, such as sodium fluoride or calcium gluconate, have been previously tested, and results have shown their ability to reverse part of the enamel mineral loss following bleaching with highly concentrated HP gels [6].

Titanium tetrafluoride (TiF_4), in the form of varnish or solution, is reported to be an effective remineralizing agent that reverses and controls the development of caries and erosive

lesions [7]. Moreover, authors have attested that TiF_4 is even more effective than NaF in reducing demineralization and increasing enamel remineralization [8]. In a previous study, an experimental bleaching gel containing Natrosol + Chemygel and 4% TiF_4 was formulated, combined with 35% HP [9], and tested on the enamel surface. According to the results, the experimental TiF_4 gel could preserve the enamel mineral content without interfering with the HP-bleaching efficacy. Even with promising results, a second study was conducted [10]. The outcomes showed that lower concentrations of TiF_4 (1%) were even more effective in maintaining the mineral content and enamel morphology while reaching the same bleaching efficacy as the commercial 35% HP gel. Furthermore, the experimental 1% TiF_4 gel did not increase the cytotoxicity of the 35% HP gel [10].

However, despite the promising outcomes documented in previous studies, the surface roughness and chemical content of enamel subjected to the experimental 1% TiF_4 gel have yet to be tested and validated. In this context, Raman spectroscopy, with its potential to determine the inorganic content of enamel submitted to the experimental bleaching treatments, could be a game-changer in this topic's investigation [11–13].

Therefore, this study aimed to evaluate the colorimetric change, surface roughness, mineral content, and morphology of the enamel submitted to bleaching with 35% HP combined with an experimental gel containing 1% TiF_4. The research hypotheses tested were that the experimental gel containing 1% TiF_4 combined with 35% HP would: (1) change the colorimetric parameters similarly to the conventional 35% HP gel; (2) maintain enamel surface roughness; (3) control enamel mineral content; (4) change enamel surface morphology.

2 | Materials and Methods

2.1 | Experimental Design

Bovine enamel blocks ($n = 12$/group) were submitted to the factor "bleaching agent" in four levels: TiF_4 (TiF), hydrogen peroxide (HP), hydrogen peroxide with TiF_4 (HPT), and the absence of the bleaching treatment (control—CT). Color parameters (ΔE_{00}, ΔL, Δa, and Δb) and whiteness changes (ΔWI_D) were measured after the staining protocol (T_0), after the 1st (T_1), 2nd (T_2), 3rd bleaching session (T_3), and 14 days elapsed from bleaching (T_4). Ra was measured at T_0 and T_3, while enamel inorganic concentration (measured by μFT-Raman spectroscopy), mineral content (energy dispersive spectroscopy [EDS]), and surface morphology (scanning electron microscopy [SEM]) were evaluated at T_4. The sample size was determined using the software G*Power (Universitat Dusseldorf: Psychologie—HHU, Dusseldorf, Germany), using the data obtained in a previous study [9]. Although 10 specimens would be necessary, 12 specimens were adopted per group.

2.2 | Specimen Preparation

Forty-eight healthy bovine incisors without enamel defects were selected, and enamel/dentin blocks (5 × 5 × 3 mm) were obtained from the buccal surface. The dentin surface was flattened with #600 silicon carbide (SiC) sandpaper to ensure parallelism to the enamel. Subsequently, the enamel was abraded with #800, #1200, and #2000 SiC sandpaper (3M Products, 411Q, Sumaré, SP, Brazil) in a polishing machine (Arotec, Cotia, SP, Brazil). Enamel was polished with a felt disk and aqueous diamond suspension (with abrasive particles of 6, 3, 1, and ¼ μm) for 2 min. Enamel blocks were stained with black tea solution (Dr. Oetker LTDA, São Paulo, SP, Brazil) for 24 h, according to a previous protocol [14, 15], brushed with pumice powder to remove the nonadherent particles and afterward, samples were stored in artificial saliva (1.5 mM CaCl, 0.9 mM NaH_2PO_4, 150 mmol/L KCl, pH 7.0) [16] at 37°C for one week, replaced every two days, for color stabilization.

2.3 | Group Division

Blocks were randomized and divided into four treatment groups ($n = 12$/group) as follows:

- CT: control, without bleaching and without application of the experimental gel containing TiF_4.
- TiF: treatment with experimental gel containing TiF_4.
- HP: bleaching with commercial 35% HP (Whiteness HP 35%, FGM Dental Group, Joinville, SC, Brazil).
- HPT: bleaching with commercial 35% HP gel combined with the experimental gel containing TiF_4.

The randomization was performed according to the baseline color evaluation (baseline time—T_0), generating ordinary random sequences in Excel by using the L^* coordinate as the parameter. A one-way ANOVA was performed to verify baseline similarity among groups regarding L^*, a^*, and b^* coordinates and Ra.

2.4 | Preparation of the Experimental gel Containing 1% TiF_4

The experimental TiF_4 gel used in this research was fabricated as described in a previous study [9]. The thickener and base vehicles used (Table 1) (2.575 g Natrosol and 2.575 g Chemygel), and 0.05 g TiF_4 (Sigma-Aldrich Brasil Ltda, Cotia, Brazil) were weighed on a precision analytical balance (Chyo JEX-200, YMC Co Ltda, Tokyo, Japan), manually mixed in a plastic container with a lid, followed by homogenization in an automatic mixer at 3500 rpm for 4 min (Speed Mixer, DAC 150.1 FVZ, Synergy Devices). After the mixture, the gels were stored in a refrigerator (4°C), and pH was measured before treatments.

2.5 | pH Measurement

An aliquot of the gels (1 g) was mixed with 10 mL of deionized water in a magnetic stirrer for 10 min at 20°C. A pH meter (Equilam, Diadema, SP, Brazil) coupled to a potentiometer (Orion Research Incorporated, Boston, MA) was previously calibrated with pH 4.0 and 7.0 standards. The pH was determined in

TABLE 1 | Composition of experimental TiF_4 gel.

Material	Composition	Manufacturer
TiF_4	Titanium tetrafluoride	Sigma-Aldrich Brasil Ltda, Cotia, Brazil
Chemygel	Mineral oil, paraffinum liquidum, liquid petrolatum, polyethylene based-gel	Chemyunion Ltda, Manalapan, Florida, USA
Natrosol	Hydroxyethyl cellulose, disodium EDTA, water, triethanolamine, methylparaben, propylene glycol	Drogal Pharmacy, Piracicaba, SP, Brazil

triplicates and determined at evaluation time points 15, 30, and 45 min, denoting the contact time of the bleaching gel on the dental surface. The mean values of the triplicates of each group were recorded [17].

2.6 | Application Protocols

TiF, HP and HPT treatments were carried out in three sessions at 7-day intervals. A 1-mm thick gel layer was applied to the buccal enamel surface, and the gel was renewed thrice every 15 min, according to the HP manufacturer's instructions, totalizing 45 min. The HP application time was used for TiF and HPT to standardize the application protocol. After treatment, the specimens were washed in distilled water and stored in artificial saliva [16] at 37°C, renewed every 24 h. The control group (CT) remained immersed in artificial saliva.

2.7 | Colorimetric Analysis

Colorimetric analysis was determined using a digital spectrophotometer (EasyShade, Vita Zahnfabrik, Bad Säckingen, Germany). The spectrophotometer was fixed on a platform with the tip positioned downward, facing the dental blocks placed on a standardized background. The device tip was kept fixed and perpendicular to the enamel surface of the samples, bearing in mind that color variations may occur if the tip is placed at a distinct angle. The specimens were placed over a white opaque ceramic background in a lifting platform (Jack lift—Q219, Quimis) to allow contact with the device tip. This set was placed in a color-matching light box using standard daylight mode (GTI Minimatcher Series, GTI Graphic Technology Inc., Newburgh, New York, USA), and color measurements were performed on each specimen in different directions, by rotating the specimen and the ceramic underneath, without moving the spectrophotometer [9, 17, 18].

Three measurements were acquired from the enamel buccal surface at T_0, T_1, T_2, T_3, and T_4. The mean values were obtained for each specimen. The color parameters (L^*, C^*, h^*, a^*, and b^*) were recorded to determine: ΔL (luminosity difference), Δa (−green/+red difference), and Δb (−blue/+yellow difference). Besides, the color difference (ΔE_{00}) according to the CIEDE2000 equation was evaluated using the formula: $\Delta E_{00}\ (T_{14}-T_0)=[(\Delta L'/\mathrm{KLSL})^2+(\Delta C'/\mathrm{KCSC})^2+(\Delta H'/\mathrm{KHSH})^2+\mathrm{RT}^*(\Delta C'/\mathrm{KCSC})^*(\Delta H'/\mathrm{KHSH})]^{1/2}$. The whiteness index for dentistry ($\Delta \mathrm{WI_D}$) was calculated using the equation: $\mathrm{WI_D}=0.511L^*-2.324a^*-1.100b^*$ [17, 19, 20]. ΔE_{00} and $\Delta \mathrm{WI_D}$ were evaluated at T_4-T_0.

The ΔE_{00} adopted for perception (PT) and acceptance (AT) limits (50%:50%) were 0.81 (PT) and 1.8 (AT) units, respectively. The $\Delta \mathrm{WI_D}$ adopted for PT and AT limits (50%:50%) were 0.7 (PT) and 2.6 (AT) units, respectively [21].

2.8 | Surface Roughness Analysis

The average two-dimensional surface roughness (Ra) was determined by a roughness meter (Surfcorder SE 1700, Kosalab), which was adapted from a previous investigation [17]. A cut-off of 0.8 mm and speed of 0.2 mm/s were used at baseline (T_0) and the end of the treatments (T_3). The specimens were individually fixed on an acrylic base and then positioned parallel to the surface of the equipment, where the measuring tip was placed perpendicular to the surface of the sample. Three readings were performed by rotating the specimen at 45°, and the average per specimen was obtained.

2.9 | μFT-Raman Spectroscopy Analysis

The enamel's inorganic concentration (phosphate and carbonate) was determined after the treatments (T_4) by means of μFT-Raman spectroscopy. The parameters used for the μFT-Raman spectroscope (Horiba Xplora Confocal micro-Raman [Minami-ku Kyoto, Japan; diode laser, 785 nm]) and subsequent analysis was performed as previously described [6, 22]. Specimens were positioned facing upward over a glass slide, and the Raman laser was focused on the enamel surface, allowing the acquisition of 64 scans (spectral range = 100 $\mathrm{cm^{-1}}$ to 3500 $\mathrm{cm^{-1}}$), from the middle point of the surface. Data were obtained according to the vibrational modes corresponding to the bands of phosphate (PO_4^{3-}) ν_2 (431–449 $\mathrm{cm^{-1}}$) and ν_4 (582–611 $\mathrm{cm^{-1}}$), and carbonate (CO_3^{2-}) ν_3 (1070 $\mathrm{cm^{-1}}$) associated with enamel hydroxyapatite. Raman spectra were fitted and normalized regarding the 960 $\mathrm{cm^{-1}}$ peak. Gaussian shapes and calculating the mean area of peak band deconvolution were performed for band deconvolution using the software Origin 5.0 (Microcal Softare Inc., Northampton, Massachusetts, USA).

2.10 | SEM and EDS

Three specimens from each group were prepared for SEM and EDS. The blocks were mounted on aluminum stubs and coated with a thin layer of carbon (Balzers-SCD 050 Sputter Coater, Liechtenstein). Surface images were obtained at 1000× and 3000× magnification (LEO-MEV 435 VP, LEO Electron Microscopy Ltd, Cambridge, UK) and the qualitative enamel

mineral content analysis (wt%) was simultaneously mapped by the EDS (Vantage System—Easymicro Noran Instruments, Middlleton, Wisconsin, USA), coupled to the SEM. The EDS images were captured at 1000× magnification, in three predefined equidistant areas from the middle point of the enamel surface (with 15kVp, 20 mm of distance, and spot size 45) [17].

2.11 | Statistical Analysis

The data were submitted to the normality and homoscedasticity test (Shapiro–Wilk/Levene). Two-way ANOVA and Bonferroni tests for repeated measures analyzed surface roughness. Color change (ΔE_{00}, transformed to $\log_{10}$), ΔL, ΔWI_D, and μFT-Raman were analyzed by one-way ANOVA and Tukey. Δa and Δb data did not exhibit normal distribution and were analyzed by Kruskal–Wallis. All analyses were performed with a significance level set at 5% in the SPSS Statistics software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The pH measurements and the images obtained from SEM and EDS were descriptively analyzed.

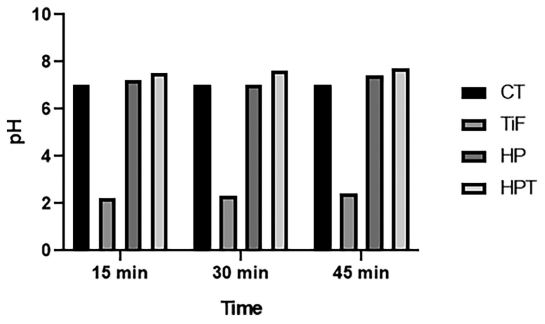

FIGURE 1 | pH means of the gels.

3 | Results

3.1 | pH Analysis

Figure 1 displays the pH means of the gels (TiF, HP, and HPT) and the control group (CT), considering 15, 30, and 45 min, corresponding to the temporal evolution of gels' application. It is possible to observe that experimental TiF gel displayed pH values (≅2.2) that were lower than HP (≅7.2) and HPT (≅7.6), indicating that only TiF displayed an acidic pH. For all groups, pH behavior over time was stable.

3.2 | Colorimetric Analysis

Figure 2 describes the ΔL, Δa, Δb, ΔE_{00}, and ΔWI_D mean values results. The luminosity increased after HP and HPT bleaching protocols, and these groups exhibited higher ΔL ($p < 0.05$) than the TiF and CT groups. No differences in ΔL were found between TiF and CT groups ($p > 0.05$).

The mean values of the a^* (reddish appearance) decreased after treatments, and HP and HPT groups exhibited greater Δa ($p < 0.05$), indicating a decrease in the reddish color in comparison with CT and TiF. The Δb coordinate (yellowish appearance) was higher (and negative) following bleaching with HP and HPT groups ($p < 0.05$) in comparison with CT and TiF. However, no differences were observed between CT and TiF ($p > 0.05$).

HP and HPT displayed higher ΔE_{00} and ΔWI_D than CT and TiF ($p < 0.05$), but no statistical differences were observed between CT and TiF ($p > 0.05$). CT and TIF groups exhibited negative ΔWI_D ($p < 0.05$), indicating that no bleaching effects were observed for these groups. HP and HPT reached mean ΔE_{00} and ΔWI_D values above the PT and AT, while CT and TiF presented only mean ΔE_{00} values above the PT and AT.

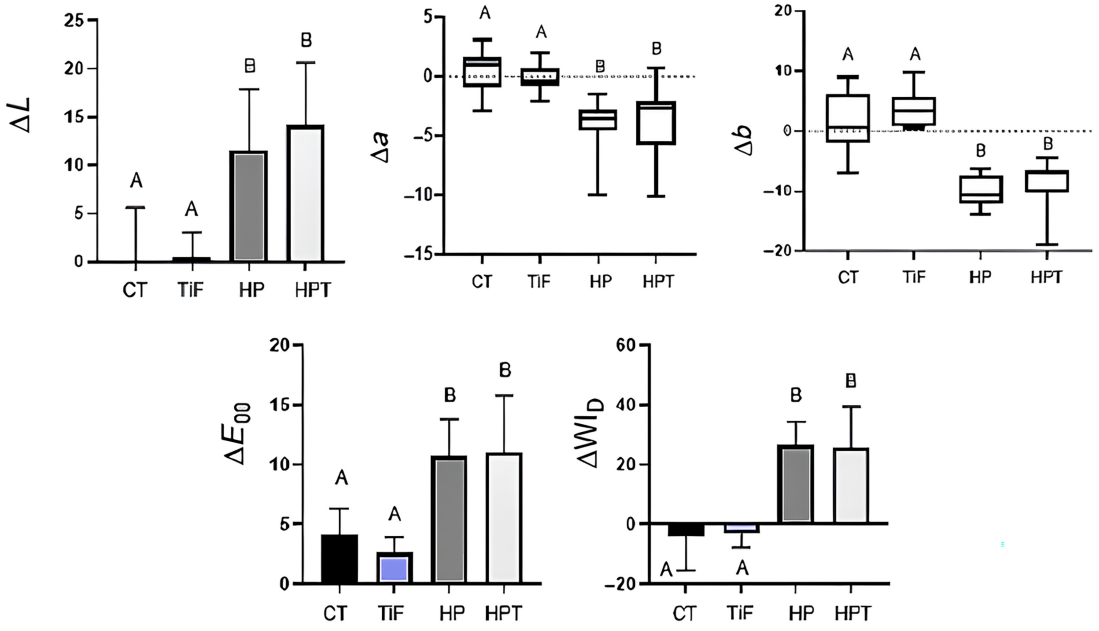

FIGURE 2 | Mean and standard deviation of colorimetric parameters ΔL, Δa, Δb, ΔE_{00}, and ΔWI_D ($\Delta = T_4 - T_0$). Different uppercase letters indicate statistically significant differences ($p < 0.05$) among groups.

3.3 | Surface Roughness Analysis

Table 2 exhibits the significant influence of treatment ($p = 0.002$), time ($p < 0.001$), and treatment*time interaction ($p < 0.001$). At baseline (T_0), no statistical differences are observed among groups ($p = 1.000$). However, at T_4, TiF treatment increased the enamel surface roughness compared with the other groups ($p < 0.001$).

3.4 | μFT-Raman Spectroscopy Analysis

Table 3 describes μFT-Raman results of mineral components obtained in the 400–2000 cm^{-1} region by comparing the area of phosphate vibrational modes peaks (ν_2, 431–449 cm^{-1} and ν_4, 582–611 cm^{-1}) and carbonate vibrational modes ν_3 (1070 cm^{-1}). No significant differences were noted between groups in the vibrational mode ν_2 ($p = 0.248$), ν_4 ($p = 0.726$), and ν_3 ($p = 0.578$ and $p > 0.05$), indicating no significant changes in the phosphate and carbonate peaks after treatments.

3.5 | SEM and EDS

Figure 3 displays the enamel surface morphology of groups CT, TiF, HP, and HPT and the percent of enamel chemical elements after treatments (T_4), according to SEM and EDS, respectively. CT group did not display any morphological alteration, except for minor sediments that could be deposits resulting from the artificial saliva immersion throughout the treatment period. Specimens from all groups, including CT, were submitted to polishing before treatments, but polishing marks were more evident in TiF, HP, and HPT groups. TiF treatment displayed significant surface defects and deeper porosities, while HP and HPT groups displayed the contours of enamel prisms, indicating a slight enamel demineralization. In the EDS analysis, Ca and P were detected in all groups with no significant dissimilarities, except for the TiF group, which exhibited lower percentage of Ca (55.07%) than the other treatments. Ti element was only detected in the TiF group (10.88%).

TABLE 2 | Mean and standard deviation of Ra (μm) values measured at T_0 and T_3. The ΔRa was the difference between $T_3 - T_0$ time points.

Group	Ra (T_0)	Ra (T_3)	ΔRa (T_3—T_0)
CT	0.028 (0.005) Aa	0.027 (0.006) Aa	−0.001
TiF	0.027 (0.005) Aa	0.050 (0.011) Bb	0.032
HP	0.028 (0.005) Aa	0.027 (0.004) Aa	−0.001
HPT	0.029 (0.005) Aa	0.030 (0.005) Aa	0.001
p	$p = 1.000$	$p < 0.05$	—

Note: Means followed by different lowercase letters indicate statistically significant difference ($p < 0.05$) within the same group over time (line). Means followed by different capital letters indicate statistically significant difference ($p < 0.05$) of time points (columns), according to two-way ANOVA for repeated measures and Bonferroni post hoc tests.

4 | Discussion

The first hypothesis was accepted because bleaching with HPT promoted significant color and whiteness change, demonstrating the effectiveness of the treatment. All tested variables for color (ΔL, Δa, Δb, ΔE_{00}, and ΔWI_D) further confirmed the superiority of the hydrogen peroxide-based groups (HP and HPT) over the nonbleached ones (CT and TiF). HP and HPT exhibited a significant luminosity change (ΔL), with a positive increase in the L^* coordinate, differently from the CT and TiF treatments. The decrease in the Δa coordinate in HP and HPT groups indicated variations from red (+80) to green (−80), corresponding with the breakdown of the black tea-chromophores within the dental blocks before treatments [10, 15]. This result attests to the ability of HP-based gels to decrease the reddish appearance promoted by black tea immersion. Furthermore, HP and HPT groups displayed an intense decrease in Δb (−10.5 and −8.5, respectively) ($p < 0.05$), which indicated variations in the b^* parameter from yellow (+80) to blue (−80), denoting bleaching effectiveness due to the decrease in the yellowish staining.

Notably, the CT and TiF groups led to negative mean ΔWI_D values (−3.9 and −3.2, respectively) since no bleaching was carried out in these groups. In contrast, HP and HPT bleaching groups promoted higher variations (26.4 and 25.5, respectively), implying an effective color and bleaching change. These results are similar to those report by a study in which the authors tested the same combination of 35% HP and TiF_4 gel but in different concentrations of TiF_4 (1%–4%) [10]. According to that research, the 1% TiF_4 gel combined with HP increased the enamel luminosity (L^* coordinate). It decreased the red and yellowish color (a^* and b^* coordinates), exhibiting behavior similar to conventional 35% HP [10]. In the present research, the evaluation of the perceptibility and acceptance thresholds indicated that only HP-containing groups achieved levels that would be considered as excellent bleaching effectiveness (>AT × 3) [21]. One might

TABLE 3 | Mean and standard deviation of enamel inorganic concentration (PO_4^{3-} and CO_3^{2-}) according to μFT-Raman spectroscopy analysis.

Vibrational bands	CT	TiF	HP	HPT	Marginal means
$(PO_4^{3-})\,\nu_2$ (431–449 cm^{-1})	2.41 (0.22)	2.46 (0.30)	2.55 (0.27)	2.79 (0.24)	A
$(PO_4^{3-})\,\nu_4$ (582–611 cm^{-1})	0.98 (0.08)	0.98 (0.08)	1.05 (0.18)	1.01 (0.11)	A
$(CO_3^{2-})\,\nu_3$ (1070 cm^{-1})	1.00 (0.16)	0.89 (0.19)	0.98 (0.18)	0.89 (0.20)	A

Note: The marginal means compare the treatment groups in each vibrational band (rows), according to one-way ANOVA and Tukey post hoc tests. Means followed by equal capital letters indicate no statistical differences ($p < 0.05$) among groups (lines).

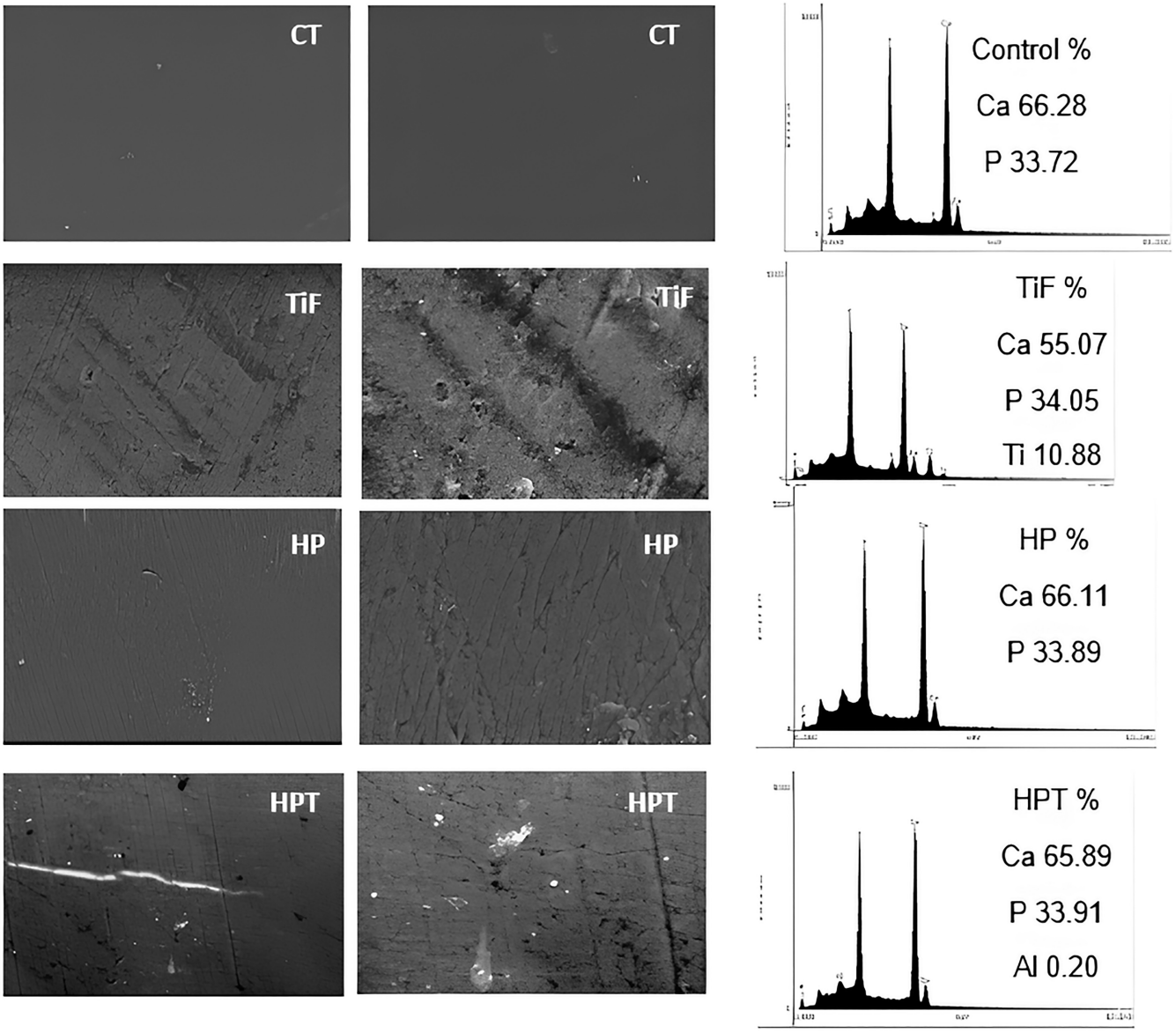

FIGURE 3 | Enamel surface morphology under SEM (1000× and 3000×) and EDS (1000×) analysis, 14 days after the last session bleaching. Al: aluminum; Ca: calcium; P: phosphorus; Ti: titanium.

question the perceivable colorimetric changes in CT and TiF groups. Still, as mentioned above, this event was verified to be a result of the specimens' darkening (mean ΔWI_D values), thereby discarding the bleaching effect on such groups.

The specimens underwent an artificial staining protocol not only to standardize the baseline tooth color among all groups but also to simulate a clinical situation. This staining protocol is consistent with previous studies using experimental bleaching gels [9, 10, 14, 15, 17, 23]. The decision to use black tea as the staining solution was based on its high pigmentation potential, as demonstrated in a previous study [24], which showed that black tea decreases luminosity and increases red and yellow tones of teeth. In their study, although stained teeth exhibited more pronounced color changes, the staining did not influence differences in color change caused by different bleaching agents compared with unstained teeth. Therefore, it is believed that the chosen staining protocol did not affect the action of HP itself nor interfere with the long-term outcomes of bleaching stability. The increase in enamel surface roughness after bleaching treatments could imply a greater susceptibility to extrinsic pigmentation, biofilm accumulation and maturation [25].

Therefore, the surface roughness analysis is an appropriate test to determine if bleaching could interfere with enamel topography and the magnitude of this interference. Before treatments, no differences in enamel surface roughness were noticed among the groups. However, after the last bleaching session, the roughness of the enamel surface increased following TiF treatment. This behavior could be explained by the low pH of the experimental gel (≅ 2.2) and, consequently, the feasible demineralizing effect triggered on the enamel surface. In other words, the enamel surface would be saturated with calcium and phosphorous ions compared with the acidic TiF gel, which would trigger the demineralization of such ions towards the undersaturated gel. However, the presence of fluoride in the gel might have influenced the gel saturation, since

there is no defined critical pH for enamel erosion (demineralization without biofilm), and low-pH drink solutions supplemented with calcium and phosphate did not erode enamel after immersion for 7 days [26]. This could explain why enamel treated with the TiF_4 gel alone (TiF group) exhibited a final surface roughness average of 0.05 mm, which is still safe and below the 0.2 mm roughness threshold to initiate biofilm accumulation [27]. However, further studies could investigate the long-term impacts of the bleaching protocols on the preservation of the enamel surface and its biofilm deposition.

Interestingly, the combination of TiF_4 gel with 35% HP resulted in a basic-pH agent, which provides a more secure application on the dental structures than the experimental TiF gel alone. Possibly because of the neutral pH, HP and HPT did not significantly increase enamel surface roughness, agreeing with a previous finding that shows that regardless of the bleaching agents' concentrations, bleaching did not considerably increase enamel surface roughness in comparison to the control group [28]. Thus, the second hypothesis was accepted since HPT did not significantly change enamel surface roughness. The pH obtained for experimental bleaching gel with HP and TiF_4 was appropriate for enamel surface. Otherwise, it could trigger decrease in the surface microhardness, mineral content, or even volume loss [29, 30] based on the aforementioned explanation. Furthermore, enamel specimens remained stored in artificial saliva during the intervals of bleaching sessions allowing remineralization of the enamel surface [15].

μFT-Raman is a molecular spectroscopy technique that uses the interaction of light with matter to determine the constitution or composition of a substrate, such as enamel, which is essentially composed of inorganic matter. The light diffusion process results in intra and intermolecular vibrations, providing a spectrum of the specific vibrations of a molecule ("molecular identity"), allowing the identification of a substance [6, 11–13, 31]. Importantly, our results showed no significant changes in enamel mineral content among groups, and the phosphate and carbonate peaks were not affected after the bleaching treatment. This finding is significant as it indicates that the pH associated with the experimental TiF_4 gel did not promote enamel demineralization, thereby reassuring the safety of the gel as it did not promote any significant change in enamel mineral content. Corroborating our findings, even without TiF_4, a previous study [32] did not find differences when using bleaching agents with Natrosol and Aristoflex thickener. Likewise, another study [22] demonstrated that the same commercial 35% HP gel did not promote impact in carbonate and phosphate content under μFT-Raman spectroscopy. On the other hand, other research [1] demonstrated that high-concentrated commercial gels, with similar composition and even with calcium or fluoride incorporation, caused significantly decrease in the calcium and phosphorous content of bleached enamel evaluated using total reflection X-ray fluorescence. Therefore, the impact of the mineral content evaluation method on the results should not be ruled out.

Titanium tetrafluoride (TiF_4) has been assessed as a remineralizing molecule capable of reversing the development of caries and erosive lesions [7, 8]. In this context, incorporating TiF_4 into a 35% HP agent may have controlled enamel mineral content while keeping the surface intact. Previously, an experimental TiF_4 gel was combined with the 35% HP [9]. According to that report, the 1% TiF_4 gel containing Natrosol and Chemygel (as thickeners) resulted in the most suitable preparation because it did not hamper the bleaching effect of 35% HP and kept enamel mineral content during bleaching [10]. The results of this study corroborate those, since 35% HP combined with the experimental TiF_4 gel could control the enamel mineral content during bleaching. Thus, the third hypothesis could be accepted.

The chemical content analyses carried out by EDS spectroscopy showed that CT, HP, and HPT exhibited standard calcium (Ca) values ranging between 65.89% and 66.28%. The same was observed for phosphorus (P), with variations from 33.72% to 34.05%, displaying no significant mineral content changes among groups. TiF group exhibited a slightly lower percent Ca in comparison with the other groups (55.07%), which was not detected as significant in the μFT-Raman spectroscopy, as previously mentioned. Additionally, the TiF group exhibited 10.88% of Ti deposition, which did not occur in the HPT group. The absence of Ti in the HPT-treated surface may denote that the available titanium must have reacted with hydroxyapatite, forming an acid-resistant vitreous Ti layer on the enamel surface. According to previous observations, it is reported that this vitreous layer is not uniformly distributed on enamel due to regional differences in Ca and P content in different enamel areas [10, 33]. However, additional analysis should be performed to identify and map Ti layer distribution over enamel.

Enamel surface morphology demonstrated irregularities in all groups but of different types: CT surface resembled deposition, possibly due to the artificial saliva immersion throughout the experiment. The HP and HPT presented a slightly demineralized-surface pattern, comparable with the initial enamel surface demineralization, in which enamel prism counters can be observed. However, since the pH of HP and HPT (≅ 7.2 and 7.6, respectively) were neutral, HP oxidation possibly promoted the slightly demineralized surface pattern. According to these findings, the fourth hypothesis can be accepted as a minor enamel surface change was observed following HPT treatment.

On the other hand, the TiF group (pH≅2.2) did not expose the enamel prism counters, but surface scratches from the polishing procedure and increased enamel surface porosity are evident. This was the only group to significantly increase surface roughness after treatments, reassuring that the application of TiF alone, without the HP combination, changed enamel surface topography. Nevertheless, as previously mentioned, this morphology change may not be clinically relevant since roughness values will not increase the risk of biofilm formation, which could happen on surfaces with roughness values higher than 0.2 μm [27].

The results of this study demonstrate that 1% TiF_4 gel combined with HP 35% did not interfere with the bleaching outcome and did not change the surface roughness or inorganic content but slightly changed the morphology of the bleached enamel. Although HPT performed similarly to HP, previous observations indicate that the main advantage of using a modified bleaching agent (HPT) could rely on mineral content maintenance over time. Also, because of the possible formation of an acid-resistant vitreous Ti layer over enamel during bleaching, future clinical

trials could assess the patient's sensitivity using this experimental bleaching agent. In this context, HPT could be an interesting alternative to decrease bleaching sensitivity since all the other parameters showed its safety in the enamel structure.

5 | Conclusion

Within the limitations of this in vitro study, it can be concluded that the combination of TiF_4 and 35% HP did not influence the bleaching effect, controlled enamel surface roughness, and kept the enamel mineral content, but slightly changed enamel surface morphology. From the clinical standpoint, the use of a TiF_4-enriched in-office bleaching gel would not negatively affect the esthetic outcomes nor impact the enamel integrity.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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