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Can TiF_4 varnish or TiF_4/NaF solution stain eroded and sound enamel?

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ABSTRACT

Objectives: This study evaluated the staining potential of TiF_4 varnish and TiF_4/NaF solution on both sound and previously eroded enamel *in vitro*.

Methods: One hundred and eighty bovine enamel samples were polished; half of them remained sound and the other half was eroded (4 × 90 s each, using a 0.1% citric acid). Thereafter, the baseline color reading was performed (T_0). The samples were then subdivided into the following treatments: TiF_4 varnish (24,500 ppm F, 1 × 6 h), NaF varnish (24,500 ppm F, 1 × 6 h), placebo varnish (1 × 6 h), TiF_4/NaF solution (500 ppm F, 6 × 1 min), Erosion Protection-*Elmex*® (500 ppm F, 6 × 1 min) and control (water, 6 × 1 min). Between the applications, the samples were exposed to artificial saliva for 30 min. The color changes were measured immediately after the applications (T_1-T_6) and after the exposure to artificial saliva (S_1-S_6) by using a spectrophotometer (Vita EasyShade®). The ΔE_{00}, L*, a* and b* data were compared using 2-way ANOVA/Tukey ($p < 0.05$).

Results: TiF_4 varnish induced significantly higher color change (ΔE_{00}) compared to NaF and placebo varnishes on sound enamel immediately after application, but not after saliva exposure. TiF_4/NaF solution induced lower ΔE_{00} values compared to control on sound enamel; however, no differences were found between TiF_4/NaF and *Elmex*®. For eroded, no differences were seen between the tested varnishes and solutions. Both types of fluoride solutions increased the yellow appearance of enamel, while the varnishes did not.

Conclusions: The color changes induced by the experimental TiF_4 products are similar to the commercial ones.

Clinical significance: Both TiF_4 varnish and TiF_4/NaF solution have shown staining potential similar to commercially available varnish (NaF) and solution (*Elmex*®) on sound and eroded enamel. These findings support the conduction of clinical trials, which, in the future, may lead to the commercialization of these products.

1. Introduction

New experimental TiF_4 products, such as varnish (4% TiF_4) and solution (TiF_4/NaF, 500 ppm F), have been tested against tooth erosion showing interesting results compared to commercial products *in vitro* and *in situ* [1–5]. The protective effect of TiF_4 is due to the formation of a glaze-like layer rich in hydrated titanium phosphate, titanium oxide and calcium fluoride on enamel [6]. Despite the promising results against tooth demineralization, it is important to know if the TiF_4 products could induce some side effects such as mucosa desquamation, tooth discoloration, and allergy, among others.

Our research group has shown that TiF_4 varnish is as cytotoxic as NaF varnish, both containing 24,500 ppm F, on murine fibroblasts [7] and they induce low levels of apoptosis in human fibroblasts [8]. With respect to the cytotoxicity of TiF_4/NaF solution, no study on this field has been done, but we would expect lower cytotoxicity compared to TiF_4 varnish since the solution presents lower fluoride concentration and higher pH value compared to the varnish.

Another important aspect to be taken into account is the staining potential of the product. Pedro et al. [9] reported a clinical case where 4% TiF_4 solution (22,500 ppm F), applied on a white spot lesion in a patient, induced a yellowish stain of enamel. We have not observed such side effect with the use of TiF_4 varnish; however, we have recently reported that 40% participants of an *in situ* study dealing with TiF_4/NaF solution complained about a temporary tooth staining induced by the tested solution [5].

There are few studies testing the staining potential of fluorides, most of them focused on silver diamine fluoride [10–12] and stannous

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fluoride [13–17]. Some evidences show that fluoride solution containing stannous is capable of staining the tooth surface [15,16,18] and even restorative materials [19]. However, no quantitative study about the staining potential of TiF_4 has been found, justifying the relevance of the present study.

Furthermore, it is possible that eroded enamel might be more susceptible to staining due to high porosity, as tested by previous authors using fluoride gel (NaF, 2%) on demineralized enamel [20,21]. Therefore, our hypothesis is that the glaze layer produced by TiF_4, which is erosion protective, could stain the sound enamel and, even more, the pre-eroded enamel. To achieve our goal, this *in vitro* study evaluated the staining potential of TiF_4 varnish and TiF_4/NaF solution on both sound and eroded enamel, comparing them with similar commercial products (NaF varnish and NaF/AmF/$SnCl_2$ solution, respectively) by using a spectrophotometer, which is commercially available to be applied in patients, allowing the comparison of the present data with values obtained clinically.

2. Materials and methods

2.1. Sample preparation

One hundred and eighty enamel samples (6 mm × 6 mm × 2.5 mm) were prepared from labial surfaces of recently extracted bovine incisors, previously maintained in 0.1% thymol solution (pH 7.0), by using two diamond-coated discs (XL 12205, "High concentration", 102 × 0,3 × 12,7 mm^3, Extec Corp.; Enfield, CT, USA) in a low-speed cutting machine (ISOMET, Buehler Ltd; Lake Bluff, USA) set at 300 rpm and cooled with deionized water.

The samples were affixed on acrylic discs using wax for the polishing. Firstly, dentin was ground flat using 320-grit SiC sandpapers (Carbimet Paper Discs, Buehler; Lake Bluff, USA); thereafter, the enamel surface was exposed to 600- and 1200-grit SiC sandpapers (Carbimet Paper Discs, Buehler Ltd; Lake Bluff, USA) by using a polishing machine under 100% humidity (Arotec; Cotia, Brazil). Between the procedures, the samples were washed in an ultrasound device containing deionized water for 2 min (T7 Thornton; Vinhedo, Brazil). Only the enamel surface was exposed; the other areas of the samples were protected with transparent nail polish.

The baseline color of the samples (T_0) was measured using a digital spectrophotometer (Vita Easyshade®, Vita Zahnfabrik; Bad Säckingen, Germany). Based on L* axis, the samples were randomly allocated to two conditions: sound (n = 90) and pre-eroded samples (n = 90). This parameter was also applied to randomly distribute the samples to the treatment groups.

The erosion was simulated under a pH cycling model with 4 × 90 s exposure to 0.1% citric acid solution (pH 2.5, v = 0.5 mL/sample), under agitation (60 rpm) and at room temperature. Between the challenges, the samples were washed in water. The color changes after erosion were measured as done at the baseline (T_0, L* axis) and the values were applied in the allocation of the samples into the treatment groups.

2.2. Treatment protocols

Fig. 1 describes the experimental design. The samples were randomly divided into the following groups: TiF_4 varnish (24,500 ppm F, pH 1.0, 1 × 6 h, FGM; Joinville, Brazil), NaF varnish (24,500 ppm F, pH 5.0 1 × 6 h, FGM; Joinville, Brazil), placebo varnish (pH 5.0, 1 × 6 h, FGM; Joinville, Brazil), TiF_4/NaF solution (500 ppm F^-, 190 ppm Ti^{+4}, pH 4.4, 6 × 1 min), Erosion Protection-*Elmex*® (500 ppm F^-, 800 ppm Sn^{+2}, pH 4.5, 6 × 1 min, GABA; Therwil, Switzerland) and control (water) (n = 15/each enamel condition). Between the applications, the samples were exposed to artificial saliva for 30 min.

The varnishes were applied once for 6 h and, during this period, the samples were exposed to artificial saliva [22]. Thereafter, the varnishes were carefully removed with a surgical blade and swabs soaked in acetone solution (1 part acetone: 1 part water) [1]. The solutions were applied 6 times of 90 s (v = 0.5 mL/sample) to simulate what was done in a previous *in situ* study [5]. The excess of the solution was removed with a cotton swab.

After the application of the varnishes and between the applications of the solutions, the samples were immersed in artificial saliva for 30 min (v = 0.5 mL/sample, 37 °C). Saliva was composed of the following reagents: 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and traces of ascorbic acid (pH 6.8) [22].

2.3. Measurement of color alteration

Color measurements were conducted after calibration, according to the manufacturer recommendations [23]. The readings were performed by one calibrated and blinded researcher three times on each sample against a white background using a digital spectrophotometer (Vita Easyshade®, Vita Zahnfabrik; Bad Säckingen, Germany) in the same room free of sunlight and under artificial standardized illumination. The CIEDE 2000 color system [24] was adopted, and the color alteration was measured according to the following equation:

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

The 50:50% perceptibility threshold (ΔE_{00} = 1.30) was adopted according to the study of Ghinea et al. [25].

The color measurements were conducted at the following moments: (T_0) baseline for sound and eroded enamel, (T_1) after the first application, (S_1) after 30 min in artificial saliva; (T_2) after the 2nd application, (S_2) after 30 min in artificial saliva; (T_3) after the 3rd application, (S_3) after 30 min in artificial saliva; (T_4) after the 4th application, (S_4) after 30 min in artificial saliva; (T_5) after the 5th application, (S_5) after 30 min in artificial saliva; (T_6) after the 6th application, (S_6) after 30 min in artificial saliva.

2.4. Statistical analysis

ΔE_{00}, L*, a* and b* values were tabulated in Excel spreadsheets. GraphPad Prism 7.04 software (San Diego, USA) was used for statistical analysis. The data were submitted to the analysis of normal distribution and homogeneity (test of Kolmogorov and Smirnov and test of Bartlett, respectively). Two-way RM ANOVA was applied, considering the factors under study: treatment (3 levels for each type of vehicle, separately) and period of analysis (T_1-T_6, S_1-S_6) for both dental substrates (sound and pre-eroded enamel, separately). The individual comparisons were performed using the Tukey post-hoc test. The sample number was 15 and the significance level adopted in all tests was 5%.

3. Results

3.1. Varnish

The ΔE_{00} values showed that the final colors were visually perceptible [25] for both sound and eroded enamel.

3.1.1. Sound enamel

On sound enamel, TiF_4 induced a higher color alteration (ΔE_{00}) compared to placebo and NaF varnish immediately after application ($p < 0.0001$), but not after saliva exposure ($p > 0.05$). NaF varnish and placebo varnish did not differ from each other in all measurements. For TiF_4 and placebo varnishes, the color was stable. However, NaF-treated samples showed significant color change after exposure to saliva compared to the change induced immediately after the application (Table 1).

All treatments similarly increased the luminosity (L*) and decrease

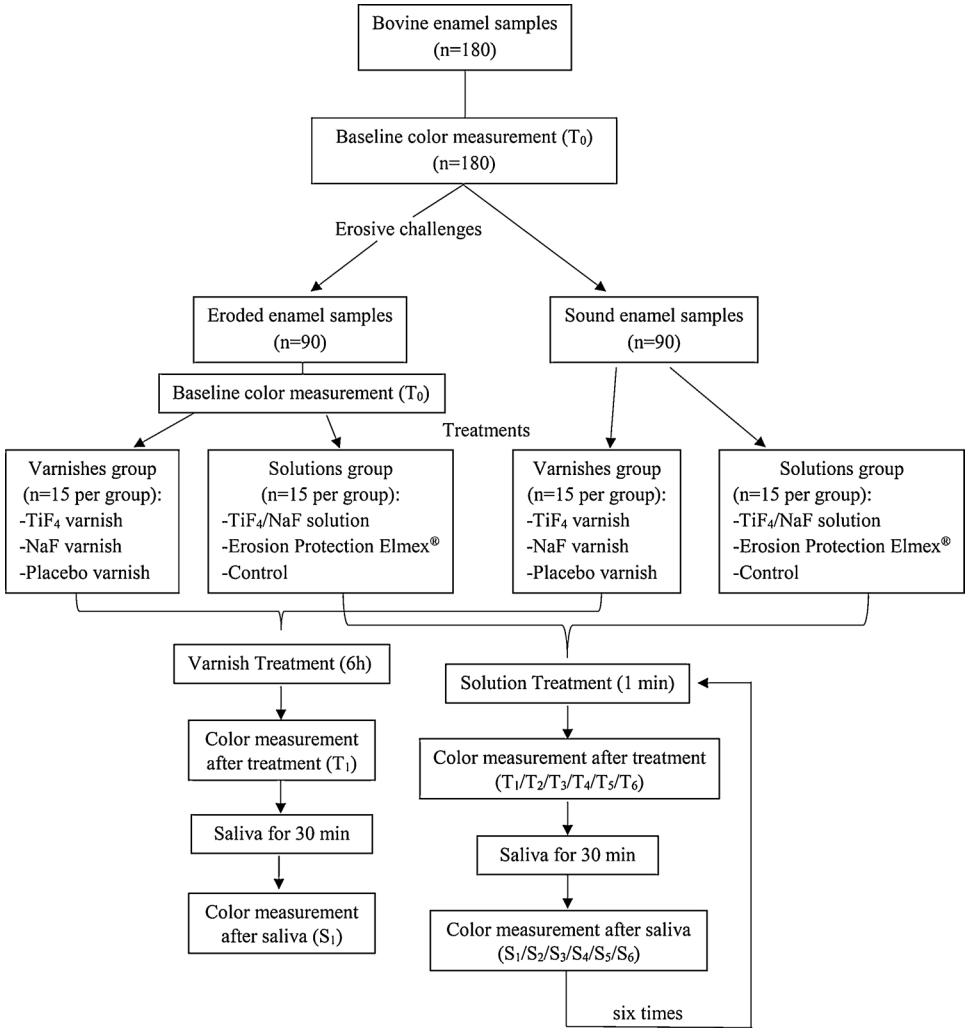

Fig. 1. Flowchart representing the experimental protocol.

a* and b* axis, *i.e.*, enhanced the bluish and greenish shades of enamel, respectively (Table 1).

3.1.2. Eroded enamel

On eroded enamel, all treatments induced similar color alteration (ΔE_{00}) regardless of the period of analysis. The color (ΔE_{00}) did not change over time (T_0 x T_1 x S_1) regardless of the treatment ($p > 0.05$, Table 2).

The erosive challenges significantly increased the luminosity (L*) and decreased a* and b* axis (Table 2). The treatments did not change the colors axis compared to the values obtained after the erosive challenge, except in case of b* axis for NaF, which decreased immediately after application and kept constant after 30 min in saliva. There was a significant difference between TiF_4 and NaF in the b* axis after erosion.

3.2. Solution

3.2.1. Sound enamel

The ΔE_{00} values showed that the color changes were visually perceptible after the first application (from T_2 until S_6). At the first application, no significant difference was found between the treatments. In the other analyses (from T_2 until S_6), the solution containing TiF_4/NaF significantly induced a lower color alteration (ΔE_{00}) compared to control ($p = 0.0016$), except at 2nd application (T_2) and after the 5th saliva exposure (S_5-S_6), where no difference was found between them. Furthermore, TiF_4/NaF induced similar color alteration (ΔE_{00}) compared to *Elmex*® except at the 4th application (T_4). At the end of the experiment, no differences were found among the groups.

With respect to L* axis, no difference was found between the treatments, except between TiF_4/NaF and *Elmex*® (higher L* value) at the 4th application only (T_4). For a* axis, TiF_4/NaF induced a significant increase in the values compared to control and *Elmex*® after the 1st and 3rd saliva exposures (S_1 and S_3) until the end of the experiment, respectively. *Elmex*® and control did not differ from each other. For b* axis, *Elmex*® significantly increased the values compared to control in all readings, while TiF_4/NaF only differed from control at the 6th application (T_6). Both fluoride treatments did not differ between them with respect to the values of the b* axis.

Generally, ΔE_{00} and L* axis increased and a* axis decreased for all treatments at the evaluated periods compared to baseline values. The b* axis significantly decreased along to the experiment (TiF_4/NaF, control and *Elmex*® – after 5th, 3rd and 4th saliva exposure, respectively). Table 3 shows the color parameters.

3.2.2. Eroded enamel

Generally, the color change values (ΔE_{00}) were visually perceptible mostly after erosion. After the treatments (T_1-T_6, S_1-S_6), ΔE_{00} values significantly decreased compared to the values found after the erosive challenge (T_0) for all groups. For control, differences were also seen when T_1 was compared to the other values (T_2-T_6, S_1-S_6). The treatments did not differ among them regarding ΔE_{00}, except TiF_4/NaF and

Table 1
Mean and standard deviation of the ΔE_{00}, L *, a * and b * values of the varnish-treated sound enamel samples obtained by the spectrophotometer analysis at the baseline, after application of the varnish and after exposure to saliva.

	TiF_4				NaF				Placebo			
	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*
T_0		67.2 ± 8.7^{Aa}	9.5 ± 2.6^{Aa}	44.8 ± 3.0^{Aa}		67.2 ± 7.7^{Aa}	9.4 ± 2.0^{Aa}	46.0 ± 2.5^{Aa}		67.2 ± 8.0^{Aa}	9.7 ± 14.1^{Aa}	45.6 ± 3.4^{Aa}
T_1	16.8 ± 3.8^{Aa}	86.8 ± 5.9^{Ab}	4.2 ± 3.1^{Ab}	42.4 ± 5.1^{Ab}	12.2 ± 6.4^{Ba}	82.9 ± 5.5^{Ab}	4.8 ± 1.8^{Ab}	43.4 ± 4.5^{Ab}	13.1 ± 4.8^{Ba}	82.0 ± 6.9^{Ab}	5.4 ± 2.1^{Ab}	43.1 ± 4.2^{Ab}
S_1	15.8 ± 4.0^{Aa}	86.5 ± 5.6^{Ab}	4.2 ± 3.0^{Ab}	42.4 ± 5.1^{Ab}	14.6 ± 6.3^{Ab}	86.0 ± 5.0^{Ab}	3.4 ± 3.1^{Ab}	42.5 ± 5.1^{Ab}	14.7 ± 4.2^{Aa}	84.5 ± 5.8^{Ab}	4.3 ± 2.1^{Ab}	42.6 ± 3.8^{Ab}

Two-way RM ANOVA ($p < 0.01$) (n = 15).
Different upper case letters mean differences between treatments (comparisons in the same line for each parameter individually) and different lowercase letters mean difference between times within the same treatment (comparison in the same column).

Table 2
Mean and standard deviation of the ΔE_{00}, L*, a* and b* values of the varnish-treated eroded enamel obtained by analysis of the spectrophotometer at the baseline, after application of the varnish and after exposure to saliva.

	TiF_4				NaF				Placebo			
	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*
Baseline T_0		66.9 ± 5.9^{A}	9.6 ± 1.7^{A}	45.2 ± 4.5^{A}		68.1 ± 6.3^{A}	9.9 ± 2.0^{A}	47.1 ± 2.8^{A}		66.2 ± 8.8^{A}	10.0 ± 2.2^{A}	47.0 ± 1.8^{A}
Erosion T_0	15.8 ± 3.7^{A}	87.5 ± 3.4^{A*}	2.5 ± 1.4^{A*}	40.0 ± 6.2^{A*}	15.7 ± 3.2^{A}	88.1 ± 3.0^{A*}	2.6 ± 1.8^{A*}	43.4 ± 3.6^{B*}	16.0 ± 4.1^{A}	86.6 ± 4.7^{A*}	2.6 ± 1.7^{A*}	42.4 ± 3.8^{AB*}
T_1	15.0 ± 3.3^{A}	85.1 ± 3.5^{A*}	2.6 ± 1.5^{A*}	38.4 ± 5.6^{A*}	15.0 ± 3.6^{AB}	85.3 ± 6.6^{A*}	2.2 ± 1.8^{A*}	40.1 ± 5.1$^{A*/**}$	14.3 ± 5.0^{A}	86.0 ± 3.8^{A*}	2.6 ± 1.7^{A*}	40.6 ± 4.3^{A*}
S_1	14.2 ± 3.3^{A}	84.9 ± 3.7^{A*}	2.8 ± 1.5^{A*}	38.4 ± 5.6^{A*}	13.6 ± 3.9^{A}	86.9 ± 3.3^{A*}	2.1 ± 1.7^{A*}	40.4 ± 3.9$^{A*/**}$	15.0 ± 6.0^{A}	85.7 ± 3.5^{A*}	2.4 ± 1.6^{A*}	39.8 ± 4.4^{A*}

Two-way ANOVA ($p < 0.005$) (n = 15).
Different capital letters mean differences between treatments (comparisons in the same line for each parameter individually).
* means significant difference with baseline value within the same treatment.
** means significant difference with value after erosion within the same treatment.

Table 3
Mean and standard deviation of the ΔE_{00}, L*, a* and b* values of the solution-treated sound enamel obtained by analysis of the spectrophotometer at the baseline, after application of the solution and after exposure to saliva.

	TiF_4				ELMEX				Placebo			
	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*	ΔE_{00}	L^*	a^*	b^*
T_0		66.9 ± 7.2^{A}	10.2 ± 2.2^{A}	46.5 ± 2.6^{AB}		68.2 ± 6.8^{A}	9.6 ± 2.0^{A}	48.0 ± 2.5^{B}		67.2 ± 6.1^{A}	9.5 ± 2.5^{A}	46.2 ± 2.5^{A}
T_1	1.8 ± 0.8^{A}	66.5 ± 6.2^{A}	10.3 ± 1.8^{A}	46.5 ± 2.5^{A}	1.8 ± 0.8^{A}	66.7 ± 6.3^{A}	10.0 ± 1.7^{A}	48.5 ± 2.4^{B}	1.7 ± 1.2^{A}	67.8 ± 6.1^{A}	9.4 ± 2.5^{A}	46.0 ± 2.5^{A}
S_1	4.6 ± 4.9^{B}	73.4 ± 8.4^{A*}	8.2 ± 2.5^{B*}	45.5 ± 3.1^{A}	5.0 ± 3.5^{B*}	75.1 ± 4.7^{A*}	8.0 ± 1.9^{B*}	47.9 ± 2.4^{B}	9.0 ± 5.2^{A*}	78.4 ± 4.4^{A*}	6.3 ± 2.2^{A*}	45.0 ± 2.6^{A}
T_2	2.4 ± 2.5^{A}	68.4 ± 6.8^{A}	9.6 ± 2.1^{B*}	46.4 ± 3.0^{A}	3.9 ± 2.6^{A}	73.6 ± 4.9^{A*}	8.4 ± 1.9^{AB}	48.3 ± 2.6^{B}	5.0 ± 3.1^{A*}	72.8 ± 5.3^{A*}	7.6 ± 2.2^{A}	46.0 ± 2.7^{A}
S_2	6.5 ± 5.4^{B*}	75.5 ± 7.3^{A*}	7.7 ± 2.8^{B*}	45.6 ± 3.3^{AB}	7.3 ± 5.3^{AB*}	78.0 ± 4.7^{A*}	6.8 ± 1.8^{AB*}	47.3 ± 2.6^{B}	9.8 ± 5.7^{A*}	79.7 ± 4.3^{A*}	5.7 ± 2.0^{A*}	44.8 ± 3.0^{A}
T_3	5.1 ± 4.4^{B*}	73.8 ± 6.9^{A*}	8.2 ± 2.6^{B*}	45.9 ± 3.3^{AB}	6.4 ± 5.2^{AB*}	76.9 ± 4.6^{A*}	7.1 ± 1.8^{AB*}	47.5 ± 2.5^{B}	8.9 ± 5.1^{A*}	78.2 ± 4.0^{A*}	6.2 ± 2.1^{A*}	45.0 ± 3.1^{A}
S_3	6.5 ± 5.2^{B*}	75.9 ± 7.7^{A*}	7.5 ± 2.8^{B*}	45.3 ± 3.5^{AB}	8.8 ± 6.5^{AB*}	79.9 ± 6.1^{A*}	5.9 ± 1.9^{A*}	46.8 ± 2.6^{B}	10.2 ± 5.1^{A*}	80.7 ± 3.9^{A*}	5.2 ± 2.1^{A*}	44.0 ± 3.3^{A*}
T_4	5.7 ± 4.5^{B*}	74.5 ± 7.8^{A*}	7.9 ± 2.9^{B*}	45.5 ± 3.4^{AB}	8.7 ± 5.4^{B*}	79.9 ± 5.6^{B*}	5.9 ± 2.0^{A*}	46.6 ± 2.9^{B}	10.1 ± 5.0^{A*}	79.7 ± 4.6^{AB*}	5.4 ± 2.3^{A*}	44.2 ± 3.2^{A*}
S_4	7.5 ± 5.2^{B*}	77.1 ± 7.8^{A*}	7.1 ± 2.9^{B*}	45.0 ± 4.0^{AB}	9.8 ± 6.1^{AB*}	81.2 ± 5.5^{A*}	5.2 ± 1.8^{A*}	46.0 ± 3.0^{B*}	10.8 ± 5.1^{A*}	81.1 ± 4.6^{A*}	4.5 ± 2.3^{A*}	43.2 ± 3.5^{A*}
T_5	6.7 ± 5.0^{B*}	76.4 ± 8.2^{A*}	7.3 ± 3.0^{B*}	44.9 ± 4.0^{AB}	9.4 ± 5.7^{AB*}	80.1 ± 5.3^{A*}	5.5 ± 2.0^{A*}	46.4 ± 2.9^{B*}	10.5 ± 5.1^{A*}	80.6 ± 4.7^{A*}	5.0 ± 2.4^{A*}	43.7 ± 3.5^{A*}
S_5	8.7 ± 5.5^{A*}	78.4 ± 8.1^{A*}	6.4 ± 2.8^{B*}	44.5 ± 4.4^{AB*}	11.0 ± 6.2^{A*}	82.5 ± 5.5^{A*}	4.5 ± 1.6^{A*}	45.7 ± 2.8^{B*}	11.5 ± 5.3^{A*}	81.4 ± 4.9^{A*}	4.3 ± 2.5^{A*}	43.0 ± 3.6^{A*}
T_6	8.5 ± 5.0^{A*}	78.3 ± 7.8^{A*}	6.4 ± 2.7^{B*}	44.2 ± 4.3^{B*}	9.8 ± 6.4^{A*}	81.7 ± 5.7^{A*}	4.7 ± 2.0^{A*}	45.8 ± 2.6^{B*}	11.3 ± 6.9^{A*}	81.1 ± 4.3^{A*}	4.7 ± 2.4^{A*}	42.0 ± 4.9^{A*}
S_6	9.3 ± 4.5^{A*}	79.2 ± 7.7^{A*}	6.1 ± 2.7^{B*}	44.5 ± 4.5^{AB*}	12.1 ± 6.0^{A*}	83.6 ± 5.3^{A*}	3.8 ± 1.8^{A*}	45.0 ± 2.7^{B*}	11.3 ± 4.6^{A*}	83.0 ± 4.0^{A*}	3.9 ± 2.3^{A*}	42.7 ± 3.8^{A*}

Two-way ANOVA ($p < 0.005$) (n = 15).
Different capital letters mean differences between treatments (comparisons in the same line for each parameter individually).
* means significant difference with baseline value (T_0) within the same treatment.

Table 4
Mean and standard deviation of the ΔE_{00}, L*, a* and b* values of the solution-treated eroded enamel obtained by analysis of the spectrophotometer at the baseline, after application of the solution and after exposure to saliva.

	TiF_4				ELMEX				Placebo			
	ΔE_{00}	L*	a*	b*	ΔE_{00}	L*	a*	b*	ΔE_{00}	L*	a*	b*
Baseline T_0		66.4 ± 6.6^{A}	12.3 ± 8.8^{A}	46.7 ± 3.1^{A}		67.9 ± 7.6^{A}	9.5 ± 2.5^{B}	45.9 ± 4.2^{A}		66.6 ± 6.7^{A}	10.1 ± 1.9^{AB}	46.7 ± 3.3^{A}
Erosion T_0	15.5 ± 4.1^{A}	86.5 ± 2.9^{A*}	3.3 ± 1.9^{A*}	44.4 ± 3.3^{A*}	16.0 ± 4.4^{A}	87.8 ± 4.2^{A*}	2.1 ± 1.7^{A*}	41.2 ± 4.5^{B*}	15.9 ± 4.1^{A}	87.6 ± 3.4^{A*}	2.9 ± 1.4^{A*}	41.0 ± 4.1^{B*}
T_1	1.8 ± 1.1^{B**}	85.0 ± 4.8^{A*}	4.4 ± 1.9^{A*}	45.1 ± 2.8^{A}	3.4 ± 3.9^{AB**}	84.6 ± 5.3^{A*}	4.3 ± 2.0^{A*}	43.6 ± 4.7^{AB*}	4.3 ± 4.2^{A**}	83.3 ± 7.6^{A*}	5.6 ± 3.6$^{A*/**}$	42.9 ± 3.7^{B*}
S_1	1.6 ± 1.0^{A**}	85.6 ± 3.5^{A*}	4.0 ± 1.5^{A*}	44.6 ± 2.8^{A*}	2.4 ± 2.1^{A**}	85.9 ± 3.6^{A*}	3.8 ± 1.5^{A*}	42.7 ± 5.1^{AB*}	1.7 ± 1.0^{A**} #	86.9 ± 4.6^{A*}	4.2 ± 2.6^{A*}	41.8 ± 3.8^{B*}
T_2	1.9 ± 1.0^{A**}	85.4 ± 4.0^{A*}	4.0 ± 1.6^{A*}	44.7 ± 2.5^{A*}	2.8 ± 3.0^{A**}	86.5 ± 3.8^{A*}	3.6 ± 1.6^{A*}	42.8 ± 5.0^{B*}	1.5 ± 1.0^{A**} #	87.1 ± 4.3^{A*}	4.3 ± 2.7^{A*}	41.8 ± 3.7^{B*}
S_2	1.5 ± 1.0^{A**}	86.2 ± 3.6^{A*}	3.9 ± 1.5^{A*}	44.5 ± 3.0^{A*}	2.3 ± 2.3^{A**}	87.8 ± 2.5^{A*}	3.1 ± 1.2^{A*}	42.1 ± 4.9^{B*}	1.4 ± 0.7^{A**} #	88.1 ± 4.1^{A*}	3.8 ± 2.4^{A*}	41.2 ± 3.9^{B*}
T_3	1.8 ± 1.2^{A**}	85.3 ± 3.9^{A*}	4.1 ± 1.6^{A*}	44.7 ± 3.1^{A*}	2.2 ± 1.5^{A**}	87.2 ± 2.7^{A*}	3.2 ± 1.3^{A*}	42.2 ± 5.2^{B*}	1.8 ± 0.9^{A**} #	87.5 ± 4.2^{A*}	3.9 ± 2.2^{A*}	41.5 ± 3.8^{B*}
S_3	1.6 ± 0.7^{A**}	85.9 ± 3.7^{A*}	3.9 ± 1.3^{A*}	44.4 ± 3.3^{A*}	1.8 ± 1.5^{A**}	87.9 ± 1.9^{A*}	2.9 ± 1.1^{A*}	41.2 ± 5.1^{B}	1.5 ± 1.1^{A**} #	87.6 ± 4.5^{A*}	3.7 ± 1.8^{A*}	41.5 ± 4.2^{B*}
T_4	1.7 ± 1.5^{A**}	85.8 ± 3.8^{A*}	4.0 ± 1.6^{A*}	44.4 ± 3.0^{A*}	2.1 ± 1.3^{A**}	87.9 ± 1.9^{A*}	2.8 ± 1.3^{A*}	41.5 ± 5.4^{B*}	1.3 ± 1.0^{A**} #	87.3 ± 3.9^{A*}	3.6 ± 1.9^{A*}	41.0 ± 4.4^{B*}
S_4	1.7 ± 1.1^{A**}	86.5 ± 3.3^{A*}	3.7 ± 1.2^{A*}	44.2 ± 3.0^{A*}	1.6 ± 0.9^{A**}	88.0 ± 2.7^{A*}	2.8 ± 1.3^{A*}	41.4 ± 5.4^{B*}	1.4 ± 0.9^{A**} #	87.6 ± 4.6^{A*}	3.5 ± 1.7^{A*}	40.9 ± 4.2^{B*}
T_5	1.8 ± 1.2^{A**}	86.4 ± 3.7^{A*}	3.6 ± 21.2^{A*}	44.4 ± 3.0^{A*}	1.6 ± 0.9^{A**}	88.1 ± 2.9^{A*}	2.6 ± 1.4^{A*}	41.0 ± 5.2^{B*}	1.4 ± 0.7^{A**} #	86.5 ± 4.0^{A*}	3.6 ± 1.7^{A*}	41.0 ± 4.5^{B*}
S_5	1.5 ± 1.2^{A**}	85.8 ± 3.5^{A*}	3.7 ± 1.5^{A*}	44.4 ± 2.7^{A*}	1.7 ± 0.9^{A**}	88.0 ± 3.4^{A*}	2.5 ± 1.4^{A*}	41.3 ± 5.3^{B*}	1.4 ± 0.9^{A**} #	87.0 ± 4.1^{A*}	3.5 ± 1.7^{A*}	41.2 ± 4.7^{B*}
T_6	1.7 ± 0.9^{A**}	86.1 ± 3.1^{A*}	3.6 ± 1.2^{A*}	44.0 ± 3.0^{A*}	1.7 ± 1.0^{A**}	87.8 ± 2.8^{A*}	2.6 ± 1.5^{A*}	41.0 ± 5.4 ^{B*}	1.8 ± 0.8^{A**} #	86.9 ± 4.4^{A*}	3.3 ± 1.6^{A*}	40.6 ± 4.6^{B*}
S_6	1.5 ± 1.0^{A**}	85.5 ± 3.4^{A*}	3.6 ± 1.2^{A*}	44.1 ± 2.9^{A*}	1.8 ± 1.2^{A**}	88.3 ± 2.3^{A*}	2.3 ± 1.4^{A*}	40.6 ± 5.1^{B*}	1.6 ± 1.1^{A**} #	86.6 ± 4.3^{A*}	3.0 ± 1.4^{A*}	40.7 ± 4.3^{B*}

Two-way ANOVA ($p < 0.005$) (n = 15).
Different capital letters mean differences between treatments (comparisons in the same line for each parameter individually).
* means significant difference with Baseline (T_0) value within the same treatment.
** means significant difference with value after Erosion (T_0) within the same treatment.
# means significant difference with value after T1 within the same treatment.

control at the 1st application (T_1, Table 4).

The erosive challenge significantly increased the luminosity (L*) and decrease a* and b* axis (Table 4). No differences were found between the treatments with respect to L* and a* axis. With respect to b* axis, TiF_4/NaF presented significantly higher values compared to control after the erosive challenge until the end of the experiment. TiF_4/NaF also presented significantly higher values compared to *Elmex*® after the erosive challenge and after the 2nd application until the end of experiment.

4. Discussion

The idea of the present study was motivated by the results of a previous *in situ* study, in which 40% of the participants reported tooth staining after using TiF_4/NaF solution [5], which is in agreement with a case report showing staining of white spot lesions after application of 4% TiF_4 solution [9].

Our results show that TiF_4 varnish induced a greater overall color change (ΔE_{00}) on sound enamel immediately after its application. However, no color difference was found after saliva exposure as well as on eroded enamel, when compared to NaF and placebo varnishes. The statistical significance should be interpreted with caution, since the numerical differences between the groups might be lower than the perceptible threshold. The erosive process itself induced a relevant color change, which was stable after the treatments with the varnishes. The color change induced by the treatments on sound enamel was compatible to those induced by the erosive challenges themselves.

On the other hand, TiF_4/NaF solution induced a lower overall color change (ΔE_{00}) compared to control on sound enamel, but it did not differ from *Elmex*®. TiF_4/NaF solution increased the shades of yellow and red; while *Elmex*® solution increased the yellow tones of the sound enamel earlier than TiF_4/NaF, which showed yellowing only after the last application compared to the control. On eroded enamel, no difference between the treatments was found, although a tendency to yellowing was seen for TiF_4/NaF, which is in agreement with previous work [5].

The analysis of tooth color staining induced by dental products is an important tool to identify possible side effects of the new formulations before they are applied routinely in patients. Information on fluoride-induced tooth pigmentation is scarce in the literature, except with respect to diamine silver fluoride [10–12] and stannous fluoride [13–17].

Recently, Vieira et al. [20] evaluated the color change induced by fluoride gels presenting different colors (transparent, pink and blue) on enamel demineralized by pH cycling simulating cariogenic challenges. After demineralization, the gels (NaF, 2%) were applied once a week for 5 weeks and the samples were immersed in water at the intervals. The authors showed significant color changes after pH cycling, as seen in the present study (after erosion), and no differences between the gels were detectable regardless of their color, in agreement with our data. Prasada et al. [21] found similar results either.

The formulated hypothesis was that the demineralized enamel, due to the high porosities, would incorporated more pigments compared to the sound one. However, our study demonstrated that the incorporation of stannous or titanium and fluoride on the eroded surface after the application of the solutions was able to fill the pores, reducing the color change previously seen for eroded enamel. The differences in the refractive index between air (RI 1.00), water (RI 1.33) and hydroxyapatite (RI 1.66) are responsible for changing in enamel color after erosive challenges. Although the varnish was more concentrated than the solution, its effect in improvement of color was more modest, probably due to the highest thickness of the glaze like-layer produced by it, which might have some color.

The erosive challenges caused a color change compatible to those induced by a single application of varnish on sound enamel. On the other hand, the application of the TiF_4 varnish on sound enamel caused a greater color change compared to the application of the TiF_4/ NaF solution on sound enamel even after 6 applications. Despite the color changes values (ΔE_{00}) increased along to the number of solution applications, the values didn't reach the level induced by the varnish application.

With respect to fluoride solution containing stannous, old clinical trials have shown that its continuous use (more than 20 applications) is capable of causing tooth staining, which can be 4-5x greater than those caused by NaF [14] and can potentiate the effect of food that are also able to cause pigmentation [15–17].

Considering the comparisons between experimental and commercial products, this work supports the use of TiF_4 varnish and TiF_4/NaF solution in future clinical trials, as the color changes are compatible to similar commercial products. Furthermore, the effect of the products containing TiF_4 on prevention of tooth erosion is promising [1–5]. Surely, this work shall be repeated *in vivo* to confirm the results in a condition where human saliva and acquired enamel pellicle may interplay in the enamel staining as discussed by Mundorff et al. [26], whose tested the effect of TiF_4 in the presence of a protein rich-pellicle.

5. Conclusion

In conclusion, the color changes induced by the experimental TiF_4 products are similar to the commercial ones, making possible to test them in clinical trials.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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