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TiF_4 and NaF varnishes as anti-erosive agents on enamel and dentin erosion progression *in vitro*

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ABSTRACT

Objective: This study assessed the effect of fluoride varnishes on the progression of tooth erosion *in vitro*. Material and Methods: Forty-eight enamel and 60 root dentin samples were previously demineralized (0.1% citric acid, pH 2.5, 30 min), leading to a baseline and erosive wear of 12.9 and 11.4 µm, respectively. The samples were randomly treated (6 h) with a 4% TiF_4 varnish (2.45%F-, pH 1.0), a 5.42% NaF varnish (2.45%F-, pH 5.0), a placebo varnish and no varnish (control). The samples were then subjected to erosive pH cycles (4x90 s/day in 0.1% citric acid, intercalated with artificial saliva) for 5 days. The increment of the erosive tooth wear was calculated. In the case of dentin, this final measurement was done with and without the demineralized organic matrix (DOM). Enamel and dentin data were analyzed using ANOVA/Tukey's and Kruskal-Wallis/Dunn tests, respectively ($p<0.05$). Results: The TiF_4 (mean±s.d: 1.5±1.1 µm) and NaF (2.1±1.7 µm) varnishes significantly reduced enamel wear progression compared to the placebo varnish (3.9±1.1 µm) and control (4.5±0.9 µm). The same differences were found for dentin in the presence and absence of the DOM, respectively: TiF_4 (average: 0.97/1.87 µm), NaF (1.03/2.13 µm), placebo varnish (3.53/4.47 µm) and control (3.53/4.36 µm). Conclusion: The TiF_4 and NaF varnishes were equally effective in reducing the progression of tooth erosion *in vitro*.

Keywords: Sodium fluoride. Titanium. Tooth erosion.

INTRODUCTION

Dental erosion is a clinical condition that is currently being studied since researchers have reported an increase in its prevalence[17]. Furthermore, dentists are becoming more aware about the etiology and early detection of this condition[23].

Acid exposure causes enamel surface demineralization, and long-term demineralization leads to erosive enamel wear as a consequence of progressive mineral loss[15]. Dentin erosion presents a different histology lesion, since the progression of demineralization is modulated by the presence of the demineralized organic matrix (DOM)[6,11]. The DOM may be affected by enzymatic and chemical degradation, which may enhance the progression of dentin wear over time[7,27]. The primary preventive measure for dental erosion is to reduce the frequency and duration of acid exposure[22]. As it is difficult to control the behavior of the patient, such as the frequency of acid intake or special drinking habits, other strategies have been proposed to control dental erosion that are rather less dependent on the compliance of the patient.

The most tested alternative is to increase the acid resistance of the teeth through the application of fluoride. Accordingly, highly concentrated fluoride applications such as oral rinses, gels and varnishes have been tested[5]. The application of NaF at high concentration is able to promote a CaF_2-like layer precipitation on enamel[26]. The CaF_2 globules behave as a physical barrier, inhibiting the contact of the acid with enamel and/or acting as a fluoride reservoir. However, this layer presents low acid resistance[10], thus, its efficacy in controlling dental erosion is restricted, at least under *in vitro* conditions[10].

On this basis, recent studies have focused on other fluorides, such as those containing polyvalent metals, which may have a higher efficacy than NaF due to surface precipitation or incorporation of ions into sound and demineralized tissue.

Accordingly, the TiF_4 has shown a promising erosion-inhibiting effect both *in vitro* and *in situ* compared to other fluorides[12-14,19,30]. The effect of TiF_4 is related to both fluoride and titanium[3]. It is hypothesized that titanium may conflate with phosphate groups, producing an acid-resistant surface coating[25].

Most studies have applied fluoride between the erosive challenges, showing a positive effect of different fluoride formulations on the progression of tooth erosion[6,28]. Recently, our research group has shown that an experimental TiF_4 varnish can be more or at least as effective as a NaF varnish in reducing erosive tooth wear when applied only once on a previously sound surface[18-21]. However, there have been no data published regarding the effect of TiF_4 and NaF varnishes on the reduction of tooth erosion progression when applied on pre-eroded tooth surfaces. Patients are usually treated with fluoride to prevent erosion when they have signs of the lesion.

Therefore, the present study aimed to analyze and compare the effect of TiF_4 and NaF varnishes on enamel and dentin erosion progression *in vitro*. The null hypothesis tested was that there is no significant difference between TiF_4 and NaF varnishes on the progression of erosive tooth wear when compared to the control.

MATERIAL AND METHODS

Sample preparation

Bovine teeth were prepared to obtain 48 enamel and 60 root dentin samples (4x4x3 mm). The teeth were stored in 0.1% buffered thymol solution (pH 7.0) at 4°C. The specimens were cut using an ISOMET low-speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond discs (Extec Corp., Enfield, CT, USA), which were separated by a 4 mm thick spacer. The surfaces of the specimens were ground flat using water-cooled silicon carbide discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), removing about 200 µm of the surface of the tooth. Thereafter, the samples were cleaned in an ultrasonic device with deionized water for 5 min. The removal of the cement from the root dentin was verified using a microscope (x40 magnification). Two-thirds of the outer surface (untreated area) of the specimens were covered with nail varnish in order to create sound areas on both sides of a central band of eroded enamel and dentin.

Initial erosive lesion and profile measurement

An initial erosive demineralization was created by immersion of the specimens in 0.1% citric acid (pH 2.5) for 30 min at room temperature under gentle agitation, using a shaking bath with horizontal movements (60 rpm). The acid solution was replaced every 5 min (30 ml/sample). After acid exposure, enamel and dentin wear (µm) were quantitatively determined using contact profilometry (Mahr Perthometer, Mahr Ltda, Göttingen, Germany). For the baseline profile measurement (initial erosive wear), the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). During the measurement, the dentin specimens were maintained in water (100% humidity) to avoid the DOM from shrinking. The diamond stylus was moved from the first reference to the exposed area and then over the other reference area (2.5 mm long and 2 mm wide). Five profile measurements were performed at intervals of 0.5 mm. The vertical distance between the horizontal line drawn on the reference areas and the one drawn on the experimental (eroded) area was defined as tooth wear using Mahr Surf XT20 (Software Mahr Surf XT20, 2009, Mahr Ltda, Göttingen, Germany). The values were averaged (µm) and used for the random allocation of the samples into the groups (treatments).

Fluoride treatment and pH cycling

Twelve enamel and 15 dentin specimens were randomly distributed according to the baseline wear of each one of the 4 groups: a 4% TiF_4 varnish (2.45% F-, pH 1.0), a 5.42% NaF varnish (2.45% F-, pH 5.0), a placebo varnish (without fluoride) and no varnish (control). All varnishes contained colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol.

The varnishes were applied in a thin layer using a microbrush, and the specimens were stored in artificial saliva. After 6 h, the varnishes were carefully removed with acetone solution (1:1) and a scalpel blade, avoiding to touch the enamel surface[19].

The samples were submitted to an erosive demineralization by immersion in 0.1% citric acid (pH 2.5) for 90 s 4 times a day for 5 d. After each demineralization, the specimens were rinsed with deionized water (10 s) and transferred into artificial saliva (pH 6.8, 30 ml/specimen, unstirred, 25°C) for 2 h. After the last daily erosive treatment, the specimens were also stored in artificial saliva overnight. The citric acid was renewed at each erosive challenge, and the artificial saliva was replaced daily. The artificial saliva consisted of 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 11 μM ascorbic acid (pH 6.8)[19].

Final profile measurement

After 5 days of pH cycling, the nail varnish was removed, and 5 profiles were recorded as done at the baseline profile measurement. The values were averaged (μm), and the increment value (final wear – baseline wear) was calculated for enamel and dentin specimens (with and without the DOM in the case of dentin) and then submitted to statistical analysis.

After the profilometric analysis, the dentin samples were submitted to degradation of the collagen fibrils in order to check the influence of the DOM on the profile measurement and comparison among the groups. A collagenase enzyme (Type VII from *Clostridium histolyticum*, product no. Co773, Sigma-Aldrich, St. Louis, MO, USA), with a collagen digestion activity of 1.98 U/μg, solid at 25°C, pH 7.5, in the presence of calcium ions, was used to remove the DOM. The collagen layer was removed by adding 100 U/ml of enzyme into artificial saliva containing 20 mM HEPES, 0.70 mM $CaCl_2$, 0.20 mM $MgCl_2.6H_2O$, 4 mM KH_2PO_4, 30 mM KCl, 0.30 mM NaN_3, and EDTA-free protease inhibitor cocktail (Complete TM protease inhibitor of Cocktail, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 5 d at 37°C[6].

Statistical analysis

The software GraphPad InStat (GraphPad Software, San Diego, CA, USA) was used. The assumptions of equality of variances and normal distribution of data were verified for all the variables tested using Bartlett and Kolmogorov-Smirnov tests, respectively. As the equality of variances was satisfied for enamel, the differences among treatments were analyzed using ANOVA followed by Tukey's test. For dentin, the equality of variances was not satisfied, and the differences among treatments were analyzed using Kruskal-Wallis followed by Dunn's test. The sample size was 12 for enamel and 15 for dentin, and the level of significance was set at 5%.

RESULTS

The baseline erosive wear means were 12.9 and 11.4 μm for enamel and dentin, respectively. The mean increment of enamel wear (μm) was similar between TiF_4 and NaF varnish. Both fluoride varnishes showed significantly less erosion (67% and 53% decrease in enamel wear, respectively) compared to the placebo varnish and the control group ($p<0.0001$), which did not differ from each other. Table 1 shows the enamel erosive wear means.

Regarding dentin, TiF_4 and NaF presented similar ability to reduce the progression of erosive wear (72.5% and 71% decrease in dentin wear, respectively), regardless of the presence or absence of the DOM, compared to placebo varnish and control group ($p<0.0001$), which did not differ from each other. Table 2 displays the average values for dentin wear.

Table 1- Mean±standard deviation of the baseline, final and increment enamel erosive wear (μm) according to each treatment

	TiF^4	NaF	Placebo	Control
Baseline	13.3±1.7	12.8±2.7	12.8±2.3	12.5±2.7
Final	14.7±2.1	14.8±2.0	16.7±2.5	17.0±2.7
Increment	1.5±1.1a	2.1±1.7a	3.9±1.1b	4.5±0.9b

*Distinct lower case letters indicate significant differences among the treatment groups (ANOVA and Tukey's test, p<0.0001, n=12)

Table 2- Baseline, final and increment dentin erosive wear means (μm), with and without DOM (interquartile range), respectively, according to each treatment

	TiF_4	NaF	Placebo	Control
Baseline	11.3	11.2	11.2	11.2
Final	12.3/13.1	12.0/13.2	14.6/16.0	14.8/15.4
Increment	0.97 (0.49)[a] 1.87 (0.95)[a]	1.03 (0.68)[a] 2.13 (0.73)[a]	3.53 (1.42)[b] 4.47 (1.91)[b]	3.53 (1.62)[b] 4.36 (2.18)[b]

*Distinct lower case letters indicate significant differences among the treatment groups (Kruskal-Wallis and Dunn, p<0.0001, n=15)

DISCUSSION

Investigations of the potential of TiF_4 to prevent tooth erosion have been performed since 1997[3]. It has been speculated that its effect against erosion is due to a high incorporation of fluoride into enamel[24] and a formation of an acid surface precipitation composed by TiO_2 or hydrated titanium phosphate[25].

Our group showed that the TiF_4 incorporated in an experimental varnish presented a higher protective potential than the TiF_4 solution on enamel erosion[19], enamel erosion-abrasion[24] and dentin erosion-abrasion *in vitro*[21]. We speculate that the better ability of the varnish to adhere to the tooth surface, allowing an increased contact time with the tooth, is responsible for its positive effect[18,19,21]. However, our previous studies have focused on the prevention of dental erosion rather than on the control of its progression. Accordingly, the varnishes were applied to previously sound surfaces in the previous studies. The question of the present study was whether TiF_4 or NaF varnishes were also able to reduce the progression of tooth wear when applied on previously eroded surfaces.

In our study, we produced a baseline enamel and dentin erosive lesion by aggressive acid exposure done under agitation. Fast stirring can increase the five-fold of the enamel wear compared to constant conditions. Clinically, this procedure simulates drinking habits such as rinsing, which may increase the risk of erosive wear[8]. The idea behind the procedure was also to simulate a patient with a history of erosive lesions, for whom a fluoride application would be indicated, according to the Basic Erosive Wear Examination (BEWE) guidelines[2]. The varnishes were then applied on the eroded surface only once and removed after 6 h in order to simulate the clinical professional application[19,20].

In the present study, all fluoridated varnishes had a similar significant potential to reduce enamel and dentin progression, therefore, the null hypothesis can be rejected. However, there was no significant difference between both fluoride varnishes.

The results observed for dentin agree with recent data from our group, in which all fluoridated varnishes tested (experimental TiF_4 and NaF, Duraphat and Duofluorid varnishes) were similarly able to significantly reduce dentin wear compared to the placebo varnish, the fluoridated solutions (TiF_4 and NaF)[21] and the control group.

A limitation of analyzing erosion in dentin is the complexity of the lesion histology, which is characterized by centripetal mineral loss starting in the peritubular areas that progresses to the intertubular area, leading to a bulk tissue loss and the presence of a completely demineralized dentin surface zone that is stable while hydrated[16]. Therefore, erosive wear is difficult to quantify since the quality of the remaining organic layer may interfere in the measurement[7]. The limitation of the wear measurement is due to the shrinkage of the DOM that may occur under different environments. Therefore, to generate reliable data, the profiles must be measured with the samples immersed in water[1]. On the other hand, Schlueter, et al.[29] (2011) advise that the DOM should be removed before the profilometric measurement to prevent this problem from occurring.

We found that the thickness of the DOM of our samples was about 1 μm, regardless of the treatment. It is important to highlight that, in the present study, the results were maintained when the wear was measured on dentin without the DOM.

Some studies have shown that the preventive effect of fluorides on dentin erosion is lower compared to enamel[5,10] and highly dependent on the presence of the organic matrix[9,27]. Only a very intensive fluoridation was effective in the prevention of dentin erosion[5]. Dentin erosion is much more complex than enamel erosion because the organic matrix plays an important role in the progression of wear. The DOM has a buffering capacity that prevents further demineralization, especially in the presence of fluoride[6]. At least for NaF, the enzymatic removal of the organic matrix substantially decreases its effect[6,11,29]. Considering that it is still unclear if what extent the organic matrix is retained under clinical conditions[27], it would be interesting to analyze the effect of TiF_4 varnish applied on eroded dentin with or without DOM.

In respect to the results of enamel, the present data are not in accordance with a previous study from our group[19], in which we found significantly less erosion for the enamel samples treated with a TiF_4 varnish than those treated with a NaF (Duraphat) or NaF/CaF_2 (Duofluorid) varnish. Based on the experimental conditions, we speculate that the glaze-like surface layer produced by TiF_4 may be thinner and with lower amounts of Ti on demineralized enamel surfaces compared to sound ones, as previously shown by Chevitarese, et al.[4] (2004).

Based on these results, we suggest that the effect of other preventive measures (professional products) should be tested on previously eroded tooth surfaces in future studies, since fluoride application is indicated only for patients with a high risk of erosion, according to BEWE guidelines[2]. The effect of the TiF_4 varnish on eroded dentin surfaces in the absence of the DOM is another important topic to be further considered. Finally, the interaction of this polyvalent metal fluoride in terms of glaze surface layer formation on an eroded tooth should be tested under conditions as close as possible to the clinical situation by simulating general tooth wear (e.g. erosion and abrasion) and using *in situ* models.

CONCLUSIONS

Under the conditions of the present study, it can be concluded that the TiF_4 varnish was as effective as the NaF varnish to reduce the enamel and dentin erosion progression *in vitro*.

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