ABSTRACT

Objective: This in vitro study assessed the anti-erosive effect of experimental mouthrinses containing TiF₄ and NaF on dentin erosive loss. Material and Methods: Bovine dentin specimens were randomly allocated into the groups (n=15): 1) SnCl₂/NaF/AmF (Erosion Protection®, pH 4.5, positive control); 2) experimental solution with 0.0815% TiF₄ (pH 2.5); 3) 0.105% NaF (pH 4.5); 4) 0.042% NaF+0.049% TiF₄ (pH 4.4); 5) 0.063% NaF+0.036% TiF₄ (pH 4.5); 6) no treatment (negative control). Each specimen was cyclically demineralized (Sprite Zero, pH 2.6, 4x90 s/day) and exposed to artificial saliva between the erosive challenges for 7 days. The treatment with the fluoride solutions was done 2x60 s/day, immediately after the first and the last erosive challenges of the day. Dentin erosive loss was measured by profilometry (μm). The data were analyzed using Kruskal Wallis/Dunn tests (p<0.05). Results: Mouthrinses containing TiF₄ or Sn/F were able to show some protective effect against dentin erosive loss compared to negative control. The best anti-erosive effect was found for experimental solution containing 0.0815% TiF₄ (100% reduction in dentin loss), followed by 0.042% NaF+0.049% TiF₄ (58.3%), SnCl₂/NaF/AmF (52%) and 0.063% NaF+0.036% TiF₄ (40%). NaF solution (13.3%) did not significantly differ from control. Conclusion: The daily application of experimental mouthrinse containing TiF₄ and NaF has the ability to reduce dentin erosion, as well as Erosion Protection® and TiF₄ alone.

Keywords: Dentin. Topical fluorides. Titanium. Tooth erosion.

INTRODUCTION

While the occurrence of dental caries has decreased during the last decades, researchers had focused on non-cariogenic lesions, including erosion. Dental erosion is an acid-induced tooth loss not involving microorganisms caused by external and/or internal acids. Recent studies indicate a meaningful increase in dental erosion prevalence, especially in dentin, due to modifications in diet, lifestyle and socioeconomic status. Early signs of erosive tooth loss have been found in children and young people. Considering that the modification in population habits and the decrease of acid exposure are very tough, alternatives to reduce the progression of tooth erosive loss have been investigated. NaF is one of the most tested fluoride salt, whose mechanism of action against erosion is based on the deposition of CaF₂-like layer on the surface promoting an additional barrier that inhibits the contact of the acid with the tooth. However, the anti-erosive effect of NaF on dentin is limited since its effect is only seen when the demineralized organic matrix (DOM) is preserved. Nevertheless, some loss of the DOM by enzymatic activity is expected in the clinical situation, especially in patients with eating disorders. Therefore, the use of other fluoride salts is likely to supply the lack of action of conventional fluoride (NaF) on dentin, and consequently, it could be more effective against erosion. Accordingly,
titanium tetrafluoride (TiF₄) has been widely studied against tooth erosive demineralization since 1997, demonstrating the enamel erosion-inhibiting effect⁶,⁹,¹²,²⁵,²⁸. On the other hand, few studies have been performed on dentin. Generally, TiF₄ has a similar effect as NaF on the prevention of dentin erosion when it is applied as varnish¹³,¹⁴, or a better effect than NaF when applied as high F concentrated solution²⁶,²⁷. Therefore, the protective effect of TiF₄ on dentin erosion is still in debate.

Considering that the application of professional fluoride, such as varnish, is not often done, patients at high risk of erosion would benefit from other alternatives to increase the frequency of fluoride exposure. Accordingly, the daily application of mouthrinses with low concentration of F, as those containing SnCl₂/AmF/NaF marketed in Europe (Erosion Protection⁹), has shown some protective effect against enamel and dentin erosion in vitro and in situ⁶,²⁰.

Therefore, the use of low concentrated TiF₄ mouthrinse by the patient could be a good alternative; however, there is a clinical limitation due to the low pH of the solution, which might cause some side effects in oral cavity, since it has cytotoxic effect on fibroblasts²¹.

This study hypothesized that the formulation of an experimental solution containing both NaF and TiF₄ would increase the pH, allowing its use in the clinical situation without losing its protective effect against dentin erosion compared to TiF₄ alone and the commercial solution Erosion Protection⁹. The null hypothesis is that there is no significant difference in the protective effect against dentin erosive loss among the tested fluoride mouthrinses.

MATERIAL AND METHODS

Preparation of the dentin samples

Ninety dentin samples, which were stored in 0.1% thymol/0.9% NaCl solution during the preparation phase, were cut from bovine dental roots. The root was separated from the crown using a water-cooled diamond saw and a cutting machine (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL, USA), and embedded in Pre-30 self-polymerized acrylic resin in cylindrical shape to facilitate the handling. Thereafter, they were serially flattened with water-cooled abrasive discs (320, 600, and 1200 grades of Al₂O₃ papers; Buehler, Lake Bluff, IL, USA), and finally polished with felt paper wetted with a diamond solution (1 μm thickness of particles; Buehler, Lake Bluff, IL, USA) on a rotating polishing machine (Arotec SA Ind. e Com, Cotia, SP, Brazil). After polishing, the samples were cleaned in an ultrasonic device with deionized water for 2 min.

The reference areas on the polished dentin surface were marked with two parallel lines made with a scalpel blade, 1.0 mm apart. Small drilling was also done on the outer area of the dentin surface to allow the correct position of the sample in the profilometer system. Prior to the experiment, the baseline profile was measured and two layers of nail varnish (Colorama, Com. Ind. Exp Ltda., São Paulo, SP, Brazil) were applied on 2/3 of the control surface (sound surfaces), leaving only 1/3 central of the exposed dentin (1.0 mm x 5 mm).

Fluoride treatment

Dentin samples were randomly allocated to each of the six treatment groups (n=15): 1) commercial SnCl₂/NaF/AmF solution (800 ppm Sn⁺², 500 ppm F⁻, pH 4.5, Erosion Protection⁹, GABA Int. AG, Basel, Switzerland, positive control); 2) experimental 0.0815% TiF₄ solution (315 ppm Ti⁺⁴, 500 ppm F⁻, pH 2.5); 3) experimental 0.105% NaF solution (500 ppm F⁻, pH 4.5 adjusted with phosphoric acid); 4) experimental 0.042% NaF+0.049% TiF₄ solution (NaF- 190 ppm F⁻, TiF₄ – 190 ppm Ti⁺⁴ and 300 ppm F⁻, pH 4.4); 5) experimental 0.063% NaF+0.036% TiF₄ solution (NaF – 285 ppm F⁻, TiF₄ – 140 ppm Ti⁺⁴ and 220 ppm F⁻; pH 4.5); 6) no treatment (untreated, negative control). All solutions had approximately 500 ppm F⁻ based on the calculation obtained from the salts concentrations diluted in deionised water, and their pH was measured using a pH electrode. The experimental fluoride solutions were prepared using the analytical grade reagents from Sigma-Aldrich (St. Louis, MO, USA).

The fluoride treatments were performed twice a day (immediately after the first and the last erosive challenges of the day; v=0.5 ml/sample) for 1 min, during 7 days of erosive challenges. Excess of the solution was removed from the surface using a cotton roll.

Erosive challenges

Samples were submitted to a 7-day erosive demineralization cycling. Erosive challenges took place by immersion in a freshly opened bottle of soft drink (Sprite Zero, Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.6, 30 ml/sample) four times a day for 90 s each, at 25°C. Then, the samples were rinsed with deionized water (5 s) and exposed to artificial saliva (pH 6.8, 30 ml/samples, 25°C) for 2h between the erosive challenges and overnight. The artificial saliva (v=500 ml) consisted of 0.001 g ascorbic acid, 0.015 g glucose, 0.290 g NaCl, 0.085 g CaCl₂, 0.080 g NH₄Cl, 0.635 g KCl, 0.080 g NaSCN, 0.165 g KH₂PO₄, 0.100 g carbamide and 0.170 g Na₂PO₄, and it was daily renewed ³².

Profile measurement

Dentin erosive loss (μm) was quantitatively determined by a contact profilometer (Mahr Perthometer, Göttingen, Lower Saxony, Germany).
before (baseline) and after 7 days of experiment. For the profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). Samples were maintained 100% wet during the measurement to avoid shrinkage of the DOM. Five profile measurements were performed at exactly the same sites as the baseline measurement, at intervals of 0.5 mm. To achieve this outcome, the dentin samples presented the identification marks (small drillings made with drill 1/4) and were inserted into a metal device, allowing the stylus to be accurately repositioned at each measurement. Baseline and final profiles were done and compared using the software MahrSurf CXR20 (Mahr, Göttingen, Lower Saxony, Germany). The scans were superposed and the average depth of the under curve area was calculated (\(N_{\text{j}}m\))\(^{12}\). For a better understanding of the treatments effect, the prevention fraction (%) of each treatment was calculated by comparing the medians (each treatment versus negative control).

### Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used for the statistical analysis. The assumptions of equality of variances and normal distribution of data were checked using the Bartlett and Kolmogorov-Smirnov tests, respectively. Once the homogeneity was not achieved, the data from dentin loss (\(\mu m\)) were analyzed using Kruskal-Wallis followed by Dunn’s test. The level of significance was set at 5%.

### RESULTS

All experimental mouthrinses promoted significantly lower dentin erosive loss when compared to the negative control (\(p<0.0001\), except NaF solution (prevention fraction of 13.3%; \(p>0.05\)). The best anti-erosive effect was found for experimental solutions containing 0.0815% TiF\(_4\) (prevention fraction of 100%) and 0.042% NaF+0.049% TiF\(_4\) (58.3%). SnCl\(_2\)/NaF/AmF (Erosion Protection\(^{\circ}\), 52%) and 0.063% NaF+0.036% TiF\(_4\) (40%) did not significantly differ from 0.042% NaF+0.049% TiF\(_4\) and NaF alone, but both were less effective than TiF\(_4\) alone. The median values (minimum-maximum) of dentin erosive loss for each group are shown in Table 1.

### DISCUSSION

Considering the increase of dental erosion’s prevalence\(^{1,2,16,17,24}\), the attention has been focused on the development of preventive approaches to reduce the progression of this dental condition. The present study investigated the protective effect of the daily application of solutions containing TiF\(_4\)/NaF. The null hypothesis tested in this study was rejected because the tested fluoride mouthrinses had a significantly different effect among them against dentin erosive loss. The solution containing pure TiF\(_4\) showed the best protective effect, differing from all other groups except from a specific combination of TiF\(_4\) and NaF.

This new approach would benefit patients with high risk of erosion presenting gingival recession due to periodontal disease, brushing habits (abrasion) or/and occlusal disorders (abfraction). The dentin, in these cases, may be likely exposed to extrinsic acid sources from the diet, and therefore, susceptible to the development of erosion.

The present study aimed to simulate the home-care application of low-concentrated fluoride (500 ppm F\(^-\)) solution, after two meals (morning and evening), in periods in which the patient could perform a rinse after the daily hygiene habit. The idea behind the combination of two fluorides, TiF\(_4\) and NaF, into an experimental mouthrinse is based on the fact that pure TiF\(_4\) has low pH, impairing its clinical use. The addition of NaF to TiF\(_4\) solution was able to increase its pH to a suitable value to be applied \(\text{in vivo}\) and to be compared with commercial products.

In this study, TiF\(_4\) alone reduced in 100% dentin loss, which might be due to the deposition of acid-resistant surface layer rich in CaF\(_2\), titanium dioxide.

| Solutions                        | Median (min; max) |
|----------------------------------|-------------------|
| Erosion Protection\(^{\circ}\) (positive control) | 0.86 (0.67; 1.85)\(^{bc}\) |
| TiF\(_4\) (0.0815%)               | -0.19 (-0.45; -0.05)\(^{a*}\) |
| NaF (0.105%)                     | 1.56 (1.01; 2.38)\(^{ad}\) |
| NaF+TiF\(_4\) (0.0424%+0.049%)    | 0.75 (0.21; 1.59)\(^{ab}\) |
| NaF+TiF\(_4\) (0.063%+0.036%)    | 1.08 (0.59; 1.66)\(^{bc}\) |
| Negative control                 | 1.80 (1.23; 4.94)\(^{d}\) |

* Negative value means increase of the surface (deposition)

Different letters show significant differences among the groups; min=minimum; max=maximum (\(p<0.0001\))

---

**Table 1** - Median (minimum-maximum) of the dentin erosive loss for different groups

---

CASTILHO ARF, SALOMÃO PMA, BUZALAF MAR, MAGALHÃES AC

2015;23(5):486-90
and hydrated titanium phosphate (unpublished data). The protocol of TiF₄ application tested in this study has not been applied in previous studies, since most of them tested high concentrated TiF₄ solution applied at once. The addition of NaF into TiF₄ solution decreased the protective effect, considering the percentage of prevention fraction, due to a likely lower precipitation of Ti and F salts. However, one of the combinations (0.042% NaF+0.049% TiF₄) was still statistically similar to pure TiF₄ solution.

The present results in respect to the daily application of fluoride mouthrinses are more promising than those found for a unique application of a product with high concentration of fluoride, as varnish, against dentin erosion and erosion-abrasion. The findings suggest the importance of a frequent low concentrated fluoride exposure rather than a unique application of a high concentrated fluoride product.

A recent study was conducted in enamel showing similar results. However, only one of the combinations (0.042% NaF+0.049% TiF₄) was effective in reducing enamel erosive loss (41% preventive fraction), while the other one did not differ from the negative control. Generally, the tested fluoride mouthrinses had better impact on dentin compared to enamel, which might be explained by the differences in the composition between the dental tissues. In case of dentin, the effect of the combinations of TiF₄ and NaF was similar to those provided by a commercial fluoride solution (positive control), which has been widely used in Europe for prevention of tooth erosion. The preventive fraction found by the application of Erosion Protection® in the present study was similar to a previous in situ study performed by other research group. Based on this finding, we can speculate that the acid-resistance of Ti and F precipitates found for our experimental solutions are similar to tin and fluoride precipitates produced by the application of Erosion Protection® on dentin.

On the other hand, NaF solution presented the worst performance, not differing from the negative control. It is widely known that NaF is ineffective to protect against tooth erosion especially in case of dentin, in which its effect depends on the presence of the DOM. In this study, the DOM was not removed, but it would be interesting to test the effect of the experimental fluoride solutions on dentin without DOM. Further studies should also test the effect of the experimental fluoride solutions on both dentin erosion and brushing abrasion to check the stability of the protective effect faced by two different challenges (chemical and mechanical).

Another point to consider is that erosion in dentin is very complex due to the role of DOM in the progression of erosive loss. Therefore, erosive loss is difficult to be quantified, since the quality of the remaining organic layer may interfere with the profilometric measurement. Shrinkage of the DOM may occur under different environments interfering in the profile analysis. Therefore, to generate reliable data, the profiles must be measured with the samples immersed 100% in water or without DOM. We have decided to perform the analysis with DOM under 100% humidity, since in previous study we have not found differences in the comparison between TiF₄ and NaF varnishes in the profile analysis of dentin with or without DOM.

The present study showed promising results for the experimental fluoride mouthrinses. Future studies, including in situ and in vivo models, must be performed to confirm the findings, since saliva can be able to buffer the pH of the fluoride solutions, which might lead to different results.

CONCLUSION

Under the conditions of the present study, we can conclude that the daily application of an experimental mouthrinse containing a specific combination of TiF₄ and NaF has the ability to reduce dentin erosion in vitro, and may be a good alternative for high-risk populations.

ACKNOWLEDGEMENTS

The authors thank FAPESP - São Paulo Research Foundation (São Paulo, São Paulo, Brazil) for the financial support (2012/20698-0).

REFERENCES

1- Aguiar YP, Santos FG, Moura EF, Costa FC, Auad SM, Paiva SM, et al. Association between dental erosion and diet in Brazilian adolescents aged from 15 to 19: a population-based study. ScientificWorldJournal. 2014;2014:818167.
2- Arnadottir IB, Holbrook WP, Eggertsson H, Gudmundsdottir H, Jonsson SH, Gudlaugsson JO, et al. Prevalence of dental erosion in children: a national survey. Community Dent Oral Epidemiol. 2010;38:521-6.
3- Büyükyilmaz T, Øgaard B, Rolla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci. 1997;105:473-7.
4- Comar LP, Cardoso CA, Charone S, Grizzo LT, Buzalaf MA, Magalhães AC. TiF₄ and NaF varnishes as anti-erosive agents on enamel and dentin erosion progression in vitro. J Appl Oral Sci. 2015;23:14-8.
5- Ganss C, Klimek J, Schäffer U, Spall T. Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res. 2001;35:325-30.
6- Ganss C, Neutard L, von Hinckeldey J, Klimek J, Schlueter N. Efficacy of a tin/fluoride rinse: a randomized in situ trial on erosion. J Dent Res. 2010;89:1214-8.
7- Ganss C, Schlueter N, Klimek J. Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions – a comparison of in vitro and in situ results. Arch Oral Biol. 2007;52:9-14.
8- Hove L, Holme B, Øgaard B, Willumsen T, Tveit AB. The protective effect of TiF₄, SnF₂ and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res. 2006;40:440-3.
9- Hove LH, Holme B, Young A, Tveit AB. The protective effect of TiF₄, SnF₂ and NaF against erosion-like lesions in situ. Caries Res. 2008;44:4-8.
10- Kreulen CM, Van’t Spijker A, Rodriguez JM, Bronkhorst EM, Creugers NH, Bartlett DW. Systematic review of the prevalence of tooth wear in children and adolescents. Caries Res. 2010;44:151-9.
11- Lussi A, Hellwig E. Risk assessment and causal preventive measures. Monogr Oral Sci. 2014;25:220-9.
12- Magalhães AC, Kato MT, Rios D, Wiegand A, Attin T, Buzalaf MA. The effect of a single application of TiF₄ and NaF varnishes and 4% TiF₄ solution on dental erosion in vitro. Caries Res. 2008;42:269-74.
13- Magalhães AC, Levy FM, Rios D, Buzalaf MA. Effect of NaF and TiF₄ varnish and solution on bovine dentin erosion. J Dent. 2010;38:153-7.
14- Magalhães AC, Levy FM, Rizzante FA, Rios D, Buzalaf MA. Effect of NaF and TiF₄ varnish and solution on dental erosion plus abrasion in vitro. J Dent. 2010;38:153-7.
15- Magalhães AC, Wiegand A, Rios D, Buzalaf MA, Lussi A. Fluoride in dental erosion. Monogr Oral Sci. 2011;22:158-70.
16- Nahás Pires Corrêa MS, Nahás Pires Corrêa F, Nahás Pires Corrêa JP, Murakami C, Mendes FM. Prevalence of dental erosion among 12-year-old children in a private dental practice. Int J Paediatr Dent. 2011;21:451-8.
17- Sales-Peres SH, Goya S, Araujo JJ, Sales-Peres A, Lauris JR, Buzalaf MA. Prevalence of dental wear among 12-year-old Brazilian adolescents using a modification of the tooth wear index. Public Health. 2008;122:942-8.
18- Schlueter N, Ganss C, Hardt M, Schegietz D, Klimek J. Effect of pepsin on erosive tissue loss and the efficacy of fluoridation measures in dentine in vitro. Acta Odontol Scand. 2007;65:298-305.
19- Schlueter N, Ganss C, Pötschke S, Klimek J, Hannig C. Enzyme activities in the oral fluids of patients suffering from bulimia: a controlled clinical trial. Caries Res. 2012;46:130-9.
20- Schlueter N, Neutard L, von Hinckeldey J, Klimek J, Ganss C. Tin and fluoride as anti-erosive agents in enamel and dentine in vitro. Acta Odontol Scand. 2010;68:180-4.
21- Sen BH, Kazemi RB, Spångberg LS. Morphologic effects on L929 fibroblasts of titanium tetrafluoride application. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86:341-6.
22- Souza BM, Lima LL, Comar LF, Buzalaf MA, Magalhães AC. Effect of experimental mouthrinses containing the combination of NaF and TiF₄ on enamel erosive wear in vitro. Arch Oral Biol. 2014;59:621-4.
23- Stenhagen KR, Hove LH, Holme B, Tveit A. The effect of daily fluoride mouth rinsing on enamel erosive/abrasive wear in situ. Caries Res. 2013;47:2-8.
24- Truin GJ, van Rijkom HM, Mulder J, van’t Hof MA. Caries trends 1996–2002 among 6- and 12-year-old children and erosive wear prevalence among 12-year-old children in The Hague. Caries Res. 2005;39:2-8.
25- Vieira AM, Ruben JL, Bronkhorst EM, Huysmans MC. In vitro reduction of dental erosion by low-concentration TiF₄ solutions. Caries Res. 2011;45:142-7.
26- Wiegand A, Hiestand B, Sener B, Magalhães AC, Roos M, Attin T. Effect of TiF₄, ZrF₄, HF₄ and AmF on erosion and abrasion of enamel and dentin in vitro. Arch Oral Biol. 2010;55:223-8.
27- Wiegand A, Magalhães AC, Sener B, Waldheim E, Attin T. TiF₄ and NaF at pH 1.2 but not at pH 3.5 are able to reduce dentin erosion. Arch Oral Biol. 2009;54:790-5.
28- Wiegand A, Waldheim E, Sener B, Magalhães AC, Attin T. Comparison of the effects of TiF₄ and NaF solutions at pH 1.2 and 3.5 on enamel erosion in vitro. Caries Res. 2009;43:269-77.