Acta Odontologica Scandinavica, 2012; 70: 160–164

informa
healthcare

ORIGINAL ARTICLE

Effect of NaF and TiF_4 varnish and solution on bovine dentin erosion plus abrasion *in vitro*

ANA CAROLINA MAGALHÃES[1], FLÁVIA MAUAD LEVY[1], FÁBIO A. RIZZANTE[1], DANIELA RIOS[2] & MARÍLIA AFONSO RABELO BUZALAF[1]

[1]*Department of Biological Sciences, and* [2]*Department of Pediatric Dentistry, Orthodontics and Public Health, Bauru School of Dentistry, University of São Paulo–Bauru, SP, Brazil*

Abstract

Objectives. This *in vitro* study aimed to analyze the effect of TiF_4 compared to NaF varnishes and solutions, to protect against dentin erosion associated with abrasion. ***Materials and methods.*** Bovine dentin specimens were pre-treated with NaF-Duraphat (2.26% F), NaF/CaF_2-Duofluorid (5.63% F), experimental-NaF (2.45% F), experimental-TiF_4 (2.45% F) and placebo varnishes; NaF (2.26% F) and TiF_4 (2.45% F) solutions. Controls remained untreated. The erosive pH cycling was performed using a soft drink (pH 2.6) 4 × 90 s/day and the toothbrushing-abrasion 2 × 10 s/day, *in vitro* for 5 days. Between the challenges, the specimens were exposed to artificial saliva. Dentin tissue loss was measured profilometrically (μm). ***Results.*** ANOVA/Tukey's test showed that all fluoridated varnishes (Duraphat, 7.5 ± 1.1; Duofluorid, 6.8 ± 1.1; NaF, 7.2 ± 1.9; TiF_4, 6.5 ± 1.0) were able to significantly reduce dentin tissue loss (40.7% reduction compared to control) when compared to placebo varnish (11.2 ± 1.3), control (11.8 ± 1.7) and fluoridated (NaF, 9.9 ± 1.8; TiF_4, 10.3 ± 2.1) solutions ($p < 0.0001$), which in turn did not significantly differ from each other. ***Conclusion.*** All fluoridated varnishes, but not the solutions, had a similar performance and a good potential to reduce dentin tissue loss under mild erosive and abrasive conditions *in vitro.* Risk patients for erosion and abrasion, especially those with exposed dentin, should benefit from this clinical preventive measure. Further research has to confirm this promising result in the clinical situation.

Key Words: *abrasion, dentin, erosion, fluoride*

Introduction

Dental erosion is defined as substance loss by exogenous or endogenous acids without bacterial involvement. The most important sources are dietary acids [1] and one acid originated from the stomach, in cases of regurgitation and reflux disorders [2]. In dentin, the erosive demineralization is mostly diffusion controlled, as the increasing exposure of organic matrix hampers ion diffusion and, thus, reduces further progression of dentin erosion [3,4]. The demineralized collagen layer might be affected by enzymatic and chemical degradation [5] as well as by abrasive influences [6].

The exposed root dentin, in the case of gingival trauma or periodontal disease, might be accompanied by an increased risk for dental hard tissue loss by different chemical (erosion) and physical (toothbrushing abrasion) processes. In order to prevent dentin tissue loss, fluoride application is recommended. However, considering the severe and chronic acid exposure in patients suffering from dental erosion, the effect of conventional fluoride, such as NaF, by the formation of a protective CaF_2 layer, is probably limited over time [7]. Therefore, fluoride compounds with a distinct potential to resist erosive challenges are required.

The potential of TiF_4 to prevent dental erosive demineralization has been investigated since 1997 [8]. Its protective effect is related to the formation of an acid-resistant surface coating, the increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice [8–11]. The glaze-like surface layer observed after the application of TiF_4 is assumed to be formed from a new compound (hydrated hydrogen titanium phosphate) or

Correspondence: Professor Dr Ana Carolina Magalhães, Al. Dr. Octávio Pinheiro Brisolla 9-75, Bauru-SP 17012-190, Brazil. Tel: 55-14-32358247. Fax: 55-14-32271486. E-mail: acm@usp.br

(Received 1 December 2010; revised 4 March 2011; accepted 12 March 2011)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2011.600711

organometallic complexes that might primarily act as a diffusion barrier [8–11].

Previous *in vitro* studies have shown that TiF_4 was as effective as or better than NaF solution on prevention of dentin erosion [12,13]; however, other works indicated a similar or lower effect of TiF_4 compared to AmF solution against dentin erosion [14,15]. The different results might be explained by the F concentration, pH and erosive challenges. It might be assumed that the preventive effect of TiF_4 against erosive demineralization is also dependent on the agent used, as it was recently shown *in vitro* that a 4% TiF_4 varnish exhibited a higher protective potential than a 4% TiF_4 solution on enamel erosion [16].

Recently, Magalhães et al. [17] showed that only NaF, but not TiF_4, varnish seems to be able to partially reduce dentin erosion. However, no data regarding the effect of this experimental varnish on dentin erosion plus abrasion is available so far. Thus, the aim of this *in vitro* study was to analyze the effect of a single application of TiF_4 varnish/solution compared to NaF varnishes/solution, to protect against dentin erosion and abrasion.

Materials and methods

Preparation of the dentin specimens

Eighty dentin specimens (4 mm × 4 mm × 3 mm) were prepared from 40 bovine dental roots, which were stored in 0.1% buffered thymol solution (pH 7.0) at 4°C. The specimens were cut from the cervical region of the root (labial and lingual surfaces), using a ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL) with two diamond disks (Extec Corp., Enfield, CT), which were separated by a 4-mm thickness spacer. The specimens surfaces were ground flat with water-cooled silicon carbide discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler), and finally polished with felt paper wet by a diamond solution (1 μm thickness; Buehler). After the polish, the specimens were cleaned in an ultrasonic device with deionized water for 2 min. Prior to the experiment, two layers of nail varnish were applied on 2/3 of the surface of each specimen to maintain reference surfaces for the dentin tissue loss determination after the experiment (1 mm of the exposed dentin area).

A sample size of 10 specimens was calculated considering an α-error level of 5% and a β-error level of 20% based on previous data [17]. Ten dentin specimens were randomly allocated to one control (untreated) and to each of the seven test groups: NaF-Duraphat varnish (Colgate, Brazil, 2.26%F, pH 4.5), NaF/CaF_2-Duofluorid varnish/(FGM-Dentscare, Brazil, 5.63%F, pH 8.0), experimental NaF varnish (FGM-Dentscare, Brazil, 2.45%F, pH 4.5), experimental TiF_4 varnish (FGM-Dentscare, Brazil, 2.45%F, pH 1.2), NaF solution (2.26%F, pH 4.5), TiF_4 solution (2.45%F, pH 1.2) and placebo varnish (FGM-Dentscare, Brazil, pH 5.0, no-F varnish control).

Treatment

The treatments were performed at the beginning of the experiment. For this, the NaF (5 g/100 mL) and TiF_4 (4 g/100 mL) powders (Sigma-Aldrich, St Louis, MO, USA) were dissolved in deionized water immediately before the application. The NaF solution was adjusted to pH 4.5 by adding 12.6 g 5 M H_3PO_4/100 mL. The pH of 1.2 was native for TiF_4. These F solutions were applied once using a microbrush and were left on the surface for 1 min [16–19]. Excess of the solution was removed from the surface by a cotton roll. After that, the specimens were immersed in artificial saliva for 6 h [16,17].

Regarding the varnishes, Duofluorid was transparent, while the other varnishes were yellow. According to the manufacturers, the varnishes from FGM-Dentscare include colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol (with or without F); while Duraphat contains 5% NaF (2.26% F), 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. The pH values of all varnishes and solutions were measured by indicator paper (± 0.5 units) and by electrode (± 0.01 unit), respectively. The pH of the solutions was checked only after the preparation, due to the fact that the solutions were immediately applied on dentin surface. On the other hand, the pH of varnishes was checked several times (after the preparation, before the application and after the experiment) and remained constant over the study.

The varnishes were applied once in a thin layer using a microbrush. The specimens remained in artificial saliva for 6 h [16,17]. The difference between the application times of the solutions and varnishes was purposely done in order to simulate the clinical condition. After this period, the varnishes were carefully removed from the surface using acetone solution (1:1 water) and a scalpel blade, taking care to avoid touching of the dental surface. Complete removal of the layer was checked microscopically (40 ×) [16,17]. After that, all specimens were immersed in artificial saliva for 6 h up to the next day when the erosive and abrasive challenges started [16,17].

Erosive and abrasive challenges

All specimens were submitted to a 5-day erosive de- and remineralization cycling. Erosion was performed with a freshly opened bottle of Sprite Zero drink (Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.6, 30 ml/specimen, unstirred, 25°C) four times daily for 90 s each. After each erosive challenge, the

specimens were rinsed with deionized water (5 s) and transferred to artificial saliva (pH 6.8, 30 ml/specimens, unstirred, 25°C) for 2 h. The artificial saliva was renewed daily and 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 ascorbic acid (pH 6.8) [20].

All specimens were also exposed to freshly made slurries of an experimental non-fluoridated toothpaste (ratio: 1 toothpaste:3 water, silica as abrasive:Crest®, Procter & Gamble, Cincinnati, OH, USA) twice daily (0.5 mL/specimen, 25°C), after the first and the last erosive challenges and then abraded using an electrical toothbrush for 10 s (166 oscillations/s, Colgate Motions Multi-Action, Sáo Paulo, Brazil). The toothbrushes were mounted in a device that was constructed to allow the heads of the toothbrushes to be aligned parallel to the specimen surface. The power of toothbrushing was standardized at 1.5 N for all specimens (1 Kg ~ 9.80 665 N, $F = 1.5$ N) [21], by weighing the toothbrushing head using a precision scale (Pesola, Baar, Switzerland) and adjusting with elastic bands. The toothbrush heads were replaced per group daily.

After the toothbrushing, the specimens were rinsed in water for 5 s, before being re-immersed in artificial saliva. At the end of the day, the specimens were also stored in artificial saliva overnight.

Profilometric measurement

After 5 days, dentin tissue loss (μm) was quantitatively determined by a contact profilometer (Mahr Perthometer, Göttingen, Germany). The dentin specimens were maintained wet until the analysis to avoid shrinkage of the demineralized organic layer. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). The specimens were slightly dried, which means that only the excess of water was gently removed with filter paper and immediately analyzed [17]. The diamond stylus moved from the first reference across the exposed area and on to the other reference area (2.5 mm length and 2.0 mm width). Five profile measurements were performed in the center of each specimen at intervals of 0.5 mm. The vertical distance between the mid-points of regression lines on the reference and experimental areas was defined as tissue loss (μm) (Software Marh Surf XR20, 2009). The accuracy of the method is ~ 0.5 μm. The standard deviation of five repeated analyses of a given sample was 0.3 μm.

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used. The assumptions of equality of variances and normal distribution of data were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. Since the assumptions were satisfied, the data were analyzed by ANOVA followed by Tukey's post-hoc tests. The level of significance was set at 5%.

Results

ANOVA showed that all fluoridated varnishes were able to significantly reduce dentin tissue loss (40.7% reduction in dentin tissue loss compared to control) when compared to placebo varnish, control and fluoridated solutions ($p < 0.0001$). There were no significant differences among the fluoridated varnishes (from 36.4 to 44.9% reduction). The placebo varnish, control and fluoridated solutions-treated specimens presented the highest dentin tissue loss means, which in turn did not significantly differ from each other (Table I).

Discussion

In general, the present study showed that fluoridated varnishes, regardless of the F salt and pH, were able to significantly reduce dentin tissue loss, while the fluoridated solutions were not effective. In the present study, the varnishes and solutions were applied only once in order to simulate the clinical situation with a single professional application, according to previous studies [16,17]. The varnishes were removed after 6 h, reproducing the *in vivo* situation in which the varnishes might be removed after some hours by toothbrushing or mastication. Thus, the present study focuses more on the chemical rather than on the mechanical effect of the varnishes. Regarding the solutions, they were applied for 1 min, since the time of application ranges from 1–4 min in the literature [8,12,16–19].

Two NaF varnishes that are frequently used in the clinic were chosen as controls. An experimental NaF

Table I. Mean dentin erosive-abrasive loss (μm) ± SD in the different groups ($n = 10$).

Treatment	Dentin tissue loss
Duraphat	7.5 ± 1.1^a
Duofluorid	6.8 ± 1.1^a
NaF varnish	7.2 ± 1.9^a
TiF_4 varnish	6.5 ± 1.0^a
NaF solution	9.9 ± 1.8^b
TiF_4 solution	10.3 ± 2.1^b
Placebo varnish	11.2 ± 1.3^b
Untreated (control)	11.8 ± 1.7^b

Distinct lower case letters indicate significant differences among the F solutions/varnishes (ANOVA and Tukey's test, $p < 0.0001$).

varnish was prepared by the same company and presented the same base composition and F concentration as the experimental TiF_4 varnish. The F concentration of the experimental TiF_4 varnish/solution was chosen in accordance to a previous study, showing favorable results for this TiF_4 varnish to prevent enamel erosion [16]. The TiF_4 agents show naturally lower pH compared to the NaF products, which in turn present a high pH range, varying from 4.5–8.0. The efficacy of TiF_4 is highly dependent on the pH of the agent, since it was shown that dentin erosion can be significantly reduced by TiF_4 (0.5 M F) at native pH (pH 1.2) but not at a pH buffered to 3.5 [13]. NaF solution with pH 1.2 was also able to significantly reduce dentin erosion [13]. However, NaF solution, in the present study, was used as vehicle control of the Duraphat varnish, because of this the pH and F concentration were not adjusted to be similar to TiF_4. A limitation of using experimental-NaF varnish with low pH (1.2) is the stability of the product over time.

In the present study, TiF_4 solution was unable to prevent dentin erosion-abrasion according to the previous data [15,17]. It was able to open dentinal tubules after the application, working as a smear layer remover (data unpublished). This result was expected, as TiF_4 agents present a low pH. However, a surface layer covering the dentinal tubules was seen after the application of TiF_4 varnish (data unpublished). The different surface effect of TiF_4 formulations seems to be related to the better ability of the varnish to adhere to the tooth surface, which allows an increased contact time and hence prolongs the reaction between the TiF_4 and dental surface. It suggests that TiF_4 incorporated into a varnish might reduce the effects of its low pH on the dentin, when in contact with saliva for 6 h.

Wiegand et al. [15] showed that titanium tetrafluoride solution induced some granular coating on dentin surfaces, which partly covered dentinal tubules. However, its protective potential did not exceed the efficacy of NaF or AmF [12,15,17], in accordance with the present study.

All F varnishes were able to significantly reduce dentin erosion-abrasion. Thus, the F concentration, type of F salt and base as well as pH might not have influenced the effect of the products on the dentin tissue loss in this case. The contact time seems to be the most important factor, as fluoridated varnishes worked better than the corresponding solutions, regardless of the other factors. Topical fluoridation induces the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in the case of conventional fluorides like NaF) or of metal-rich surface precipitates (in the case of TiF_4). It can also be speculated that the CaF_2-layer or organometallic glaze like-surface formed by the application of NaF and TiF_4 varnishes, respectively, might have had the same impact on the prevention of dentin erosion and abrasion.

In a previous study, Duraphat was the only treatment able to reduce dentin erosion [17]. The differences between the previous data and the present study might be related to the profilometric analyses. Besides, it is speculated if the abrasion could have removed some particles of the varnishes that might be inside of the tubules from the demineralized dentin, allowing a better differentiation among the fluoridated varnishes and placebo varnish/control.

The preventive effect of fluorides on dentin erosion is highly dependent on the presence of the organic matrix [5,22]. Initial studies showed that a very intensive fluoridation was most effective in the prevention of dentin erosion [23,24]. However, after enzymatic removal of the organic matrix, fluoride was ineffective [3,5]. It was assumed that the demineralized organic dentin matrix has a buffering capacity sufficient to prevent further dentin demineralization, especially in the presence of high amounts of fluoride [3]. Moreover, the exposed organic matrix of etched dentin involves an increased surface area and increased diffusion pathways, enhancing the amount of KOH-soluble fluoride uptake [25]. However, it remains unclear to what extent the organic material is retained under clinical conditions, when the collagen layer might be affected by enzymatic and chemical degradation [5] as well as by abrasive forces [6]. From the clinical appearance of dentin erosive lesions it seems likely that the collagenous layer is at least partly removed.

Therefore, before F varnishes start to be frequently used in the clinical situation, the next step is to test the effect of these treatments on human enamel and dentin erosion and abrasion *in situ*, a condition that more closely resembles the clinical situation.

Under the conditions of the present study it can be concluded that all fluoridated varnishes, but not the solutions, had a similar performance and seem to have a good potential to reduce dentin tissue loss under mild erosive and abrasive conditions *in vitro*.

Acknowledgement

The authors are grateful to FAPESP (Proc. 08/07105-4 and 08/03727-0) for the financial support and FGM-Dentscare Ltda, Brazilian Dental Products Company, for the preparation of the varnishes.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res 2004;38(Suppl 1):34–44.

[2] Bartlett D. Intrinsic causes of erosion. Monogr Oral Sci 2006; 20:119–39.
[3] Ganss C, Klimek J, Starck C. Quantitative analysis of the impact of the organic matrix on the fluoride effect on erosion progression in human dentine using longitudinal microradiography. Arch Oral Biol 2004;49:931–5.
[4] Hara AT, Ando M, Cury JA, Serra MC, González-Cabezas C, Zero DT. Influence of the organic matrix on root dentine erosion by citric acid. Caries Res 2005;39:134–8.
[5] 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.
[6] Addy M, Shellis RP. Interaction between attrition, abrasion and erosion in tooth wear. Monogr Oral Sci 2006; 20:17–31.
[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] Büyükyilmaz T, Øgaard B, RØlla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci 1997;105:473–7.
[9] Mundorff SA, Little MF, Bibby BG. Enamel dissolution. 2. Action of titanium tetrafluoride. J Dent Res 1972;51:1567–71.
[10] Wei SH, Sobooff DM, Wefel JS. Effects of titanium tetrafluoride on human enamel. J Dent Res 1976;55:426–31.
[11] Ribeiro CC, Gibson I, Barbosa MA. The uptake of titanium ions by hydroxyapatite particles - structural changes and possible mechanisms. Biomaterials 2006;27:1749–61.
[12] Schlueter N, Ganss C, Mueller U, Klimek J. Effect of titanium tetrafluoride and sodium fluoride on erosion progression in enamel and dentine in vitro. Caries Res 2007;41:141–5.
[13] Wiegand A, Magalhães AC, Sener B, Waldheim E, Attin T. TiF(4) 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.
[14] Wiegand A, Hiestand B, Sener B, Magalhães AC, Roos M, Attin T. Effect of TiF(4), ZrF(4), HfF(4) and AmF on erosion and erosion/abrasion of enamel and dentin in situ. Arch Oral Biol 2010;55:223–8.
[15] Wiegand A, Magalhães AC, Navarro RS, Schmidlin PR, Rios D, Buzalaf MA, et al. Effect of TiF4 and AmF treatment combined with CO2 laser-irradiation on enamel and dentin erosion. Photomed Laser Surg 2010;28:219–26.
[16] Magalhães AC, Kato MT, Rios D, Wiegand A, Attin T, Buzalaf MAR. The effect of an experimental 4% TiF4 varnish compared to NaF varnishes and 4% TiF4 solution on dental erosion in vitro. Caries Res 2008;42:269–74.
[17] Magalhães AC, Levy FM, Rios D, Buzalaf MAR. Effect of a single application of TiF(4) and NaF varnishes and solutions on dentin erosion in vitro. J Dent 2010;38:153–7.
[18] Tezel H, Ergucu Z, Onal B. Effects of topical fluoride agents on artificial enamel lesion formation in vitro. Quintessence Int 2002;33:347–52.
[19] Van Rijkon H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibition effect of sodium fluoride and titanium tetrafluoride treatment in vitro. Eur J Oral Sci 2003; 111:253–7.
[20] Klimek J, Hellwig E, Ahrens G. Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. Caries Res 1982;16:156–61.
[21] Voronets J, Jaeggi T, Buergin W, Lussi A. Controlled toothbrush abrasion of softened human enamel. Caries Res 2008; 42:286–90.
[22] Ganss C, Lussi A, Sommer N, Klimek J, Schlueter N. Efficacy of fluoride compounds and stannous chloride as erosion inhibitors in dentine. Caries Res 2010;44:248–52.
[23] Ganss C, Klimek J, Schaffer U, Spall T. Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res 2001;35:325–30.
[24] Ganss C, Klimek J, Brune V, Spall T. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561–6.
[25] Buchalla W, Lennon AM, Becker K, Lucke T, Attin T. Smear layer and surface state affect dentin fluoride uptake. Arch Oral Biol 2007;52:932–7.