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The erosion-inhibiting effect of TiF_4, SnF_2, and NaF solutions on pellicle-covered enamel *in vitro*

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Abstract

Objective. The aim of this study was to compare the protective effect of TiF_4, SnF_2, and NaF treatment on the development of erosion-like lesions in pellicle-covered human enamel. ***Material and Methods.*** Twelve human molars were each divided into 5 specimens, 4 of which were immersed in saliva for 2 h. Three pellicle-covered specimens from each tooth were treated with a TiF_4, SnF_2, or NaF solution (all 0.5 M F) for 2 min. Control specimens, one with and one without pellicle, were included. Immersion in acid (0.01 M HCl) was carried out stepwise (2+2+2+2 min). The etching depths (in μm) were measured using white light interferometry. ***Results.*** Compared with the control with pellicle, TiF_4 reduced enamel loss by 100% after 2 min and by 24% after 8 min of acid exposure. The corresponding values for SnF_2 were 45% and 14%. NaF provided no significant protection of the surface. The pellicle-covered specimens showed reduced lesion depths after 6 and 8 min compared to the controls without pellicle. ***Conclusions.*** TiF_4 gave the best protection against acid attack. SnF_2 provided significant protection only after 2 min of acid exposure, while NaF had no significant protective effect.

Key Words: *Dental erosion*, *sodium fluoride*, *tin fluoride*, *titanium fluoride*, *White Light Interferometry (WLI)*

Introduction

In recent years, much focus has been placed on extrinsic causes of dental erosion, such as acidic dietary beverages and citric and phosphoric acids, which are often added to soft drinks. These erosive solutions have been widely tested in both *in vitro* [1,2] and *in situ* studies [3]. Few studies have focused on the erosive effect of intrinsic acids even though gastric acids have been shown to have a higher erosive potential than a standard cola drink [4]. Gastric content can severely affect dental surfaces when present in the oral cavity. Furthermore, prolonged acid exposure can cause dental erosion and in severe cases result in pathological tooth wear with loss of both function and esthetics. It has been shown that people suffering from eating disorders or gastric reflux are at increased risk of developing dental erosion [5–7].

The inhibiting effect of sodium fluoride (NaF) on caries is well documented, and a protective effect against dental erosion has been shown *in vitro* [8,9]. Tin fluoride (SnF_2) has also been used in caries prevention and has shown a promising erosion-inhibiting effect both *in vitro* [9,10] and *in situ* when combined with NaF [11]. *In vitro* studies have demonstrated a considerable erosion protection ability of titanium fluoride (TiF_4) on enamel [12–14] and an acidified fluoride gel (NaF and amine fluoride, pH 4.5) improved the surface hardness of enamel against abrasion [15]. In previous studies using a scanning electron microscope, a coating or glaze was observed when tooth surfaces were treated with TiF_4 [12,16]. This coating could be one explanation for the superior effect of TiF_4 compared to SnF_2 and NaF in inhibiting the erosive effect of HCl on non-pellicle-covered human enamel *in vitro* [17].

In the oral cavity, saliva plays an important role in the erosive process by means of buffering capacity, diluting effect on acids, remineralizing properties, and formation of a pellicle. It has been demonstrated that salivary pellicle protects the underlying enamel against erosive challenge [2,18,19]. The thickness of the pellicle may vary, but even after a procedure with sponge-rubbing, a thin basal layer was preserved on

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the surface [20]. Until now, few erosion studies involving fluoride treatments have been performed with a pellicle-covered enamel surface [11,21]. In order to study the extent to which the pellicle influences the effect of topical application of TiF_4, Hove et al. [21] measured Ca loss from enamel specimens after HCl exposure *in vitro*. The results showed that the 2-h pellicle formed on the enamel surface did not interfere with the protective effect of TiF_4. However, a protective effect of the pellicle itself could not be demonstrated. The technique used in that study, atomic absorption spectroscopy, measures Ca loss from a surface's area, but gives no information about the roughness or the depths of the lesions created. Furthermore, since other authors have found a significant protective effect of an *in vitro* pellicle using other measurement techniques, in the present study White Light Interferometry was used to measure differences between pellicle-covered and pellicle-free controls. WLI provides the opportunity to register the true etching depth of the erosion-like lesions. Since the technique is fast, accurate, and non-destructive, an effect of the pellicle itself could possibly be demonstrated. The method has been described by Holme et al. [22].

In the clinical situation, pellicle is usually present on tooth surfaces. Therefore, in the search for the clinically best suited fluoride agent for inhibiting dental erosion, it is important to test the erosion-inhibiting effect of different fluoride solutions on enamel surfaces covered with pellicle. The aim of the present study was to compare the effect of TiF_4, SnF_2, and NaF treatment on the development of erosion-like lesions in pellicle-covered enamel measured by WLI and, furthermore, to register the protective influence of a 2-h pellicle.

Material and methods

Preparation of enamel specimens

Twelve freshly extracted human permanent molars were used in this study. The enamel surfaces were polished with polishing paste, wiped free of debris, and rinsed in tap water. Five circular amalgam fillings (ca. 1 mm in diameter) were made in each tooth approximately 2 mm above the enamel–cement junction. The amalgam was used as a reference surface during the analysis. Each tooth was then divided with a high-speed turbine into 5 specimens each including 1 amalgam filling, and the specimens were randomly assigned to one of 5 groups. All enamel surfaces and restorations were carefully examined and only specimens with intact surfaces were used. Prior to the experiment, the specimens were stored in a 100% humid environment.

Preparation of specimen blocks

All five specimens from one tooth were mounted on an epoxy resin block using a light curing resin. During analyses, the resin block was fixed in a prefabricated device inside the WLI, which allowed the block to be removed for pellicle formation, fluoride treatment, and acid exposure and repositioned in exactly the same location. Prior to fluoride application, and after fluoride and acid exposure, all specimens were analyzed using the WLI.

Pellicle formation

The *in vitro* salivary pellicle used in this study was based on the procedure described by Nekrashevych & Stösser [2]. Saliva was collected 1 h after breakfast from 6 volunteers without active carious lesions, erosions, or salivary dysfunction. Salivary secretion was stimulated by chewing a block of paraffin and expectorated directly into ice-chilled test tubes. The saliva samples were then pooled and clarified (12,000 rpm at 4°C, 20 min). Four enamel specimens from each tooth were immersed in the clarified saliva at room temperature for 2 h. One specimen from each tooth was protected against saliva contamination. This control specimen without pellicle was later compared with the control with pellicle.

Fluoride treatment

Three different specimens with pellicle from each tooth were treated with one of the three fluoride solutions: SnF_2, TiF_4, or NaF (all 0.5 M F) for 2 min. Fluoride application was performed with a pipette dropper and the solution was applied as droplets with a constant flow (one per second). The other specimens were protected with plastic shields to avoid contamination. Two control specimens were included: one with pellicle ("control with pellicle") and one without ("control without pellicle"). After fluoride treatment, the specimen surfaces were dried with a gentle blast of air.

Hydrochloric acid exposure

After fluoride treatment, the entire block with specimens was immersed and stirred in 500 ml of 0.01 M HCl (pH 2.2) for 4 consecutive 2-min periods at 23°C. After each exposure to the acid solution, the specimens were rinsed in deionized water for 30 s and analyzed in the WLI.

Analytical method

All specimens were analyzed using a WLI (WYKO™ NT-2000; Veeco, USA) both before and after each acid exposure. The enamel surfaces were washed in 20% ethanol and rinsed in tap water in order to remove traces of organic debris prior to the analysis.

During analysis of one surface, the other specimens on the same block were covered with a cotton pellet soaked in water to avoid dehydration. Analysis time for one surface was approximately 1 min, and the topographic image was produced immediately. The WLI is a computed optical microscope that uses interference to produce a topographic image of the surface. Digital images from WLI are typically shown as topographic maps where various colors denote different heights for the picture elements (pixels). The pixel heights are recorded by the WLI software (Vision 32™; Veeco, USA). By subtracting the original image from the image obtained after etching, a difference image was created which showed how much enamel had been removed during the erosive challenges. The mean heights with standard deviations of the amalgam reference surfaces and the enamel regions were calculated, thus quantifying the material loss with high accuracy (<50 nm). The mean roughness change (R_q) of the different images was calculated to express the increase in surface roughness due to etching of the enamel. This method, used in analysis of erosion-like lesions, has been described in more detail previously by Holme et al. [22].

Statistical analysis

Statistical procedures were performed with the Statistical Package for Social Sciences for Windows (SPSS, v. 13.0, Chicago, Ill., USA). Paired *t*-tests were used to assess the significance of differences between the various treatments and the controls with pellicle (baseline), between the different treatments, and between the controls with and without pellicle. For multiple comparisons, *p*-values were adjusted using the Bonferroni method. The level of significance was set at 0.05.

Results

The mean etching depths for fluoride-treated enamel surfaces and controls with and without pellicle for different acid exposure intervals are given in Table I. Mean values and standard deviations are based on 12 repetitions for each treatment. After 2 min of acid exposure the TiF_4 treatment reduced the etch depth by 100%. This effect gradually decreased to 75% after 4 min ($p<0.001$), 42% after 6 min ($p<0.001$), and 24% after 8 min ($p=0.012$) compared to the control with pellicle. The corresponding values for SnF_2 were 45% after 2 min ($p=0.018$), 23% after 4 min (not significant), 18% after 6 min (not significant), and 14% after 8 min (not significant) compared to the control with pellicle. TiF_4 was significantly more effective than the other fluoride solutions, except for SnF_2 after 6 and 8 min etching. NaF provided no reduction in etch depth after any acid exposure intervals compared to the control with pellicle, but compared to the control without pellicle a difference was found after 4, 6, and 8 min (not shown in Table I). Compared to the control without pellicle, the control with pellicle provided a significant 8% reduction in etch depth after both 6 min ($p=0.027$) and 8 min ($p=0.006$) of acid exposure.

The mean (SD) etch rates from 2 to 8 min for the TiF_4, SnF_2, and NaF samples were all 1.4 (0.4) μm/min. The corresponding etch rates for the controls with and without pellicle were 1.5 (0.2) μm/min and 1.6 (0.2) μm/min, respectively.

Surface roughness change

The mean roughness change (R_q) after 8 min of HCl exposure for the control with pellicle was 0.8 (0.2) μm and 0.8 (0.3) μm for the NaF sample. The R_q levels for the SnF_2, control without pellicle, and TiF_4 were 0.9 (0.5) μm, 1.0 (0.5) μm, and 2.2 (1.1) μm, respectively. It was not possible to detect a distinct pattern in the surface roughness levels and no correlations were observed between etching duration, etch depth, and the increase in surface roughness.

Discussion

In the present *in vitro* study, the TiF_4 treatment was more effective than both the NaF and SnF_2 treatments in protecting the enamel surface against erosion-like lesions. In fact, after 2 min of acid exposure the TiF_4 treatment completely protected

Table I. Mean etching depths in μm (SD) for various fluoride treatments and etching times for the *in vitro* study ($n=12$ specimens/group). Etching depth is the height differences between the amalgam reference surface and the natural enamel surface.

	Mean etching depth (μm)				
Acid exposure (min)	TiF_4	SnF_2	NaF	Control with pellicle	Control no pellicle
2 min	0.0 (0.4)	1.2 (0.9)	2.1 (0.7)[a]	2.2 (0.6)[ab]	2.4 (0.7)[b]
2+2 min	1.3 (1.2)	4.0 (1.7)[a]	4.7 (1.3)[a]	5.2 (1.0)[ab]	5.5 (1.1)[b]
2+2+2 min	4.7 (1.7)	6.6 (2.5)[a]	7.6 (1.7)[a]	8.1 (1.3)[a]	8.8 (1.5)
2+2+2+2 min	8.4 (2.1)	9.5 (3.2)[a]	10.6 (1.9)[a]	11.0 (1.5)[a]	12.0 (1.5)

[a, b]Values are not significantly different from control with pellicle ($p>0.05$).

the enamel surface. SnF_2 was the second most effective fluoride treatment showing a protective effect after 2 min exposure to acid, but thereafter the effect gradually decreased. Similar trends were observed in ground pellicle-free enamel specimens in the previous *in vitro* study by Hove et al. [17]. The present study aimed to further imitate the clinical situation, not only by the presence of a pellicle but also by using natural tooth surfaces.

The formation of CaF_2 on enamel surfaces after application of fluorides (SnF_2, NaF) has been described in many studies. Furthermore, it has been speculated that a CaF_2-like layer provides some additional mineral that could be dissolved during an acid exposure before the underlying enamel is attacked [9]. This layer may initially reduce the erosive attack on the enamel but the effect will be reduced after repeated and prolonged acid exposures. It has also been shown (by Larsen & Richards [23]) that in the presence of saliva a higher amount of CaF_2 was formed on the enamel surface after fluoride application. However, in the literature, there is no description about formation of a CaF_2 layer after application of TiF_4. The superior protective effect of TiF_4 shown in our previous study [17] may be due to the strong binding of TiF_4 with a subsequent formation of a glaze on the enamel surface, described by Tveit et al. [24]. When TiF_4 is dissolved, hydrolysis of TiF_4 will result in a low pH of the solution. Under these conditions the Ti ion will exhibit a strong tendency to complex with a phosphate group and a complex binding between the metal fluoride and the tooth surface may occur.

TiF_4 has been shown to form a protective surface layer or glaze [12,17] on enamel surfaces and it has been speculated that this glaze is responsible for the protective effect against acid. As indicated in Table I, from 4 to 8 min, the data from all specimen groups showed approximately linear trends. The topographic images revealed that the protective effect of the glaze gave no etching in the regions where the glaze was intact, but a normal etch rate where it was not present. This gave rise to the slow average etch rate for the first 4 min, when most of the surface glaze was intact. However, after subsequent etching an increasing area had lost the glaze and etched at a normal rate. A coating has also been described following SnF_2 treatment of enamel [17], but in both *in vitro* studies the coating was not as resistant as the TiF_4 glaze.

The protective ability of the salivary pellicle is regulated by factors such as composition, thickness, and maturation of the pellicle, but will also be affected by the nature of the erosive challenge such as the acid exposure time [25], type of acid [1,26] and acid concentration [1,2]. The superior protective effect of an *in situ* compared to an *in vitro* pellicle has been shown in a study by Hall [27], in which an *in vitro* formed pellicle significantly prevented mineral surface loss although an *in situ* pellicle protected the enamel surface even better. In a study by Nieuw-Amerongen et al. [28], different fractions of saliva showed different protective properties. However, it was stated that in the oral environment "the participation of mucins in pellicle formation is initially slight as a result of competition with, e.g. acidic proline rich proteins, but their involvement may increase with the age of the pellicle". Therefore, on enamel surfaces repeatedly exposed to acid (e.g. in individuals with frequent regurgitation of gastric acids) the presence of mucin in the pellicle may be minor and the basal pellicle may be the pellicle formed between the attacks. The purpose of the present study was not to use a matured highly protective pellicle before fluoride application. The intention with this *in vitro* study was to find out to what extent the interaction between the fluoride compound and the enamel would be disturbed or changed by the basal layer of pellicle present on the tooth surface. In individuals with frequent regurgitation of gastric acids, the pellicle present before fluoride application is most likely immature due to repeated acid exposure.

In our recent *in vitro* study [21], no protection could be registered from a 2-h *in vitro* formed pellicle against acid attack as measured by calcium loss in an atomic absorption spectroscope. This is in accordance with the results presented in a previous study [2] on 24-h pellicle, whereby erosion was also determined by measurement of calcium loss from enamel. However, in the same study the 24-h pellicle significantly reduced the surface roughness and prevented surface microhardness loss. The measured calcium loss is an indirect measurement for quantifying changes in the surface enamel, and the method is not capable of excluding calcium released from the pellicle. Furthermore, it gives no information about the roughness or the depths of the lesions created. In the present study using WLI, the initial 2-h *in vitro* formed salivary pellicle showed a significant protective effect after 6 and 8 min of acid exposure.

In previous erosion studies, the surface roughness has been measured and related to the erosiveness of an acid or an acidic product [2,18]. The mean surface roughness change measurements (R_q) in the present study cannot be related directly to the actual mineral loss or etch depth. In fact, the initially most resistant surfaces (TiF_4-treated) showed the highest mean R_q after the second acid exposure. The WLI images of these samples showed cracks in the protective surface layer or glaze, with a resulting "terrace effect" of flat parts with deep etched pits between them; these cracks grew in size and depth after repeated acid exposures. Furthermore, after 4 min acid exposure the highest average etch rate was measured for TiF_4 due to breakdown of the glaze.

Quantification of the protective effect of pellicle will depend upon which technique is used and whether tooth surface solubility or tooth surface loss/roughness/hardness is measured. Different measurement techniques will undoubtedly provide different results. Vieira et al. [14] stressed the need for a technique better suited for quantification of dental erosion, as have other authors [29,30]. In our previous *in vitro* study [17], WLI was used to test the protective effect of three different fluoride solutions on acid resistance on ground enamel surfaces. The advantage of this technique is that the specimens will not desiccate and the progression of a lesion can be measured. Using this method, both surface roughness and the progression of substance loss following the acid attack can be analyzed. Furthermore, a WLI provides a topographic image of the surface, a quantified three-dimensional evaluation of the whole surface, i.e. not just linear measurements along a selected axis.

Many erosion measurement techniques require polished enamel surfaces (for example surface microhardness measurements, surface profilometry, and confocal laser scanning microscopy) for acceptable precision and accuracy [31]. In order to simulate intra-oral erosion, the use of native tooth surfaces is desirable. WLI has been successfully applied previously on native enamel surfaces and in the present study the analyses were not disturbed by the pellicle formed on the specimens. These observations are important in validating the reliability of a measuring technique suitable for *in vivo* conditions.

In the oral cavity, constant saliva flow, temperature, different enzymes, and also micro-organisms play a role in pellicle maturation and may therefore affect the protective properties of the pellicle. Therefore, studies with a focus on the protective nature of fluorides *in situ* or *in vivo* should be performed. The results from this study strongly indicate that local fluoride treatment of enamel in the form of SnF_2 and in particular TiF_4 solution may be a promising method for preventing loss of tooth substance associated with acid exposure.

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References

[1] West NX, Hughes JA, Addy M. Erosion of dentine and enamel in vitro by dietary acids: the effect of temperature, acid character, concentration and exposure time. J Oral Rehabil 2000;27:875–80.

[2] Nekrashevych Y, Stösser L. Protective influence of experimentally formed salivary pellicle on enamel erosion. An in vitro study. Caries Res 2003;37:225–31.

[3] Hooper SM, Hughes JA, Newcombe RG, Addy M, West NX. Methodology for testing the erosive potential of sports drinks. J Dent 2005;33:343–8.

[4] Bartlett DW, Coward PY. Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. J Oral Rehabil 2001;28:1045–7.

[5] Moazzez R, Bartlett D, Anggiansah A. Dental erosion, gastro-oesophageal reflux disease and saliva: how are they related? J Dent 2004;32:489–94.

[6] Gregory-Head BL, Curtis DA, Kim L, Cello J. Evaluation of dental erosion in patients with gastroesophageal reflux disease. J Prosthet Dent 2000;83:675–80.

[7] Rytömaa I, Järvinen V, Kanerva R, Heinonen OP. Bulimia and tooth erosion. Acta Odontol Scand 1998;56:36–40.

[8] Sorvari R, Meurman JH, Alakuijala P, Frank RM. Effect of fluoride varnish and solution on enamel erosion in vitro. Caries Res 1994;28:227–32.

[9] 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.

[10] Willumsen T, Øgaard B, Hansen F, Rølla G. Effects from pretreatment of stannous fluoride versus sodium fluoride on enamel exposed to 0.1 M or 0.01 M hydrochloric acid. Acta Odontol Scand 2004;62:278–81.

[11] Ganss C, Klimek J, Brune V, Schurmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561–6.

[12] Buyukyilmaz 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.

[13] van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment in vitro. Eur J Oral Sci 2003; 111:253–7.

[14] Vieira A, Ruben JL, Huysmans MCDN. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371–9.

[15] Attin T, Deifuss H, Hellwig E. Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999;33:135–9.

[16] Wei SH, Soboroff DM, Wefel JS. Effects of titanium tetrafluoride on human enamel. J Dent Res 1976;55:426–31.

[17] Hove L, Holme B, Øgaard B, Willumsen T, Tveit AB. The protective effect of TiF_4, SnF_2 and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res 2006;40:440–3.

[18] Nekrashevych Y, Hannig M, Stösser L. Assessment of enamel erosion and protective effect of salivary pellicle by surface roughness analysis and scanning electron microscopy. Oral Health Prev Dent 2004;2:5–11.

[19] Wetton S, Hughes J, West N, Addy M. Exposure time of enamel and dentine to saliva for protection against erosion: a study in vitro. Caries Res 2006;40:213–7.

[20] Hannig M, Khanafer AK, Hoth-Hannig W, Al Marrawi F, Acil Y. Transmission electron microscopy comparison of methods for collecting in situ formed enamel pellicle. Clin Oral Investig 2005;9:30–7.

[21] Hove LH, Young A, Tveit AB. An in vitro study on the effect of TiF_4 treatment against erosion by hydrochloric acid on pellicle-covered enamel. Caries Res 2007;41:80–4.

[22] Holme B, Hove L, Tveit AB. Using White Light Interferometry to measure etching of dental enamel. Measurement 2005;38:137–47.

[23] Larsen MJ, Richards A. The influence of saliva on the formation of calcium fluoride-like material on human dental enamel. Caries Res 2001;35:57–60.

[24] Tveit AB, Hals E, Isrenn R, Tøtdal B. Highly acid SnF_2 and TiF_4 solutions. Effect on and chemical reaction with root dentin in vitro. Caries Res 1983;17:412–8.

[25] Hara AT, Ando M, Gonzalez-Cabezas C, Cury JA, Serra MC, Zero DT. Protective effect of the dental pellicle against erosive challenges in situ. J Dent Res 2006;85:612–6.
[26] Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. Arch Oral Biol 2005;50: 541–52.
[27] Hall AF, Buchanan CA, Millett DT, Creanor SL, Strang R, Foye RH. The effect of saliva on enamel and dentine erosion. J Dent 1999;27:333–9.
[28] Nieuw Amerongen AV, Oderkerk CH, Driessen AA. Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. Caries Res 1987; 21:297–309.
[29] Lussi A, Hellwig E. Risk assessment and preventive measures. Monogr Oral Sci 2006;20:190–9.
[30] Hughes JA, West NX, Addy M. The protective effect of fluoride treatments against enamel erosion in vitro. J Oral Rehabil 2004;31:357–63.
[31] Attin T. Methods for assessment of dental erosion. Monogr Oral Sci 2006;20:152–72.