Original Paper

Caries Research

Caries Res 2008;42:68–72
DOI: 10.1159/000112816

Received: May 3, 2007
Accepted after revision: October 25, 2007
Published online: December 21, 2007

The Protective Effect of TiF_4, SnF_2 and NaF against Erosion-Like Lesions in situ

L.H. Hove[a] B. Holme[b, c] A. Young[a] A.B. Tveit[a]

[a]Faculty of Dentistry and [b]Department of Physics, University of Oslo, and [c]SINTEF Materials and Chemistry, Oslo, Norway

Key Words
Dental erosion, in situ · Fluoride · Hydrochloric acid · White light interferometry

Abstract
The aim of this in situ study was to compare the protective effect of TiF_4, SnF_2 and NaF on the development of erosion-like lesions in human enamel. Fourteen human molars were each divided into 4 specimens, mounted on acrylic mouth appliances and worn by 7 volunteers for 9 days. In order to mimic a feasible treatment procedure for patients with voluntary or involuntary gastric reflux, the specimens were etched for 2 min twice a day (0.01 M HCl) and fluoride applications were performed every third day (2 min). The controls were treated as the other specimens except for the fluoride applications. Etch depths and surface roughness changes (R_q) were measured by white light interferometry. Compared with the control, TiF_4 and SnF_2 reduced the etching depth by 100% ($p < 0.001$) and 91% ($p < 0.001$), respectively, and both treatments resulted in an observable surface layer. NaF had no significant protective effect ($p = 0.46$). It can be concluded that although SnF_2 provided significant protection for the enamel surfaces, TiF_4 showed the best protection against acid attack, while NaF had no significant protective effect in this study.
Copyright © 2007 S. Karger AG, Basel

The erosion-inhibiting effects of different fluoride solutions and varnishes have been investigated in many in vitro studies. The protective effect of high concentrations of NaF against dental erosion has been demonstrated by several authors [Sorvari et al., 1994; Ganss et al., 2001; van Rijkom et al., 2003]. High concentrations of fluoride are believed to promote the formation of CaF_2 on the enamel surface which may result in a tooth mineral surface less prone to dissolution [Ganss et al., 2001]. SnF_2 has also shown a promising erosion-inhibiting effect both in vitro [Ganss et al., 2001; Willumsen et al., 2004] and in situ when combined with NaF [Ganss et al., 2004]. In a recent study by Young et al. [2006] toothpaste with SnF_2 reduced the dissolution of enamel in an in vivo model. In vitro studies have also demonstrated a considerable erosion protection ability of TiF_4 on enamel [Büyükyilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005; Hove et al., 2006; Hove et al., 2007a, b], and an acidified fluoride gel (NaF and amine fluoride, pH 4.5) improved surface hardness against abrasion in an erosion study [Attin et al., 1999]. Scanning electron microscope studies have demonstrated a coating or glaze when tooth enamel surfaces were treated with TiF_4 [Wei et al., 1976; Büyükyilmaz et al., 1997]. This coating could explain the superior effect of TiF_4 compared to NaF in inhibiting the erosive effect of HCl on enamel, found in a study by Hove et al. [2006]. Schlueter et al. [2007] also found that the over-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2007 S. Karger AG, Basel
0008–6568/08/0421–0068$24.50/0

Accessible online at:
www.karger.com/cre

Lene Hystad Hove
Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo
Box 1109 Blindern
NO–0317 Oslo (Norway)
Tel. +47 228 521 42, Fax +47 228 523 44, E-Mail lenehh@odont.uio.no

all effect of TiF_4 exceeded that of NaF in an in vitro study where the solutions had a pH of 1.2.

Saliva plays an important role in the erosive process by diluting and buffering the effect of acids, promoting tooth surface remineralization, and in the formation of a salivary pellicle. It has been demonstrated that the pellicle protects the underlying enamel against erosive challenge both in vitro [Nekrashevych and Stösser, 2003; Nekrashevych et al., 2004; Wetton et al., 2006] and in vivo/in situ [Hannig et al., 2004; Hara et al., 2006]. However, the extent to which the pellicle influences the effect of topical application of different fluoride solutions is not fully known. In an in vitro study by Hove et al. [2007b] a 2-hour pellicle formed on natural enamel surfaces did not negatively influence the inhibiting effect of TiF_4 on calcium loss following acid exposure. In another in vitro study, also with a 2-hour pellicle present on the enamel surface, TiF_4 reduced enamel loss by 100% after 2 min of acid exposure while the corresponding value for SnF_2 was 45% [Hove et al., 2007a]. Despite the fact that in vitro studies can only attempt to imitate the clinical situation, these results provide valuable information about the influence of an initial pellicle on the efficacy of fluoride.

Given that the composition of saliva (including enzymes and micro-organisms), the saliva flow rate and the temperature will influence pellicle maturation, erosion studies involving fluoride treatment of enamel especially in the form of SnF_2 and TiF_4 should preferably be performed in vivo. However, in order to simulate the biological condition in the mouth using currently available measuring techniques, in situ studies are still more feasible. Investigations with a focus on the efficacy and durability of these fluoride treatments in the oral environment are required. This in situ study was performed in order to measure the effect of TiF_4, SnF_2 and NaF treatment on the development of erosion-like lesions measured by a white light interferometer.

Materials and Methods

Subjects

The study was approved by the Regional Committee for Medical Research Ethics, Norway. Seven healthy volunteers working at the University of Oslo (1 male, 6 females, age 25–55 years) participated after having given informed and written consent. The participants received written instructions and schedules. Inclusion criteria were no sign of hyposalivation or xerostomia and good oral health. Exclusion criteria were general/systemic illness, pregnancy and active caries or erosion lesions. The participants wore the mouth appliances at all times for 9 days, except while eating, during oral hygiene procedures and treatment procedures. After meals the participants rinsed their mouths with tap water before reinsertion. During these periods the mouth appliances were stored in 100% humidity. The participants used non-fluoridated toothpaste 3 days prior to and during the entire experimental period.

Preparation of Enamel Specimens

Fourteen extracted human permanent molars were polished with pumice in water, wiped free of debris with ethanol (20%) and rinsed in tap water. The teeth were stored in a sealed container with a thymol crystal until use. Four circular amalgam fillings (approx. 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 sectioned with a high-speed turbine into 4 enamel specimens each including 1 amalgam filling, and the specimens from each tooth were randomly assigned to 1 of 4 groups (56 specimens). 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 Mouth Appliances

Seven mandibular acrylic mouth appliances were fabricated and all 4 specimens from 1 tooth were mounted buccally on each side of the mouth appliance. The appliances, each with 2 sets of teeth (8 specimens), were retained by clasps in the molar region. The appliances were stabilized during white light interferometer analysis by a rubber replica compound that ensured that no rotation or tilt of the samples occurred between the images made before and after the erosive challenges.

Fluoride Treatment and Acid Exposure

The appliances with specimens were inserted and worn for 2 h prior to the first application of fluoride treatment in order to form a basal pellicle, generally present on enamel surfaces in vivo.

Prior to each fluoride treatment, the specimens were rinsed in tap water for approximately 30 s and dried with a gentle blast of air. From each tooth, 1 specimen served as a control and the remaining 3 were treated separately with 1 of the 3 fluoride solutions: 3.9% SnF_2, 1.5% TiF_4 and 2.1% NaF (all 0.5 M F) for 2 min, every third day, for a total of 3 times during the experimental period. Fluoride application was performed with a pipette dropper, and the solution was applied as droplets with a constant flow (1 per second). The specimens on the same side were separated with a Permadyne® impression material barrier in order to avoid contamination. The specimens were then rinsed in tap water for approximately 30 s and returned to the test subjects. The enamel surfaces were exposed to acid twice a day for 2 min to simulate two gastric reflux episodes by immersing the appliances in 300 ml 0.01 M HCl. The demineralization solution was renewed after each exposure and all the treatments and analyses were performed extra-orally.

White Light Interferometry Analysis

All specimens were analysed by white light interferometry (WLI; WYKO NT-2000, Veeco Instruments Inc., New York, N.Y., USA) both before and after the 9-day study period. Prior to the second analysis, the appliances were washed with NaClO (1%) in order to remove traces of organic debris. The person doing the WLI analysis was not involved in the running of the experimental study or the fluoride treatment procedure.

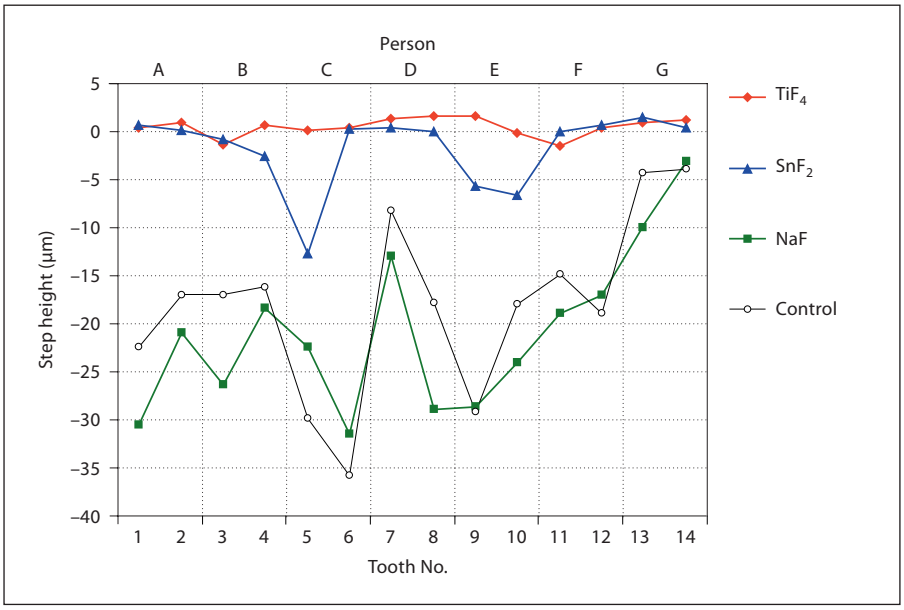

Fig. 1. Etching depths (height differences between the amalgam reference surface and the natural enamel surface) for the various fluoride treatments. Specimens of teeth No. 1 and 2 were mounted on 1 appliance (left and right side of the mouth) and worn by person A.

A white light interferometer is a computerized optical microscope that uses interference to produce a topographic image of the surface. Digital WLI images are typically shown as a topographic map where various colours denote different heights for the pixels, as recorded by the WLI software (Vision 32). By subtracting the original image from the image obtained after the experiment, 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). Using WLI it was also possible to calculate the mean roughness change in the difference images (R_q) as a measure of the increase in surface roughness due to etching of the enamel. The roughness value also has a contribution from the computerized fitting of the images before subtraction. However, the corresponding roughness values from the amalgam regions suggested that the precision of fitting was reasonably constant, so that the differences observed in the R_q values stem from the increasing roughness of the enamel. The difference image WLI technique used for the analysis of erosion-like lesions on dry, polished surfaces has previously been described in more detail by Holme et al. [2005]. There, the precision of repeated step measurements was found to be 10 nm. The accuracy of the measured height differences between amalgam and enamel was estimated to be 20 nm if all possible sources of systematic errors were properly accounted for. An estimate of the accuracy in the present study can be found by determining how close to zero the measured 'step heights' were at zero etching time. The mean and standard deviation were –1 nm and 45 nm, respectively, based on 46 difference images where the image subtraction was properly done. This means that on a pellicle-covered, naturally curved human enamel surface we have a 'noise level' for the step height measurement of about 50 nm. The limit of detection is defined as 3 times the zero-signal noise level. Thus, for pellicle-covered, natural enamel, the WLI technique should be able to detect etching depths as small as 150 nm.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 13.0; SPSS Inc., Chicago, Ill., USA) for Windows. A multi-level paired t test was used to assess the significance of differences between the specimen groups. Multi-level analysis was performed using the Number Cruncher Statistical System (version 4.1), with participant as level 1 and specimens within participants as level 2. The level of significance was set at 0.05. Positive 'etching depths' were adjusted to zero.

Results

All volunteers completed the study and a total of 56 samples were analysed. The etch depths for each enamel specimen and the mean etch depths for the different treatments after the 9-day experimental period are shown in figure 1. The mean etch depth in the control group was 18.1 ± 9.2 µm and 21.0 ± 8.3 µm in the NaF group. All TiF_4- and SnF_2-treated samples showed positive 'etching depths' (an additional protective surface layer) on the difference images but in some regions this layer had fractured locally. This allowed etching of the enamel at a rate comparable to the control sample. Local fracturing of the glaze was even more common on the SnF_2-treated samples, giving a larger overall etch depth with a larger spread in the values. The mean etch depth in the SnF_2 group was 1.7 ± 4.0 µm, and a positive mean etch height of 0.5 ± 0.9 µm was registered for TiF_4. The mean etch depth in the control group was significantly greater than for the TiF_4- ($p < 0.001$) and SnF_2-treated specimens ($p < 0.001$),

but not significantly different from the NaF group ($p = 0.46$). There was no significant difference between the TiF_4- and SnF_2-treated specimens ($p = 0.62$).

The mean R_q for the control was 3.3 ± 2.0 μm. The TiF_4-treated surfaces showed the lowest mean R_q level of all specimens at 0.8 ± 0.7 μm, while the SnF_2- and NaF-treated specimens recorded mean R_q values of 1.8 ± 1.5 and 4.3 ± 2.6 μm, respectively. The lower values for the SnF_2 and TiF_4 samples follow the overall lower etching depths after these treatments, and no severe break-up of the surface layer (coating or glaze) took place during this in situ experiment. For the NaF and control sample it was not possible to correlate roughness with etch depth, as for example an R_q near 2.5 μm was found for etch depths between 4 and 36 μm. There were significant differences in the R_q between the control and TiF_4-treated specimens ($p < 0.001$) and SnF_2-treated specimens ($p = 0.01$). No significant differences were found between the control and NaF-treated specimens ($p = 0.56$) and between the TiF_4- and SnF_2-treated specimens ($p = 0.28$).

Discussion

In the present study the TiF_4 solution was more effective than the NaF solution in protecting the enamel surface against erosion-like lesions. Similar results were observed in ground enamel specimens in a previous in vitro study [Hove et al., 2006] and also in an in vitro study with native enamel specimens covered with a 2-hour pellicle [Hove et al., 2007a]. Interestingly, in the present in situ study both SnF_2 and TiF_4 provided an almost complete protective effect, a better result than seen in the 2 in vitro experiments. In contrast, the etch depths in the control and NaF-treated samples were greater in situ than in vitro.

In the present study both the fluoride treatments and the acid challenges were repeated several times in order to imitate the clinical situation. The results indicate that the protective effect of SnF_2 and TiF_4 was enhanced and not reduced by the physiological/biological conditions in the mouth. Furthermore, the repeated application of SnF_2 and TiF_4 resulted in an irregular but measurable increase in surface height of the enamel relative to the amalgam. The observed positive step height may be related to: (1) etching of the amalgam reference surfaces, or (2) an actual build-up of material on the enamel surface. Since previous studies [Holme et al., 2005] have shown that the amalgam is virtually inert under the given etching conditions, the measured step height is most likely caused by the repeated treatments of the samples with SnF_2 or TiF_4 solutions, which allowed the formation of a coating or a glaze on top of the enamel. It was not within the scope of this study to examine this glaze further with microscopic techniques, but this will be undertaken in future studies. During the statistical analysis of the data, these positive 'etching depths' were adjusted to zero, since this surface layer is clearly not enamel.

In previous studies TiF_4 has also been shown to form a protective surface layer or glaze [Wei et al., 1976; Büyükyilmaz et al., 1997; Hove et al., 2006] on both pellicle-free and pellicle-covered enamel surfaces, and it has been speculated that this glaze is responsible for the protective effect against acid. A coating has also been described following SnF_2 treatment of enamel [Hove et al., 2006], but in that in vitro study the coating was not as resistant as the TiF_4 glaze.

The present study demonstrates that the natural pellicle formed in situ did not have any negative influence on the protective effect of these fluoride solutions. Under clinical conditions a basal pellicle layer will be present on enamel prior to fluoride application. The salivary pellicle per se has been shown to protect enamel against dissolution caused by acids [Hannig et al., 2004; Hara et al., 2006], but there is sparse information about the retention of fluorides under acidic conditions and the reaction mechanism including chemical binding of TiF_4 and SnF_2 to the enamel surface.

In the present study there were major differences in the etch depths among the control samples, and the severity of the mineral loss seemed to depend largely on the oral environment. The results in subjects C and G clearly show that there are large individual variations in the ability to reduce dissolution of enamel after acid exposure. Since the acidic challenges were performed extra-orally and the appliances were rinsed after exposures, the major differences in protective effect could be related to oral biological factors.

Different measurement techniques will undoubtedly provide different results. Many authors have stressed the need for better techniques for quantification of dental erosion [Hughes et al., 2004; Vieira et al., 2005; Lussi and Hellwig, 2006]. It has been demonstrated in this and previous studies that WLI can be used to measure erosion-like lesions on pellicle-covered [Hove et al., 2007a], ground [Hove et al., 2006] and native enamel surfaces that are mounted on mouth appliances and worn by subjects for several days between the initial and final topographic imaging.

The mean R_q in the present study could not be related directly to the actual mineral loss or etch depth. In the in

vitro study by Hove et al. [2007a], the initially most resistant surfaces (TiF_4-treated) showed the highest mean R_q after the second acid exposure. The white light interferometer images showed cracks in the protective surface layer or glaze and these cracks grew in size and depth after repeated acid exposures. Furthermore, the highest average etch rate was measured for TiF_4 when the total area of unprotected enamel grew quickly, due to a breakdown of the glaze. In contrast, the TiF_4 and SnF_2 groups showed low R_q levels in the present in situ study. The reason for this could be that these surfaces were without cracks and more or less unaffected by the acid challenge.

Schlueter et al. [2007] found that a 2.2% w/v NaF solution with a pH of 1.2 markedly reduced enamel mineral loss compared to the control. The low pH of the NaF may be the explanation for this significant effect. In the study by Hove et al. [2007a] and in the present study, a 2% NaF solution with a pH of 8 gave no significant protection of enamel. In future studies, it would be interesting to perform investigations with acidified fluoride solutions in situ and compare them with the excellent effect of TiF_4 and SnF_2 found in the present study.

The experimental setting with 2 HCl exposures per day was selected to mimic a voluntary or involuntary gastric reflux situation, and, in order to imitate a possible clinical approach, fluoride application was performed every third day. Comparing previous studies with the present study it would appear that TiF_4 becomes more durable after repeated applications and prolonged experimental periods. Therefore, in patients at risk of dental erosion, it is crucial to repeat the fluoride applications in order to minimize the progression of the lesions.

The results of this study strongly indicate that topical fluoride treatment of enamel, in the form of either highly concentrated SnF_2 or in particular TiF_4 solution, is a promising method for preventing loss of tooth substance associated with intra-oral acid exposure. However, more in situ and in vivo investigations with a focus on the long-term effect of different fluoride preparations are needed.

Acknowledgement

The authors would like to thank Dr. Leiv Sandvik for his assistance in the statistical analysis of the data.

References

Attin T, Deifuss H, Hellwig E: Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999;33:135–139.

Büyükyilmaz T, Øgaard B, Rølla G: The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci 1997;105:473–477.

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;38:561–566.

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;35:325–330.

Hannig M, Fiebiger M, Guntzer M, Dobert A, Zimehl R, Nekrashevych Y: Protective effect of the in situ formed short-term salivary pellicle. Arch Oral Biol 2004;49:903–910.

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;85:612–616.

Holme B, Hove L, Tveit AB: Using white light interferometry to measure etching of dental enamel. Measurement 2005;38:137–147.

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;40:440–443.

Hove LH, Holme B, Young A, Tveit AB: The erosion-inhibiting effect of TiF_4, SnF_2 and NaF solutions on pellicle-covered enamel in vitro. Acta Odontol Scand 2007a;65:259–264.

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 2007b;41:80–84.

Hughes JA, West NX, Addy M: The protective effect of fluoride treatments against enamel erosion in vitro. J Oral Rehabil 2004;31:357–363.

Lussi A, Hellwig E: Risk assessment and preventive measures. Monogr Oral Sci 2006;20:190–199.

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.

Nekrashevych Y, Stösser L: Protective influence of experimentally formed salivary pellicle on enamel erosion. An in vitro study. Caries Res 2003;37:225–231.

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;41:141–145.

Sorvari R, Meurman JH, Alakuijala P, Frank RM: Effect of fluoride varnish and solution on enamel erosion in vitro. Caries Res 1994;28:227–232.

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;111:253–257.

Vieira A, Ruben JL, Huysmans MC: Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371–379.

Wei SH, Soboroff DM, Wefel JS: Effects of titanium tetrafluoride on human enamel. J Dent Res 1976;55:426–431.

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;40:213–217.

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;62:278–281.

Young A, Thrane PS, Saxegaard E, Jonski G, Rølla G: Effect of stannous fluoride toothpaste on erosion-like lesions: an in vivo study. Eur J Oral Sci 2006;114:180–183.