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ORIGINAL ARTICLE

May caries-preventive fluoride regimes have an effect on dental erosive wear? An *in situ* study

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Abstract

Objective. High and low concentration NaF regimes have shown caries protective properties, but the preventive effect against erosive/abrasive wear is unclear. ***Aim.*** To measure the inhibiting effect on enamel wear of low and highly concentrated sodium fluoride (NaF) toothpastes and a stannous (SnF_2) fluoride gel in a single-blind, randomized *in situ* study, using a White Light Interferometer. ***Materials and methods.*** Sixteen human molars were each divided into four specimens, mounted on acrylic mouth appliances and worn by eight volunteers for 9 days. Experimental procedures were performed in the laboratory. The enamel specimens were brushed every day with fluoride-free toothpaste. Treatments; group 1: no fluoride treatment (control), group 2: SnF_2 gel 2500 ppm F (5 min) every third day, group 3: NaF toothpaste 5000 ppm F 5 min every third day and 2 min the other days, group 4: NaF toothpaste 1450 ppm F (2 min) every day. In order to mimic gastric reflux/vomiting, the specimens were etched with 0.01 M HCl for 2 min twice a day. ***Results.*** The mean step height (μm) for the control specimens was −32.9 (SD = 6.8). The mean values for the other groups were −22.2 (SD = 8.4) (group 2), −30.8 (SD = 7.8) (group 3) and −31.4 (SD = 7.7) (group 4). Compared with the control, the SnF_2 treated specimens showed significantly lower wear. The NaF toothpastes gave no significant protective effect. ***Conclusions.*** Application of SnF_2 gel every third day gave protection against erosive–abrasive challenges. Daily application of both low concentration and high concentration NaF toothpaste provided no protection.

Key Words: *dental erosion, in situ, NaF toothpastes, SnF_2 gel*

Introduction

Daily use of fluoride toothpastes is considered a main contributor to the caries decline over the last decades [1] and the caries-preventive effect increases with higher fluoride concentration and frequency of application [2]. This is illustrated by a significantly lower caries progression [3] and improved effect in caries reduction found for a 5000 ppm F toothpaste compared to toothpaste with lower concentrations [4–6]. Therefore, the current clinical practice for patients with increased caries risk often includes a highly concentrated fluoride toothpaste or an intensive fluoride regime with topical fluoride vehicles such as gel, mouth rinse or varnish [2]. In a clinical trial by Gisselsson et al. [7], 1% NaF gel and 1% SnF_2 gel applied 4-times a year resulted in 30% and 39% caries reduction compared to a placebo gel, respectively.

Dental erosion is a condition of growing concern and it has been reported that this type of wear is more frequent in adolescents with caries experience [8–10], possibly due to a cariogenic and erosive diet [8]. It has also been shown that fluoride treatments may inhibit erosive tooth wear both *in vitro* and *in situ* [11]. Local applications with high concentration fluoride solutions have also been shown to increase abrasion resistance and to decrease the development of erosion in enamel [12,13] and to increase remineralization [14]. In addition, it has been shown that, under both erosive and abrasive challenges, two highly concentrated solutions (5000 ppm NaF and 19000 ppm NaF) afforded a significant protection to enamel from attrition and erosion compared to a low (1450 ppm NaF) concentration solution [15]. However, contradictory results have been published regarding the effect of traditional fluoride toothpastes (~1500 ppm F) [16–21] and high

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concentration toothpaste (5000 ppm F) on dental erosions [22–24]. In addition, local applications of highly concentrated stannous fluoride solutions (up to 9500 ppm F and 30000 ppm Sn^{2+}) have shown significant erosion-inhibiting properties [25–30]. Even though 1% SnF_2 gel and high concentration NaF toothpaste have shown good effect on caries, it is not clear whether they are effective against erosive wear. The pathogenesis and mode of action of fluoride is different in the two conditions, but it would be of high importance if caries preventive fluoride regimes could add an inhibiting effect on erosive–abrasive wear.

Therefore, the aim of the present *in situ* study was to investigate the erosive–abrasive inhibiting effect of three different fluoride regimes with known caries-preventive properties. The hypothesis was that fluoride toothpastes with 'low' and 'high' fluoride concentrations and SnF_2 gel may or may not inhibit enamel erosive/abrasive wear.

Materials and methods

This study was a prospective, paired, randomized and single-blind *in situ* study performed according to the Declaration of Helsinki and the Guidelines of Good Clinical Practice and was approved by the Regional Committee for Medical Research Ethics, Norway. Three dentists employed at the University of Oslo (UiO) performed all technical and clinical procedures. The White Light Interferometer (WLI) measurements and analyses were performed by a certified physicist at SINTEF, Oslo not involved in the experimental procedures.

Volunteers/participants

The study included eight healthy volunteers (22–28 years). Six females and two males 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 dental erosions. The participants wore the mouth appliances at all times for 9 days, except while eating, during oral hygiene and experimental procedures. During these periods the mouth appliances were stored in a plastic container (100% humidity). After meals, the participants rinsed their mouths with tap water before re-inserting the appliances. The participants used non-fluoridated toothpaste 3 days prior to and during the entire experimental period.

Preparation of enamel specimens

Sixteen extracted human permanent molars from 16 different anonymous patients were polished with pumice in water, wiped free of debris with ethanol (20%) and rinsed in tap water. The teeth were stored under humid conditions in individual sealed containers with Thymol crystals until use. Four circular amalgam fillings (~1 mm in diameter) were made in each tooth ~2 mm above the enamel–cement junction. The amalgam was used as a reference surface during the analysis. Every tooth was then sectioned with a high-speed turbine into four enamel specimens, each including one amalgam filling, and the specimens from each tooth were randomly assigned to one of four groups ($4 \times 16 = 64$ specimens). All enamel surfaces and restorations were carefully examined and only specimens with intact surfaces were used. The specimens were stored for at least 2 weeks in a Thymol solution [31–33] prior to the experiment. On the first day of the experiment the specimens were brushed with a detergent soap (Zalo, Lilleborg, Norway) to remove remnants of organic debris and immersed in 70% Ethanol for 30 min. The specimens were stored in a 100% humid environment.

Preparation of mouth appliances

Eight mandibular acrylic (Triad® Visible Light Curing System, Dentsply, International) mouth appliances were fabricated and all four specimens from one tooth were mounted buccally on the right side of the mouth appliance and another tooth (four specimens) was mounted on the left side by Z 250/Filtek™ Flow, 3M ESPE™ and the resin was wiped off with a cotton pellet soaked in 20% Ethanol. The appliances, each with two sets of teeth (eight specimens), were retained by clasps in the molar region. During WLI analyses the appliances were stabilized by a two-component silicone polymer compound (Microset® 101TH, Microset products Ltd. Warwickshire, UK) to ensure that no rotation or tilt of the samples occurred between the images made before and after the experimental period.

Experimental design

Each of the four specimens on each side was individually treated with one of the different test compounds. The experimental unit was the tooth and the sample size was 16 teeth (16 in four groups = 64 specimens).

The experimental factors were three treatments and one control. The fluoride treatments and acid exposures were performed in the laboratory and the volunteers were blinded. The study outcome was the mean enamel surface loss in μm, relative to the amalgam reference surfaces, for each specimen and for each treatment (16 specimens).

Fluoride treatment, abrasion and acid exposure

During the 9 days experimental period, the specimens were brushed manually once a day (one stroke per second for 30 s) with fluoride-free toothpaste (Solidox, Lilleborg), rinsed in tap water for ~30 s and dried with a gentle blast of air. Before applying the fluoride compound, the specimens on the same side were separated with a Permadyne® impression material barrier in order to avoid cross contamination. The enamel surfaces were then moistened with water and covered with a 1 mm thick layer of toothpaste or gel. The compounds were applied according to protocol with different fluoride regimes and tooth brushing once a day, simulating a clinically applicable procedure.

From each tooth one specimen served as a negative control and the remaining three specimens were randomly assigned to the other groups:

- Group 1; Fluoride-free toothpaste (Solidox® without fluoride, Lilleborg);
- Group 2; 1% SnF_2 gel, 2500 ppm F (Stannous fluoride 1.0 g, Hypromellose 4000 2.5 g, Propylene glycol 20.0 g, sterilized water to 100.0 g) for 5 min (on day 1, 4 and 7);
- Group 3; Colgate Duraphat® (5000 ppm Fluoride Toothpaste, Sodium Fluoride 1.1% w/w) 2 min daily and additionally 3 min every third day (on day 1, 4 and 7, 5 min); and
- Group 4; Colgate Karies Kontroll® (1450 ppm F, Sodium Fluoride 0.320% w/w), 2 min daily.

The excess compound was removed from the surface with compressed water/air flow and then the surfaces were rinsed in tap water for ~5 s, dried with a gentle blast of air and then the appliances were returned to the volunteers. The abrasion (tooth brushing) and application of fluorides was performed 2 h prior to the acid exposure. The appliances were immersed twice a day for 2 min (midday and afternoon) in freshly made 300 ml 0.01 M HCl to simulate two gastric reflux/vomiting episodes, rinsed in tap water, air dried and returned to the volunteers/participants. Prior to the analysis, remaining debris on the enamel surface was carefully removed by tap water. All experimental procedures were performed in the laboratory and the volunteers were blinded.

Toothpaste abrasivity R_a (μm) (arithmetic mean deviation of the profile) was tested at the Nordic Institute of Dental Materials (NIOM) according to ISO/TR14569-1-2007 including 5000 brushing cycles on a poly methyl methacrylate (PMMA) material and subsequent roughness measurements with a Mitutoyo profilometer, Surftest SJ-201 J. Abrasivity wear on the PMMA surfaces was tested by White Light Interferometry (WYKO® NT9800, Veeco, NY, USA) at SINTEF, Oslo, Norway. In the test, the mean wear value was 7.13 μm and the R_a was 1.54 μm for the Fluoride free toothpaste.

Analytical techniques

White light interferometry analysis. Topographic images were made from all specimens by White Light Interferometry (WLI, WYKO® NT9800, Veeco) both before (baseline) and after the 9-days study period. This analytical technique has been tested [34] and used in previous studies [25,35]. By subtracting the baseline image from the image obtained after the experiment, a 'difference image' (Figure 1) is created which shows a step when enamel has been lost during the erosive/abrasive wear challenges. The mean step heights between the amalgam reference surface and the enamel region was calculated, thus quantifying the material loss with high accuracy. The reproducibility of the measurements on natural, curved enamel surfaces (standard deviation for repeated imaging on the same specimen) was 0.2 μm on average. The accuracy of the WLI instrument was verified by repeated

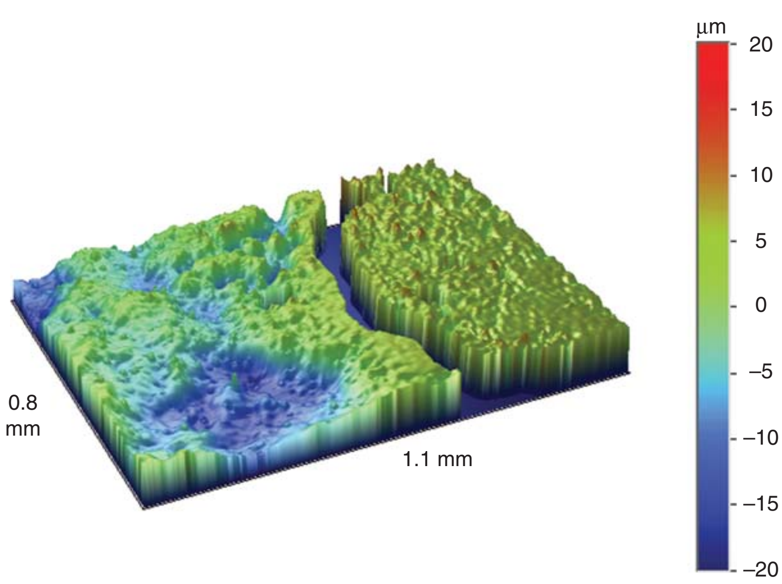

Figure 1. Image (3D) of a specimen treated SnF_2 and after acid exposures. Enamel (left) with unetched areas can be seen.

measurements on a calibration standard. The surface loss outcome for each specimen was based on the average step across the analysed area (0.9 mm^2). The mean step height for the 16 specimens was calculated.

Statistics

Statistical procedures were performed with the Statistical Package for Social Sciences (SPSS 16.0; SPSS Inc., Chicago, IL) for Windows and 16 specimens were used for each regimen. Paired *t*-tests were used to assess the differences between the various treatments and the control. For multiple comparisons, *p*-values were adjusted using the Bonferroni adjustment method for multiple comparisons. Differences with *p*-values below 0.009 were considered significant (0.05/6 = 0.009) at 5% significance level.

The sample size of 16 teeth was chosen based on results and standard deviations (SDs) of differences in surface loss between pairs of regimens found in a previous *in situ* study [35] with the same study design. Calculations were performed based on considerations of clinically relevant etch depth differences (control, SnF_2) being above 5 µm, with a significance level of 0.05 and 80% power.

Results

All participants completed the study according to the protocol, no side-effects were reported, no specimens failed and 64 specimens were analysed for enamel loss. Figure 2 shows the average enamel height change relative to the amalgam for each tooth and treatment after 9 days experimental period. Each participant wore specimens from two teeth which were exposed to the same oral environment. Mean enamel step depths (SD) in µm for the control specimens with no fluoride treatment (group 1) was 32.9 (6.8) and range = 19.6–41.7 µm. The mean values for the other groups were; group 2 (SnF_2 gel) = 22.2 (8.4) and range = 5.5–36.7; group 3 (Colgate Duraphat®) = 30.8 (7.8) and group 4 (Colgate Karies Kontroll®) = 31.4 (7.7). Compared with the control, application of SnF_2 gel reduced enamel wear by 27%. This was significantly lower enamel wear compared with the control group and also compared with the other groups. None of the NaF toothpastes (5000 ppm F and 1450 ppm F) gave significant reduction in enamel wear compared with the control, $p = 0.2$ and $p = 0.4$, respectively.

Discussion

The main findings in the present study were that caries preventive regimes using NaF toothpaste with high concentration and a toothpaste with lower concentration provided no protective effect against erosive and abrasive wear, while a SnF_2 gel offered significant protection of the surface.

An explanation for this finding may be that the mechanisms of fluoride action could be different for the two conditions, caries and erosive wear. The caries preventive effect of fluoride is dependent on retention and accumulation of CaF_2 precipitates in lesions and on surfaces where the process is most prominent. The long-lasting cariostatic efficacy of highly concentrated fluoride products may be due to the formation of structurally bound fluoride where CaF_2 acts as an efficient source of free fluoride ions that may subsequently be incorporated into the enamel as hydroxyfluorapatite or fluorapatite.

In pathological dental erosion, tooth surfaces will be subjected to a combination of erosive and abrasive wear. Unlike the caries process; occlusal, labial and palatinal surfaces are predominantly affected. On these sites, long-term retention of the globular precipitates is less likely and may rather be dissolved and eliminated from the surface during prolonged and repeated acid attacks leading to a deficient erosion inhibiting effect [36]. Most conventional toothpastes

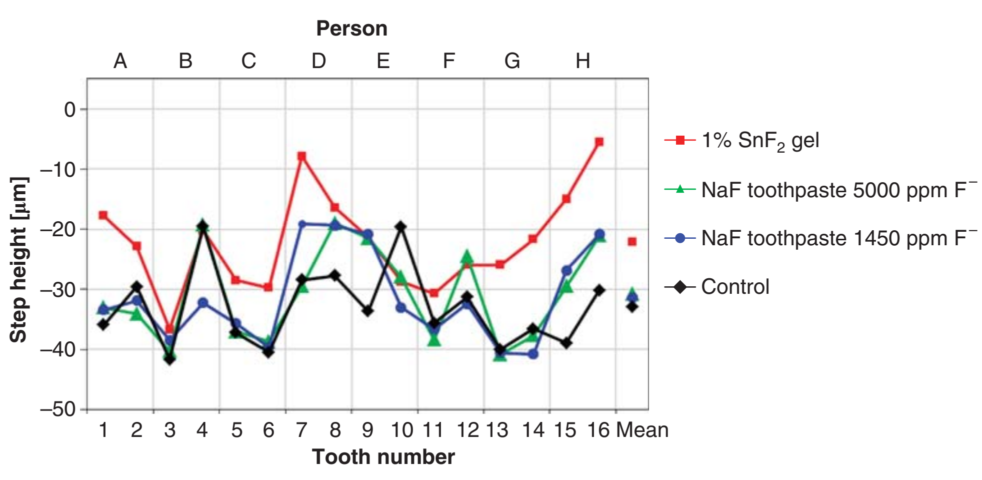

Figure 2. Average enamel depth change for each tooth and treatment after 9 days experimental period. Each participant (A–H) wore two sets of teeth.

contain fluoride in concentrations ~1000–1450 ppm and conflicting results regarding their ability to inhibit erosive wear have been published. In an *in vitro* study by Bartlett et al. [16], less wear was produced in the presence of a fluoride toothpaste than in the presence of a non-fluoride toothpaste and, in an *in situ/ex vivo* study by Magalhães et al. [17], a fluoride dentifrice (1098 ppm F) had a protective effect on eroded enamel. However, results from other studies are in accordance with the present study and show no protective effect [19,20]. This suggests that conventional toothpastes alone may not be capable of inhibiting acid-induced tooth wear. Experimental conditions such as different application and brushing procedures, different enamel, *in vitro/in situ* conditions, as well as different pH of the erosive solution may influence the results.

It has been proposed that the effect of the active ingredients may be counteracted by the abrasive component in the toothpaste [37,38]. In several erosion studies investigating the effect of fluoride toothpastes, enamel specimens have either been immersed in a toothpaste-slurry or the toothpaste has been applied to the enamel surfaces without abrasion. This is not a clinically relevant study design since erosion-softened enamel has been shown to be susceptible to mechanical impacts such as tooth brushing [39,40] and, in an *in vitro* study by Ganss et al. [21], conventional NaF toothpastes were able to reduce erosive tissue loss, but had limited efficacy regarding the prevention of both erosive and abrasive wear. In the present study all specimens were brushed with the same fluoride-free toothpaste prior to application of the product in order to investigate the protective effect of the ingredients and not the abrasivity of the product.

Regarding toothpastes, a clinically-proven dose-dependent relationship between the level of fluoride and the degree of clinical anti-caries efficacy has been shown [6]. It may be anticipated that, by increasing the concentration, a potential protective effect of the enamel against erosive/abrasive wear may be enhanced. A fluoride toothpaste (5000 ppm F as NaF) has been launched for caries control and it is uncertain if this should be recommended for patients at risk for pathological progression of dental erosion as well. A few studies support the use of high concentration fluoride toothpaste also for these patients; a protective effect was found in an *in vitro* study by Moretto et al. [23] using bovine enamel. Ren et al. [22] showed that high fluoride toothpaste provided a significantly improved resistance to enamel erosive wear *in situ* compared to a control (1450 ppm fluoride), but the enamel was not subjected to abrasive wear. However, no protective effect was reported by Rios et al. [24], studying the possible inhibiting effect of a highly concentrated F dentifrice (5000 ppm F) on enamel erosive and erosive/abrasive wear *in situ*, *ex vivo*. These results are in accordance with findings in the present study.

The present SnF_2 gel provided a significant protection of the surfaces against erosive and abrasive wear which is consistent with results from previous studies using SnF_2 solutions. Both high concentration and low concentration solutions with SnF_2 have shown significantly better protective effects against erosive wear compared to NaF solutions *in vitro* [26,27,30] and *in-situ* [25,41–43]. In an *in-situ* study by Stenhagen et al. [35] a SnF_2 solution (0.05 M F, 0.4% SnF_2), applied daily for 9 days, showed a superior erosion/abrasion inhibiting effect compared to a NaF solution (0.05 M F, 0.2% NaF). The preventive effect of SnF_2 has been explained by the formation of a tin containing coating on the enamel surface [44]. It is claimed that the tin containing coating and the formation of CaF_2 precipitates may favour incorporation of fluoride in the hydroxyapatite lattice and, thereby, the formation of fluorhydroxyapatite. However, while CaF_2 may dissolve under erosive conditions, tin precipitates have shown to be acid resistant [45]. In a study by Baig et al. [46], NaF and SnF_2 enhanced the acid resistance of hydroxyapatite, but resistance was significantly greater after SnF_2 treatment. In addition, Schlueter et al. [44] found deposition of tin on the surface after SnF_2 treatment, but also incorporated deeper into the enamel after acid exposure in a cyclic de- and re-mineralization model. It may be speculated that the long-lasting effect is due to incorporation of tin into enamel and may be an additional explanation for the better effect of SnF_2 compared to NaF. Although the protective effect of SnF_2 solutions seems promising, the applicability of a solution with low pH and a relatively high concentration of metal ions may cause side-effects such as an astringent feeling in the mucosa [41]. Therefore, other application forms and vehicles should be considered, like a SnF_2 gel used in a mouth tray. A SnF_2 gel with the same fluoride concentration as in the present study has shown a good caries preventive effect in a caries risk study [7] and may be an alternative for patients suffering from severe dental erosive wear as well. A gel base may promote prolonged retention of the fluoride compound on the surface and with the use of a plastic dental retainer/mouth tray; side-effects on the oral mucosa could be reduced.

Conclusions

In the present study, only the SnF_2 gel showed a protective effect on erosive wear *in situ.* In a clinical situation for patients with severe erosive wear, 2 min brushing daily with conventional fluoride toothpaste and an application with SnF_2 gel 3-times a week may be a feasible regime. The present findings support the results from studies concluding that high and low concentration NaF toothpastes seem to be unable to provide a protective effect against erosive wear.

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

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Notice of correction

The Early Online version of this article published online ahead of print on 8 Sep 2014 contained errors in the figure legends.
The corrected version is shown here.