Reduction of Erosive Wear in situ by Stannous Fluoride-Containing Toothpaste

C.P.A.H. Klijna  A.M. Vieirab

aCollege of Dental Science, Radboud University Nijmegen Medical Centre, Nijmegen, and bCenter for Dentistry and Oral Hygiene, University Medical Center Groningen, Groningen, The Netherlands

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Abstract
Background/Aims: Stannous fluoride (SnF) has been suggested as a dental erosion-preventive agent. The aim of this single-centre, randomized, double-blind, in situ study was to evaluate the effect of toothpastes with SnF in the prevention of erosive enamel wear. Methods: A combined split-mouth (extra-oral water or toothpaste brushing) and crossover (type of toothpaste) set-up was used. Twelve volunteers wore palatal appliances containing human enamel samples. Three toothpastes were used, in three consecutive runs, in randomized order: two toothpastes containing SnF (coded M and PE) and one toothpaste containing only sodium fluoride (coded C). On day 1 of each run the appliances were worn for pellicle formation. On days 2–5 the samples were also brushed twice with a toothpaste-water slurry or only water (control). Erosion took place on days 2–5 extra-orally 3 times a day (5 min) in a citric acid solution (pH 2.3). Enamel wear depth was quantified by optical profilometry. The effect of toothpastes was tested using General Linear Modeling. Results: Average erosive wear depth of control samples was 23 µm. Both SnF toothpastes significantly reduced erosive wear: M by 34% (SD 39%) and PE by 26% (SD 25%). The control toothpaste reduced erosive wear non-significantly by 7% (SD 20%). Both SnF-containing toothpastes significantly reduced erosive wear compared to the sodium fluoride toothpaste. Conclusion: We conclude that SnF-containing toothpastes are able to reduce erosive tooth wear in situ.

Increasing scientific effort is being invested in identifying optimal fluoride formulations for the prevention of erosive tooth wear. Common formulations used in caries prevention, neutral solutions of sodium fluoride (NaF) at 250–500 mg/kg F and NaF toothpastes at 1,000–1,500 mg/kg F, have been shown to have limited effect [Attin et al., 1998; Lussi et al., 2004, 2008]. The added concern when using toothpaste is that the brushing action with the toothpaste on previously erosion-softened enamel increases abrasion [Attin et al., 2000].

D.E.M.F. Unk and C.P.A.H. Klijn are master students at the College of Dental Science, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.
Acidic solutions, such as native solutions of stannous fluoride (SnF₂), titanium tetrafluoride (TiF₄) and hydrofluoric acid (HF), have been shown to be effective in vitro and in situ [Hove et al., 2008; Hjorstsjö et al., 2010]. Of the above, SnF₂ has recently been considered the most promising, as both TiF₄ and HF are probably too acidic for clinical use (pH <2), and pH adjustment of TiF₄ reduces its protective effect [Wiegand et al., 2009]. SnF₂ is already being used in toothpastes and mouth rinses, and its effect on plaque and gingivitis is well recognized [Paraskevas and van der Weijden, 2006].

Recently, most research has focused on solutions containing different concentrations of SnF₂ or combinations of different fluorides with SnCl₂. In an in vivo model, a 1-min exposure to a 0.78% w/v SnF₂ solution reduced enamel dissolution during a 1-min exposure to citric acid by 67% [Hjorstsjö et al., 2009a]. However, the effect did not last for more than 1 day [Hjorstjö et al., 2009b]. A novel approach to tin-containing solutions has been studied extensively, using SnCl₂ as the source of tin with amine fluoride and/or NaF as the source of fluoride. In an in vitro erosive cycling model, such solutions reduced tissue loss significantly, even when using a severe erosion regime [Schlueter et al., 2009a, c]. In an in situ study, an experimental mouth rinse containing 1,900 mg/kg Sn (from SnCl₂) and 1,000 mg/kg F (from NaF and amine fluoride) used once a day reduced erosive wear of enamel and dentine by 73 and 50%, respectively [Schlueter et al., 2009d]. The tin is thought to work through uptake into the surface enamel and/or the formation of a tin-containing surface layer on top of the enamel [Schlueter et al., 2009b; Yu et al., 2010].

It is less is known about the erosion-preventive effect of SnF₂ in toothpastes. The concentration of SnF₂ in toothpastes is usually lower than those used in solutions, and the abrasive effect of the toothpaste may interfere with the protective effect. A study where specimens were immersed into toothpaste slurries showed reduced microhardness loss during erosion in vitro, but there was no significant difference following treatment with a SnF₂-containing toothpaste or a NaF-containing toothpaste [Lussi et al., 2008]. In the in vivo model mentioned above as used by Hjorstjö et al. [2009b], enamel dissolution was significantly reduced by 4-min application of SnF₂ toothpaste applied with a soft brush [Young et al., 2006]. It is unclear whether this method mirrored real toothbrushing. Moreover, tissue loss due to the brushing alone was not measured. In vitro [Attin et al., 2000] and in situ studies [Jaeggi and Lussi, 1999] have shown that eroded enamel and dentine are susceptible to toothbrush abrasion.

The presence of toothpaste during brushing is more important than the brushing action itself [Voronets et al., 2008; Voronets and Lussi, 2010], whilst fluoride in toothpaste reduces abrasion [Ganss et al., 2007]. Although increasing the time period between erosion and brushing reduces the effect of abrasion, it still has an effect at least up to 2 h following erosion [Attin et al., 2000; Ganss et al., 2007].

We hypothesized that in an in situ set-up with palatal sample placement, brushing samples twice a day with a stannous fluoride-containing toothpaste would not increase erosive wear, compared to brushing with water only, and would reduce erosive wear compared to brushing with a sodium fluoride toothpaste.

Subjects and Methods

This study was a single-centre, randomized, double-blind in situ study. A combined split-mouth (extra-oral water or toothpaste brushing) and crossover (type of toothpaste) set-up was used. Ethical approval was obtained from the regional accredited Medical Research Ethics Committee (MREC code: NL28303.091.09). Twelve healthy volunteers from the staff and students of the Radboud University Nijmegen Medical Centre provided written informed consent to participate. Exclusion criteria were: current systemic disease and all use of medication, presence of caries or periodontal disease, inability to wear a palatal appliance and/or to concur with the study protocol. The participants wore acrylic palatal appliances, each containing 2 acrylic blocks with 2 embedded human enamel samples [Vieira et al., 2007]. The samples were prepared from recently extracted human (pre)molars obtained from patients who provided verbal informed consent, as approved by the regional MREC. The samples were prepared from the facial or lingual surface of the teeth (approximate dimensions: 3 × 3 × 2 mm) and were embedded in groups of 2 in acrylic resin (De Trey, Self-Cure Acrylic, England) using a mould that produced blocks of 5 × 9 × 3 mm. Subsequently the embedded enamel samples were ground flat on a rotating polishing machine under water cooling (Phoenix Beta grinder/polisher, Buehler, Germany) using SiC grinding paper P1200. The samples were sterilized with ethylene oxide (WIMAC Kliniekdiensten B.V., Rotterdam, The Netherlands, ISO 9001:2000 and EN 13485:2003). Prior to insertion in the appliance, the blocks were partially covered with PVC tape leaving an exposed enamel window approximately 1 mm wide, thus protecting enamel reference areas for measurement of surface loss. One of the blocks from each subject was brushed with toothpaste slurry twice a day, whilst the samples from the other block were brushed with water and served as control. All brushing took place extra-orally, using a brushing machine.

Three toothpastes were used, each in one of three consecutive runs, in randomized order. Two of these contained stannous fluoride: Meridol (coded M; GABA Benelux, Weesp, The Netherlands; slurry pH = 4.7) containing 1,050 ppm fluoride from stannous fluoride and 350 ppm from amine fluoride, and Oral B

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Toothpastes
Pro-Expert Enamel Protection (coded PE; Procter & Gamble, Weybridge, UK; slurry pH = 5.8) containing 1,100 ppm fluoride from stannous fluoride and 350 ppm from sodium fluoride. The third toothpaste was a sodium fluoride control: Oral B 123 (coded C; Procter & Gamble; slurry pH = 7.0) containing 1,450 ppm fluoride. Thus all toothpastes contained 1,400–1,450 ppm fluoride.

**Study Design**

The participants wore the appliances for 3 experimental runs (1 run for each toothpaste) of 5 working days from 9.00 until 17.00 (times could vary ± 30 min). During the entire study period, starting 1 week before the first run, the subjects were instructed to use the NaF toothpaste (C) for normal home brushing. With the appliances in situ the participants were instructed not to eat and were only allowed to drink coffee or black tea without sugar. From 12.00 till 13.00 (lunch break) and from 17.00 till 9.00 the next day, as well as during the weekends, the appliances were stored in saline at room temperature. On day 1 of every run, the appliances were worn and no erosive challenges took place in order to allow pellicle formation on the enamel surfaces. From day 2 till day 5 the experimental procedure was as follows (all times ± 15 min): 8.15 samples brushed with toothpaste or water (controls); at 10.30, 13.00 and 15.00 samples exposed to erosion challenge, 17.30 samples brushed as before. Between the runs, a washout period of at least 2 days was observed. During this time the sample blocks were polished to remove the top layer of enamel amounting to 100 ± 20 μm thereby providing a fresh surface for the next experimental run. This was controlled by digital caliper.

The erosion challenge consisted of immersing the appliance with the samples for 5 min in 100 ml of a 0.05 M citric acid solution (pH = 2.3), with no agitation and at room temperature. A fresh volume was used for every exposure. After the exposure, the appliances were rinsed for 10 s under running tap water and immediately reinserted. The brushing was carried out by removing the sample blocks from the appliance and inserting them into a brushing machine. Toothpaste slurries (1:3 toothpaste to demi-water ratio) were freshly prepared and poured into the individual wells for each sample block. For control samples, the wells were filled with demi-water. Samples were exposed to the slurry/water for 2 min, and within that time period, were brushed (10 strokes, 150 g). After 2 min the samples were rinsed under running tap water for 10 s and replaced in a saline storage container until further use.

**Wear Measurement**

Enamel surface loss was measured using light profilometry (Proscan 2100, Scantron, England). A baseline measurement was performed on each sample before PVC tape application in order to evaluate the flatness of the polished enamel surfaces. If baseline curvature was higher than 1 μm the samples were polished again.

After each run, the PVC tape was removed and scans were taken of the exposed surface and reference surfaces (step size 10 μm). The function ‘3 point step height’ of the equipment’s software package was used to determine surface height loss for each sample. Two areas of approximately 0.25 × 2 mm were selected on the scan, at the edges of the two reference surfaces. A third area of about 0.7 × 2 mm was selected in the centre of the exposed surface. The enamel surface loss was calculated as the difference between the average height of the reference surfaces and that of the exposed surface. The results for the 2 samples in each block were averaged before further statistical analysis.

Scans were analysed twice, with a time interval of 2 weeks, in order to evaluate measurement reproducibility, showing Limits of Agreement (95% CI of repeated measurement) of ±0.5 μm.

**Scanning Electron Microscopy**

Following the last run, a sample from each group (water control and toothpastes) was prepared for scanning electron microscopy (SEM). Organic deposits were removed using immersion in 1 M sodium hydroxide for 18.5 h (of which 30 min with ultrasonication). Subsequently, samples were dehydrated using ethyl alcohol, dried in an incubator at 37 °C, and gold-sputtered.

**Statistical Analysis**

General Linear Modeling was used to statistically analyse the data (GLM, SAS 92, Cary, N.C., USA). Control samples were first analysed separately, to check for a run effect or a crossover effect of the toothpaste on the water-brushed controls. Subsequently, the effect of the toothpaste compared to water brushing was analysed, and the effects of the toothpastes mutually compared. A significance level of p = 0.05 was used.

**Results**

Twelve healthy volunteers were included, 11 female and 1 male, aged between 20 and 50 years, all with normal salivary flow rates (unstimulated flow >0.2 ml/min; stimulated flow >0.7 ml/min). All subjects completed the study without problems. One appliance during 1 run was accidentally exposed for 17 min. This was partly compensated for by omitting the subsequent exposure, and the results for this run were included in the effects analyses. One scan was lost and could not be replaced (toothpaste C, run 2).
Average erosive wear depth of water-brushed control samples in the three runs was 22.3, 23.4, and 24.7 μm, respectively, showing a small but significant run effect (effect 1.1 μm; p = 0.01). No crossover effect of the toothpaste on the surface loss of the control samples could be observed.

The results grouped by toothpaste, each with their own water control results, can be seen in figure 1. Both stannous fluoride toothpastes significantly (p ≤ 0.01) reduced erosive wear: M by 34% (SD 39%) and PE by 26% (SD 25%) compared to the water-brushed controls. The sodium fluoride toothpaste reduced erosive wear by 7% (SD 20%), but this was not statistically significant. Mutually comparing the toothpastes showed a significant difference (p < 0.05) between group C and both M and PE, who were not significantly different from each other.

SEM images (fig. 2) show a typical honeycomb structure of etched prisms for the water-brushed control sample. A similar, though slightly less distinct appearance is seen for the sample from group C. Samples from groups M and PE both show a mixed appearance, with areas of etched prisms combined with areas where a surface layer appears to cover the enamel.

Discussion

In this in situ study we showed that brushing twice daily with SnF₂-containing toothpastes had a preventive effect on the development of erosive wear. The erosive cycling model was rather severe, as can be seen from the tissue loss in the control group: on average 23 μm in 4 days. It is noteworthy that a basic home care product like toothpaste may influence and reduce even such severe wear.

The model was designed to include maximum pairing of data: a split-mouth design was used for the water-brushed control, and samples were re-used after each run, so different toothpaste results were obtained using the

Fig. 2. Scanning electron micrographs (each a composition of two magnification level images) of the surface of a water-brushed sample (A) and a sample from each of the toothpaste groups M, C and PE.
same samples. This design carried with it the risks of contamination: toothpaste effects carried over from one side of the mouth to the other side (water-brushed control), and of a run effect: samples changing from one run to the next. A crossover effect could not be shown, nor could a run effect be shown when the complete sample group was analysed. Only for the separate analysis of the control samples could a significant run effect be observed: for each new run, an extra 1.1 μm of wear was seen. The most likely explanation for this is the combined tissue removal during erosion and polishing of about 125 μm in each run. This exposes deeper layers of enamel, expected to have a lower degree of mineralization and higher solubility. However, this effect was not relevant for the analysis, as each run had its own water-brushed control and as the run order was randomized for the toothpastes.

The range of individual erosive wear results for control samples was between 13 and 33 μm. This is more uniform than previously reported [Vieira et al., 2007; Wetton et al., 2007]. However, the range in preventive effects of the toothpastes was great: toothpaste M showed that the effect ranged from a 69% increase to a 86% decrease in wear. The individual with the lowest wear from water-brushed samples showed increased wear for all toothpaste-treated samples. Neither the factors involved in individual susceptibility to erosive wear, nor those involved in the individual response to preventive agents have as yet been identified. This aspect of erosion (prevention) urgently needs more research.

In this study we found on average no added wear from tooth brushing with toothpaste. The palatally placed samples were exposed to tongue friction after each erosive challenge, which has previously been shown to remove a softened enamel layer [Gregg et al., 2004; Vieira et al., 2007].

One study reporting the effect of SnF₂ toothpaste on erosion failed to find a significant effect or difference when compared to a NaF-containing toothpaste [Lussi et al., 2008]. Elsewhere, calcium loss was reduced immediately after a 4-min application of a SnF₂ toothpaste [Young et al., 2006]. Such single erosion challenge studies may not adequately model the complex situation leading to erosive tooth wear, where abrasion is a significant factor. Also, a cycling of both erosion and SnF₂ exposure may be important, as Schlueter et al. [2009b] showed that the incorporation of tin in the enamel is related to the preventive effect. The SEM images support the theory that SnF₂, like TiF₄, may work through the formation of a protective surface layer, limiting or delaying the direct contact of the acid with the enamel mineral [Schlueter et al., 2009b].

As we used commercially available toothpastes, which differed in more aspects than the fluoride source, we cannot conclude unequivocally that the stannous fluoride was the effective agent in this study. Two common factors may have influenced the toothpaste effects: pH and abrasivity. Toothpaste M, with the largest effect, also had the lowest pH. Although this may seem counterintuitive, it has been shown before that acidic fluoride applications reduce erosive wear more effectively than neutral ones [Hove et al., 2008; Wiegand et al., 2009]. The RDA values of the toothpastes were not determined, and they may have had an unknown modifying effect on the results. However, as toothpaste brushing did not contribute on average to the erosive wear, the influence of this factor is doubtful. In a previous in situ study, using an experimental toothpaste which is probably closely related to toothpaste PE, a similar erosive wear-reducing effect was mainly attributed to the sodium hexametaphosphate which this toothpaste also contains [Hooper et al., 2007]. However, given the similar, possibly even larger, effect of toothpaste M in our study, containing stannous but no sodium hexametaphosphate, the evidence points more strongly towards the stannous.

In situ research is the closest we can get to clinical studies on erosive wear, and on this basis we conclude that toothpaste, and particularly toothpaste containing SnF₂, may play a role in its prevention. A clinical study is needed, however, to support a translation to the clinical situation.

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