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A clinical study to measure anti-erosion properties of a stabilized stannous fluoride dentifrice relative to a sodium fluoride/triclosan dentifrice

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West NX, Seong J, Hellin N, Eynon H, Barker ML, He T. A clinical study to measure anti-erosion properties of a stabilized stannous fluoride dentifrice relative to a sodium fluoride/triclosan dentifrice.

Abstract: *Objective:* To compare the enamel protection efficacy of a stabilized stannous fluoride (SnF_2) dentifrice to a sodium fluoride (NaF)/triclosan dentifrice following acidic erosive challenge. *Methods:* In this *in situ*, randomized, controlled, double-blind, two-treatment, four-period crossover clinical trial, subjects wore an appliance fitted with human enamel samples 6 h day^{-1} during each 15-day treatment period. Twice each treatment day they swished with their assigned dentifrice slurry: 0.454% SnF_2/0.077% NaF or 0.32% NaF/0.3% triclosan. After each treatment and two other times daily, subjects swished with 250 ml of orange juice over a 10-min period (acidic erosive challenge). Enamel samples were measured for tooth surface loss using contact profilometry at baseline and days 10 and 15. *Results:* Thirty-six subjects (mean age 44.8 years, range 23–65 years) were randomized to treatment; 33 subjects completed the final study visit. There were no statistically significant baseline differences ($P > 0.44$) in the specimen surfaces of the two dentifrice treatment groups via profilometry. At day 10, the SnF_2 dentifrice provided a statistically significant ($P < 0.0001$) reduction in enamel loss by 67% versus the NaF/triclosan dentifrice with estimated medians of 1.22 and 3.68 μm, respectively. At day 15, the SnF_2 dentifrice again provided a significantly greater benefit ($P < 0.0001$) against tooth surface loss versus the NaF/triclosan dentifrice, with 68% less erosion, and estimated medians of 1.60 and 5.03 μm, respectively. Both dentifrices were well tolerated. *Conclusion:* A stabilized SnF_2 dentifrice provided superior protection against the initiation and progression of tooth enamel surface loss *in situ* after erosive challenge compared to a NaF/triclosan dentifrice.

Key words: dentifrice; erosion; sodium fluoride; stannous fluoride; triclosan

Introduction

Significant strides in dental public health have occurred in the last half-century, driven in great measure by the inclusion of fluoride in dentifrices to reduce dental caries, along with greater consumer appreciation of the importance of plaque control for optimal oral health. Yet one widespread trend threatens to mar these preventive gains: a burgeoning overconsumption of acidic – and often sugar-laden – beverages that can lead not only to decay, but also to the surface loss of tooth enamel. Soft

drinks, fruit juices, sports drinks and increasingly popular energy drinks now account for a concerning proportion of beverages regularly consumed by children, teens and adults (1–3), and their typically low pH composition renders them especially egregious among dietary sources of acid attack in the promotion of tooth erosion. Erosion has been defined as pathologic, non-bacterial dental hard tissue loss induced by extrinsic or intrinsic acids or chelators acting on plaque-free tooth surfaces (4). With tooth erosion prevalence quite high (some surveys indicate upwards of 80% of children and adults may be affected) (5–7), there is growing awareness by dental clinicians and researchers of erosion as a notable threat to the integrity of tooth structure, and the need for effective intervention strategies.

Clearly, preventive approaches that protect against enamel dissolution from acid and potentially permanent damage are a dental public health priority worthy of considerable focus. Fluorides as a class have definitively been proven to be effective anticaries agents (8), with sodium fluoride (NaF), stannous fluoride (SnF_2) and sodium monofluorophosphate (SMFP) being commonly used actives in commercially available global dentifrices today. However, as the use of fluoride dentifrices is ubiquitous, tooth erosion prevalence is nonetheless on the rise, suggesting not all marketed fluoride toothpastes are sufficiently formulated to protect against enamel loss in the face of substantial acidic insult.

One anticaries agent – SnF_2 – has demonstrated significant efficacy in reducing enamel erosion as measured by tooth surface loss relative to other fluoride systems following erosive challenge in several studies of both *in vitro* and *in situ* designs (9–19). Research suggests that the ability of stabilized stannous fluoride to produce significantly greater reductions in erosion compared to other fluorides is due to its ability to increase enamel resistance to acid attack as well as deposit a protective barrier layer on the pellicle-coated surface of the enamel which is retained for hours, blocking acid that would otherwise initiate erosive damage. This research further suggests that the barrier layer left on the tooth surface following use of stannous fluoride is likely linked to calcium and phosphate sites on enamel, improving the ability of the complex to remain attached to the acid-challenged tooth surface (13, 15–21).

To further the body of knowledge regarding stannous fluoride and enamel loss prevention relative to other dentifrice formulations, the present randomized and controlled clinical investigation compared the ability of a marketed stannous fluoride dentifrice to protect against human enamel erosion relative to a marketed sodium fluoride/triclosan dentifrice control, utilizing an *in situ* model with an orange juice erosive challenge in a population of adult subjects.

Methods and materials

Study population

Prior to subject recruitment, the study protocol and participant consent form were reviewed and approved by the National Research Ethics Service Committee South West – Exeter (12/SW/0178), United Kingdom, and the study was conducted according to Good Clinical Practice (GCP) guidelines. Generally, healthy volunteers at least 18 years of age from the University of Bristol Dental School and Hospital were assessed for study eligibility at a pretrial screening visit. After providing written informed consent, potential subjects were clinically screened via an oral soft tissue examination. Additionally, demographic, medical and concomitant medication information was obtained to determine whether they met the study entrance criteria. Qualified subjects were required to have no evidence of the following: susceptibility to acid regurgitation; recurrent or regular aphthous ulcers; dental erosion or a previous history of susceptibility to high dental erosion after drinking sports drinks/juices; excessive gingival inflammation; severe periodontal disease; and unremovable mouth or tongue jewellery. Subjects who were unwilling to delay elective dentistry and/or refrain from the use of non-study assigned products or from use of acidic medications (pH < 5.3) during the course of the study were not eligible for enrolment. If all aforementioned study entrance criteria were met, subjects were enrolled in the clinical trial.

Study design

This clinical investigation was a single-centre, randomized, double-blind, two-treatment, four-period crossover study, involving supervised usage of two treatments to evaluate the enamel protection efficacy of a stannous fluoride dentifrice versus a sodium fluoride dentifrice in an *in situ* erosion model based on Hooper *et al.* (11). The sodium fluoride dentifrice containing 1450 ppm fluoride is considered a regular fluoride control for erosion. Each of the four study periods spanned approximately 3 weeks and was comprised of 15 (generally) consecutive weekday treatment days. Following enrolment, subjects were randomly assigned to one of the four treatment sequences specifying the order of use of each of the dentifrice treatments, such that each subject ultimately used the two test products two times each. Subjects were then provided with a non-treatment toothpaste and toothbrush which they were instructed to use until the start of the study (a minimum of 2 days) and their first assigned treatment test kit.

An outline of treatment-day procedures is provided in Fig. 1. On each treatment day, participants brushed their teeth at home in their typical manner using the non-treatment toothpaste and manual toothbrush supplied at the screening visit. Subjects then presented to the clinical site, where they collected their previously disinfected custom-made palatal intraoral appliance fitted with two enamel samples (Fig. 2). After insertion, subjects wore the appliance for an hour before their first treatment and for a total period of approximately 6 h over the course of each treatment day. During this time and under site supervision, subjects swished twice daily (at baseline, and 3 h post-treatment) with their assigned treatment toothpaste slurry for 60 s and then rinsed with 10 ml of water. The erosive challenge occurred after each slurry treatment and at 2

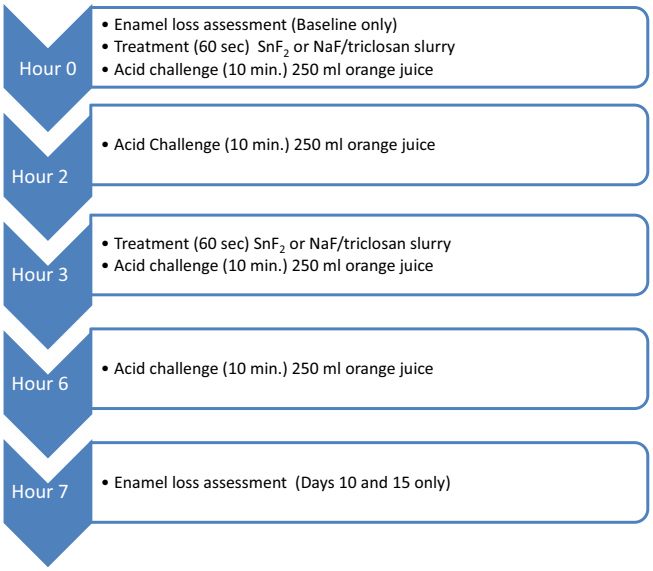

Fig. 1. The sequence of events during treatment days 1–15 of each study period is detailed. After inserting their intraoral appliance fitted with human enamel samples, subjects swished at baseline and hour 3 with their randomly assigned dentifrice slurry: either 0.454% stannous fluoride plus 0.077% sodium fluoride [1450 ppm fluoride] or 0.32% sodium fluoride [1450 ppm fluoride] with 0.3% triclosan. After each slurry treatment and at hours 2 and 6, subjects swished with orange juice for an acidic erosive challenge. Enamel samples were measured for tooth surface loss using contact profilometry at baseline and days 10 and 15.

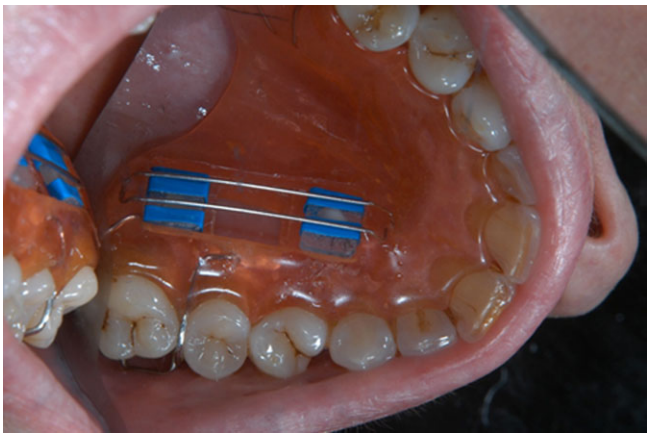

Fig. 2. Palatal intraoral appliance.

and 6 h post-baseline with the appliance in the mouth: subjects were required to sip 25 ml of orange juice (Sainsbury's, UK) over a timed minute, swishing it around their mouth, and then expectorating. The process was replicated 10 consecutive times, so that the enamel samples were exposed to a total of 250 ml of orange juice over a 10-min period each time. In between challenges and treatments, while wearing the appliance, subjects refrained from eating or drinking anything, other than small sips of water. During a one-hour lunch break midday, subjects were permitted to remove their appliance, which was stored in a moist container.

At study baseline and at the end of treatment days 10 and 15 of each treatment period, the enamel samples were assessed for tissue loss using a calibrated contact surface profilometer. Fresh enamel samples were placed in the intraoral appliance at the beginning of each study period. Subjects attended an assessment visit within 2 weeks of completing the last treatment period, where they received a final clinical oral assessment and medical interview prior to study exit.

Test products

Subjects were randomly assigned at study initiation to a treatment sequence ordering their use of the two commercially available test dentifrices:

- 0.454% stannous fluoride plus 0.077% sodium fluoride (1450 ppm fluoride), marketed in the United Kingdom as Oral-B® Pro-Expert dentifrice (Procter & Gamble Company, Gross-Gerau, Germany);
- 0.32% sodium fluoride (1450 ppm fluoride) with 0.3% triclosan, marketed in the United Kingdom as Colgate® Total® Advanced Dentifrice (Colgate-Palmolive, Dublin, Ireland).

Assigned test dentifrices were administered orally by means of a slurry on treatment days, prior to the erosive challenge. Clinical site personnel prepared subject dentifrice slurries by mixing three grams of dentifrice with 10 ml of water. Subjects were unaware of the product identity of their assigned dentifrice slurry and were instructed not to discuss the physical properties of their assigned products with other study subjects or clinical site personnel. Additionally, the investigator and personnel performing and recording the surface profilometry assessments were prohibited from access to the product dispensing room during treatment to maintain study blinding. For subject toothbrushing during weekends, off-treatment days, in the morning and evening prior to and after treatment day visits, and before and after wearing of the dental appliances during treatment phases, participants were assigned a non-treatment 0.32% sodium fluoride (1450 ppm fluoride) marketed dentifrice (Crest® Decay Protection; The Procter & Gamble Company, UK) and an Oral-B 35 manual toothbrush (The Procter & Gamble Company, Cincinnati, OH, USA), with written and oral product usage instructions provided.

Evaluation methodology

Preparation and maintenance of enamel samples

Prior to study initiation, enamel samples were prepared at the clinical site, with recently extracted and donated caries-free adult human third molars of either gender – consent was obtained following ethical approval – serving as enamel samples. Following donation, the teeth underwent sterilization via sodium dichloroisocyanurate (20 000 ppm available chlorine), sectioning and hand polishing. The surface that was to be

exposed for treatment had any epoxy resin flash removed with 1200 grit carborundum paper mounted on a glass slab. The enamel samples were polished with an aluminium oxide powder (350 nm) slurry on a lap which was mounted on a glass slab.

Two baseline readings of each enamel sample were obtained using a contact profilometer (Surftest SV-2000; Mitutoyo Corporation, Japan), with the samples masked on either side of a two- to three-millimetre-wide window of exposed enamel. The samples were placed in the intraoral appliances, with each sample identified on its reverse side with a unique number for identical replacement after removal for future profilometry measurements.

The palatal appliances containing the enamel samples were disinfected in Corsodyl® mouthrinse with 0.2% w/v chlorhexidine gluconate (GlaxoSmithKline, Brentford, Middlesex, UK) at the start and end of each treatment day. Prior to profilometry measurements, the samples were removed from the appliance and disinfected by soaking in a mixture of 0.5% chlorhexidine and 70% aqueous ethanol; this combination was also used for disinfection post-profilometry after the samples were retaped and replaced in the appliances. During the one-hour lunch period and overnight when the palatal appliances/samples were absent from the mouth, they were stored in a 'moist pot', that is a container with a water-moistened cotton wool pad, to prevent dehydration.

Surface (contact) profilometry measurements

The measurement of any enamel loss due to erosion challenge during each treatment period was accomplished using a surface (contact) profilometer (Planer Products Ltd, Windmill Road, Sunbury-On-Thames, Middlesex, UK). The profilometer was calibrated prior to each measuring period. A stainless steel jig constructed to the exact dimensions of the prepared enamel samples was used to hold them in place during the profilometry assessment. Operating in a vibration-free environment, the measuring head was fitted with a diamond stylus to follow the surface of the enamel being tested and transversed the specimen at a constant speed of 10 mm min^{-1}. The signals from the measuring head were processed on an electrical control unit and displayed on a monitor screen.

For each enamel sample, two baseline readings were obtained, with readings taken across a demarcated two- to three-mm treatment area to be exposed to the test treatments. Post-exposure profilometry readings were measured in the same demarcated area at treatment days 10 and 15 of each treatment period.

Safety assessments

Safety was evaluated by the presence or absence of side effects associated with use of the test product. Safety evaluations included a clinical assessment of the oral soft and hard tissues. Assessment of the oral soft tissues was conducted via a visual examination of the oral cavity and perioral area, including the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips and perioral area.

Statistical analyses

The target number of subjects for recruitment was 36, providing at least 80% power to detect a difference between the treatment dentifrices in two-sided testing at the 5% significance level. This calculation was based on previous research (18) in which a natural logarithm transformation was applied to the data prior to data analysis, and assumed the effect size (mean treatment difference divided by the error standard deviation) was approximately 0.50 or higher in a two-treatment four-period crossover design study.

For each subject and treatment period, the average of four erosion profilometry measurements was calculated using two replicate measurements from each of two enamel sections. The change from baseline in surface loss was calculated as the value at baseline minus the value at post-baseline. As the day 10 and day 15 enamel loss distributions were right-skewed, the data were transformed using the natural log function to make the distribution bell-shaped before performing between-treatment analyses that assume normality. A general linear mixed model was used to compare treatments, and the final model included period and treatment as fixed effects and subject as a random effect. Neither the carry-over effect nor the baseline covariate was statistically significant ($P > 0.76$), and each was removed from the statistical model. From the final statistical model, estimated means on the natural log scale were back-transformed using the exponential function (e^{mean}) to obtain the estimated medians or 50th percentiles on the original scale (μm), and 95% confidence intervals (CI) were calculated. All statistical comparisons were two-sided with a significance level of 0.05. Adverse events were summarized.

Results

A total of 36 subjects were randomized to a test product sequence at study initiation, and 33 participants completed the trial through treatment period 4, day 15. One subject withdrew voluntarily after treatment period 1, and two subjects were not able to complete all treatment periods within the study duration. Therefore, for each of the day 10 and day 15 visits, all 36 subjects had measurements available for analysis from some portion of the study. Subjects ranged in age from 23 to 65 years, averaging 44.8 years (standard deviation 12.15), with female volunteers comprising 81% of the subject population. Ninety-two per cent of all subjects were of Caucasian ethnicity.

Efficacy results

At baseline, there were no statistically significant differences ($P > 0.44$) in the surfaces of specimens assigned to the two

Table 1. **Treatment comparisons for enamel loss (μm) by day**

	Original scale in μm estimated median* (95% CI)	% Benefit versus NaF/ triclosan†	Two-sided *P*-value
Day 10‡			
SnF_2 dentifrice	1.22 (1.07, 1.39)	67%	<0.0001
NaF/triclosan dentifrice	3.68 (3.23, 4.19)		
Day 15§			
SnF_2 dentifrice	1.60 (1.40, 1.82)	68%	<0.0001
NaF/triclosan dentifrice	5.03 (4.42, 5.72)		

μm, micrometer; SE, standard error; SnF_2, stannous fluoride; Log scale, natural logarithm scale.
*Estimated medians in μm were obtained using the exponential function on the means from the natural logarithm scale (e^{mean}), and 95% confidence intervals (CI) were calculated.
†Calculated from estimated medians in μm as (NaF/triclosan – SnF_2)/(NaF/triclosan).
‡Day 10 variance components: subject = 0.034, residual = 0.230.
§Day 15 variance components: subject = 0.047, residual = 0.201.

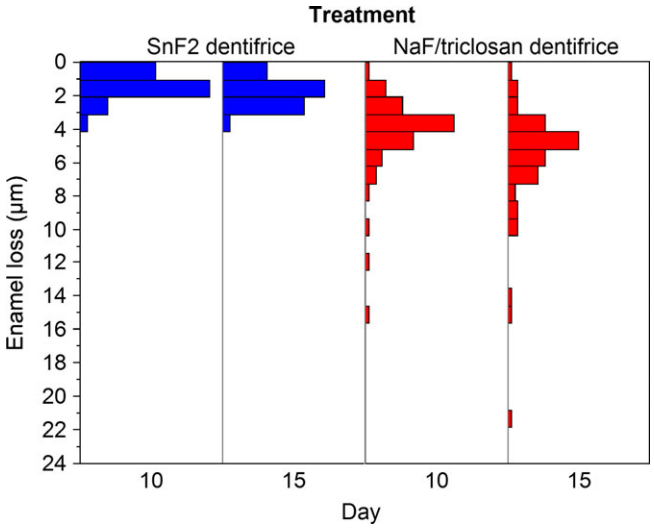

Fig. 3. Histograms of the subject- and period-level enamel loss (μm) measurements by treatment and day.

dentifrice treatment groups, as measured by profilometry, and all measurements were near zero within ± 0.2 μm. As shown in Table 1, at treatment day 10, profilometry results revealed that the stannous fluoride dentifrice provided a statistically significant ($P < 0.0001$) 67% greater protection against enamel loss than the sodium fluoride/triclosan dentifrice, with an estimated median of 1.22 μm with 95% CI 1.07–1.39 for the stannous fluoride dentifrice and 3.68 μm with 95% CI 3.23–4.19 for the sodium fluoride/triclosan dentifrice.

At treatment day 15, the use of the stannous fluoride dentifrice again resulted in significantly ($P < 0.0001$) lower enamel loss following erosive challenge, with 68% less erosion compared to the use of the sodium fluoride/triclosan dentifrice. Estimated medians were 1.60 μm with 95% CI 1.40–1.82 for the stannous fluoride dentifrice and 5.03 μm with 95% CI 4.42 –5.72 for the sodium fluoride/triclosan dentifrice (Table 1). Figure 3 displays the distribution of the individual subject and period data values by treatment and day.

Safety results

Both dentifrices were well tolerated. One adverse event, a mouth ulcer rated mild in severity, was reported in a subject who withdrew following period 1. Upon questioning and clinical examination, the event was deemed not related to test product use.

Discussion

The unfortunate practice of liberally consuming highly acidic and sometimes sugary beverages is reportedly growing in frequency in both children and adults, raising concerns over potentially irreversible tooth surface loss/erosion (1–3, *22*). As dietary acid attack is a key source of erosion to susceptible softened enamel, and achieving sustained individual dietary behaviour change can be difficult, the development of products that will mitigate adverse outcomes by protecting against the initiation and furthering of erosive acid damage is thus of substantial importance on a population level, particularly in the light of increasing lifespan which requires the longevity of a functional dentition for quality of life. Products such as dentifrices that are readily available and affordable, potentially offer multiple benefits and are easy to incorporate into one's daily routine, are the most desirable way for providing an effective intervention strategy to help curb both the initiation and progression of dental erosion.

Fluoride use has been shown to be of some value in erosion protection, but research has demonstrated that the benefit is dependent upon dosage and type; not all fluoride dentifrices are equally efficacious for tooth surface loss prevention (13–15, 17–19, 23). One fluoride has shown repeated superior anti-erosion efficacy relative to other fluoride salts in numerous laboratory and clinical *in situ* investigational designs: stannous fluoride (9–19, 21, 23).

At the dentine level, stannous salts have been observed to occlude patent dentinal tubuli via the chemical precipitation of a stannous-rich layer resisting acid-based disclosure of tubules (24–26). Deposition of acid-resistant smear layers may be an important mechanism for protection of dentine against both erosive acid attack and hypersensitivity. Smear layers are soluble in acid, and will preferentially dissolve prior to the acid attacking the dentin itself, in effect serving as a sacrificial source of mineral to help neutralize the acid attack. Studies by Rees *et al.* (27) and Pinto and colleagues (28) assessed the impact of various erosive beverages on smear layer removal. In general, both studies found that more aggressive beverages (measured in terms of pH, acid type, acid content and titratable acidity) resulted in faster removal of the smear layer and more rapid opening up of

occluded tubules. By deposition of a more acid-resistant smear layer, the overall aggressiveness of the acid challenge against the dentin can be reduced. White *et al.* reported that dentin was more resistant to acid dissolution and tubule exposure with *in vitro* use of a stabilized SnF_2 dentifrice, measured by microhardness testing and scanning microscopy analyses (29). Zsiska and colleagues further demonstrated the rapid formation of environmental-resistant smear layers with the use of SnF_2-based dentifrices (30).

The mechanism of action for the enamel erosion protection benefits of SnF_2 may be multifactorial. As reported by Faller *et al.*, 'It has been hypothesized that stabilized SnF_2 provides enhanced protection compared to other fluoride actives due to both the high level of available stannous fluoride in the formula and the ability of the active to deposit on and attach to the tooth surface'. (15) Earlier research by Addy and Mostofa had demonstrated SnF_2 to be deposited onto dental tissue, providing acid protection benefits (24). In a recent *in vitro* study using laser ablation, Khambe *et al.* demonstrated the deposition of an invisible, relatively continuous stannous-containing barrier layer onto pellicle-coated human enamel surfaces, following treatment with a SnF_2-containing dentifrice. In this study, enamel samples presoaked in human saliva to form a pellicle layer were divided into four groups and treated with a slurry mixture containing SnF_2 for varying durations/cycles. The findings indicated that the SnF_2 treatment deposited an acid-protective barrier layer onto the enamel surface after the initial treatment, that increased following multiple treatments, and was retained for many hours after product use (20).

Other laboratory research has focused on the ability of SnF_2 to bind to hydroxyapatite and inhibit dissolution of this primary enamel component during acid challenges. Baig *et al.* found SnF_2 to be significantly more advantageous in this regard over NaF in studies that assessed both the raw fluoride ingredients as well as after both ingredients were incorporated into complete dentifrice formulations (16). In multiple *in vitro* studies using an erosion challenge cycling method that is designed to closely approximate the human clinical environment, the relative erosion protection ability of a wide range of marketed toothpastes was compared versus a stabilized SnF_2 dentifrice (13, 17, 21). The stabilized SnF_2 dentifrice produced statistically significantly greater erosion protection benefits compared to all the non-SnF_2 treatments tested. With the exception of SnF_2, other commonly used fluoride sources, when formulated at levels commonly found in over-the-counter dentifrices, have not been demonstrated to provide a high level of erosion benefits (31). Some researchers believe, however, that higher levels of these fluoride actives, such as those found in prescription products, might provide some level of enhanced benefit (23). The stabilized SnF_2 dentifrice included in the current study has even been demonstrated to provide significantly greater protection against erosive acid challenge than prescription strength preparations formulated at 5000 ppm F (32, 33).

An *in situ* study design with human dietary acid exposure in an oral environment is an important validation of the findings of *in vitro* testing. A model originally developed by West *et al.* (34) in which prepared enamel samples are worn by study participants via an intraoral appliance and then measured for surface loss change following an erosive acid challenge was utilized by Hooper *et al.* for *in situ* testing (11). A ten-minute acid challenge is used as this is a reasonable time to consume a juice beverage and the juice is swished to ensure contact with the specimen in the palatal appliance (11). Therefore, the comparative ability of a stabilized SnF_2 and NaF-only dentifrices to prevent erosive damage under those conditions was assessed, with the former dentifrice providing significantly ($P < 0.0001$) greater enamel loss protection (by an estimated 67–68%), and the authors noting this provided further support for toothbrushing with a proven enamel protection toothpaste before meals. Similarly, in the study detailed in this current paper, in a randomized and controlled four-period crossover design with a well-established evaluation measure of tooth surface loss (profilometry) (11, 18), subjects wore intraoral appliances containing human enamel samples while following the prescribed protocol that included dentifrice treatments (stabilized SnF_2 or NaF/triclosan) and erosive acid challenges (orange juice). The results were in agreement with the previously discussed *in vitro* and *in situ* research, with the stabilized SnF_2 dentifrice yielding significantly superior acid protection compared to the dentifrice formulated with NaF. One could argue that the *in situ* benefit should be manifested in long-term human erosion clinical trials; however, the slow progression of this condition renders those studies impractical.

Conclusion

In conclusion, a stabilized stannous fluoride dentifrice provided superior protection against the initiation and progression of tooth enamel surface loss *in situ* after erosive challenge compared to a sodium fluoride/triclosan dentifrice.

Clinical relevance

Scientific rationale for study

Dental erosion is a growing oral health concern. Delivering agents to prevent the condition via dentifrice is an economical and efficient solution. This *in situ* study was conducted to evaluate the anti-erosion effects of two marketed dentifrice products.

Principal findings

The study showed that the stannous fluoride dentifrice provided greater protection from dental erosion compared to the sodium fluoride/triclosan dentifrice.

Practical implications

Dental professionals should consider recommending the stannous fluoride dentifrice for patients who need protection from dental erosion.

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Conflict of interest and funding statement

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