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A randomised *in situ* trial, measuring the anti-erosive properties of a stannous-containing sodium fluoride dentifrice compared with a sodium fluoride/potassium nitrate dentifrice

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Objectives: To determine if a stabilised, stannous-containing sodium fluoride dentifrice provides greater enamel protection *in situ* against intraoral dietary erosive challenges compared with a sodium fluoride/potassium nitrate dentifrice. **Methods:** A single-centre, investigator blind, randomised, supervised, two-treatment, non-brushing, four-period crossover *in situ* study was undertaken, with each test period being 15 days. Thirty-five healthy adult subjects were recruited to participate in the study, which included four erosive acid challenges per day. Subjects were randomised to product treatment, which included either: (1) a stannous-containing sodium fluoride dentifrice (Oral-B® Pro-Expert Sensitive) or (2) a sodium fluoride/potassium nitrate dentifrice (Sensodyne® Pronamel®). Each study subject wore an intraoral appliance retaining two sterilised, polished human enamel samples for 6 hours/day. Subjects swished with an allocated dentifrice slurry twice a day and with 250 ml of orange juice for 10 minutes (25 ml/minute over a 10-minute period) four times per day. The primary and secondary outcomes for this study were enamel loss measured using contact profilometry at days 15 and 5, respectively, using parametric analysis methods. **Results:** At day 15, a 38% lower enamel loss ($P < 0.0001$) was observed, with estimated medians of 2.03 μm (SE 0.247) and 3.30 μm (SE 0.379), in favour of the stannous-containing dentifrice. At day 5, specimens treated with the stannous-containing sodium fluoride dentifrice demonstrated 25% less enamel loss than those treated with the sodium fluoride/potassium nitrate dentifrice. Treatment differences at day 5 were also statistically significant ($P < 0.05$), with estimated medians of 1.37 μm (SE 0.177) and 1.83 μm (SE 0.223), respectively. **Conclusions:** Results of this *in situ* study suggest the stabilised, stannous-containing sodium fluoride dentifrice could be used to provide significantly greater protection to enamel from erosive acid challenge compared with that provided by conventional fluoride-containing products.

Key words: *Erosion, toothwear, toothpaste, stannous, clinical trial*

INTRODUCTION

The continual increase in consumption of acidic soft drinks[1], combined with copious clinical evidence that frequent acidic challenges can result in erosive tooth wear[2–5] and dentine hypersensitivity[6], reinforces the need to develop approaches that prevent, or at least impede, erosive tooth surface loss.

Individual acids, such as citric acid, and acidic soft drink products have been used in many *in vitro* and *in situ* studies to investigate the aetiology and mechanisms of dental erosion[7–13]. When dietary acid comes into contact with enamel, two inter-related processes occur: dissolution and demineralisation (softening). The former process is irreversible whereas the latter is potentially reversible, provided that that physical wear processes, such as abrasion, do not remove the softened layer before rehardening can occur[13–15]. Successful attempts have been made to reduce the potential erosivity of acid-containing products by modifying factors such as pH, titratable acidity and the chelation potential of the specific acid[12,16–19]; some success has also been observed using food-approved polymers[20,21]. One obvious approach to reduce dental erosion has been the use of fluoride, in view of its enamel strengthening and anti-caries potential[22,23].

In vitro studies testing the effects of adding fluoride to soft drinks have proven to be somewhat disappoint-

ing[24,25]. Greater success, however, has been achieved when focused toward proactively 'protecting' enamel with fluoride before erosive challenge. Sodium fluoride is thought to have a protective effect because of the *in situ* formation of CaF_2 acting as a mineral reservoir[23,26]. *In vitro* and *in situ* studies have suggested that increased protection can be achieved when using product regimens that provide intensive fluoride treatment (paste, rinse, gel)[27,28]. A number of other fluoride-containing products, such as those containing stannous fluoride (SnF_2), titanium fluoride (TiF_4) varnishes/solutions and amine fluoride (AmF) compounds, have also shown successful anti-erosive effects[29–34].

Although many studies have demonstrated that various fluoride-containing products are able to provide some level of erosion protection effects, the fluoride compound itself appears to have the greatest influence on the level of protection provided. While fluoride plays two roles in enamel erosion – first to reduce dissolution and second to promote remineralisation – SnF_2 has been demonstrated to form complexes[35] that have the potential to enhance the resistance of the tooth surface to acid challenges. Indeed, the polyvalent stannous metal cation, in combination with fluoride, appears to be far more effective in reducing erosion than other fluoride sources in *in vitro*[36–38] and *in situ* studies investigating both toothpastes[39] and rinses[40]. The mode of action of stannous is thought to be through deposition of a tin-rich surface on the enamel, with a number of different stannous compounds being detected depending on a number of factors such as concentration of the ions, pH and mode of application[35].

Stannous-containing dentifrices have also been shown to reduce dentine hypersensitivity[41] by occlusion of dentinal tubules as well as other influences. Reducing the effect of one of the aetiological components of tooth sensitivity, namely erosion, by providing a protective surface layer also plays a role in its anti-sensitivity benefits[42]. The stabilised stannous-containing dentifrice included in this study (Oral B® Pro Expert Sensitive) contains NaF (1,450 ppm F) as the active ingredient and stannous chloride ($SnCl_2$) as a key excipient. During toothbrushing, NaF and $SnCl_2$ combine synergistically to form a bioavailable stannous-fluoride complex[43]. This product is also marketed for its ability to reduce dentine hypersensitivity. The product comparison dentifrice, Sensodyne® Pronamel® was chosen because it was deemed the most suitable comparative product available at the time of the study, being marketed as both a desensitising and an anti-erosive product[44]. This product contains sodium fluoride and potassium nitrate.

The aim of this *in situ* clinical study was to determine if a stannous-containing, sodium fluoride dentifrice provided greater enamel protection against intraoral dietary erosive challenges than a sodium fluoride/potassium nitrate dentifrice in the absence of toothbrushing. The null hypothesis tested was that there was no difference between the effects of the two dentifrices with regard to anti-erosive protection properties, with dentifrice treatment preceding an intraoral erosive acid challenge.

METHODS

A single-centre, investigator-blind, randomised, supervised-usage, two-treatment, non-brushing, four-period crossover study was performed. Subjects presented for four 15-day study periods and were randomised to receive one of the two dentifrice products during each period. Ethical approval was granted from a UK research ethics committee (South West – Exeter Research Ethics Committee, 13/SW/0039) and all subjects gave oral and written consent to participate. The study was conducted according to Good Clinical Practice (GCP) guidelines[45].

Subjects aged 18 years or older were recruited from staff working at the research site, but were not members of the clinical trials unit. A register of staff members who have expressed an interest in taking part in a clinical trial is held by the unit. During the recruiting phase, those on the register were contacted by the study coordinator to see if they were interested in participating. Potential subjects who expressed an interest were invited to the clinical trials office, where the study was explained to them and they were provided with a subject information sheet to take away. When a screening date had been confirmed, those who expressed an interest in the study were contacted again to make a screening appointment. For this trial, 35 potential subjects attended screening.

Randomisation, interventions and data collection were carried out by study staff trained in GCP and study methodology at the clinical trials unit. At screening, subjects were allocated a unique number, assigned in ascending numerical order according to their appearance at the study site, as dictated by their availability. All 35 subjects met the inclusion and exclusion criteria for the study and were distributed by study staff according to randomised schedules of treatment sequences that were generated by a computer program approved by the statistician. Exclusion criteria were pregnancy, erosive medications, medical conditions with reflux, allergies, and anyone wearing orthodontic appliances. An alginate impression of the upper dental arch was taken to enable construction of an intraoral palatal appliance to hold the study specimens.

Subjects were given a standard dentifrice [Crest® Decay Protection Dentifrice, 0.32% sodium fluoride (1,450 ppm fluoride); Procter & Gamble UK, Weybridge, UK] and toothbrush (ADA Soft Manual Toothbrush; American Dental Association, Chicago,

IL, USA) for home use for the duration of the study. Written instructions on how to use these products twice a day, morning and night on both treatment and non-treatment days instead of their normal toothbrush/dentifrice, were provided and reviewed verbally.

During the study periods subjects wore a palatal intraoral appliance that retained two sterile, polished flat, human enamel samples (*Figure 1*). Caries-free human third molars that had been recently extracted and donated from patients aged over 18 years to an NHS research ethics committee approved tooth tissue bank (HSC REC1, ref 11/NI/0145) located at the study site were used to prepare the enamel specimens. The teeth had been collected with informed consent in accordance with the UK Human Tissue Act 2004[46]. Following extraction, each tooth was soaked in a 20 g/l available chlorine solution (Haz tabs; Guest Medical, Aylesford, UK) for at least 24 hours and scraped clean of any remaining tissue. Using a water-cooled diamond precision annular saw (Microslice; Ultratec, Santa Ana, CA, USA) teeth were sectioned at the cemento-enamel junction and pulpal tissue was removed before being further soaked in 20 g/l available chlorine solution for a minimum of 24 hours. Before use, the teeth were thoroughly rinsed in deionised water. The crowns of the teeth were subsequently sectioned (obtaining between two and four enamel samples per tooth), set in Epoxy resin (Hitek, UK), then hand polished to produce a smooth enamel surface. The enamel samples then had baseline profilometry measurements taken, were masked to expose a 2–3 mm zone of enamel, then placed into the palatal appliances.

On each treatment day subjects wore the appliances for 6 hours/day, excluding 1 hour for lunch. No food or drink could be consumed while the appliance was in the mouth, with the exception of water. At the end of each treatment day, the subjects returned their appliance to the study site where they were stored overnight in a moist closed container to prevent dehydration of the enamel samples. On day 1 of the study, current and concomitant medications were reviewed and recorded, continuance criteria were assessed and each appliance was checked for comfort and fit. This information was reviewed daily by study staff to ensure that subjects were aware of these requirements. At the beginning of each treatment day, and 3 hours later, subjects visited the clinic and, under the supervision of study staff, swished for a timed 60 seconds with the dentifrice slurry allocated for that period according to the randomisation schedule. Fresh slurries (3 g of dentifrice to 10 ml of Volvic bottled water (Danone, UK)) were prepared for each treatment. The balance for weighing the dentifrice was calibrated daily. Following swishing with the dentifrice slurry, subjects were allowed to rinse their mouth once using 10 ml of Volvic bottled water. To maintain blinding to the treatments, neither the investigator nor the profilometry analyst were permitted in the dispensing room for the study.

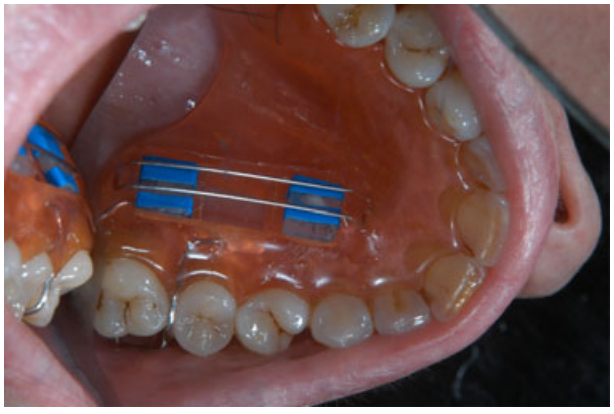

Figure 1. Intraoral palatal appliance.

The key ingredients of the two test dentifrices were:

- Marketed Test Dentifrice: 0.32% sodium fluoride (1,450 ppm fluoride) as active ingredient, and stannous chloride as a key excipient. (Oral-B® Pro-Expert Sensitive; The Procter & Gamble Company, Egham, Surrey, TW20 9NW UK)
- Marketed Comparator Dentifrice: 0.32% sodium fluoride (1,450 ppm fluoride) and 5% potassium nitrate (Sensodyne® Pronamel®, GlaxoSmithKline Consumer Healthcare, Brentford, UK)

The erosive challenge was administered at the study site each treatment day, shortly after each dentifrice slurry application, and 2 hours and 6 hours after the initial visit each day. Visit timings were monitored and recorded by study staff and subjects were reminded when they should attend for their next visit. Subjects were trained to swish the orange juice (Sainsbury's Supermarkets Ltd, London, UK), average pH 3.4, before the start of the study to ensure standardisation of effect and each erosive challenge was supervised by study staff. A total of 250 ml of orange juice was swished at each time-point, resulting in the tooth samples being exposed to 1 l of juice each day. At each time-point, subjects swished with 25 ml of orange juice for 1 minute before expectoration. This procedure was performed a total of 10 times over a 10-minute period without any water rinses between swishes in order to obtain a steady, controlled flow of acidic challenge over the tooth samples for all subjects throughout the study and to minimise variation.

At days 5 and 15 of each study period, samples were removed from the appliances and readings from the enamel samples were measured by contact profilometry (Surftest SV-2000, Mitutoyo Instruments, UK). The profilometer was calibrated at the start of each assessment day using a standard metal calibration block. Two baseline readings of each sample were

taken, measuring across the area to be exposed to the study treatment. The readings needed to be a maximum of ±0.3 from 0.00 μm in deflection. The designated treatment area was marked on the enamel sample and polyvinyl chloride (PVC) tape placed over the samples, leaving approximately a 2- to 3-mm-wide zone of enamel exposed for treatment.

At days 5 and 15 contact profilometry readings from this exposed area were taken after the PVC tape was removed. Two values (measuring tissue loss) were obtained for each of the two samples to provide a representative value of tissue change in the treatment area after day 5. Samples were measured in the same position located on the specimen using magnification of markers on the specimen. It was not necessary to define the exact position for retracing each sample as this was a representative two-dimensional tracing of surface loss across the sample, performed twice in two areas from two samples in 35 subjects. Multiple enamel samples were measured over the study period in order to obtain the order of tissue loss. The samples were then re-taped with PVC tape and returned to the palatal appliance. Samples remained in the appliance for the next 10 days of the treatment period and were then measured again. The primary measure of efficacy was enamel tissue loss (μm) taken as the average of all values from two samples at day 15. The measurements at days 5 and 15 were taken from masked area across treated area to masked area. If a preset safety limit of 20 μm enamel loss was recorded on an enamel sample in any phase of the trial, the subject was withdrawn from that study period[39].

Disinfection of appliances and specimens during the study

At the beginning and end of each study day, the palatal appliances (containing the enamel samples) were soaked for 3 minutes in a 0.2% chlorhexidine mouth rinse (Corsodyl; GlaxoSmithKline) to prevent plaque accumulation. Enamel specimens were also soaked in 0.5% chlorhexidine gluconate and 70% aqueous ethanol (Hydrex Pink, Ecolab, Leeds, UK) before and after profilometry readings to minimise the potential for contamination.

Sample size

A total of 35 subjects were projected to provide 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 in which a natural logarithm transformation was applied to the data before data analysis. This estimate assumes that the effect size (mean treatment difference divided by the error standard deviation) is approximately 0.50 or higher in a two-treatment, four-period crossover design.

Statistical analysis

Profilometry measurements of each enamel sample at days 5 and 15 were averaged for each subject separately by visit. These measurements were then averaged across the two enamel sections on a per subject basis separately by visit. The enamel loss score was measured as baseline minus post-treatment so that higher numbers indicated more enamel loss. As a few enamel loss scores were small negative numbers, a normalising constant was added ($c = 0.472$) to all of the enamel scores in order to make all changes (baseline minus post-treatment) positive numbers and then the natural logarithm transformation was applied. The optimal constant was chosen to minimise the absolute skewness and kurtosis for model residuals simultaneously for days 5 and 15 in order to make the residuals normally (Gaussian) distributed[47]. A general linear mixed model (a parametric method) for crossover designs was performed on the data by visit, with period and treatment as fixed effects and subject as a random effect. From the final statistical model, estimated means on the natural log scale were back-transformed by using the exponential function (e^{mean}) to obtain the estimated medians or 50th percentiles on the original scale (μm); further, the normalising constant described above was subtracted ($e^{mean} - 0.472$). Back-transformed standard errors of the estimated medians in μm were obtained using the statistical delta method.

Statistical analyses of the individual measurements on the natural logarithm scale were also conducted to investigate different sources of variability from the random effects factors. Factors assessed included enamel sections, replicates, subject, day and residual variances using a general linear mixed model with treatment and period as fixed effects.

RESULTS

Thirty-five subjects were enrolled in the study and 32 subjects completed it. Subjects ranged in age from 19 years to 62 years, with a mean (SD) age of 41.9 (12.7) years. There were 23 (66%) females, and 30 subjects were Caucasians. Three subjects withdrew from the study, one voluntarily, one as recommended by the investigator as a result of the enamel loss observed on the samples allocated to the subject and one as a result of a moderate adverse event.

No statistically significant differences were observed between the two groups at baseline ($P > 0.85$) with means of 0.15 (SE 0.02) and 0.15 (SE 0.02) for specimens allocated to the sodium fluoride/potassium

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

Visit/treatment	Original scale in μm, estimated median* (SE)	Log scale, mean (SE)	Two-sided *P*-value
Day 5†			
NaF/KNO_3 dentifrice	1.83 (0.233)	0.834 (0.097)	0.0169
$NaF/SnCl_2$ dentifrice	1.37 (0.177)	0.612 (0.096)	
Day 15‡			
NaF/KNO_3 dentifrice	3.30 (0.379)	1.328 (0.100)	<0.0001
$NaF/SnCl_2$ dentifrice	2.03 (0.274)	0.919 (0.099)	

*Estimated medians in μm were obtained by using the exponential function on means from the natural logarithm scale, and subtracting the normalizing constant ($e^{mean} - 0.472$). Back-transformed standard errors were estimated using the statistical delta method.
†Day 5 variance components: subject = 0.180, residual = 0.280.
‡Day 15 variance components: subject = 0.171, residual = 0.325.
Log scale = natural logarithm scale.

Table 2 Enamel loss (μm) summary statistics by day and treatment

Summary statistic	Day 5 (n = 35)		Day 15 (n = 35)	
	NaF/KNO_3 dentifrice	$NaF/SnCl_2$ dentifrice	NaF/KNO_3 dentifrice	$NaF/SnCl_2$ dentifrice
Number of Observations*	67	69	65	68
Median	1.917	1.186	3.001	1.636
Mean	2.707	1.965	4.390	3.009
Standard deviation	3.256	2.990	3.554	4.925
5th, 95th percentiles	−0.039, 8.102	0.285, 7.301	0.871, 12.628	0.508, 8.907

*Number of observations where each subject used each treatment generally twice, and 35 subjects were analysed for both day 10 and day 15.

nitrate dentifrice and the stannous-containing sodium fluoride dentifrice test groups, respectively. These starting values were normally distributed and no data transformation was needed.

The enamel loss results on days 5 and 15 for the two treatment toothpastes were obtained using a statistical model and are shown in *Table 1*. The natural log transformation was used in the statistical model because of the right-skewed data distribution and estimated medians were calculated in μm using the back-transformation described previously. At day 5, the stannous-containing dentifrice demonstrated 25% lower enamel loss than sodium fluoride/potassium nitrate dentifrice, with estimated medians of 1.37 μm (SE 0.177) and 1.83 μm (SE 0.223), respectively, which was statistically significant ($P < 0.05$). At day 15, the stannous-containing dentifrice demonstrated 38% lower enamel loss than the sodium fluoride/potassium nitrate dentifrice, with estimated medians of 2.03 μm (SE 0.247), and 3.30 μm (SE 0.379), respectively, which was statistically significant ($P < 0.0001$). For the sake of completeness, basic summary statistics of the subject and period-level observations are also provided in *Table 2*.

No evidence of a carryover effect was detected for the natural log difference at either day 5 ($P = 0.91$) or at day 15 ($P = 0.34$) and was eliminated from the statistical model. Similarly, the baseline covariate was also not statistically significant at either day 5 ($P = 0.40$) or day 15 ($P = 0.92$) and was removed from the statistical model.

Statistical analysis of the individual measurements on the natural logarithm scale was conducted to investigate the contribution of different sources of variability for enamel loss (*Table 3*). The factors with the largest variances in the statistical model were specimen to specimen (52.9%) and subject to subject (24.3%). Variances for day to day (12.5%) and sample replication (<1%) were also noted, with the residual (or unexplained) variance accounting for 10.2% of the total variance.

Both test products were well-tolerated. In total, three subjects reported adverse events, all of which were hyperaesthesia and had a possible causal relationship to the toothpaste investigated. Two of the adverse events were moderate and one mild; the mild event and one moderate event resolved between treatment periods.

Table 3 Evaluation of different sources of variability for enamel loss on the natural log scale

Variable	Statistical model variance (SE)	% of total variance
Repeat sample reading	0.0007 (0.001)	0.95
Sample differences	0.3900 (0.038)	52.93
Subject	0.1791 (0.058)	24.31
Day	0.0921 (0.131)	12.50
Residual	0.0749 (0.004)	10.16

DISCUSSION

Based on limited epidemiological data, it is suggested that 20–45% of the population may show signs of hard tooth tissue wear through multifactorial aetiology of erosive, abrasion and attrition processes[9]. Delivering erosion protection benefits to a population as a whole is a difficult challenge. Preventing the initiation and progression of dental erosion with a vehicle such as dentifrice is an attractive proposition, as dentifrices are already used by the majority of most populations. Anti-erosive dentifrices, specifically formulated to help reduce erosive tooth wear and dentine hypersensitivity, have recently been introduced into the market.

The objective of this study was to compare the protection afforded by two fluoride-based products – a stannous-containing sodium fluoride formula and a sodium fluoride/potassium nitrate product – that are claimed to provide erosion protection benefits. The present model cycled treatments and challenges with the propensity to have both demineralisation and remineralisation impacting upon the measurements, although the order and time-frame of dentifrice application and acid challenges was such that the model is focused more on protection against demineralisation rather than encouragement of remineralisation by the agents in the products. Measurement of effectiveness in this study was thus based primarily on measuring the ability of each dentifrice to protect against the initiation of irreversible erosive damage. Using the *in situ* appliance model with an intraoral dietary acidic challenge, the stannous-containing sodium fluoride dentifrice demonstrated significantly greater protection against dietary erosion compared with the sodium fluoride/potassium nitrate dentifrice in the absence of brushing.

A previous *in situ* study investigating a stannous fluoride dentifrice with *ex vivo* acid challenge[48], and another study utilising a dentifrice containing stannous fluoride with an *in vivo* acid challenge[39], have both shown that stannous fluoride compounds deliver greater protection than sodium fluoride alone. Another *in situ* study demonstrated contrasting views[44]. That study, however, based its efficacy assessment on the use of surface microhardness and failed to recognise that the SnF_2 dentifrice included in the study was also formulated to provide anti-tartar benefits. Anti-tartar dentifrices work by reducing mineralisation on the surface of enamel (but not within the enamel). By using surface microhardness as the measure of effectiveness, any effective anti-tartar dentifrice would, by definition, be expected to result in little, if any, surface microhardness change, which is exactly what the authors found, although they incorrectly related the result to a lack of remineralisation potential for the dentifrice tested.

Regarding the design of the current study, negative and positive controls were not specifically included, although the sodium fluoride/potassium nitrate dentifrice included in this study has been previously reported to provide a positive result in an *in situ* erosion model[44] and the stabilised stannous-containing NaF dentifrice has been demonstrated to provide erosion protection benefits that were not significantly different from a clinically proven stabilised SnF_2 dentifrice in an *in vitro* erosion-cycling model study[49]. Toothbrushing was not included in the current *in situ* study to enable the effect of the anti-erosive components to be assessed without other additional wear factors associated with an intraoral challenge. The fluoride dentifrice application preceded the erosive challenge rather than following it, which was designed to assess primarily preventive, protective benefits rather than remineralising effects, rendering toothbrushing far less relevant to the study design.

In the analysis of this study, factors causing variance in the statistical model were determined. Limited data are available on where the variation arises in these models. This study demonstrated that sample to sample differences were the greatest variable. This result was not unexpected, as human enamel is known to exhibit variation in structure, not just from tooth to tooth, but also from site to site on a single tooth surface, which carries the potential for some level of variability[50]. Subject-to-subject differences were found to represent the next largest variances, which again one would hypothesise would be high because of biological influences, for example variability in pellicle and neutralisation capacity of the individual[51]. Data suggest repeat tracings of the sample show low variance, validating the profilometry techniques and reproducibility of the measurements at each timepoint. These findings will provide important perspectives when designing future studies using this *in situ* clinical model.

The current study supports the position that stannous fluoride, whether formulated directly into a dentifrice or formed *in situ* during product use, inhibits the initiation and progression of erosive acid damage, and may be able to deliver a substantial benefit to potential sufferers of erosive tooth wear.

CONCLUSION

Results of this *in situ* study suggest that the stabilised, stannous-containing sodium fluoride dentifrice could be used to provide significantly greater protection to enamel from erosive acid challenge compared with that provided by conventional fluoride-containing products.

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Conflicts of interest

Dr. He and Dr. Barker are employees of The Procter & Gamble Company. The other authors have no conflicts to disclose.

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