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Effect of toothbrushing duration and dentifrice quantity on enamel remineralisation: An *in situ* randomized clinical trial

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ABSTRACT

Objectives: The influence of toothbrushing duration and dentifrice quantity on fluoride efficacy against dental caries is poorly understood. This study investigated effects of these two oral hygiene factors on enamel remineralisation (measured as surface microhardness recovery [SMHR]), enamel fluoride uptake (EFU), and net acid resistance (NAR) post-remineralisation in a randomized clinical study using an *in situ* caries model.

Methods: Subjects (n = 63) wore their partial dentures holding partially demineralised human enamel specimens and brushed twice-daily for two weeks, following each of five regimens: brushing for 120 or 45 s with 1.5 g of 1150 ppm F (as NaF) dentifrice; for 120 or 45 s with 0.5 g of this dentifrice; and for 120 s with 1.5 g of 250 ppm F (NaF) dentifrice.

Results: Comparing brushing for 120 s against brushing for 45 s, SMHR and EFU increased by 20.0% and 26.9% respectively when 1.5 g dentifrice was used; and by 22.8% and 19.9% respectively when 0.5 g dentifrice was used. Comparing brushing with 1.5 g against brushing with 0.5 g dentifrice, SMHR and EFU increased by 35.3% and 51.3% respectively when brushing for 120 s, and by 38.4% and 43.0% respectively when brushing for 45 s. Increasing brushing duration and dentifrice quantity also increased the NAR value. The effects of these two oral hygiene factors on SMHR, EFU, and NAR were statistically significant ($p < 0.05$ in all cases).

Conclusion: Brushing duration and dentifrice quantity have the potential to influence the anti-caries effectiveness of fluoride dentifrices. Study NCT01563172 on ClinicalTrials.gov.

Clinical significance: The effect of two key oral hygiene regimen factors – toothbrushing duration and dentifrice quantity – on fluoride's anticaries effectiveness is unclear. This 2-week home-use *in situ* remineralisation clinical study showed both these factors can influence fluoride bioactivity, and so can potentially affect fluoride's ability to protect against caries.

1. Introduction

Regular brushing with a fluoride-containing dentifrice has been convincingly demonstrated to reduce the development of dental caries [1]. Its mode of action is at least two-fold: firstly, fluoride can reduce demineralisation when bound to the enamel surface, as fluoridated enamel is less acid-soluble than native enamel; secondly, it can promote remineralisation of partially demineralised enamel in the presence of saliva-derived calcium and phosphate ions, both when bound to the enamel surface and when present in solution in fluid overlying the lesion [2,3]. Fluoride is delivered from a dentifrice into the oral cavity only during the brief periods of toothbrushing. Evidence indicates fluoride is incorporated into enamel during the time of brushing [4] driven by high concentration in the dentifrice slurry, but is also taken up by oral soft tissues and plaque [5]. These reservoirs slowly liberate fluoride into the oral fluids over several hours post-brushing, boosting its protective effect on enamel [6,7].

The protection fluoride provides against caries will therefore be influenced by the intra-oral fluoride concentration achieved during brushing and by the frequency and duration of fluoride

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exposure. Simply increasing fluoride concentration in the dentifrice is an effective way to decrease caries incidence [8,9]; however, fluoride concentration is limited in mass-market dentifrices to 1500 ppm or less. Other ways to potentially increase fluoride protection include increasing frequency of use, decreasing post-brushing rinsing, increasing the amount of dentifrice used, and increasing brushing duration [10,11]. Consequently, how an individual uses a fluoride dentifrice – *i.e.* their oral hygiene routine – is very important to how much protection toothbrushing can afford against caries [12].

Surprisingly, to date only frequency of brushing and degree of rinsing have been extensively studied in relation to fluoride effectiveness. Several correlation studies have between them indicated that these two factors are important in determining caries risk [12–16], though it should be noted a later, prospective study did not show an effect of post-brushing rinsing regimen on caries incidence [17].

The quantity of dentifrice used must at some point affect fluoride efficacy. However, in the conventional usage range of approximately 0.5–1.5 g (*i.e.* from a typical pea-sized amount to an amount sufficient to cover the length of a typical toothbrush head [18,19]), the effect on fluoride efficacy *in vivo* is controversial. Clinical studies utilizing the proxy caries measures of fluoride delivery and remineralisation *in situ*, in which quantity was prospectively controlled or carefully monitored, have consistently shown positive correlations with dentifrice quantity [11,20,21]. In contrast, two caries clinical studies that investigated the correlation of dentifrice quantity with caries prevention [15,22], and a study by Sjögren and Birkhed correlating oral hygiene practice with caries incidence [16], found no evidence for a link, though in none of the clinical studies was dentifrice quantity controlled, nor measured on more than one occasion.

The effect of toothbrushing duration on fluoride effectiveness has received little attention. As noted for dentifrice quantity, at some point toothbrushing duration must influence fluoride effectiveness, but it is not clear whether there is any benefit from increasing brushing time from the typical value of about 45 s [23] to the recommended 2 min or more (*e.g.* British Dental Association recommendation [24]). Fluoride penetration through plaque *in situ* [25], uptake to demineralised enamel *in vitro* [4], and fluoride delivery to the oral soft tissues *in vivo* [11,26] are all time-dependent in this range. Consistent with these results, a pilot single-use study by this group demonstrated that brushing duration can influence *in situ* enamel fluoride uptake and remineralisation in the absence of plaque [11].

Building on this limited evidence base, the aim of this study was to determine whether two key aspects of dentifrice usage regimen, brushing duration and dentifrice quantity, can – in ranges relevant to typical oral hygiene practice – affect fluoride performance using an *in situ* clinical design as representative as practicable of the *in vivo* oral situation. 'Fluoride performance' encompassed measuring effects on enamel remineralisation (by surface microhardness recovery, SMHR [27]); enamel fluoride uptake (by EFU [28]); and enamel acid resistance post-remineralisation (by net acid resistance, NAR [29]). The primary objective was to determine whether brushing for 120 s *versus* 45 s with 1.5 g of 1150 ppm F dentifrice can increase SMHR. The effect of dentifrice dose was also examined by brushing with 0.5 g of this dentifrice for the same time periods. The null hypotheses were that neither brushing duration nor dentifrice quantity would have an effect on the study end-points.

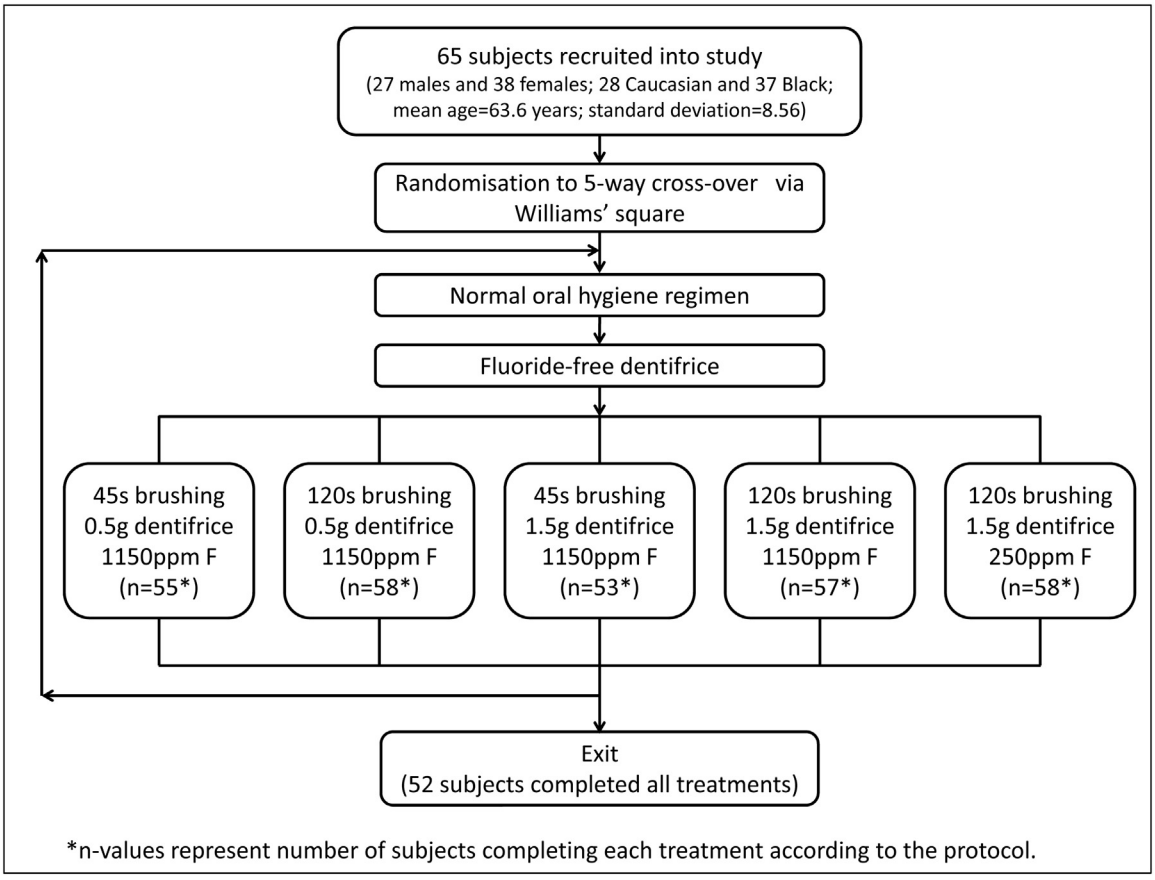

Fig. 1. Subject study flow chart showing recruitment and treatment details.

2. Materials and methods

This was an examiner- and analyst-blind, five-treatment regimen, cross-over, *in situ* model study [30], performed at the Oral Health Research Institute, Indiana, USA. The study was approved by the Indiana University Institutional Review Board (approval number 0812-55) and carried out according to guidelines set out in the Declaration of Helsinki. This study is registered on ClinicalTrials.gov, study number NCT01563172. There was one amendment to the study to more explicitly explain brushing instructions to subjects.

2.1. Study population

Individuals 18–80 years old were recruited to the study from the Indianapolis area (where community water contains about 1 μg/mL fluoride). Inclusion criteria included wearing a removable mandibular partial denture, being otherwise in good general and dental health and having unstimulated saliva flow ≥0.2 mL/min, stimulated saliva flow ≥0.8 mL/min. Subjects were recruited who granted written informed consent, demonstrated understanding of the protocol and were considered willing, able and likely to comply with all study procedures. Potential subjects were excluded if they reported they were pregnant, were intending to become pregnant during the study period, or were lactating. Subjects were enrolled and allocated to treatment by clinical personnel under supervision of the Principal Investigator (DTZ).

The recruitment sample size target of 65 was based on 45 subjects completing all treatments assuming a drop-out rate of 30%. This sample size gave 90% power at $p < 0.05$, using two-sided testing, to detect a mean difference in % SMHR of 8.36, based on within-subject standard deviation of 11.96 (determined from previous, unpublished data). Randomisation was performed *via* a Williams' square approach, generated by GlaxoSmithKline Consumer Healthcare Biostatistics department.

2.2. Experimental design

The study flow is summarized in Fig. 1. Each subject undertook treatments in a cross-over design in five successive 3-week cycles. At the start of each 3-week cycle, subjects followed their normal oral hygiene regimen, then, following a prophylaxis, they used a fluoride-free 'washout' dentifrice twice daily during the final 2–3 days of the first week. The treatment phase started at the beginning of Week 2 and involved subjects using their assigned dentifrice (1150 ppm F or 250 ppm F) twice daily, brushing for the specified brushing duration and using the specified dentifrice quantity. Treatment continued until the end of Week 3, after which subjects started the next cycle. Adverse events (AEs) were recorded at each visit to the clinic.

2.3. Treatment regimens

Three dentifrices were used in this study: (i) an 1150 ppm fluoride (as NaF) dentifrice (Aquafresh® Advanced 2 x Enamel Strengthening Action, silica-based; GSK Consumer Healthcare, Weybridge, Surrey, UK); (ii) a 250 ppm fluoride (as NaF) dentifrice, prepared in the same base; (iii) a variant of this base with no added fluoride, used as a washout product. For (ii) and (iii), sodium fluoride was replaced with water.

The single experimental factor analysed was treatment regimen, as follows:

i. 45 s brushing, 0.5 g of 1150 ppm F dentifrice;
ii. 120 s brushing, 0.5 g of 1150 ppm F dentifrice;
iii. 45 s brushing, 1.5 g of 1150 ppm F dentifrice;
iv. 120 s brushing, 1.5 g of 1150 ppm F dentifrice;
v. 120 s brushing, 1.5 g of 250 ppm F dentifrice.

A new Aquafresh® Flex soft toothbrush was supplied to each subject for each treatment cycle.

2.4. Preparation and use of in situ devices

Measurements on enamel blocks were made *in vitro*, separated from the clinical environment. Human enamel specimens were cut and polished, and their SMH measured as previously described [27,30]. Five baseline indentations were placed on each specimen; only those with mean indentation lengths of 43 ±3 μm were accepted. Specimens were then subjected to pre-treatment demineralisation by immersion in 0.05 M/L lactic acid, 50% saturated with hydroxyapatite, containing 0.1% w/v Carbopol® 907 (BF Goodrich Co., Cleveland, OH, USA), pH 5.0, at 37 °C for 24 h, following which SMH was again measured by placing five indentations to the left of the baseline indentations. This process created very shallow lesions (25–30 μm depth) in which a surface layer had not completely formed [31]. Specimens with mean indentation lengths of 120 ±20 μm were accepted for the study.

The specimens were randomly divided into five equally sized groups and graded by post-demineralisation SMH, then randomly assigned to the 65 subjects, who were considered as statistical blocks. Prior to insertion, specimens were sterilized by ethylene oxide. Two specimens were mounted in the subjects' appliances, as shown in Fig. 2, and covered with gauze (Polyester Knit Fabric; Item# 401628, Impra, Tempe, USA). Subjects wore their appliance continuously throughout each 2-week treatment period [30], but could remove it to clean it with water and to rinse their mouth after meals and snacks. Prior to brushing, subjects weighed the dentifrice to within 0.1 g of their assigned target weight. They spent one quarter of their assigned brushing time brushing each of the four quadrants of their dentition, taking care not to brush the enamel specimens. Subjects expectorated immediately following the end of their timed brushing period and without delay rinsed their mouths by swilling with 15 mL tap water continuously for approximately 10 s.

After the 2-week period, subjects removed their appliance and the enamel specimens were excised. The specimens were re-analysed for SMH and then tested for EFU using micro-drill enamel biopsy as previously described [28], taking four 100 μm cores per specimen. Core diameters were determined using a calibrated microscope. The specimens were then subjected to post-treatment

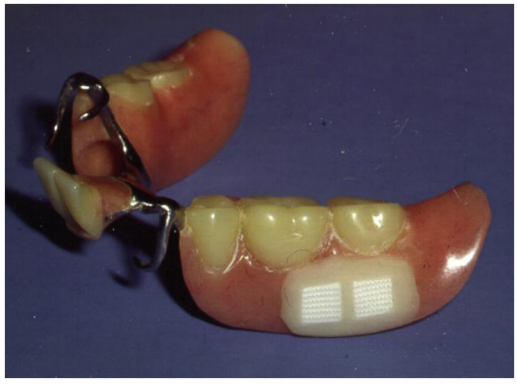

Fig. 2. An example partial denture with gauze-covered enamel specimens as used in this study.

demineralisation using the same acid challenge as for the pre-treatment demineralisation and SMH was again recorded.

2.5. Subject adherence

Subjects undertook an extensive training and tracking program to promote protocol adherence. Five pre-study training sessions covered the use of study weighing scales and timers, which were subsequently used by each subject at home to weigh dentifrice and time each brushing during treatment periods. Subjects recorded the time of day, dentifrice weight and brushing duration in a diary. Each subject also performed a single supervised brushing at the study site at the beginning and on the eighth day of each test period (±1 day). Compliance was assessed by weighing of dentifrice tubes before and after each treatment period and comparison to product usage diaries.

2.6. Data analysis

The response factor for the primary objective was SMHR, calculated as:

$$\%\ \text{SMHR} = [(D1 - R)/(D1 - B)] \times 100$$

Where B = indentation length (μm) of sound enamel specimen at baseline; D1 = indentation length (μm) after pre-treatment *in vitro* demineralisation; R = indentation length (μm) after *in situ* remineralisation [30]. For each indentation measurement, the five indentations within each of the two demineralised enamel blocks were averaged and used in the calculation of % SMHR on an enamel block level. These results were averaged over the two blocks to give the subject-level value. If a subject was missing an enamel block, the data from the remaining block was used to perform calculations.

Secondary objective response factors included EFU and NAR of the experimental specimens. EFU was calculated based on the amount of fluoride divided by the area of the enamel cores, expressed as $\mu g\ F/cm^2$. NAR was calculated as:

$$\%\ \text{NAR} = [(D1 - D2)/(D1 - B)] \times 100$$

Where D1 and B are defined as above and D2 = indentation length (μm) after post-treatment *in vitro* demineralisation [29].

Statistical analysis was performed on the per-protocol (PP) population, which included all randomized subjects who had no major protocol deviations. For each efficacy parameter, the study treatment regimens were compared using a mixed-model analysis of variance (ANOVA). The model included fixed effects for study period and treatment regimen, and a random effect for subject. The analysis of the data included the estimation of the least-square means for the treatments and the comparison of the least-square means for the study treatment regimes. Post-ANOVA, selected pair-wise treatment comparisons were performed. All statistical tests of hypotheses were two-sided and employed a level of significance of $\alpha = 0.05$. No adjustment was made for multiplicity as the primary endpoint and comparisons were pre-defined.

3. Results

Of 74 subjects screened, 65 were recruited into the study. They were aged 43–79 (mean 63.6), and 58.5% were female. Recruitment started in January 2009 and the experimental phase involving subjects ended in June 2009. *In vitro* analysis and subject follow-up was completed by end July 2009. Fig. 1 provides details of study design and subject flow. Data from 2 subjects were completely excluded from the analysis. Partial data from 11 more subjects were excluded because of protocol violations (in all cases, subjects either took prohibited medication or were non-compliant with the treatment regimen requirements).

Enamel SMH readings across the different phases of the experimental procedure are shown as a function of treatment regimen in Table 1. The values calculated from these data for SMHR are presented in Fig. 3a; values for NAR are shown in Table 1. The EFU data are shown in Fig. 3b.

3.1. Effects of brushing duration

Brushing duration had a significant influence on all study measures: SMHR, EFU and NAR. Increasing duration from 45 s to 120 s, with a 1.5 g dose of 1150 ppm F dentifrice, increased SMHR from 34.7% to 41.6% ($p < 0.001$), a relative increase of 20.0%. This achieved the primary objective of the study. Correspondingly, EFU rose from 1913 to 2427 $\mu g/cm^2$, a relative increase of 26.9% ($p < 0.001$), and % NAR (which is a complex measure, so simple calculation of relative change has little meaning) increased from 14.38 to 18.43 ($p = 0.043$).

For the 0.5 g dose, increasing brushing duration from 45 s to 120 s had a similar effect in relative terms as for the 1.5 g dose: SMHR rose by 22.8% and EFU by 19.9% (both $p < 0.05$). NAR also increased significantly (from 4.03% to 8.70%; $p = 0.018$).

3.2. Effects of dentifrice quantity

The dentifrice quantity used also had a significant influence on all study measures. In relative terms, increasing the quantity of the dentifrice from 0.5 g to 1.5 g when brushing with 1150 ppm F dentifrice for 120 s increased SMHR by 35.3%; EFU rose by 43.0% (both $p < 0.001$). Again in relative terms, increasing dentifrice quantity from 0.5 g to 1.5 g when brushing for 45 s with this dentifrice increased SMHR by 38.4% and EFU by 51.3% (both $p < 0.001$).

For NAR, when the quantity of paste increased from 0.5 g to 1.5 g, the value rose from 8.70% to 18.43% when brushing for 120 s and from 4.03% to 14.38% when brushing for 45 s (both $p < 0.001$).

Table 1
Effect of the different study treatments on indent length measurements of surface microhardness (SMH), and on net acid resistance (NAR; mean ± standard deviation of the mean).

Dentifrice	Dose	Duration	No.	Mean indent length, μm				% NAR ± SD
				B	D1	R	D2	
1150 ppm F	0.5 g	45 s	55	44.405	111.676	94.495	108.375	4.03 ± 15.531
		120 s	58	44.307	111.345	90.637	105.265	8.70 ± 15.262
	1.5 g	45 s	53	44.321	111.662	89.062	102.090	14.38 ± 15.417
		120 s	57	44.380	112.979	84.209	99.825	18.43 ± 15.224
250 ppm F	1.5 g	120 s	58	44.258	111.252	93.172	107.561	5.02 ± 15.262

B = baseline; D1 = after pre-treatment *in vitro* demineralisation; R = post *in situ* remineralisation; D2 = after post-treatment *in vitro* demineralisation.

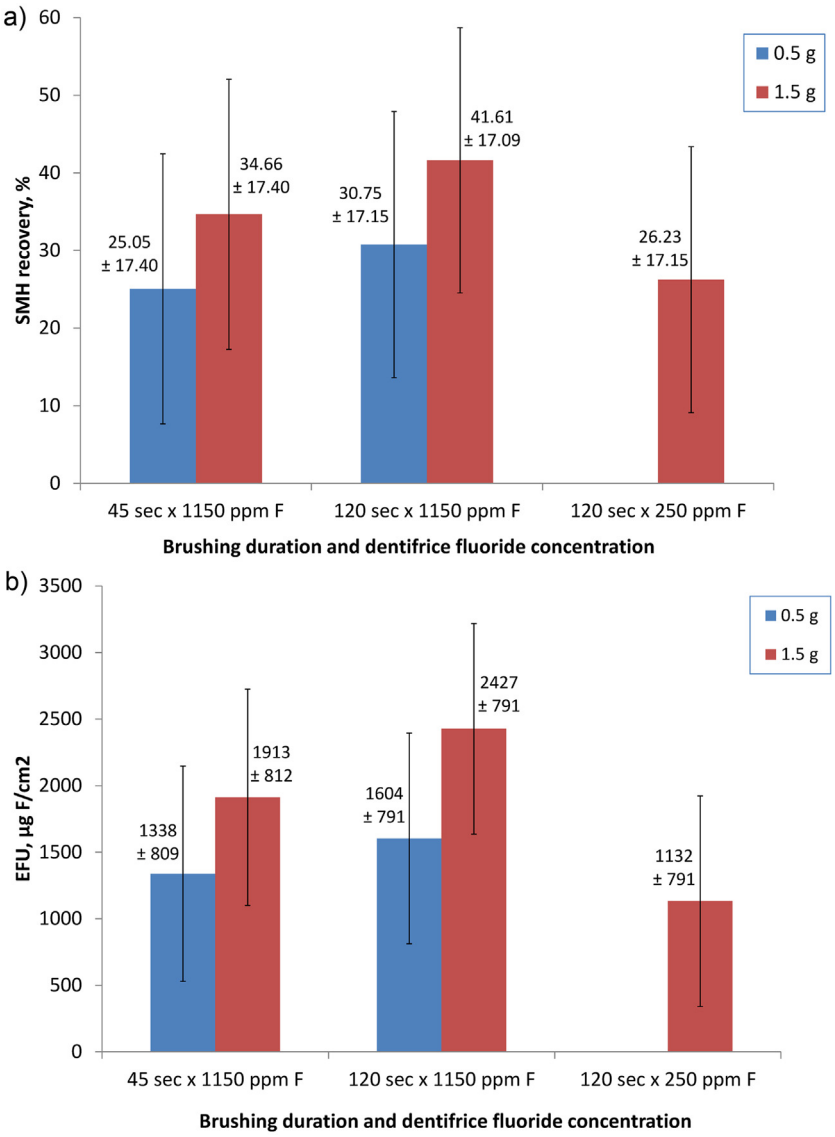

Fig. 3. Effect of brushing duration and quantity of dentifrice on % surface microhardness (SMH) recovery (3a) and enamel fluoride uptake (EFU, 3b) *in situ*. Dentifrices contained either 1150 ppm or 250 ppm fluoride as sodium fluoride. Values are means ± standard deviation of the mean.

3.3. Effect of dentifrice fluoride concentration

Increasing fluoride concentration from 250 ppm to 1150 ppm had a strong effect on all study measures: in relative terms, SMHR rose by 58.6% and EFU rose by 114% when brushing for 120 s (both $p < 0.001$). The % NAR rose from 5.02 to 18.43 ($p < 0.001$).

3.4. Relative strength of effect of treatment factors on SMHR and EFU

The effects of treatment factors on EFU and on SMHR followed a very similar pattern. However, the sensitivity of EFU to changes in the treatment regimen was generally greater than for SMHR. The correlation between EFU and SMHR at an individual enamel block level was $r = 0.520$, in the 'fair to good' range [32].

3.5. Safety

There were 56 treatment-emergent AEs (in 37 subjects), 21 of which were oral (in 18 subjects). All were mild in nature; three (in three subjects) were recorded as treatment-related (two defined as 'tongue coated', one defined as 'chapped lips'). Treatment-emergent AEs were followed up until resolved, the condition stabilized, was otherwise explained, or contact with the subject was lost.

4. Discussion

The preceding pilot single-use remineralisation study [11] showed a positive relationship between SMHR and brushing duration across the window 30–180 s that was of borderline statistical significance ($p = 0.048$). However, there were no significant differences in any pair-wise comparisons of different brushing durations. That study also demonstrated a significant increase in EFU, but not in SMHR, when brushing with 1.5 g *versus* 0.5 g dentifrice (comparison only performed at 60 s brushing duration).

The present study set out to answer more definitively whether brushing duration and dentifrice quantity are important to fluoride's effects in the oral cavity. The *in situ* model chosen was designed to better simulate the caries process than the single-use model by allowing near-normal intra-oral exposure conditions for

the test enamel specimens while maintaining a high degree of control over treatment conditions and flexibility in how the specimens are prepared and analysed [27]. The model achieved this by employing a 14-day treatment period of normal twice-daily brushing without restrictions on diet. The intra-oral appliance used promotes the development of a biofilm, derived from the host's natural oral flora, over the surface of the enamel specimen. The surface is therefore subjected to repeated, essentially normal, diet-induced remineralisation-demineralisation cycles. The superficial lesions induced in the enamel surface prior to insertion into the appliance represent the very early stages of the caries process. SMH was used as it is the most sensitive and precise technique available to measure fluoride-mediated changes in mineralisation in such lesions [33].

In this study, 13 of the 65 subjects initially recruited did not successfully complete all treatments, which was not unexpected as a high level of compliance was required for the oral hygiene procedures. The minimum sample size target of n = 45 subjects was achieved.

The sensitivity of the model was demonstrated in this study by the highly significant increase in SMHR, EFU and NAR after use of the 1150 ppm F dentifrice compared to the 250 ppm F dentifrice; existing studies show that this increase in dentifrice fluoride concentration should give an increase in caries protection of clinical significance [9].

The current study found that both brushing duration and dentifrice quantity had clear effects on both enamel remineralisation and fluoride uptake *in situ*. We believe that clearer effects were observed in the present study *versus* the pilot study due primarily to this improved methodology.

4.1. Relative influence of test factors on SMHR and EFU

The results reported here indicate that, in the normal range of use, dentifrice quantity has a greater impact than brushing duration on SMHR and EFU (notwithstanding the slight difference in the ratios between the two durations and the two quantities: 2.67:1 (120 s/45 s) and 3:1 (1.5 g/0.5 g) respectively). Fluoride concentration in the dentifrice was found to be a stronger influence on SMHR and EFU than either brushing duration or dentifrice quantity, but note that the ratio between the two concentrations tested was greater (4.67:1, *i.e.* 1150 ppm/250 ppm).

There was little evidence that remineralisation or fluoride uptake was approaching saturation for the higher-efficacy treatments in this study. For example, if fluoride uptake were approaching saturation, then the effect of brushing duration on EFU would not be as strong at the higher dentifrice dose as it would be at the lower dose, because there would be more 'headroom' for improvement at the lower dose. In this study, the effect of brushing duration on EFU was as strong at the higher dentifrice dose as at the lower. This conclusion is consistent with clinical evidence demonstrating that the anti-caries effect of fluoride dentifrices continues to increase at concentrations above the 1150 ppm F used here [8,9]. Given the toxicological situation restricting the fluoride content of general-sale dentifrices, this situation should encourage continued efforts to improve effectiveness of fluoride dentifrices by enhancing fluoride bioavailability in formulations in addition to efforts to improve individuals' oral hygiene regimens.

4.2. Correlation between SMHR and EFU

Given the close relationship at a population level between treatment effects on SMHR and EFU (Fig. 3a and b), the correlation at a specimen level was not strong. One factor that could have reduced the correlation between SMHR and EFU was that fluoride can be taken up preferentially in demineralised enamel without any remineralisation necessarily occurring [34].

4.3. Effect of brushing duration and dentifrice quantity on NAR

The NAR is a more complex parameter than SMHR or EFU: the numerator in the calculation of the NAR ratio is the sum of three numbers (the hardness change during the pre-treatment acid challenge, plus that during the remineralisation phase, plus that during the post-treatment acid challenge). Hence, geometric comparisons of NAR values between treatments do not have a straightforward physico-chemical meaning (in contrast to such comparisons for SMHR or EFU values), so are presented in tabular form.

The NAR is, nevertheless, a useful measure of the overall efficacy of a fluoride treatment (the NAR calculation encompasses both the remineralisation and demineralisation phases, cf. the relative erosion resistance measure for erosive lesions [35]). As observed for SMHR and EFU, the NAR dependence on dentifrice quantity (0.5 g *vs* 1.5 g) was consistently numerically greater than the dependence on brushing duration (45 s *vs* 120 s) in this study.

Interestingly, in this study, treatment effects on the NAR value appeared to be driven solely by treatment effects during the remineralisation phase: *i.e.*, the enamel hardness change during the post-treatment acid challenge (which is the difference between R and D2) showed no relation to treatment regimen. This observation should be treated with some caution, as differences in the degree of remineralisation due to treatment create an unbalanced starting point for the post-treatment acid challenge. Nevertheless, it is perhaps surprising that even treatment with the 1150 ppm fluoride dentifrice, which led to a substantial increase in both enamel fluoride content and mineralisation level compared to treatment with the 250 ppm variant (1.5 g dose/120 s duration in each case), did not create a more acid-resistant enamel surface [36].

4.4. Clinical relevance of brushing duration to caries risk

It is well-established that duration of normal toothbrushing has an effect on the amount of plaque removed [37], and that improved plaque removal may reduce caries risk [38]. The present study demonstrates that longer brushing with fluoride dentifrice has the potential to reduce caries risk by a second mechanism, *i.e.*, by increasing the effectiveness of fluoride. This *in situ* model rules out benefits due to enhanced plaque removal because the test specimens are not brushed. The evidence showing that (i) increased exposure time to dentifrice-strength fluoride solutions leads to greater fluoride penetration through plaque during brushing [25], and (ii) increasing brushing duration increases the amount of fluoride bound to oral soft tissues [11,26], suggest two independent mechanisms by which this increase in fluoride effectiveness could occur.

4.5. Clinical relevance of dentifrice quantity to caries risk

Results reported here support the conclusion that dentifrice quantity also has the potential to affect caries experience *in vivo*. This conclusion is in agreement with previous intra-oral studies of effects on fluoride delivery and *in situ* enamel remineralisation [11,20,21,26]. So how can the disparity be explained between the conclusions of these studies and the conclusions of the studies of correlation with caries incidence, in which no relationship with dentifrice quantity was detected [15,16,22]? It is possible that the effects of dentifrice quantity seen in this and the existing fluoride delivery/remineralisation studies may have over-estimated the impact on caries itself, but these measures have previously

established clinical relevance, and clearly at some level the amount of dentifrice used must become important. Alternatively, the quantity estimates made in the studies of correlation with caries incidence may have been too crude or unreliable, thereby masking a true link with caries. These caries studies were not designed primarily to determine the effects of behavioural factors; however, they were each able to identify a link between caries and rinsing behaviour, suggesting that any meaningful link with dentifrice quantity should also have been detected. It is possible there is some confounding factor, such as an interaction between aspects of brushing behaviour, which could rationalise this discrepancy. Further study is needed in this area.

Considering the effects of brushing duration and dentifrice quantity together, it is intriguing that the SMHR value for the 'minimum exposure' regimen (45 s brushing/0.5 g dose) for the 1150 ppm dentifrice was very similar to that for the 'maximum exposure' regimen with the 250 ppm fluoride dentifrice (120 s brushing/1.5 g dose). So, when using an 1150 ppm dentifrice, improving the regimen by increasing brushing duration and dentifrice quantity together increased enamel remineralisation in this study by the same amount as did increasing the fluoride concentration in the dentifrice from 250 ppm F to 1150 ppm in constant brushing conditions. This increase in concentration has been shown to be clinically meaningful [9].

In conclusion, in this study of early enamel caries lesions *in situ*, both duration of toothbrushing and quantity of dentifrice used were found to have potentially important effects on the enamel remineralisation process. The scale of the effects of these oral hygiene behavioural factors is sufficient to suggest that they could influence an individual's caries experience in real life.

Declaration of interests

J.E.C., R.K., R.J.M.L. are employed by GSK Consumer Healthcare, and M.L.B. was employed by GSK Consumer Healthcare at the time of the study. D.T.Z. has received compensation from GSK Consumer Healthcare as a consultant. This study was supported by GSK Consumer Healthcare.

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