

A randomised clinical evaluation of a fluoride mouthrinse and dentifrice in an *in situ* caries model

Short title: Fluoride mouthrinse and dentifrice in an *in situ* caries model

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Abstract

Objectives. Fluoride mouthrinses provide advantages for fluoride delivery by maintaining elevated intra-oral fluoride concentrations following fluoride dentifrice use. This *in situ* caries study investigated potential anti-caries efficacy of a 220 ppm fluoride mouthrinse

Methods. This was an analyst-blinded, four-treatment, randomised, crossover study using partially demineralised, gauze-wrapped, human enamel samples mounted in a mandibular partial denture. Participants brushed twice daily for 14 days with either a 1150 ppm fluoride or fluoride-free placebo dentifrice and either rinsed once daily with the 220 ppm fluoride mouthrinse or not. Following each treatment period, percent surface microhardness recovery (%SMHR) and enamel fluoride uptake (EFU) were assessed.

Results. Fifty three participants completed the study. Compared with the placebo dentifrice/no rinse treatment, the fluoride-containing regimens demonstrated greater enamel remineralisation (%SMHR) and fluoridation (EFU): fluoride dentifrice/fluoride rinse (%SMHR difference: 21.55 [95% CI: 15.78,27.32]; EFU difference 8.35 [7.21,9.29]); fluoride dentifrice/no rinse: 19.48 [13.81,25.15]; 6.47 [5.35,7.60]; placebo dentifrice/fluoride rinse: 16.76 [11.06,22.45]; 5.87 [4.72,7.00]) (all $P < .0001$). There were no significant differences in %SMHR between fluoride regimens. The fluoride dentifrice/fluoride rinse regimen was associated with higher EFU than the fluoride dentifrice/no rinse (1.88 [0.75,3.01], $P = .0013$) and placebo dentifrice/fluoride rinse regimens (2.48 [1.34,3.62], $P < .0001$). Treatments were generally well-tolerated.

Conclusions. The *in situ* caries model demonstrated that the fluoride mouthrinse is effective in promoting enamel caries lesion remineralisation and fluoridation whether used following a fluoride or non-fluoride dentifrice. Additive (potential) anti-caries benefits of a fluoride rinse after a fluoride dentifrice were confined to enhancements in lesion fluoridation (EFU).

Clinical Significance. In conjunction with a fluoride dentifrice, fluoride mouthrinses enhance enamel fluoridation, which may be useful in caries prevention.

Key Words: caries; dentifrice; fluoride; *in situ* model; mouthrinse; remineralisation

1. Introduction

Brushing with fluoride-containing dentifrice products has been shown in numerous clinical trials to be effective in reducing dental caries [1,2]. Fluoride has two relevant mechanisms of action: inhibition of acid-induced demineralisation (that could lead to dental caries), which is beneficial as fluoridated enamel is more acid-resistant than native enamel, and enhancement of remineralisation of partially demineralised enamel during the early stages of caries in the presence of calcium and phosphate ions from saliva [3,4].

For individuals at high risk of developing dental caries, fluoride mouthrinses are recommended in addition to fluoride dentifrices [5,6]. Cochrane Collaboration systematic reviews of fluoride mouthrinses have reported that the supervised use of fluoride mouthrinse by children is associated with a clear reduction in caries increment based on a meta-analysis of 35 trials [7], and that use of fluoride mouthrinse can reduce dental caries irrespective of exposure to fluoridated water [8]. A systematic review of fluoride mouthrinses in populations of various ages found a caries-preventive effect in the permanent teeth of schoolchildren and adolescents with no additional fluoride exposure [9]. While the authors found a caries-preventive effect of fluoride mouthrinses on root caries in older adults, they questioned the additional benefit in children using fluoride dentifrice daily. Although a number of clinical studies have explored the adjunctive benefit of fluoride mouthrinses, few studies have explored the role of fluoride mouthrinses in the fundamental aspects of the caries process.

The use of *in situ* surrogate caries models as an approach to evaluate the anti-caries efficacy of fluoride dentifrices and other fluoride-containing dental products, such as mouthrinses, is generally well-recognised and accepted [10]. In particular, modifications of the Koulourides intra-oral model [11] have led to the development of an *in situ* caries model [10] with sufficient sensitivity and reproducibility to respond in a dose-dependent manner to meet the requirements for model validation [12]. For the current study, the potential anti-caries efficacy of dentifrices and mouthrinses in remineralising previously demineralised enamel specimens was investigated using the surface microhardness (SMH) test to accurately determine the changes occurring at the enamel surface during the early stages of the caries process [10]. The SMH test has been used widely to evaluate enamel remineralisation in studies involving *in situ* caries models and has been shown to have greater sensitivity in comparison to other techniques such as cross-sectional microhardness and transverse microradiography to evaluate enamel remineralisation of shallow caries-like lesions. [10,11,13–16].

The primary objective of this study was to evaluate and compare the potential anti-caries efficacy of a regimen consisting of a fluoride mouthrinse once daily plus brushing with a fluoride-free placebo dentifrice twice daily versus only twice daily brushing with the placebo dentifrice, to remineralise previously demineralised enamel specimens, as measured by percent SMH recovery (%SMHR). Secondary objectives were to compare the potential anti-caries efficacy of other treatment regimens comprising the fluoride mouthrinse plus a fluoride dentifrice and the fluoride dentifrice alone. Further secondary objectives were to evaluate and compare treatments with respect to enamel fluoride uptake (EFU), and pre- and post-treatment changes in salivary fluoride concentrations, and to explore the relationship between EFU and salivary fluoride concentrations and the results of enamel remineralisation based on %SMHR.

2. Materials and Methods

This was a single-centre, analyst-blind, four-treatment, crossover, randomised *in situ* model study performed at the Oral Health Research Institute, Indiana University School of Dentistry, USA. It was approved by the Indiana University Institutional Review Board (# 1503890832) and was conducted in accordance with the Declaration of Helsinki. This study is registered at clinicaltrials.gov; study number NCT02399163.

2.1. Participants

Healthy participants aged 18–85 years were recruited from the Indianapolis area (where community water contains approximately 1 µg/mL fluoride). All participants provided written informed consent prior to screening. Participants were required to have a removable mandibular partial denture suitable to retain two enamel specimens and be willing and capable of wearing their denture 24 hours/day during the experimental periods. They were required to be in good general and dental health with an unstimulated and stimulated saliva flow rate of at least 0.2 mL/minute and at most 0.8 mL/minute, respectively, and not to have had a professional fluoride treatment within 14 days before the first treatment visit.

Participants could not have any active caries or periodontal disease that in the opinion of the investigator could compromise the study. Participants were excluded if they were pregnant, intending to become pregnant, or were breastfeeding; had a known or suspected intolerance to the study materials; were taking antibiotics or had taken antibiotics in the two weeks

before the screening visit; or if they were taking or had taken a bisphosphonate drug for treatment of osteoporosis.

2.2. Experimental design and study procedures

At the screening visit, participants underwent an oral soft tissue (OST) and oral hard tissue (OHT) examination and their salivary flow rate was measured. An OST examination was also performed during the visit before and after each treatment period; an additional OHT examination was performed at the first prophylaxis visit before the first treatment period.

Each participant undertook treatments in a crossover design in four successive 2-week treatment periods. Between each treatment period, participants used their usual dentifrice for at least 4 days, and then reported to the study site 2–3 days before the start of each of the four treatment periods, where they had an OST examination and underwent dental cleaning using a fluoride-free prophylaxis dentifrice. The fluoride-free dentifrice formulation used during the study period and a study toothbrush (Aquafresh® Toothbrush 3-Way head; GSK Consumer Health, Weybridge, Surrey, UK) were dispensed to participants for use before starting the next treatment period.

At the start of the first treatment period, eligibility to continue in the study was assessed and then participants were randomised to the sequence in which they received the four study-treatment regimens. The order in which each participant received the treatment regimens was determined by a randomisation schedule provided by the Biostatistics Department of GSK Consumer Healthcare. A Latin square was used to ensure uniform design (Williams square design). Randomisation numbers were assigned in ascending numerical order as each participant was determined to be fully eligible for the study. The following dentifrices and mouthrinse were used in the study:

- **Fluoride dentifrice:** Aquafresh® Extreme Clean® Pure Breath Action fluoride dentifrice containing 1150 ppm fluoride as sodium fluoride (GSK Consumer Healthcare, Weybridge, Surrey, UK; USA marketed product);
- **Fluoride mouthrinse:** containing 220 ppm of fluoride as sodium fluoride (non-marketed formulation);
- **Control placebo dentifrice:** non-fluoride dentifrice (non-marketed formulation).

In each treatment period, participants were assigned to one of the following treatment regimens: fluoride dentifrice/fluoride rinse; fluoride dentifrice/no rinse; placebo dentifrice/fluoride rinse; placebo dentifrice/no rinse. By the end of the study, all participants

experienced all four treatment regimens. Supplied dentifrices were overwrapped to blind as far as possible participants to dentifrice allocation; however, study group could not be fully blinded as participants would know whether or not they were in a fluoride rinse group. The site laboratory analyst, study statistician, data management staff and other employees of the Sponsor who could have influenced study outcomes were blinded to treatment allocation.

Study product(s) were dispensed to the participants, who completed the initial brushing/rinsing under supervision at the study site then used the study products at home for the rest of the 2-week treatment period. Participants were provided with a diary card to record brushing and/or rinsing times, any adverse events (AEs), and concomitant medications until their next visit. The diary cards were used to assess compliance to study procedures.

At the start of each treatment period, two partially demineralised enamel specimens were mounted in the participant's mandibular partial denture. During the 2-week treatment periods, participants brushed twice daily (after breakfast and just before bedtime) and wore their mandibular partial denture continuously for 24 hours, except as specified during the brushing procedure and for cleaning. At each brushing, participants removed their partial denture and cleaned their natural teeth with water and the study toothbrush. They cleaned the denture outside of the mouth with the study toothbrush and water only, taking care not to brush the enamel specimens, and then reinserted the denture. The participants then brushed their natural and denture teeth for 1 timed minute using a full ribbon of assigned test dentifrice on the study toothbrush, again taking care not to brush the enamel specimens. Participants then rinsed with 10 mL of tap water for 5 seconds, and (where allocated) rinsed with mouthrinse for 1 minute immediately after the night-time brushing, with their denture in place.

Between each treatment period, participants used their usual dentifrice for at least 4 days, and then switched to the wash-out fluoride-free dentifrice for 2–3 days before starting the next treatment period. This process was repeated until all participants had used all four test treatment regimens, as specified in the randomisation schedule.

Two unstimulated saliva samples for fluoride concentration assessment were collected during the visit at the beginning of each study period; one before supervised brushing/rinsing and one immediately after. At the end of each 2-week treatment period, participants returned to the study site where an OST examination was performed and an unstimulated saliva sample for fluoride assessment was collected. The enamel specimens were removed from

the mandibular partial denture and analysed. At the participant's final study visit an OHT exam was also conducted.

2.3. Lifestyle restrictions

Participants were instructed not to use any fluoride dentifrices or other fluoride-containing oral-hygiene products for a minimum of 2 days before the start of each treatment and to refrain from rinsing with water for 30 minutes after use of the mouthrinse. During the treatment and wash-out periods, participants were instructed not to use other oral hygiene products with the exception of interproximal cleaners, e.g., dental floss, if this was their normal practice. Participants could remove their partial denture for short periods to rinse their mouth with tap water after meals and snacks. No denture adhesive could be used in the mandibular partial denture; a zinc-free adhesive could be used for an upper denture if needed. Participants were asked to refrain from eating canned sardines during the course of the study, as these may have high fluoride levels.

2.4. Preparation of enamel specimens and in situ devices

2.4.1. Enamel specimens

Specimens obtained from human permanent teeth were prepared as previously described [17]. Briefly, enamel specimens (4 mm × 4 mm) were cut, ground and polished to provide a minimum surface of 3 mm × 3 mm in the centre of the enamel surface for testing of SMH [18]. Five baseline indentations, 100 µm apart, were placed in the centre of each specimen using a Knoop diamond under a 50 g load. Only those specimens with a mean indentation length of 43 ± 3 µm were used. For assessment of SMHR, the specimens were first partially demineralised using a modification of the method described by White [19] by immersion in an acid buffer (0.05 mol/L lactate), 50% saturated with respect to hydroxyapatite, containing 0.2% (w/v) polyacrylic acid (Carbopol® C907; BF Goodrich, Cleveland, OH, USA), pH 5.0, for 24 hours at 37°C. Following demineralisation, the SMH was again measured by placing a further five indentations 100 µm to the left of the sound enamel indentations on each specimen. Specimens with a mean indentation length of 120 ± 20 µm were used in the study. Before insertion into the participant's partial dentures, all enamel specimens were sterilized with ethylene oxide gas.

2.4.2. Intra-oral appliance

The participants' mandibular partial dentures were modified for study use by creating a hollow in the buccal flange area to accommodate the enamel specimens. Two partially demineralised enamel specimens were placed in the buccal flange area and covered with polyester knit fabric (Item #R01628, Impra, Tempe, AZ, USA) to encourage plaque formation [11,20]. The specimens were mounted such that the enamel surface of the specimen was flush with the surface of the buccal flange of the participant's partial denture and luted in place using a light-cured dental composite (Triad VLC material, Dentsply Int., York, PA, USA). On completion of the study, the participant's partial denture was permanently repaired with acrylic.

2.5. Assessments

2.5.1. Assessment of enamel remineralisation

Changes in the mineral status of the enamel specimens were assessed by measuring SMH using a Wilson 2100 Hardness Tester, as previously described [21]. After the *in situ* phase, five indentations spaced 100 µm apart were placed 100 µm to the right of the sound enamel indentations and the length of each indentation was measured.

The extent of remineralisation was calculated based on the method of Gelhard and colleagues [13]:

$$\%SMHR = [(D1-R)/(D1-B1)] \times 100$$

Where B = indentation length (µm) of sound enamel specimen at baseline; D1 = indentation length (µm) after *in vitro* demineralisation; and R = indentation length (µm) after intra-oral exposure.

2.5.2. Assessment of enamel fluoride bioavailability

Following completion of the SMH procedures, enamel specimens were analysed for EFU using the microdrill enamel biopsy technique as described by Sakkab and colleagues [22]. Four 100 µm cores were taken per specimen; core diameters were determined using a calibrated microscope interfaced with an image analysis system. The amount of fluoride uptake by enamel was calculated based on the amount of fluoride in the pooled powder sample divided by the total cross-sectional area of the enamel cores, and expressed as µg F/cm².

2.5.3. Salivary fluoride concentration

Salivary fluoride concentration was analysed using the Martinez-Mier and colleagues [23] modification of the hexamethyldisiloxane microdiffusion method of Taves [24], and expressed as $\mu\text{g F/mL}$.

2.6. Safety

The safety population included all randomised participants who received study treatment(s). The safety and tolerability of the study treatment regimens were assessed with respect to treatment-emergent AEs recorded from the start of the prophylaxis procedure until 5 days following last administration of the investigational product. AEs were graded as mild, moderate or serious according to the clinical judgment of the investigator. Any AEs occurring during the washout period between treatments were assigned to the last experimental treatment administered.

2.7. Data analysis

2.7.1 Sample size determination

A sufficient number of participants were screened so that a maximum of 62 participants could be randomised to participate in the study to ensure that approximately 50 participants were evaluable for the efficacy analysis. This sample size was calculated to have 90% power to detect a mean difference of 7.91% in %SMHR between treatment groups, based on an estimated within-participant standard deviation of 11.96% (determined from previous unpublished results), and with a significance level of 0.05 using a two-sided paired t-test.

2.7.2 Efficacy analyses

The intent-to-treat (ITT) population was defined as all participants who were randomised, received at least one dose of study product and had at least one post-baseline efficacy assessment. Efficacy analyses were conducted on the per-protocol (PP) population, defined as participants in the ITT population who had no protocol violations deemed to affect efficacy during the study. All outcome variables were analysed under a null hypothesis of no difference between study treatments against an alternate hypothesis of a difference between study treatments. All significance testing was conducted at the two-sided 5% significance level with no adjustments for multiple testing.

The primary efficacy variable was the %SMHR in the PP population. The mean %SMHR was computed by averaging the two within-participant specimen-level results for each treatment. Percent SMHR, EFU and salivary fluoride concentration were evaluated using an analysis of variance (ANOVA) model with %SMHR as the dependent variable, fixed effects of treatment and study period, and a random effect of participant. The assumptions underlying the ANOVA model for each assessment were examined and the homogeneity of variance assumption was violated in that the variability in the placebo group was much less than in the other groups. As a consequence, Wilcoxon signed-rank tests were also performed to investigate the effect of this violation on between-treatment group inferences.

The correlations between mean %SMHR, EFU, and salivary fluoride differences were examined by treatment. Pearson's correlation coefficients (95% confidence intervals and P-values) were calculated between each pair of endpoints.

All analyses were performed using SAS 9.2 with Proc Mixed for ANCOVA (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Participants

A total of 80 participants were screened and 62 were randomised to treatment (Figure 1). The first participant was enrolled on April 20 2015 and the last participant completed the study on August 5 2015. All randomised participants were included in the safety population, which had a mean age of 64.1 years (standard deviation 10.45 years, range 35–83 years); 35 (56.5%) were female and the participants were predominantly white (n=30; 48.4%) or black/African American (n=30; 48.4%).

3.2. Efficacy

Endpoints were analysed for the PP population as less than 5% of participants (n=2) were excluded from this population (Figure 1). These participants were excluded due to non-compliance with the rinsing period or the washout period or their treatment period being outside the acceptable range. A further 10 participants had a protocol violation that led to exclusion of data from at least one, but not all analysis. Final participant numbers are shown

in Figure 2. Wilcoxon signed-rank test p-values are given for comparison between treatments.

3.2.1. %SMH recovery

Baseline values did not vary significantly between the groups. All three active-treatment regimens (fluoride dentifrice/fluoride rinse, fluoride dentifrice/no rinse, placebo dentifrice/fluoride rinse) were associated with statistically significantly greater %SMHR than the placebo dentifrice/no rinse treatment (Figure 2a; Table 1; all comparisons $P < .0001$). There were no statistically significant differences in %SMHR between the three active (fluoride) treatment groups. Analysis from the ANOVA model provided similar results (Table 1).

3.2.2. Enamel fluoride uptake

In all three active treatment groups, EFU was statistically significantly higher than in the placebo/no rinse group (Figure 2b, Table 2; $P < .0001$ for all comparisons). The fluoride dentifrice/fluoride rinse regimen was associated with a statistically significantly higher EFU than both the fluoride dentifrice/no rinse regimen ($P = .0010$) and the placebo dentifrice/fluoride rinse treatment ($P < .0001$). There was no statistically significant difference in EFU between the fluoride dentifrice/no rinse and placebo dentifrice/fluoride rinse treatment groups. Analysis from the ANOVA model provided similar results (Table 2).

3.2.3. Salivary fluoride (Table 3)

The changes in salivary fluoride concentration between pre- and post-treatment samples on Day 1 were statistically significantly different ($P < .0002$) between all groups except between the fluoride dentifrice/fluoride rinse and placebo dentifrice/fluoride rinse groups. Analysis from the ANOVA model showed similar results. The changes in salivary fluoride concentration between Day 1 (pre-treatment) and Day 14 were statistically significantly different ($P < .002$) between either the fluoride dentifrice/with or without fluoride rinse groups and the placebo dentifrice/no rinse group only. Analysis from the ANOVA model did not show any significant differences. The changes in salivary fluoride concentration from Day 1 (post-treatment) to Day 14 were statistically significantly different ($P < .0002$) between all treatment groups with the exception of between the fluoride dentifrice/fluoride rinse and placebo dentifrice/fluoride rinse groups. Analysis from the ANOVA model showed similar results.

3.2.4. Correlations

Moderate positive correlations of the order of $r=0.45$ to $r=0.51$ (all $P < .001$) were observed between %SMHR and EFU in the three active-treatment groups, with a slightly negative ($r = -0.27$) but non-significant correlation in the placebo group. Salivary fluoride concentration correlated poorly with either %SMHR or EFU across all treatment groups, with statistical significance only shown for Day 14 salivary fluoride concentration and %SMHR in the fluoride dentifrice/no rinse group ($r=-0.47$; $P = .0002$) and EFU in the placebo dentifrice/no rinse group ($r=0.31$; $P = .0285$).

3.3. Safety

Treatment-emergent and treatment-related AEs are summarised in Table 4. There were three serious AEs: hematemesis in the fluoride dentifrice/fluoride rinse group, asthma in the placebo dentifrice/fluoride rinse group, and hypertension in the placebo dentifrice/no rinse group. None were related to study treatment; two (hematemesis and asthma) resulted in study withdrawal. All AEs subsequently resolved.

4. Discussion

The present *in situ* study was designed to investigate potential additive effects of an adjunct fluoride therapy and to explore the potential anti-caries benefits a fluoride mouthrinse can provide in its own right. The chosen *in situ* caries model has been extensively used to successfully study the effects of a range of fluoride delivery vehicles [10,12,21]. The study employed surface softened lesions covered in gauze to provide insight into the potential efficacy of anticaries agents during the early stages of caries by reproducing the conditions found in interproximal areas [10]. In this design, there was no abrasion component during the *in situ* phase as specimens were wrapped in gauze, thereby protecting the enamel surface from abrasive forces in the oral cavity. In such a design, it is anticipated that surface loss is an insignificant variable. While not confirmed in this study, a previous *in vitro* study employing more severe caries lesions demonstrated that no surface loss occurred [25].

The present study demonstrated that a 220 ppm fluoride mouthrinse has predicted anti-caries efficacy and that it should provide additive benefits to a conventional fluoride dentifrice even if used only once a day. In general, these results support those from other studies that demonstrate a fluoride mouthrinse can be a useful adjunct to a fluoride dentifrice [7]. The additional remineralisation achieved with the fluoride mouthrinse, as shown by

%SMHR, was directional to that of the fluoride dentifrice, which in itself is a potentially clinically important anti-caries benefit. However, the response effect between the three active (fluoride) treatment groups was small which may indicate that the level of cariogenic challenge in subjects participating in this study was not sufficient to demonstrate an additional benefit for the fluoride mouthrinse. In this model, subjects with modifiable partial dentures were employed, and, while it is considered that this study population is representative of adults, it is possible that the oral ecology of such subjects is subtly different compared to fully dentate individuals, resulting in less demineralisation following a cariogenic challenge and an under estimation of treatment effects for adjunctive treatments. Further studies on larger, more advanced lesions, and/or under more demineralising conditions, are warranted to elucidate the potential adjunctive benefit of fluoride mouthrinses.

The fluoride mouthrinse (220 ppm fluoride) was used once daily in this study, which for fluoride mouthrinses above 90 ppm fluoride is according to its indication in the USA [26]. It was used only after night-time brushing as fluoride exposure at night has been shown to be more effective than rinsing in the morning [27] due to fluoride's greater ability to enhance remineralisation than prevent demineralisation and its greater substantivity due to decreased salivary flow at night [28,29]. Comparative studies (e.g. day versus night, once versus twice daily usage, intermittent usage, and comparison between fluoride concentrations) are warranted to provide better clinical recommendations. Nonetheless, the present study has provided further evidence for the beneficial use of fluoride mouthrinses in caries prevention.

The EFU data showed greater sensitivity and ability to discern between treatments than the %SMHR data. This is in agreement with previous studies employing this model [16,21,30] and can be explained by the greater capacity of the lesions to retain fluoride than their ability to be remineralised. Furthermore, and by virtue of the microbiopsy technique, the fluoride analysis captures surface and subsurface-bound fluoride, fluoride that may potentially be of relevance during a more severe cariogenic attack.

A limitation of this study is that although the identity of the dentifrices was masked, participants could not be blinded as to whether or not they were using the fluoride mouthrinse; hence, observed differences may be due to this lack of blinding.

In summary, this *in situ* caries study has shown that a 220 ppm fluoride rinse provides similar anti-caries benefits to that of an 1150 ppm fluoride dentifrice. The combined regimen of rinse and dentifrice provided further directional, although non-significant, benefits in terms of lesion remineralisation; however, statistically significant enhancements were observed in

lesion fluoride uptake. A relatively high number of adverse events were observed in this study; however, the number of adverse events across treatment groups was similar.

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References

1. Stookey GK. Critical evaluation of the composition and use of topical fluorides. *J Dent Res.* 1990;69 Spec Iss:805–812.
2. Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev.* 2010;(1):CD007868.
3. Featherstone JD. Dental caries: a dynamic disease process. *Aust Dent J.* 2008;53(3):286-291.
4. ten Cate JM. Contemporary perspective on the use of fluoride products in caries prevention. *Br Dent J.* 2013;214(4):161-167.
5. Burrell CH, Chan JT. Systemic and topical fluorides. In: Cianco SG, ed. *ADA Guide to Dental Therapeutics.* Chicago: ADA Publishing, 2000: 230-241.
6. Centers for Disease Control and Prevention. Recommendations for using fluoride to prevent and control dental caries in the United States. *MMWR Recomm Rep.* 2001;50(RR-14):1-42.
7. Marinho VC, Chong LY, Worthington HV, Walsh T. Fluoride mouthrinses for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev.* 2016;(7):CD002284.
8. Marinho VC, Higgins JP, Logan S, Sheiham A. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev.* 2003(4):CD002782.
9. Twetman S, Petersson L, Axelsson S, et al. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. *Acta Odontol Scand.* 2004;62(4):223-230.
10. Zero DT. *In situ* caries models. *Adv Dent Res.* 1995;9(3):214-230.
11. Koulourides T, Phantumvanit P, Munksgaard EC, Housch T. An intraoral model used for studies of fluoride incorporation in enamel. *J Oral Pathol.* 1974;3(4):185-196.

12. Proskin HM, Chilton NW, Kingman A. Interim report of the ad hoc committee for the consideration of statistical concerns related to the use of intra-oral models in submissions for product claims approval to the American Dental Association. *J Dent Res.* 1992;71 Spec Iss:949-952.
13. Gelhard TB, ten Cate JM, Arends J. Rehardening of artificial enamel lesions in vivo. *Caries Res.* 1979;13(2):80-83.
14. Corpron RE, More FG, Clark JW, Korytnicki D, Kowalski CJ. In vivo remineralization of artificial enamel lesions by a fluoride dentifrice or mouthrinse. *Caries Res.* 1986;20(1):48-55.
15. Pearce EI, Nelson DG. In vivo comparison of caries inhibition by a plaque mineral enriching mouthrinse and a fluoride dentifrice. *Caries Res.* 1988;22(6):362-370.
16. Zero DT, Zhang JZ, Harper DS, et al. The remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. *JADA.* 2004;135(2):231-237.
17. Zero DT, Fu J, Anne KM, Cassata S, McCormack SM, Gwinner LM. An improved intra-oral enamel demineralization test model for the study of dental caries. *J Dent Res.* 1992;71 Spec Iss:871-878.
18. Zero DT, Rahbek I, Fu J, Proskin HM, Featherstone JD. Comparison of the iodide permeability test, the surface microhardness test, and mineral dissolution of bovine enamel following acid challenge. *Caries Res.* 1990;24(3):181-188.
19. White DJ. Use of synthetic polymer gels for artificial carious lesion preparation. *Caries Res.* 1987;21(3):228-242.
20. Featherstone JD, Zero DT. An *in situ* model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization. *J Dent Res.* 1992;71 Spec Iss:804-810.
21. Lippert F, Martinez-Miler EA, Zero DT. An *in situ* caries study on the interplay between fluoride dose and concentration in milk. *J Dent.* 2014;47(7):883-890.
22. Sakkab NY, Cilley WA, Haberman JP. Fluoride in deciduous teeth from an anti-caries clinical study. *J Dent Res.* 1984;63(10):1201-1205.

23. Taves DR. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. *Talanta*. 1968;15(9):969-974.
24. Martinez-Mier EA, Cury JA, Heilman JR, et al. Development of gold standard ion-selective electrode-based methods for fluoride analysis. *Caries Res*. 2011;45(1):3-12.
25. Nassar HM, Lippert F, Eckert GJ, Hara AT. Dentifrice fluoride and abrasivity interplay on artificial caries lesions. *Caries Res*. 2014;48(6):557-65.
26. US Food and Drug Administration. Anticaries drug product for over the counter human use. Final monograph 21 CFR Part 355. <https://www.gpo.gov/fdsys/pkg/FR-1995-10-06/pdf/95-24693.pdf>. Accessed Feb. 01, 2017.
27. Kusano SC, Tenuta LM, Cury AA, Cury JA. Timing of fluoride toothpaste use and enamel-dentin demineralization. *Braz Oral Res*. 2011;25(5):383-387.
28. Zero DT, Fu J, Espeland MA, Featherstone JD. Comparison of fluoride concentrations in unstimulated whole saliva following the use of a fluoride dentifrice and a fluoride rinse. *J Dent Res*. 1988;67(10):1257-1262.
29. Thie NM, Kato T, Bader G, Montplaisir JY, Lavigne GJ. The significance of saliva during sleep and the relevance of oromotor movements. *Sleep Med Rev*. 2002;6(3):213-227.
30. Zero DT, Kelly SA, Proskin HM, Bosma ML, Buch M, Smith SR. Comparison of marketed NaF and MFP dentifrice products using an *in situ* caries model. *Caries Res*. 2007;41:Abstract #130

Figure legends

Figure 1. Flow diagram of participant disposition

ITT, intent to treat; PP, per protocol

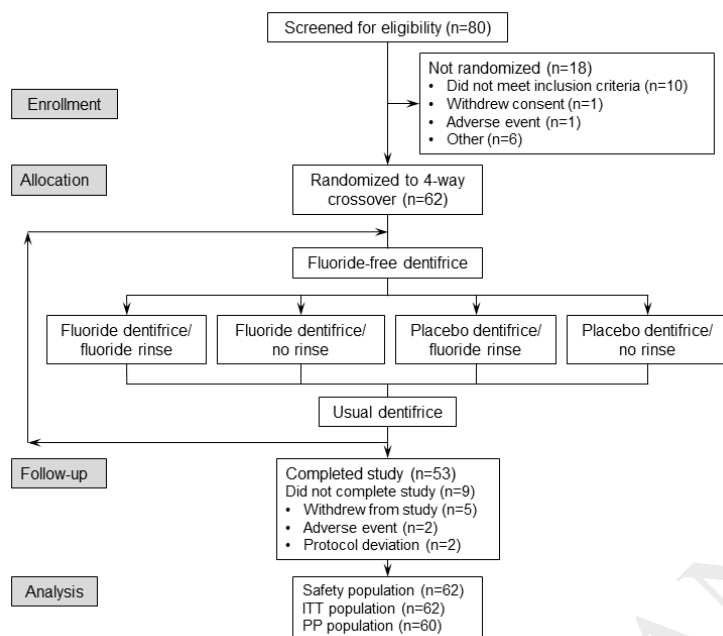


Figure 2. Effect of dentifrice and mouthrinse regimen on (left axis) SMH recovery and (right axis) EFU (per-protocol population)

Data plotted are adjusted means and standard errors of the mean. Adjusted means and 95% confidence intervals from the ANOVA model are shown above the figures.

EFU, enamel fluoride uptake; SMH, surface microhardness

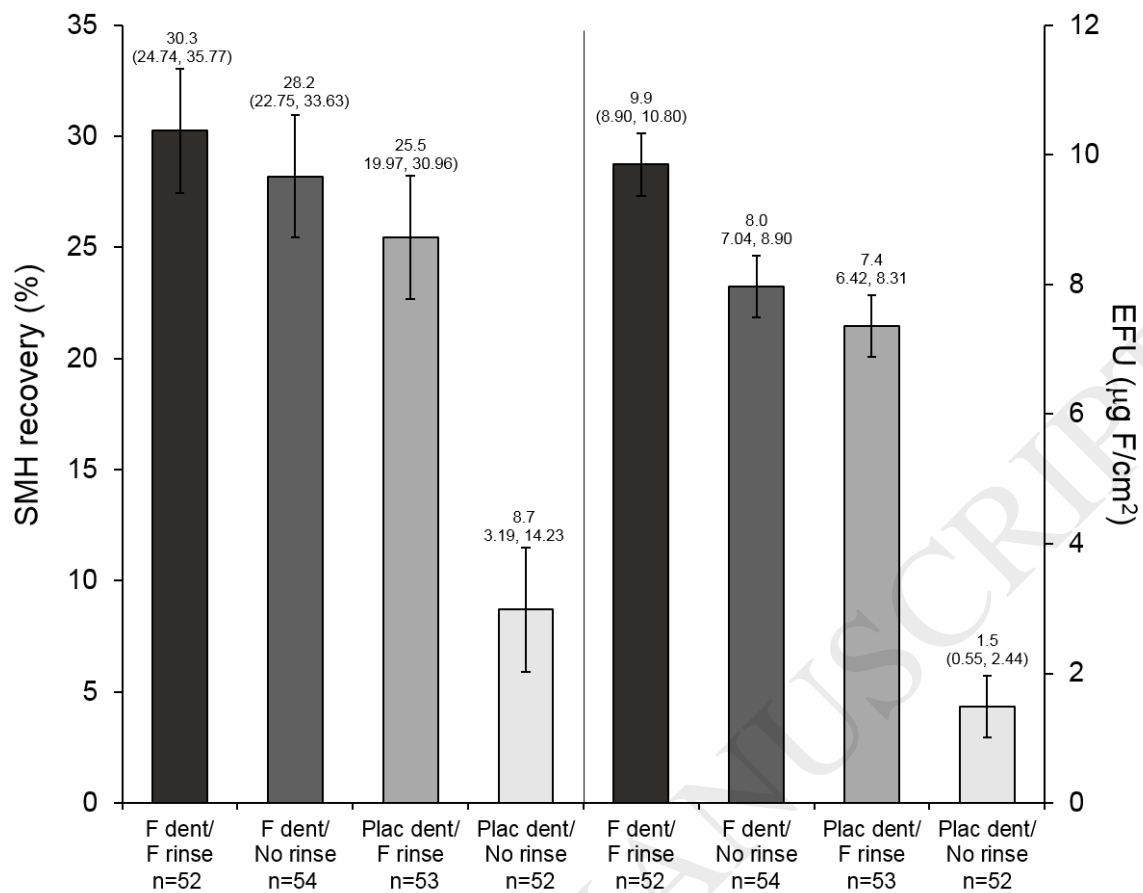


Table 1. Between-treatment comparison of %SMHR (per-protocol population).

Treatment comparison		Between-treatment	Wilcoxon	
		difference ^a (95% CI)	P-value ^a	P-value ^b
Fluoride dentifrice/fluoride rinse vs	Fluoride dentifrice/no rinse	2.07 (-3.64, 7.78)	0.4752	0.0868
	Placebo dentifrice/fluoride rinse	4.79 (-0.99, 10.57)	0.1035	0.0906
	Placebo dentifrice/no rinse	21.55 (15.78, 27.32)	<.0001	<.0001
Fluoride dentifrice/no rinse vs	Placebo dentifrice/fluoride rinse	2.72 (-2.92, 8.37)	0.3420	0.1713
	Placebo dentifrice/no rinse	19.48 (13.81, 25.15)	<.0001	<.0001
Placebo dentifrice/fluoride rinse vs	Placebo dentifrice/no rinse	16.76 (11.06, 22.45)	<.0001	<.0001

^aFrom ANOVA: Difference is first-named treatment minus second-named treatment such that a positive difference implies a larger response value for the first-named treatment

^bp-value from Wilcoxon Signed-Rank Test

%SMHR, percent surface microhardness recovery

Table 2. Between-treatment comparison of EFU (per-protocol population).

Treatment comparison		Between-treatment	Wilcoxon	
		difference ^a (95% CI)	P-value ^a	P-value ^b
Fluoride dentifrice/fluoride rinse vs	Fluoride dentifrice/no rinse	1.88 (0.75, 3.01)	0.0013	0.0010
	Placebo dentifrice/fluoride rinse	2.48 (1.34, 3.62)	<.0001	<.0001
	Placebo dentifrice/no rinse	8.35 (7.21, 9.49)	<.0001	<.0001
Fluoride dentifrice/no rinse vs	Placebo dentifrice/fluoride rinse	0.61 (-0.51, 1.72)	0.2862	0.5326
	Placebo dentifrice/no rinse	6.47 (5.35, 7.60)	<.0001	<.0001
Placebo dentifrice/fluoride rinse vs	Placebo dentifrice/no rinse	5.87 (4.74, 7.00)	<.0001	<.0001

^aFrom ANOVA: Difference is first-named treatment minus second-named treatment such that a positive difference implies a larger response value for the first-named treatment

^bp-value from Wilcoxon Signed-Rank Test

EFU, enamel fluoride uptake

Table 3. Between-treatment comparison of fluoride in saliva samples (per-protocol population).

Treatment comparison		Day 1 pre- to Day 1 post-treatment			Day 1 pre-treatment to Day 14			Day 1 post-treatment to Day 14		
		Diff ^a (95% CI)	P-value ^a	Wilcoxon P-value ^b	Diff ^a (95% CI)	P-value ^a	Wilcoxon P-value ^b	Diff ^a (95% CI)	P-value ^a	Wilcoxon P-value ^b
F dent/f rinse vs	F dent/no rinse	5.2 (2.64, 7.67)	<.0001	<.0001	0.1 (-0.03, 0.15)	0.1605	0.1386	5.1 (2.66, 7.44)	<.0001	<.0001
	Plac dent/F rinse	0.9 (-1.67, 3.4)	0.5016	0.4395	-0.0 (-0.09, 0.09)	0.9846	0.0665	0.9 (-1.56, 3.28)	0.4820	0.4395
	Plac dent/no rinse	13.8 (11.28, 16.36)	<.0001	<.0001	0.7 (-0.02, 0.16)	0.1190	0.0001	13.3 (10.85, 15.72)	<.0001	<.0001
F dent/no rinse vs	Plac dent/F rinse	-4.3 (-6.78, -1.81)	0.0008	0.0001	-0.1 (-0.15, 0.02)	0.1524	0.5645	-4.2 (-6.55, -1.82)	0.0006	0.0001
	Plac dent/no rinse	8.7 (6.16, 11.16)	<.0001	<.0001	0.0 (-0.08, 0.09)	0.8562	0.0017	8.2 (5.85, 10.63)	<.0001	<.0001
Plac dent/F rinse vs	Plac dent/no rinse	12.9 (10.45, 15.46)	<.0001	<.0001	0.1 (-0.02, 0.16)	0.1125	0.0559	12.4 (10.02, 14.82)	<.0001	<.0001

^aBetween-treatment difference from ANOVA: Difference is first-named treatment minus second-named treatment such that a positive difference implies a larger response value for the first-named treatment

^bp-value from Wilcoxon Signed-Rank Test

F, fluoride; dent, dentifrice; Plac, placebo

Table 4. Treatment-emergent adverse events (safety population).

	Fluoride dentifrice/ Fluoride rinse (n=57)		Fluoride dentifrice/ No rinse (n=59)		Placebo dentifrice/ Fluoride rinse (n=60)		Placebo dentifrice/ No rinse (n=56)	
	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE
	Participants with ≥1 AE	17 (29.8)	20	18 (30.5)	22	15 (25.0)	22	13 (23.2)
Participants with ≥1 oral AE	10 (17.5)	12	10 (16.9)	12	9 (15.0)	12	9 (16.1)	14
Participants with ≥1 TRAE	3 (5.3)	4	1 (1.7)	1	2 (3.3)	4	6 (10.7)	8
Treatment-related AEs								
Dry mouth	1 (1.8)	1	0	0	0	0	0	0
Glossodynia	1 (1.8)	1	1 (1.7)	1	0	0	2 (3.6)	2
Lip dry	1 (1.8)	1	0	0	0	0	3 (5.4)	3
Parasthesia oral	1 (1.8)	1	0	0	1 (1.7)	1	1 (1.8)	1
Cheilitis	0	0	0	0	1 (1.7)	1	1 (1.8)	1
Oral discomfort	0	0	0	0	1 (1.7)	1	0	0
Throat tightness	0	0	0	0	1 (1.7)	1	0	0
Gingival ulceration	0	0	0	0	0	0	1 (1.8)	1

AE, adverse event; TRAE, treatment-related AE; n (%), number (percent) of participants in treatment group; nAE, number of AEs.