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ORIGINAL ARTICLE

In situ remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices

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Abstract The aim of this study was to evaluate the remineralization potential of three silica-containing NaF dentifrice systems in an intraoral model. Subjects (N=30) in this randomized, three-phase, 28-day, crossover study served as their own control. Each participant wore a customized orthodontic appliance attached to a mandibular molar and contained one tooth block with caries-like lesion. For each phase, participants engaged in twice-daily brushing for 2 min with one of the following dentifrices: 500 ppm F, 500 ppm F plus functionalized β-tricalcium phosphate (fTCP), and a clinically proven 1,100 ppm F. After each phase, appliances were removed, and specimens were analyzed using surface microhardness (SMH), transverse microradiography (TMR), and cross-sectional microhardness (CSMH). Statistically significant ($p<0.05$) remineralization of white-spot lesions relative to baseline occurred for each dentifrice as determined with SMH and TMR. No significant differences ($p>0.05$) in SMH were found among the three groups, but trending revealed the 500 ppm F plus fTCP produced 26% and 27% greater SMH recovery relative to 500 and 1,100 ppm F, respectively. Similarly, no significant differences ($p>0.05$) in TMR were found among the groups. However, the 500 ppm F plus fTCP dentifrice produced 10% and 38% greater mineral recovery relative to 500 and 1,100 ppm F, respectively, while reducing the lesion depth 30% and 52%, respectively. Significant differences ($p<0.05$) in CSMH existed among the three dentifice groups at different enamel depths, but statistical differences ($p<0.05$) in relative lesion size were only found between 500 ppm F plus fTCP and 500 ppm F. The combination of fTCP and fluoride in a single-compartment, water-based dentifrice can cooperate with fluoride to produce significant remineralization. These results suggest that the combination of 500 ppm F with fTCP may provide comparable anticaries benefits relative to a 1,100 ppm F dentifrice.

Keywords Fluoride · fTCP · Caries · Dentifrice · Remineralization

Introduction

Supported with years of clinically proven research demonstrating inhibition of white-spot lesions formation or arrestment of early caries lesions, it is undisputed that fluoride remains the most clinically effective anticaries agent [1]. However, the recommended levels needed to demonstrate and maintain clinical efficacy have been challenged [2]. And this is especially true with respect to children, where higher levels of fluoride can increase the risk of developing dental fluorosis [3]. For various reported reasons, such as increased hip fractures, osteofluorosis or skeletal fluorosis (crippling bone disease), increased general skeletal fragility, and osteomalacia [4], adults may also opt

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for a low-fluoride alternative that still provides sufficient protection. Because dentifrices are one of the most successful methods of delivering fluoride to the teeth, some advocate the use of a dentifrice containing less fluoride than is normally available over-the-counter (i.e., between 1,000 and 1,500 ppm F) [2, 3]. Although clinical data involving normal (e.g., 1,000 ppm F) and reduced (e.g., 400–600 ppm F) levels in dentifrices demonstrate there may be no statistical advantage with a higher-fluoride toothpaste, directional trending suggests the risk of caries progression increases when lower-dose fluoride toothpastes are used over a longer time [5, 6]. Further to this point, the Cochrane Oral Health Group reviewed data from various clinical trials and concluded that fluoride dentifrices with less than 1,000 ppm F currently do not provide sufficient protection against the caries process [7].

One way of achieving a lower-dose fluoride alternative, but not compromising on anticaries efficacy, may be the inclusion of other mineralizing agents, such as calcium, phosphate, strontium, etc. [1, 8, 9]. In particular, there have been several reports on the promising benefits observed through a combination of fluoride and calcium [1, 8, 10–12]. A unique approach has been through the use of a functionalized β-tricalcium phosphate (fTCP), which is a low-dose calcium phosphate system that cooperates with fluoride to build stronger, more acid-resistant mineral relative to fluoride alone [13–15]. Previous studies suggest the calcium oxide polyhedra manifested in the β-tricalcium phosphate lattice, which are protected with specific organic molecules such as fumaric acid or sodium lauryl sulfate, cooperate with fluoride to bond with loosely bound or broken enamel lattice constituents, including orthophosphate [16, 17]. Using in vitro pH cycling models as a screening method, evaluation of developmental dentifrices with and without the fTCP ingredient has been performed [18–22]. Therefore, it was the aim of this study to determine whether a developmental 500 ppm F plus fTCP dentifrice can provide at-least-as-good-as, if-not-better-than remineralization of white-spot lesions relative to a clinically proven 1,100 ppm F dentifrice in an intraoral model. Each study participant served as his/her own control in this three-phase crossover study and brushed twice daily for 2 min for 28 days. Dentifrice performance was then assessed using surface microhardness (SMH), transverse microradiography (TMR), and cross-sectional microhardness (CSMH). The null hypotheses were that (1) each of the three treatments promotes white-spot lesion remineralization, as determined by SMH, TMR, and CSMH analyses, that is significantly greater than zero, and (2) remineralization of white-spot lesions would not differ between a 500-ppm F+fTCP dentifrice and a 1,100-ppm F dentifrice.

Methods

Subject recruitment This randomized, crossover in situ model contained three phases, with each subject receiving each of the three dentifrices for a period of 28 days. The study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio (Approval #, HSC20100313H). Thirty healthy adults (12 males, 18 females) aged 18 to 50 years old and from different ethnic origins and socioeconomic status participated in this study. The subjects were identified with code numbers. After providing informed written consent, subjects underwent a complete intraoral examination and completed a medical history questionnaire. The inclusion criteria included having at least 22 teeth and a past history of dental caries but no clinically active caries, periodontal disease, or other oral pathology, and having a mandibular first molar with sound, unrestored buccal surface. The mean (SD) unstimulated flow rate was 0.3 (0.2)ml/min, and the mean (SD) stimulated flow rate was 1.8 (0.6)ml/min. Qualified subjects were assigned sequentially a unique randomization number which determines the treatment assignment for each subject according to a randomization schedule. Our power analysis and sample size calculation were performed using nQuery Advisor software (Statistical Solutions, Cork, Ireland) and were based on previous results obtained in this group [10]. In that study, the mean pretreatment% ΔZ was equal to 28.5 with a standard deviation equal to 31.2. For our null hypothesis that white-spot lesion remineralization will be significantly greater than zero, the proposed sample size of $n=30$ will have power greater than 0.95 with a 0.05 one-sided significance level to detect a difference between a null hypothesis mean of zero and a sample mean% ΔZ equal to or greater than 10%. The primary end point of the present study was disease treatment, i.e., remineralization of early caries lesion.

Study procedures Freshly extracted human molar teeth were collected and sterilized with ethylene oxide (ETO) gas (Sterile Technologies, NY, USA) with a fumigating time of 18 h. ETO was used due to its proven ability to kill bacteria, fungi, spores, and viruses [23]. Following sterilization, teeth were stored in 0.1% thymol solution prior to use. Thirty teeth without caries, cracks, or enamel malformations were selected and cleaned with pumice to remove the remnants of pellicle and debris/stains from the buccal surface. The buccal surface of each tooth was ground and polished to produce a flat surface, with a mean enamel reduction of approximately 100 μm. Each specimen was painted with two coats of acid-resistant nail varnish (Revlon, NY, USA), except for a window of exposed

enamel measuring approximately 9×2 mm, on the flat buccal surfaces of the tooth. In these specimens, artificial lesions were created in each exposed window through immersion in an acidified gel (0.1 M sodium hydroxide, 0.1 M lactic acid, and 6% *w*/*v* hydroxyethyl cellulose, pH 4.5) for 7 days at 37°C [24]. Following exposure, the nail varnish on all teeth was carefully and totally removed with acetone (GPR, Aldrich, Milwaukee, USA). Using a water-cooled diamond wire saw (Buehler, Germany), three lesion-bearing blocks (approximately 3 mm long×2 mm wide×1.5 mm thick) to be used for the remineralization promotion test were cut from each window.

SMH was tested on each test-block surface using Vicker's diamond indenter (Tukon 2100; Wilson-Instron, Norwood, MA, USA), with a load of 25 g applied for 15 s. Compared to the penetration depth of a Knoop indenter (~3 μm), the Vickers indenter penetrates into the white-spot lesion about twice as deep; thus, SMH was assessed using the Vickers indenter in order to bridge the subsequent cross-sectional microhardness measurements, which could not be effectively measured at depths below 10 μm without extensive cracking. Three total indentations were made at the middle, upper, and lower ends of the enamel surface, and the Vickers hardness numbers (VHN) were automatically calculated and averaged for each block. This established the pre-test SMH (SMH^i) for the white-spot lesion. Next, one tooth section (control) of approximately 150 μm thickness was cut from each experimental block. These control slices were processed, microradiographed, and visualized using the TMR analysis software version 3.0.0.11 (Inspektor Research Systems, Netherlands) as described previously [25]. The microradiographic images were used only for selection of the suitable lesions for the study. Only the controls that showed caries-like lesion with subsurface lesion and pseudointact surface layer [24], which display a fairly uniform width throughout its length, were selected for the remineralization process, and their "test blocks" were used for construction of the in situ appliance.

Each tooth block was then covered with polyester gauze (Bard Peripheral Vascular, AZ, USA) and mounted within a customized orthodontic bracket to create the intraoral appliance, which was then carried inside each subject's mouth. The appliance is based on the design of brackets used in orthodontics, and consists of an orthodontic molar pad with retentive mesh backing, which has a rectangular stainless steel band welded to it to form a box within which the test block is retained using fluoride-free IRM cement. In order to control the plaque thickness and thus have a more natural plaque on the enamel surface [26], the specimens were mounted flush with the edges of the band. Each tooth successfully completing the fabrication process produced three in situ appliances (one for each dentifrice group). All appliances were sterilized with ETO (Sterile Technologies, NY, USA) prior to intraoral application for reason stated above.

This study was comprised of three distinct phases lasting 28 days each, and was preceded by a 7-day washout period to balance for residual effects of the previous product. During the washout periods, subjects used the next assigned product without wearing any appliance. The assignments of the test products were based on a randomization scheme devised by the Biometrics and Clinical Data Systems Department of the Investigator. Indiana Nanotech provided blinded test products. A one-part label was affixed to each product. The label contained the following information: randomization number, net contents, warnings, protocol number, and study site identification. The examiner did not know which treatment has been administered, and the examiner, recorder, and subject did not have access to the treatment code. Personnel dispensing the test products or supervising their use did not participate in any evaluations of subjects in order to minimize potential bias. The three in situ appliances made out of the three tooth blocks originating from the same tooth were assigned to one subject. Following this, and in accordance with current principles of orthodontic practice, each appliance was bonded onto the buccal surface of the right first mandibular molar chosen randomly to carry the appliances. Transbond™ XT light cure adhesive paste (3M Unitek, Monrovia, CA, USA) was used to bond the appliance on the tooth following etching of the tooth surface with 37% phosphoric acid for 30 s, and was cured using 3M Unitek™ Ortholux XT visible Light Curing Unit (3M Unitek, Monrovia, CA, USA) applied for 20 s. Following the fitting of the appliances, subjects were provided with their appropriate toothpaste and a toothbrush. Three water-based sodium fluoride (NaF), silica-containing dentifrices were evaluated in this study: 500 ppm F, 500 ppm F plus 50 ppm fTCP, and 1,100 ppm F Crest® Cavity Protection. The subjects were asked to brush their teeth with their respective toothpaste two times daily for 2 min on each occasion, preferably morning and last thing before bed. In order to monitor product usage, a diary was provided to each subject to record the number of toothbrushing events performed each day and the time it was done. Further, subjects were instructed to return the remaining toothpaste after each study, where the weight of the toothpaste was measured before and after the study phase. All subjects were asked to maintain their normal dietary habits. The use of any other oral hygiene product was prohibited. These measures were to ensure uniformity in the use of oral hygiene product which may otherwise unduly influence the de-/remineralization cycle during the study periods. Sub-

jects were instructed to avoid brushing directly on the appliance. They were supplied with a toothbrush designed for use with orthodontic brackets. At the end of each 28-day period, the appliance was detached, and after the washout period, the next sets of appliances were cemented in place on the same tooth and on the same dental arch as the first sets. This procedure was repeated until the three phases were completed. At the detachment of the appliance, any bonding agent left on the tooth surface was carefully and completely removed with composite-removing burs (CompoSite; Shofu Dental, NJ, USA).

After detachment, the blocks were removed from their respective appliances and measured for SMH by making three fresh indentations on the free surfaces of the block. These indentations were then averaged for each block and established the post-test SMH (SMH^p). Following SMH measurements, an enamel slice (about 150 μm thick) was cut from each block and processed for TMR as described above for the control. Although the pre-test control sections had been microradiographed for selection of the appropriate lesions for the study, they were microradiographed again, together with the post-test sections, and were then analyzed together for quantification of the lesion parameters of mineral loss (ΔZ) and lesion depth (LD) using the TMR analysis software version 3.0.0.11 (Inspektor Research Systems, Netherlands). This enabled both control and test sections to be microradiographed, processed, and analyzed under the same conditions, to obtain the pre-test (ΔZ^i and LD^i) and the post-test (ΔZ^p and LD^p) TMR parameters of the lesions as well as pre-test and post-test microradiograms of the lesions. In addition to directly comparing the pre- and post-test SMH and TMR data, the percent change in SMH and TMR was also calculated as follows [25]:

$$\%\text{Hardness recovery} = \left(SMH^p - SMH^i\right)/\left(SMH^i\right) \times 100 \quad (1)$$

$$\%\text{Mineral recovery} = \left(\Delta Z^i - \Delta Z^p\right)/\left(\Delta Z^i\right) \times 100 \quad (2)$$

$$\%\text{Reduction in lesion depth} = \left(LD^i - LD^p\right)/\left(LD^i\right) \times 100 \quad (3)$$

CSMH was then performed on the remaining half of each specimen. The sections were mounted with ClaroCit methylmethacrylate-based cold mounting resin (Struers, Cleveland, OH, USA) with the freshly cut surfaces exposed. The mounted specimens were serially ground with 100, 600, and 1,000 grit sandpaper (3M, St. Paul, MN, USA), and then serially polished using a Leco Spectrum System 1000 grinder/polisher with 3 μm microid diamond compound and compound extender for lubricant. Due to the delicacy of the enamel in the white-spot lesion zone, which can lead to undesirable cracking upon indentation, along with the spatial limitations of multiple indents, we selected the Knoop indenter over the Vickers indenter. A series of three indentation lanes per specimen are made under a load of 10 gf at 12.5 μm, 25 gf at 25, and 37.5 μm, and 50 gf at 50, 75, and 100 μm below the specimen surface [16, 22]. Measurements closer to the enamel were not feasible at the given load limits due to the delicacy of the specimens. This resulted in a total of 18 indents per specimen. The Knoop indentation lengths were then converted to Knoop Hardness Numbers (KHN). Relative to KHN of sound enamel, relative lesion sizes in units of square root of KHN ($\sqrt{KHN}$) times enamel depth (micrometer) were then calculated using Simpson's Composite Rule [27, 28].

Statistical analysis of the data was conducted using SPSS statistical software (PASW Statistics 18.0), with the level of significance (α) selected at 0.05. The mean (SEM) values of SMH and TMR parameters were calculated for the pre- and post-test groups of each of the dentifrices. All data were examined for normality and homogeneity of variance. The pre-test and post-test parameters within each group were compared using paired t tests at the 95% confidence level. Intergroup comparisons were performed using one-way analysis of variance ($p<0.05$), followed by post hoc multistep comparisons (Tukey WSD).

Results

Comparing the pre-test and post-test microradiograms (images) side-by-side, it was clearly observed that in all the three products, there was an increase in mineral density in both the subsurface lesion and pseudointact surface layer, depicted by increased opacity in these layers. This increased mineral density was homogeneously distributed throughout the two layers, and was reflected on the TMR mineral distribution graph as an increased thickness and height of the pseudointact surface layer and reduced depth of the subsurface lesion layer. This change in mineral density in these two layers is in agreement with the increased SMH values recorded post-test with all the three dentifrices. SMH results for the enamel slabs treated with each of the three dentifrice groups in situ are summarized in Table 1. Formation of the white-spot lesions softened the enamel slabs by about 150 VHN, leading to initial SMH values of about 180 VHN. After 28 days in situ, each dentifrice produced statistically greater surface strengthening (SMH^p) compared to its respective initial SMH^i. However, among the three groups, no statistical differences were found ($p>0.05$). Trending indicates, however, that addition fTCP provides some additional benefits relative to fluoride alone. The ΔSMH for the 500 ppm F+fTCP group was 23% and 27% greater relative to the 500 and

Table 1 Mean (standard error of the mean) surface microhardness (SMH) results

Groups	SMH^i (VHN)	SMH^p (VHN)	ΔSMH (VHN)	Relative% hardness recovery
500 ppm F	180.7 (4.6)[a]	237.6 (7.3)[b]	56.9 (7.1)	32.8 (4.2)
500 ppm F+fTCP	174.1 (5.0)[a]	244.3 (9.6)[b]	70.2 (8.6)	41.4 (5.1)
1,100 ppm F	180.0 (4.5)[a]	235.2 (113)[b]	55.2 (11.9)	32.7 (6.9)

For each group, initial SMH (SMH^i) was significantly different relative to the post 28-day in situ SMH (SMH^p), as denoted with a<b. No significant differences were found within each column. ΔSMH is the difference between SMH^p and SMH^i

1,100 ppm F groups, respectively. Additionally, relative percent hardness recovery for the 500 ppm F+fTCP group was 26% and 27% greater relative to 500 and 1,100 ppm F groups, respectively. In contrast, the data in Table 1 do not reveal trending between the two fluoride-only groups.

The enamel slabs analyzed for SMH were then sectioned with one half section assessed using TMR. These results are summarized in Tables 2 and 3. For each group, the initial integrated mineral loss and lesion depth (ΔZ^i, LD^i) were statistically larger ($p<0.05$) relative to the post 28-day integrated mineral loss and depth (ΔZ^p, LD^p), indicating all three dentifrices produced significant white-spot remineralization. The relative percent mineral recovery was calculated based on the integrated mineral loss of the white spots before and after the 28-day in situ study period. Similarly, the relative percent reduction in lesion depth was also calculated. With respect to both metrics, no significant differences were found ($p>0.05$) among the groups. Trending shows that the 500 ppm F+fTCP dentifrice confers 10% and 38% greater mineral recovery relative to both the 500 and 1,100 ppm F dentifrices. With respect to percent reduction in lesion depth, trending shows the 500 ppm F+fTCP reduces lesion size by 30% and 52% relative to both the 500 and 1,100 ppm F dentifrices.

The other half section of the enamel slab was then analyzed using CSMH as a function of enamel depth from 12.5 to 100 μm. These results are shown in Fig. 1 and Table 4. The brackets shown in Fig. 1 indicate statistical differences do not exist ($p>0.05$). Based on the CSMH values in Fig. 1, the size of the white-spot lesions appears to extend down to about 50 μm from the enamel surface, at which point the KHN values are consistent with those of sound enamel [22, 29]. Statistically significant ($p<0.05$) differences were found near the outer surface of the lesion at 12.5 μm, with both the 500 ppm F+fTCP and the 1,100 ppm F dentifrices leading to 141% and 134% greater microhardness relative to 500 ppm F, respectively. Statistical separation was also observed deeper within the sound enamel region at the 100-μm depth, where 500 ppm F+fTCP and 1,100 ppm F produced 43% and 41% greater strengthening relative to 500 ppm F, respectively. Also, within the sound enamel region at the 75-μm depth, 500 ppm F+fTCP produced statistically stronger ($p<0.05$) mineral relative to the 500 ppm F and 1,100 ppm F groups (58% and 37%, respectively), which were not statistically different ($p>0.05$). Within the body of the white-spot lesion, trending at the 25 and 37.5-μm depths indicates 1,100 ppm F generates stronger mineral relative to both 500 ppm F (77% and 90%, respectively) and 500 ppm F+fTCP (62% and 12%, respectively) dentifrices. Near the bottom of the lesion, however, all three dentifrice groups trended to a similar microhardness. To summarize the CSMH effects of the three dentifrices on white-spot lesion remineralization, a lesion size relative to sound enamel for each group was determined and is shown in Table 4. A significant difference ($p<0.05$) was found between the two 500 ppm F groups; however, 500 ppm F+fTCP was not significantly different ($p>0.05$) from 1,100 ppm F. The relative lesion size for groups treated with 500 ppm F+fTCP was about 41% and 10% smaller compared to those treated with both 500 ppm F and 1,100 ppm F dentifrices, respectively. There were no statistical differences ($p>0.05$)

Table 2 Mean (standard error of the mean) initial and post integrated mineral loss (ΔZ^i, ΔZ^p) and relative percent mineral recovery as determined using TMR

Groups	ΔZ^i (vol.%μm)	ΔZ^p (vol.%μm)	Relative% mineral recovery
500 ppm F	604.1 (16.4)[a]	487.0 (22.4)[b]	24.0 (3.4)
500 ppm F+fTCP	667.9 (28.5)[a]	483.2 (29.4)[b]	26.5 (4.0)
1,100 ppm F	652.8 (31.9)[a]	525.5 (30.4)[b]	19.2 (3.2)

For each group, ΔZ^p was significantly different relative to ΔZ^i, as denoted with a>b. No significant differences were found within each column

Table 3 Mean (standard error of the mean) initial and post lesion depth (LD^i, LD^p) and relative percent reduction in lesion depth as determined using TMR

Groups	LD^i (μm)	LD^p (μm)	% Reduction in lesion depth
500 ppm F	34.9 (1.3)[a]	28.0 (1.3)[b]	18.2 (3.7)
500 ppm F+fTCP	37.7 (1.4)[a]	28.1 (1.8)[b]	23.6 (5.2)
1100 ppm F	35.5 (1.4)[a]	30.0 (1.9)[b]	15.5 (4.9)

For each group, LD^p was significantly different relative to LD^i, as denoted with a>b. No significant differences were found within each column

between the 500 ppm F and 1,100 ppm F groups, although a 52% smaller relative lesion size was produced using the 1,100 ppm F dentifrice.

Discussion

This study (funded by the National Institutes of Health, NIH) was performed to test the hypothesis of whether a calcium-containing 500 ppm F dentifrice could produce at-least-as-good, if-not-better-than performance relative to a clinically proven 1,100 ppm F dentifrice (i.e., Crest® Cavity Protection, Procter & Gamble). The present in situ study was based on our prior research assessing the surface and cross-sectional microhardness of white-spot lesions evaluated with these pastes in vitro [22]. In the prior research, the change in SMH relative to baseline as well as the calculated CSMH lesion size together served to determine the primary end points in characterizing the effects of the treatments near the surface and within the body of the white-spot lesion. We have observed that the combination of fluoride and calcium leads to improved surface and, in particular, subsurface effects relative to fluoride alone. Continuing to build on this research, we held that SMH (both ΔVHN and percent recovery) and CSMH lesion size serve to determine the primary end points in the present study, but TMR was also performed to further characterize the effects of the dentifrice systems. The statistical similarity among the SMH and CSMH data (i.e., mean ΔVHN, percent recovery, and calculated lesion size) demonstrates that the null hypothesis that the remineralization of white-spot lesions would not differ between a 500 ppm F plus fTCP dentifrice and the 1,100 ppm F dentifrice was upheld. And so also is the null hypothesis that the test products promote remineralization greater than zero, since the mean percentage change is significantly greater than zero. Upon recommendation by the NIH, this study did not include a fluoride-free dentifrice (i.e., negative control) due to possible adverse dental health; however, each subject served as his/her own control. While a lower-dose fluoride group could have been used (e.g., 250 ppm F), instead, we included two 500 ppm F dentifrices: one with fTCP and one without. In doing so, this also added another control as we were then able to evaluate the "baseline" effect of the 500 ppm F dentifrice formulation on remineralization in the absence of fTCP. Relative to baseline SMH and TMR measurements, all three dentifrices significantly remineralized white-spot enamel lesions at the end of the 28-day in situ period. This outcome demonstrates the in situ model is sensitive to 500 ppm F, and is consistent with a wide body of research demonstrating cariostatic

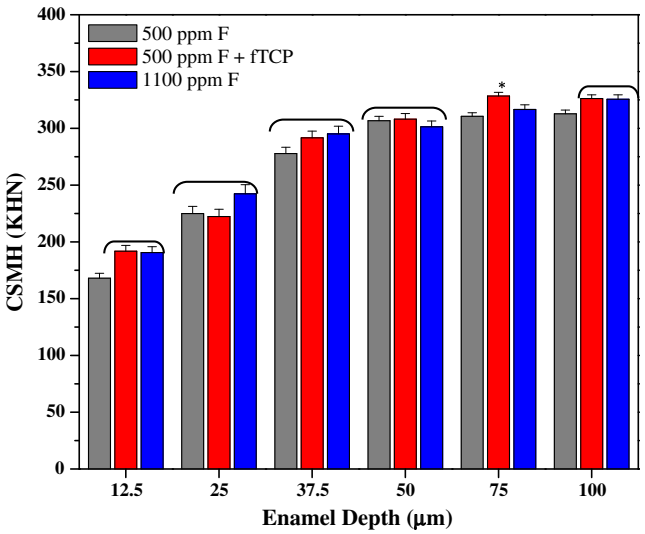

Fig. 1 Mean (standard error of the mean) cross-sectional microhardness (CSMH) at enamel depths ranging between 12.5 and 100 μm. *Brackets* indicate no statistical differences were found ($p>0.05$), and the *asterisk* for the 75-μm data set indicates a statistical difference ($p<0.05$) from the other two groups

Table 4 Mean (standard error of the mean) lesion size (i.e., relative hardness difference between white-spot lesion and sound enamel) determined using cross-sectional microhardness (CSMH)

Groups	Lesion size (√KHN μm)
500 ppm F	69.9 (8.2)[a]
500 ppm F+fTCP	41.1 (8.5)[b]
1,100 ppm F	45.9 (9.0)[a, b]

Significant differences ($p<0.05$) are indicated, with a<b

benefits of dentifrices containing at least 500 ppm F [3, 5, 7, 30]. Though the purpose of this study was to test whether 500 ppm F dentifrice containing fTCP could provide at-least-as-good benefits relative to an established 1,100 ppm F dentifrice, model improvements could be made for a larger follow-on study, such as increasing the duration of the study (e.g., the residual time of the appliance extended to 6 or 8 weeks) to allow for longer-term use of the dentifrice systems, recruiting more study participants in order to expand representation, implementing a lower-fluoride (e.g., 250 ppm F) dentifrice as a negative control, or large-scale in vivo randomized controlled clinical trial. Still, the present study agrees very well with reported findings of 500 and 1,100 ppm F dentifrice studies [3, 5–7, 30]. A 2003 study reported a lack of a dose response at 9 or 21 months for participants receiving either a 500 ppm F or 1,450 ppm F dentifrice in vivo [31]. Separately, a clinical research report on 677 children found that over a 2-year period, there were no statistical differences in anticaries benefits between two groups using either 1,000 or 400 to 500 ppm F dentifrices [3]. Recently, a report found no statistical differences in the remineralization of white-spot lesions in deciduous enamel using a 500, 1,000, or 1,500 ppm F dentifrice in situ [30]. Though not significantly different in any of those studies, in each case, trending of the data favored the higher-fluoride dentifrice groups. Although in our study we also observed that the remineralization potential was not significantly different among the 500 and 1,100 ppm F dentifrices with respect to the SMH and TMR data, we observe trending that favors the 500 ppm F+fTCP dentifrice over the 500 and 1,100 ppm F dentifrices. Notably, comparisons of the relative lesion size obtained from cross-sectional microhardness demonstrate a statistical advantage of the 500 ppm F dentifrice containing fTCP relative to the 500 ppm F control dentifrice.

The distinction between integrated mineral loss from TMR and relative lesion size from CSMH requires some commentary. For instance, the integrated mineral loss assessed by TMR is based on optical density differences of the white-spot lesion relative to sound enamel, and is qualitatively evaluated by an analyst. On the other hand, microhardness measurements are based on the physical strength of the substrate (i.e., the enamel), with the ensuing microhardness measurement resulting from a combination of mineral densities, bonding within the enamel framework as well as prism orientation. This means that while TMR can detect whether mineral has been deposited into a white-spot lesion, this technique cannot accurately assess the quality of the resultant mineralization. This might contribute to the observations, for instance, that clinically observed passive [32] and active [33] white-spot lesions were found to be relatively insensitive to fluoride therapy when assessed using QLF. Alternately, microhardness interrogations are able to assess the quality of mineralization (i.e., integration of newly deposited mineral with the existing enamel framework). It may not be surprising then that although relationships have been proposed to link data obtained using TMR and CSMH, the correlation between the two techniques is not perfect [27, 28, 34]. Since TMR is not intended to assess the nature of the mineral formation within the enamel framework, CSMH therefore provides important information regarding the quality of the remineralization. Additionally, because remineralization processes are often complex [35], and likely even more so when agents (e.g., zinc, tin, titanium, calcium, etc.) other than fluoride are imparting a role, it may be recommended to characterize the remineralization of an enamel specimen using more than one technique [26]. In the present study, although the TMR data trends favor the 500 ppm F+fTCP dentifrice, the statistical separation achieved between the two 500 ppm F dentifrices assessed by CSMH suggests the strength of the white-spot enamel framework is affected differently in the presence of fTCP. This view is supported with previous in vitro and in situ observations [10, 14, 16, 19, 22].

Conclusion

Based on the results from this in situ study, the combination of fTCP and fluoride in a single-compartment, water-based dentifrice can cooperate with fluoride to produce significant remineralization.

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Conflict of interest/disclosure Indiana Nanotech provided coded dentifrice formulations to UTHSCSA, who remained blinded to the dentifrice identifications throughout the course of the study and specimen analyses. Dr. Bennett T. Amaechi is an Associate Professor and Director of Cariology in the Department of Comprehensive Dentistry at UTHSCSA, and was principal investigator for the design and execution of this in situ study. Drs. Poornima K. Mensinkai, Renzo A. Ccahuana-Vasquez, and Irene Chedjieu are research staff of Dr. Amaechi. At Indiana Nanotech, Mr. Mackey is the Quality Director and Laboratory Manager, Dr. Robert L. Karlinsey is the CEO and Principle Investigator, and Trenton J. Walker and Douglas D. Blanken are research technicians.

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