Carious lesion remineralizing potential of fluoride- and calcium-containing toothpastes
A laboratory study

Frank Lippert, PhD; Karmjeet K. Gill, BDS

ABSTRACT

Background. The authors conducted a laboratory study to determine the carious lesion remineralization and fluoridation potential of fluoride (F)- and calcium-containing toothpastes.

Methods. The authors created early carious lesions in bovine enamel specimens and assigned them to 7 treatment groups on the basis of their surface Vickers microhardness: Clinpro Tooth Crème (Clinpro) (3M ESPE), CTx4 Gel 1100 (CTx4) (Oral Biotech), Enamelon Fluoride Toothpaste (Enamelon) (Premier Dental), MI Paste ONE (MI-One) (GC America), Crest Cavity Protection Toothpaste (Crest) (Procter & Gamble), and 2 F-dose controls (low F, high F). The authors pH cycled the specimens for 10 days by using an established model, determined changes in surface microhardness, calculated percentage of surface microhardness recovery (%SMHr; primary outcome variable), and measured enamel F uptake (EFU). The authors used a 1-way analysis of variance for data analysis.

Results. Study results showed an F-dose response for both %SMHr (low-F control: mean, 9.8; 95% confidence interval [CI], 5.7 to 13.8); Crest: mean, 26.2; CI, 21.8 to 30.6; high-F control: mean, 33.5; CI, 29.4 to 37.5) and EFU (low-F control: mean, 47; CI, 12 to 83; Crest: mean, 225; CI, 189 to 260; high-F control: mean, 307; CI, 271 to 342; all micrograms of F per cubic centimeter). For %SMHr, Clinpro (mean, 26.5; CI, 22.5 to 30.6) and CTx4 (mean, 27.3; CI, 23.1 to 31.5) were similar to Crest, all being superior to Enamelon (mean, 15.6; CI, 11.6 to 19.7), which was superior to MI-One (mean, 4.3; CI, 0.3 to 8.3). For EFU, there were no differences between Clinpro (mean, 189; CI, 153 to 224), CTx4 (mean, 177; CI, 142 to 213), Enamelon (mean, 196; CI, 161 to 232), and Crest, all being superior to MI-One (mean, 66; CI, 30 to 102).

Conclusions. This study’s results failed to show superior remineralizing efficacy of any of the toothpastes compared with those of a calcium-free F toothpaste, with 2 of the 4 toothpastes being inferior. Clinical testing will be required to establish conclusive evidence.

Practical Implications. Clinicians should be aware of the remineralizing potential of new anticaries products.

Key Words. Carious lesion; remineralization; fluoride; calcium; laboratory testing; dentifrices; toothpastes.

Several fluoride- (F-) and calcium-containing toothpastes with claims of enhancing carious lesion remineralization have become commercially available. These products are over-the-counter F toothpastes with the difference being that they are not distributed via conventional paths, such as grocery and drug stores. Instead, they are available exclusively via oral health care professionals or directly from the manufacturer. These toothpastes contain agents such as functionalized β-tricalcium phosphate (fTCP), stabilized casein phosphopeptide—amorphous calcium phosphate (CPP-ACP) nanocomplexes, or (nano)-hydroxyapatite or amorphous calcium phosphate. Little evidence exists as to their ability to enhance remineralization in the presence of F. Furthermore, a 2018 expert panel convened by the American Dental Association Council on Scientific Affairs and Center for Evidence-Based Dentistry advised against the use of 10% CPP-ACP (in the absence of F), with investigators in an earlier, similar review concluding that there was insufficient evidence to recommend the use of calcium phosphates in caries prevention.
Consequently, we conducted this laboratory study to shed some light on the ability of these toothpastes to remineralize and fluoridate early carious lesions. Information gained from our study will inform oral health care professionals about the predicted remineralization efficacy, which will aid in the counseling of their patients and the development of treatment plans.

METHODS

Study design

In our laboratory study, we compared the carious lesion remineralization and fluoridation ability of 4 F- and calcium-containing toothpastes with those of a calcium-free F toothpaste and 2 F-dose controls. We used a 10-day pH cycling remineralization model to assess surface microhardness (SMH) change, as a percentage of SMH recovery (%SMHr), and enamel F uptake (EFU) in artificially induced early carious lesions. We analyzed data by using 1-way analysis of variance. The primary outcome variable was %SMHr.

Specimen preparation, lesion formation, and characterization

We prepared enamel specimens obtained from bovine teeth (West Michigan Beef Company). We cut the tooth sections into round specimens (4-millimeter diameter) by using a hollow-core diamond drill bit (DD86, Continental Diamond Tool). We then ground and polished the specimens to create planar parallel dentin and enamel surfaces. We ground the dentin side flat by using 500-grit silicon carbide paper (Struers Inc.), followed by grinding and polishing the enamel side with 1,200-grit silicon carbide paper, followed by 2,400- and then 4,000-grit silicon carbide papers. As a final polishing step, we used a 1-micrometer diamond suspension (DP Suspension P, Struers) on a polishing cloth. Resulting specimens had a thickness range of 1.9 to 2.1 mm. The center of the enamel surface had a minimum polished area of 2.5-mm diameter. We then mounted each specimen onto a 1-inch square acrylic block with sticky wax and covered the sides of each specimen with an acid-resistant nail polish (Advanced Hard As Nails, Sally Hansen) so that only the enamel surface was exposed. We prepared and used 13 specimens per treatment group for our study (n = 13).

We used the SMH test to assess the mineral status and changes thereof in the enamel specimens. We measured SMH by using a hardness tester (2100 HT, Wilson Instruments) by placing 5 sound enamel baseline indentations spaced vertically 100 µm apart with a Knoop diamond under a 50-gram load in the center of the specimen as shown elsewhere. We determined SMH by measuring the length of the indentations by using image analysis software (Clemex CMT.HD, Version 6.0.011, Clemex). For enamel specimens to be acceptable for use in the study, the mean of the 5 baseline indentation lengths had to be between 40 and 46 µm with a standard deviation of 3 µm or less.

We used a modification of the method described by White to create early carious lesions in the specimens. We immersed the enamel specimens for 18 hours at 37°C under static conditions in 40 milliliters of a demineralization solution with the following composition: 50.0 millimolar lactic acid (Fisher Scientific), 50% saturated with respect to hydroxyapatite, 0.2% (weight per volume) polyacrylic acid (Carbopol 907, BF Goodrich), pH adjusted to 5.0 with potassium hydroxide.

After in vitro demineralization of the samples, we measured the SMH of the enamel specimens again by placing 5 indentations 100 µm to the left of the sound enamel indentations. To qualify for inclusion into the study, the mean (n = 5) indentation lengths of the partially demineralized specimens had to be between 140 and 180 µm with a standard deviation of 11 µm or less. We then assigned specimens to their test toothpastes ensuring that there were no differences in mean postdemineralization indentation lengths between treatment groups.

Test and control toothpastes

Table 1 shows the detail of the 5 test and 2 control toothpastes. We used all test toothpastes as aqueous slurries prepared at a ratio of 1 part toothpaste to 2 parts deionized water, thereby mimicking the dilution occurring during toothbrushing. We included 2 F-dose controls using Crest Cavity Protection Toothpaste (Crest) (Procter & Gamble): for the low-F control, we used an aqueous slurry of Crest at a ratio of 1:100, resulting in an estimated F concentration of approximately 11 parts per million (ppm) F. For the high-F control, we prepared a slurry of Crest with an aqueous solution containing 1,950 ppm F as sodium F at a ratio of 1:2, thereby mimicking a 5,000 ppm F prescription-strength toothpaste. We prepared all slurries immediately before treatment.

ABBREVIATION KEY

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>MEANING</th>
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<tbody>
<tr>
<td>CPP-</td>
<td>Casein phosphopeptide—amorphous calcium phosphate.</td>
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<tr>
<td>ACP-</td>
<td>Functionalized β-tricalcium phosphate.</td>
</tr>
<tr>
<td>EFU</td>
<td>Enamel fluoride uptake.</td>
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<tr>
<td>F</td>
<td>Fluoride.</td>
</tr>
<tr>
<td>TCP</td>
<td>Tricalcium phosphate.</td>
</tr>
<tr>
<td>NA</td>
<td>Not applicable.</td>
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<tr>
<td>ppm</td>
<td>Parts per million.</td>
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<tr>
<td>%</td>
<td>Percentage of surface microhardness recovery.</td>
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<tr>
<td>SMHr</td>
<td>Surface microhardness recovery.</td>
</tr>
<tr>
<td>SMH</td>
<td>Surface microhardness.</td>
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We based our cost estimation (Table 1) on twice daily use with 1.5 g of toothpaste per tooth-brushing session. We determined the cost of each product at Benco Dental (Clinpro Tooth Crème [Clinpro], 3M ESPE; Enamelon Fluoride Toothpaste [Enamelon], Premier Dental; MI Paste ONE [MI-One], GC America), CariFree (CTx4 Gel 1100 [CTx4], Oral Biotech), and CVS (Crest) online in October 2018 and did not consider tax, shipping costs, special offers, quantity discounts, coupon codes, and so on that may affect the actual cost.

**pH cycling phase**

We used a modified version of an established pH cycling model. The main modification was that instead of 4 daily toothpaste treatments we treated the specimens only twice daily to mimic actual human usage. Briefly, we used the following sequence every day for 10 days: a 1-minute treatment with the assigned toothpaste, 2 hours of remineralization, 4 hours of cariogenic challenge, 2 hours of remineralization, a 1-minute treatment with the assigned toothpaste, followed by remineralization overnight. We used artificial saliva (1.5 mM calcium chloride dihydrate [Fisher Scientific]; 0.9 mM monopotassium phosphate; 130.0 mM potassium chloride; and 20.0 mM hydroxyethyl piperazineethanesulfonic acid, pH adjusted to 7.0 with potassium hydroxide) as the remineralization medium. We used a demineralizing solution (100 mM lactic acid [Fisher Scientific]; 4.1 mM calcium chloride dihydrate; 8.0 mM monopotassium phosphate; and 0.2% [weight per volume] polyacrylic acid, pH adjusted to 5.0 with potassium hydroxide) as the cariogenic challenge. We conducted the experiment at room temperature under static conditions. To prevent carryover, we rinsed all specimens with deionized water after each slurry treatment and solution change. We renewed the remineralization solution and cariogenic challenge daily. Our pH cycling procedure mimics in vivo caries with alternating periods of demineralization and remineralization with a twice daily use of F toothpaste, albeit its limitations need to be considered in the interpretation of our findings.

**Post-pH cycling lesion characterization**

After 10 days of pH cycling, we SMH tested the specimens again as described earlier by placing 5 indentations 200 μm to the right of the baseline indentations. We calculated the extent of remineralization or further demineralization on the basis of the method of Gelhard and Arends as follows:

\[
\%\text{SMHer} = \frac{(D - R)}{(D - B)} \times 100
\]

Table 1. Test and control toothpastes.

<table>
<thead>
<tr>
<th>NAME</th>
<th>SHORT NAME</th>
<th>MANUFACTURER</th>
<th>FLUORIDE COMPOUND AND CONCENTRATION</th>
<th>OTHER NOTEWORTHY INGREDIENTS</th>
<th>LOT OR BATCH NO.</th>
<th>ANNUAL COST ESTIMATION, $</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Toothpaste</strong></td>
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<tr>
<td>Clinpro Tooth Crème</td>
<td>Clinpro</td>
<td>3M ESPE</td>
<td>Sodium F, 0.21% (950 ppm F)</td>
<td>Tricalcium phosphate</td>
<td>60151</td>
<td>99</td>
</tr>
<tr>
<td>CTx4 Gel 1100</td>
<td>CTx4</td>
<td>Oral Biotech</td>
<td>Sodium F, 0.24% (1,086 ppm F)</td>
<td>(Nano-)hydroxyapatite, sodium bicarbonate, xylitol</td>
<td>111705</td>
<td>307</td>
</tr>
<tr>
<td>Enamelon Fluoride Toothpaste</td>
<td>Enamelon</td>
<td>Premier Dental</td>
<td>Stannous F, 0.45% (1,091 ppm F)</td>
<td>Calcium sulfate, monosodium phosphate</td>
<td>PD7341-1</td>
<td>112</td>
</tr>
<tr>
<td>MI Paste ONE</td>
<td>MI-One</td>
<td>GC America</td>
<td>Sodium F, 0.24% (1,086 ppm F)</td>
<td>Casein phosphopeptide-amorphous calcium phosphate (10%), xylitol, potassium nitrate (5%)</td>
<td>180117A</td>
<td>405</td>
</tr>
<tr>
<td>Crest Cavity Protection Toothpaste</td>
<td>Crest</td>
<td>Procter &amp; Gamble</td>
<td>Sodium F, 0.243% (1,099 ppm F)</td>
<td>NA</td>
<td>610673</td>
<td>17</td>
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<tr>
<td><strong>Control Toothpastes</strong> (Fluoride-Dose Controls)</td>
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<tr>
<td>Crest Cavity Protection Toothpaste</td>
<td>Low-F control</td>
<td>Procter &amp; Gamble</td>
<td>Sodium F, 0.002% (11 ppm F)</td>
<td>NA</td>
<td>610673</td>
<td>NA</td>
</tr>
<tr>
<td>Crest Cavity Protection Toothpaste</td>
<td>High-F control</td>
<td>Procter &amp; Gamble</td>
<td>Sodium F, 1.1% (5,000 ppm F)</td>
<td>NA</td>
<td>610673</td>
<td>NA</td>
</tr>
</tbody>
</table>

* For cost estimation, see Test and control toothpastes in the Methods section. † F: Fluoride. ‡ ppm: Parts per million. § NA: Not applicable. ¶ Crest Cavity Protection Toothpaste was used to prepare both control toothpaste slurries.
After completion of the SMH testing, we determined the F content of each enamel specimen by using the microbiopsy technique to a depth of 200 μm.9 We placed 2 drill holes in the bottom right and left corners of the specimen, avoiding the center area, and determined the diameter of the drill holes. We collected the enamel powder from the drill holes and dissolved (20 microliters of perchloric acid [Fisher Scientific], 40 μL citrate—ethylenediaminetetraacetic acid buffer and 40 μL deionized water) and analyzed it for F by means of comparison with a standard curve obtained with a similarly prepared sample. We calculated the F data as micrograms of F per cubic centimeter as follows: μg F × dilution factor/volume of drilling.

**Statistical analysis**

We tested the data for normal distribution (Shapiro-Wilk test). We then calculated the variables %SMHr and EFU for each specimen and analyzed the data by using a 1-way analysis of variance with the factor toothpaste. We considered %SMHr the primary outcome variable. Where significant differences were indicated, we used a Fisher least significant difference test to determine differences between treatment groups. On the basis of previous data, we estimated the within-group standard deviations for %SMHr to be 7.0 and for EFU to be 90.0. With a sample size of 13 specimens per group, the study had 80% power for detection of a difference of 6.8 for %SMHr and 86 μg F/cm³ in EFU between any 2 groups, assuming 2-sided tests each conducted at a 5% significance level.

**RESULTS**

There were no statistically significant differences between treatment groups for sound enamel microhardness (P = .90) and lesion baseline microhardness (P > .999). Table 2 presents the means (standard deviations) and results of the statistical analyses for both %SMHr and EFU and for all treatment groups.

**%SMHr**

The model’s results showed an F-dose response for %SMHr; the high-F control provided greater remineralization than did Crest (P = .018) and the low-F control (P < .0001), with Crest also affording greater remineralization than did the low-F control (P < .0001). In comparison with Crest, Clinpro (P = .915) and CTx4 (P = .724) provided similar extents of remineralization. However, both Enamelon (P = .01) and MI-One (P < .0001) were inferior to Crest. MI-One was comparable only with the low-F control (P = .06) and provided the numerically least extent of remineralization.

**EFU**

The EFU data also showed an F-dose response; the high-F control provided the greatest F uptake, followed by Crest (P = .02) and the low-F control (P < .0001), with Crest being superior to the
DISCUSSION

We designed this laboratory study to evaluate the remineralization potential of several F toothpastes that contain a range of calcium compounds to enhance carious lesion remineralization (Table 1). Because not every new toothpaste can be evaluated for its ability to prevent caries in an in vivo caries clinical trial, a wide array of model systems to determine the predicted efficacy of F toothpastes has been developed over the past decades. These model systems include in situ (intraoral), in vitro (laboratory), and animal caries models. Although none of these models is a like-for-like surrogate for the complexity of in vivo caries, in vitro models generally are considered suitable tools to simulate specific aspects of the caries process. We chose a modified White model for our study because it is one of the most widely used models for studying the ability of toothpastes to remineralize early carious lesions. The primary outcome variable was the change in SMH, which we previously have shown to be more sensitive in determining changes in the mineralization status of early carious lesions than the reference standard transverse microradiography.

One of the key requirements for any laboratory caries model is its ability to show an F-dose response, which we also observed in our study (Table 2). Although no clinical data for coronal caries prevention exists, to our knowledge, for the comparison between 1,100 and 5,000 ppm F (as tested in our study), there is a plethora of studies in which the investigators compared placebo and 1,100 ppm F toothpastes, and there are several studies in which the investigators compared with toothpastes of higher F concentrations. These studies’ results have shown that the relative caries-preventive effect of toothpastes increases with increasing F concentrations. In vitro caries models generally have been able to help predict the clinical performance of F toothpastes of different concentrations. However, these models often lead to overestimation of potential differences because of their comparatively higher sensitivity. For example, only F concentrations of 1,000 ppm or more afford caries prevention in children and adolescents, whereas results with pH cycling models have shown caries-preventive effects for concentrations as little as 250 ppm F. Furthermore, in vitro caries models also can be designed to yield a net demineralization or remineralization outcome. Here, we deliberately chose the latter because the manufacturers of the tested F- and calcium-containing toothpastes claimed to provide remineralization.

Our results indicate that none of the F- and calcium-containing toothpastes provided additional benefits over an F toothpaste not containing calcium, with 2 being inferior (Table 2). Among the calcium compounds contained in the test toothpastes, CPP-ACP is the most studied and controversial non-F agent. CPP-ACP has been commercialized and can be found in a 5% sodium F varnish, in a gel for prolonged topical application with or without added F (most studied product), and in the F toothpaste evaluated in our study. Although results from earlier reviews have been conflicting, the consensus based on clinical data is that 10% CPP-ACP (topical gel without F) is not recommended for caries prevention or management. To our knowledge, in our study, we were the first to evaluate the 2017 launched CPP-ACP-containing F toothpaste. Its poor performance in our study may be the result of inadequate F bioavailability as indicated also by the low EFU (Table 2). Although CPP-ACP nanocomplexes can be stable, their disruption will result in the release of ionic calcium. Calcium then can react with F ions and form largely insoluble calcium F–like compounds before reaching the tooth surface or other intraoral binding sites. This mechanism applies not only to CPP-ACP but also to other calcium compounds formulated with F in a single, aqueous base.

We found both Clinpro and CTx4 to be similar to a calcium-free F toothpaste in their ability to remineralize early carious lesions. Clinpro contains fTCP, which is also available in a 5% sodium F varnish. A presumably similar toothpaste containing this agent was equivalent to an F toothpaste in an in situ remineralizing study, thereby mirroring the observations in our study (those results with CPP-ACP, however, did not). Results from a comparable in situ caries study showed some synergy between sodium F and fTCP in remineralizing early carious lesions, which we did not observe in our study. This discrepancy can be attributed to inherent model differences (for example, remineralization and demineralization media, sequence of treatments, type and severity of the baseline carious lesion). Unlike Clinpro, CTx4 contains a range of compounds in addition to...
sodium F (Table 1). Some clinical data exist on the efficacy of xylitol to prevent caries when delivered with sodium F in a toothpaste, whereas the author of a 2017 review considered sodium bicarbonate a promising plaque-buffering agent. Some anecdotal clinical evidence suggests that a toothpaste containing F-free (nano-)hydroxyapatite exhibits anticaries benefits. However, there are no credible studies on its potential in the presence of or comparison with F.

Enamelon, the only test toothpaste containing stannous instead of sodium F, has the amorphous calcium phosphate technology of the earlier and now unavailable Enamelon toothpaste. In contrast to the newer offering, it contained sodium F and used a dual-chamber approach—sodium F and phosphate in 1, calcium sulfate in the other, with both mixed during toothbrushing. Investigators previously tested a similar dual-chamber approach successfully; results from 2 caries clinical trials showed significant caries reduction for a sodium F–dicalcium phosphate dihydrate dual-chamber toothpaste compared with that of a sodium F–only toothpaste. Neither the original Enamelon nor the toothpaste tested in vivo are commercially available anymore. Furthermore, we were unable to retrieve any data on the Enamelon toothpaste we used in our study. Nonetheless, the sequential delivery or co-delivery of calcium and F has attracted considerable attention by researchers in the past. Results of short-term in vivo studies highlighted that calcium can enhance intraoral F retention greatly and in particular in dental plaque. This finding is not surprising given that plaque F concentrations are strongly dependent on plaque calcium concentrations. However, despite more than 20 years of research on this topic, commercialization of a dual-delivery or co-delivery product containing calcium and F has been challenging.

Although we were concerned primarily with determining the predicted remineralization efficacy of the test toothpastes, we also wanted to highlight the potential cost associated with their everyday use (Table 1). There is an approximate 4-fold difference in cost between the F- and calcium-containing toothpastes, with all being substantially more expensive than a calcium-free F toothpaste.

There are limitations to consider when interpreting our findings. As pointed out earlier, no model can replace the complexity of in vivo caries. Here, we investigated the carious lesion remineralization potential, and results may have been different if we would have chosen a laboratory model with a net demineralization outcome, such as that developed by Featherstone and colleagues. Irrespective of the model, the subtle pH fluctuations occurring at the dental plaque-enamel interface or the gradual release of F and other agents from the toothpaste during toothbrushing, which depend on the formulation’s inherent properties, cannot be mimicked sufficiently in vitro. Furthermore, the inclusion of a dental plaque surrogate (in vitro biofilm) also may be advantageous in future research because F retention is affected by calcium. Lastly, we should not forget that patient compliance and, in particular, oral health care habits, such as toothbrushing frequency and duration, is key to the caries prevention afforded by F toothpaste. Paying a premium price for an oral health care product potentially may be a motivator to adhere more strictly to professional advice and instructions.

CONCLUSIONS
None of the tested F- and calcium-containing toothpastes provided enhanced carious lesion remineralization or fluoridation in comparison with an F toothpaste that does not contain calcium. However, further clinical research will be needed to provide comprehensive recommendations as to their usefulness in everyday at-home caries prevention.

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Dr. Gill is a volunteer, Oral Health Research Institute, Indiana University School of Dentistry, Indianapolis, IN.

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