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*Full Length Research Paper*

# Remineralization potential of 5,000 ppm fluoride dentifrices evaluated in a pH cycling model

**Robert L. Karlinsey[1]*, Allen C. Mackey[1], Emily R. Walker[1], Bennett T. Amaechi[2], Ramalingam Karthikeyan[2], Kaveh Najibfard[2] and Aaron M. Pfarrer[3]**

[1]Indiana Nanotech, LLC, 351 West 10th Street, Suite 309, Indianapolis, IN 46202, USA.
[2]University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA.
[3]3M ESPE, 3M Center, St. Paul MN 55144, USA.

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**Prescription 1.1% sodium fluoride (NaF) dentifrices designed to either have fast dispersion for improved enamel fluoride uptake (that is, PreviDent® Booster 5000) or contain an innovative tricalcium phosphate system for enhanced remineralization (that is, Clinpro® 5000) were evaluated for anticaries potential in an *in vitro* pH cycling model. Polished bovine enamel specimens were initially softened in a white-spot-forming solution comprising 0.1 M lactic acid plus 100 kDa polyacrylic acid (PAA, pH = 5.0) for 26 h at 37°C. Specimens were then measured for baseline Vickers microhardness and stratified (N = 12) into the following groups: Group A: Tom's of Maine fluoride-free dentifrice (negative control); Group B: Colgate PreviDent® Booster 5000 (5000 ppm fluoride) and Group C: 3M Clinpro® 5000 (5000 ppm fluoride). The groups were then cycled for 10 days in a pH cycling model consisting of four one-minute treatment periods (diluted 1:3 with distilled water) and one four-hour acid challenge (lactic acid-PAA , pH = 5.0) per day. Between these events, specimens were immersed in artificial saliva (pH = 7.0). After 10 days of cycling, the specimens were evaluated for Vickers surface microhardness, mineral loss and lesion depth using microindentation, transverse microradiography and polarized light microscopy. For all analyses, statistical differences (t-tests, $p<0.05$) were found to exist among the groups, with Clinpro® 5000 conferring superior surface and subsurface remineralization potential relative to both PreviDent® Booster 5000 and Tom's of Maine fluoride-free paste. Due to this superiority, these results suggest the combination of 5,000 ppm fluoride plus the tricalcium phosphate system may provide significant anticaries benefits relative to fluoride-only and fluoride-free dentifrices.**

**Key words:** Dental caries, clinpro 5000, prevident booster 5000, 5000 ppm fluoride, 1.1% NaF, tricalcium phosphate, microhardness, transverse microradiography, polarized light microscopy, pH cycling, remineralization.

## INTRODUCTION

Although dental caries is one of the most preventable diseases known to man, caries experience is on the rise (Dye et al., 2007). In the US, for instance, the National Institute of Dental and Craniofacial research reports that dental restorations are needed in almost four out of every five children by the age of 17. Thus, despite widespread water fluoridation, installation of protective dental sealants and increased understanding of caries management, additional measures may be required to improve caries prevention.

One way to thwart the caries experience may be through the application of a 5,000 ppm (1.1% sodium fluoride, NaF) fluoride dentifrice. Several studies have shown 5,000 ppm fluoride dentifrice can provide significant anticaries benefits relative to 1,100 ppm fluoride dentifrices (Baysan et al., 2001; Tavss et al., 2003). In the US, most over-the-counter (OTC)

*Corresponding author. E-mail: rkarlins@gmail.com. Tel: 317-278-7892.

formulations contain about 1,100 ppm fluoride, while toothpastes containing 5,000 ppm fluoride are only available through the dental office or by prescription. Dental professionals typically recommend 5,000 ppm fluoride dentifrices for high-risk caries patients, including those with orthodontic brackets, prostheses and restorations, those with xerostomia, or those genetically susceptible to tooth decay. One highly recommended 5,000 ppm fluoride dentifrice is Colgate's PreviDent® Booster 5000. This fluidic, or gel-like, formulation has been reported to provide faster dispersion in saliva and higher fluoride uptake into enamel saliva relative to a comparable 5,000 ppm fluoride paste formulation, PreviDent® 5000 Plus (Joziak et al., 2003). This may be considered important since PreviDent® 5000 Plus has been shown to significantly reduce caries incidence by as much as 59% after six months of use (Baysan et al., 2001). Separately, a novel 5,000 ppm fluoride dentifrice, Clinpro® 5000, was recently introduced by 3M ESPE. This 1.1% NaF silica-containing paste contains an innovative functionalized tricalcium phosphate (fTCP) ingredient that, when evaluated in development formulations, has been shown to boost remineralization performance relative to fluoride-only systems (Karlinsey et al., 2009b; Karlinsey et al., 2008; Karlinsey et al., 2009c). Many studies have shown that combinations of calcium and fluoride can significantly boost remineralization relative to either mineral alone (Feagin et al., 1971; Karlinsey et al., 2009a; LeGeros et al., 1999; Reynolds, 2008). However, the ability to formulate a dentifrice with both bioavailable calcium and fluoride has remained elusive due to the rapid reaction kinetics that lead to premature calcium fluoride formation within the dentifrice tube. The fTCP technology solves this problem by protecting its bioavailable calcium with a fluoride-repelling surfactant (sodium lauryl sulfate) and as a result, can be readily combined in an aqueous dentifrice formulation with NaF.

Because of the creative approaches that have led to the development of the PreviDent® Booster 5000 and Clinpro® 5000 dentifrices, there exists an opportunity to investigate the remineralization potential in a head-to-head comparison involving a placebo dentifrice (that is, Tom's of Maine fluoride-free dentifrice). In this manuscript, we performed a 10-day *in vitro* remineralization/demineralization (remin/demin) pH cycling study evaluating the reversal of 'white-spot' (that is, non-cavitated) lesions in bovine enamel treated with a placebo paste (Tom's of Maine), PreviDent® Booster 5000, or Clinpro® 5000. The endpoint evaluations included surface Vickers microhardness measurements, transverse microradiography (TMR) and polarized light microscopy (PLM). Altogether these measurements provided intimate insight into the nature of surface and subsurface mineralization responses from each dentifrice. Furthermore, these observations ought to aid the dental practitioner in understanding the potential options and benefits of novel 5,000 ppm fluoride systems.

## MATERIALS AND METHODS

### Specimen preparation and White-spot Lesion formation

Three millimeter enamel cores were drilled from the lingual and labial surfaces of bovine incisors and molars using a bench top drill press (Power Glide) affixed with a hollow core drill bit. The cores were embedded into hollowed out acrylic rods using DuraBase resin (Dental Mfg. Co.). Each specimen was ground by hand with 600 grit SiC sandpaper under water cooling for 30 sec using a Leco Spectrum System 1000 Grinder/Polisher set to 300 rpm. Then, with the unit set to 200 rpm, each specimen was polished by hand for 1 min using 3 μm diamond compound in conjunction with microid extender solution (Leco). Using a Leco LM247AT microhardness tester, Vickers surface hardness was performed with a 200 gf load and 15 sec dwell time to confirm the presence of sound enamel in the polished specimen as indicated by a Vickers hardness number (VHN) of 300 VHN or greater. Artificial lesions were then formed through immersion in a solution comprising 0.1M lactic acid/1.0 wt. % 100 kDa polyacrylic acid (PAA) solution (pH = 5.0) for 26 h at 37°C.

The white-spot lesion depth extends to about 70 μm, as determined previously with cross-sectional microhardness and reflective microscopy. Upon white-spot formation, acceptable baseline surface microhardness ranged from 20 to 50 VHN (200 gf for 15 seconds made using a Leco 247AT indenter).

### Treatment groups

Enamel specimens were then divided into the following three groups:

Group A: Tom's of Maine fluoride-free toothpaste (placebo);
Group B: Colgate PreviDent® Booster 5000 (5,000 ppm fluoride);
Group C: 3M Clinpro® 5000 (5,000 ppm fluoride plus 800 ppm functionalized tricalcium phosphate, fTCP);

The fluoride source used in Groups B and C was sodium fluoride (NaF).

### pH cycling model

The three groups of enamel specimens were then subjected to a remin/demin pH cycling model as described in Table 1. The model includes two one-minute treatment periods performed an hour apart in the morning, followed by one four-hour lactic acid-PAA acid challenge and finally two more one-minute treatment periods in the afternoon, administered daily for 10 days. In between the daily treatments and acid challenge, specimens were immersed in artificial saliva (Ten Cate et al., 1988).

The treatments were diluted three-fold with distilled (DI) water (5 grams dentifrice: 10 ml DI water). The treatments and saliva events were magnetically agitated at 300 rpm, while the acid challenge was static. We note that PreviDent® Booster 5000 was much more fluidic relative to both Tom's of Maine and Clinpro® 5000 dentifrices; however, all dentifrices slurried readily when stirred for 5 min prior to treatment. After each treatment and acid challenge, the specimens were rinsed with DI water prior to placement into artificial saliva. Four fresh treatment slurries and fresh acid solution were used daily, with the artificial saliva solution changed once daily after the third treatment.

### Surface microhardness measurements

After 10 days of cycling, the enamel specimens were examined for

**Table 1.** Outline of daily events and duration employed in the remin/demin dental model. *On Day one, specimens were pre-conditioned for one hour in artificial saliva prior to the first treatment. **Artificial saliva was refreshed daily after the acid challenge.

| Event | Duration |
|---|---|
| Treatment #1* | 1 min |
| Saliva, pH =7.0 | 1 h |
| Treatment #2 | 1 min |
| Saliva, pH =7.0 | 1 h |
| Acid Challenge, pH =5.0 | 4 h |
| Saliva,** pH =7.0 | 1 h |
| Treatment #3 | 1 min |
| Saliva, pH = 7.0 | 1 h |
| Treatment #4 | 1 min |
| Saliva, pH = 7.0 | Overnight |

Vickers surface hardness (200 gf, 15 s dwell time). The change in Vickers hardness number ($VHN^{10}$) was determined as the difference between the post and baseline values ($VHN^{10} = VHN_{post} - VHN_{base}$).

#### Transverse microradiography

Transverse microradiography (TMR) was used to assess the lesion parameters (integrated mineral loss (∆Z, vol. %•µm) and lesion depth (LD, µm)) as follows. A tooth slice (~150 µm thick) was cut from each tooth block using a water-cooled diamond wire saw (Wells Diamond saw, Switzerland). Both sides of each slice were polished using Plain Back Polishing film in a MultiPrep™ Precision Polishing machine (Allied High Tech, USA) to achieve planoparallel surfaces as well as to reduce the thickness of the slice to 80-100 µm (the appropriate thickness for TMR). Then the polished slices were placed in a specially fabricated microradiographic plate-holding cassette, incorporating an aluminium step wedge (10 steps of 24.5 µm thickness). The cassette was loaded with type IA high resolution glass X-ray plates (Microchrome Technology, CA, USA) and microradiographed using a Phillips x-ray generator system set up for this purpose.The plates were exposed for 10 min at an anode voltage of 20 kV and a tube current of 10 mA and then processed. Processing consisted of a five minute development in Kodak HR developer and 15 min fixation in Kodak Rapid-fixer before a final 30 min wash period. After drying, the microradiographs were subjected to visualization and image analysis using a computer program (TMR2006 version 3.0.0.6, Inspektor Research Inc., Netherlands). The hardware was a Leica DMR optical microscope linked via a Sony model XC-75CE CCTV camera to a 90 MHz Dell™ Pentium Personal Computer. The enhanced image of each microradiograph was analyzed under standard conditions of light intensity and magnification and processed, along with data from the image of the step wedge, by the TMR program. By this method, the parameters of ∆Z and LD were quantified for each caries lesion.

#### Polarized light microscopy

Each tooth slice was imbibed with water and examined with Carl Zeiss polarized light microscopy and image analysis software using a Nikon, optiphot® light microscope (Nikon, Tokyo, Japan) with rotating stage, polarizer and analyzer at a magnification of 450x.

The microscope was connected via a Zeiss digital camera to a 90 MHz Dell™ Pentium Personal Computer, housing the Axiovision 4™ image analysis software. The image from the microscope was captured in the computer and using the software, the depth (µm) of the caries lesion was quantified. Three slices (as outlined in the TMR section) per specimen were made and analyzed using this technique, resulting in a total of 36 slices and images. The most representative slice from each specimen was then used for TMR analysis.

#### Statistics

The statistics were determined using Sigma Stat Version 3.1. The microhardness data were analyzed for normality using the Kolmogorov-Smirnov test with p=0.05. Means and standard deviations of the means were calculated and the outliers were evaluated using either Dixon's Q-test for non-parametric data sets or Peirce's criterion for parametric data sets. Pairwise t-test comparisons between groups ($p<0.05$) were then performed to test for significance.

## RESULTS

The baseline and post-cycle Vickers microhardness numbers (VHN), TMR and PLM data (mean ± SEM) are summarized in Table 2. Statistical analyses of all data were performed using a one-way analysis of variance ($p < 0.05$) and multiple pair wise t-tests ($p < 0.05$) were used to determine significant differences. The softened enamel specimens were stratified into the groups with respect to baseline VHN. After 10 days of cycling, the VHN values changed significantly, with $VHN^{10}$ ranked from lowest to highest as follows: Group A < Group B < Group C.

Representative TMR images are shown in Figure 1, with quantitative results summarized in Table 2. Based on Figure 1, a clear difference in subsurface lesion size is observed between Group A and the fluoride-containing groups. Between the two fluoride-containing groups, the TMR image for Group C shows a relatively thinner and less pronounced lesion relative to that for Group B, indicating a difference in mineralization. Quantitative calculations in mineral density and lesion size based on 12 radiographs collected on each group are shown in Table 2. The integrated mineral loss, ∆Z, is ranked from highest to lowest as follows: Group A > Group B > Group C. The mean LD values were found to be statistically larger for specimens in Group A relative to those in Groups B and C, which were found to be equivalent. Representative PLM images are shown in Figure 2, with quantitative results summarized in Table 2. The images in Figure 2 reveal that a distinct lesion zone (as supported with black arrows) exists in the specimens treated with each of the three groups. We were unable to accurately measure distinct surface and subsurface layers for all three groups, so the mean total depth (TD) in Table 2 represents the combination of surface and subsurface layer thicknesses. The depth sizes are ranked remin/demin model employed in this study is sensitive to enamel specimens treated with dentifrices with and

**Table 2.** Quantitative summary of surface micro hardness, TMR and PLM data for the three dentifrice groups. A) Tom's of Maine fluoride-free dentifrice (placebo); B) PreviDent® Booster 5000; C) Clinpro® 5000.

| Group | $VHN^0$ | $VHN^{10}$ | ΔZ (vol. %•µm) | LD (µm) | TD (µm) |
|---|---|---|---|---|---|
| 1 | 39.4 ± 2.4[a] | 47.1 ± 4.3[a] | 721.7 ± 21.0[a] | 33.9 ± 0.5[a] | 201.0 ± 2.2[a] |
| 2 | 39.7 ± 2.3[a] | 64.8 ± 5.8[b] | 466.7 ± 26.0[b] | 22.5 ± 0.6[b] | 150.4 ± 6.2[b] |
| 3 | 39.7 ± 2.1[a] | 105.6 ± 5.6[c] | 330.8 ± 24.7[c] | 22.0 ± 0.9[b] | 114.1 ± 10.8[c] |

$VHN^0$ = baseline Vickers Hardness Number (VHN) ± SEM (N=12); $VHN^{10}$ = VHN ± SEM (N=12) after 10 days of cycling; ΔZ = integrated mineral loss ± SEM (N=12) of the white-spot lesion, determined by TMR; LD = Lesion Depth (LD) ± SEM (N=12) is the measured body of the demineralized zone, determined by TMR; TD = Total Depth (TD) ± SEM (N=12) determined by PLM and measured from the outer surface of the tooth extending to the bottom of the demineralized region (that is, surface + lesion). Superscripts (a-c) indicate significant differences (*t*-test comparisons, $p < 0.05$). Data are presented in order of increasing $VHN^{10}$.

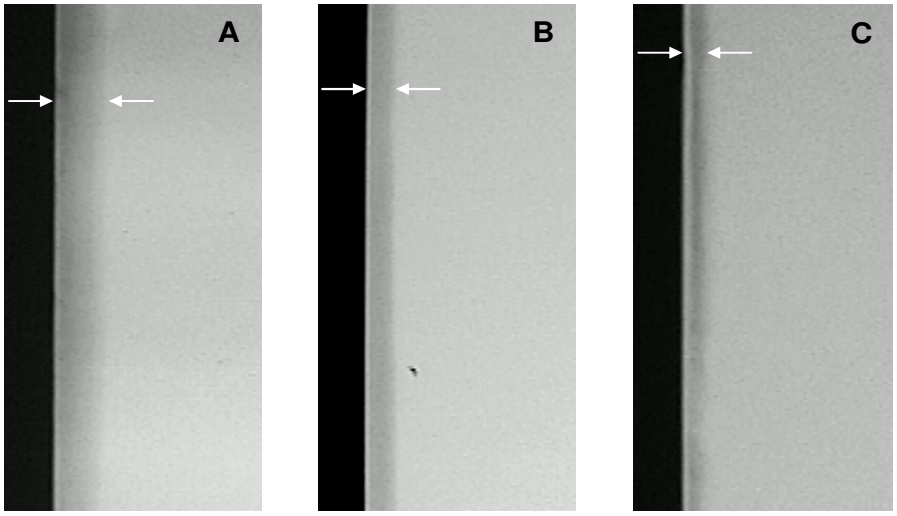


**Figure 1.** Representative TMR images of sectioned enamel specimens treated A: Tom's of Maine fluoride-free dentifrice (placebo); B: PreviDent® Booster 5000; and C: Clinpro® 5000. White arrows are shown to help indicate the subsurface lesion.

without fluoride and is consistent with our previous laboratory investigations. Between the two fluoride-containing groups, Clinpro® 5000 (Group C) provided superior remineralization properties relative to PreviDent® Booster 5000 (Group B).

## DISCUSSION

We have previously used this in vitro remin/demin cycling model to evaluate the remineralization ability of NaF dentifrices (Karlinsey et al., 2009b; Karlinsey et al., 2008; Karlinsey et al., 2009c) and the present results further demonstrate the model's sensitivity to both 5000 ppm fluoride (PreviDent® Booster 5000) and 5000 ppm fluoride plus an innovative calcium phosphate remineralizing agent (Clinpro® 5000). The microhardness, TMR and PLM examinations of white-spot enamel lesions revealed that the PreviDent® formulation exhibited inferior remineralization relative to Clinpro® 5000. As part of the Clinpro® 5000 remineralization benefits, the system appears to penetrate deeper into the subsurface lesion than PreviDent® Booster 5000, as shown in the contrasting TMR and PLM images B and C in Figures 1 and 2. This suggests the two formulations, despite having the same 1.1% NaF content and bioavailability (Karlinsey et al., 2009c), manifest ingredients that influence remineralization. Both 1.1% NaF formulations evaluated in this study contain unique ingredients, such as a pyrophosphate agent in PreviDent® and fTCP in Clinpro® 5000. Pyrophosphate, an anti-calculus agent, has been shown to possibly encumber surface remineralization in laboratory studies (Featherstone et al., 1990; Sullivan et al., 1995),

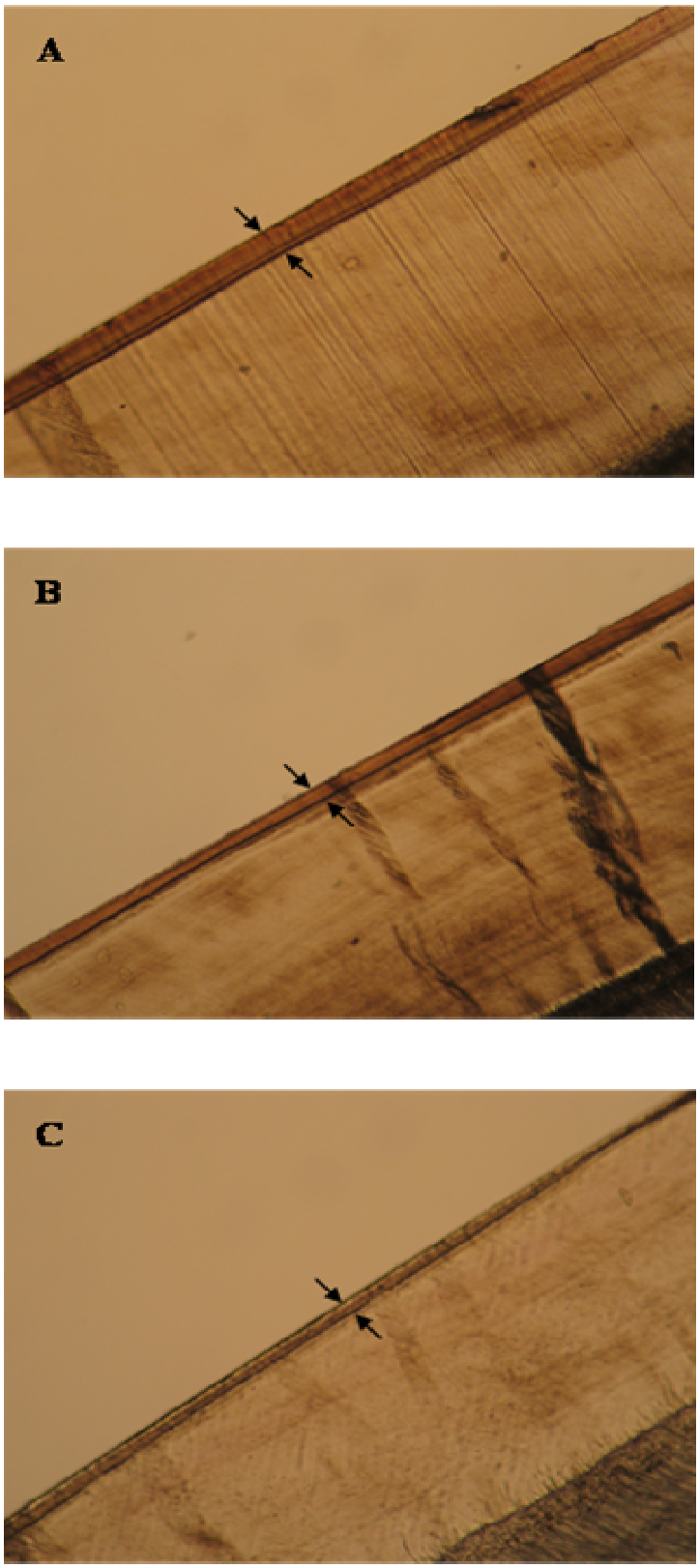


**Figure 2.** Representative PLM images of sectioned enamel specimens treated with A: Tom's of Maine fluoride-free dentifrice (placebo); B: PreviDent® Booster 5000; and, C: Clinpro® 5000. Black arrows are shown to help indicate the surface and subsurface layers.

even though bulk remineralization will still occur. (Sullivan, 1995) We previously reported on the ability of fTCP to react with fluoride at the enamel surface as well as penetrate into subsurface enamel lesions (Karlinsey et al., 2009a; Karlinsey et al., 2009b; Karlinsey et al., 2009c; Karlinsey et al., 2009d). Thus, enamel surface strengthening might be limited for the PreviDent® formulation due to the mineralization-competing presence of pyrophosphate, while fTCP in Clinpro® 5000 cooperates with fluoride to boost enamel surface strength.

In addition to the sensitivity of the enamel surface to treatment by each of the three dentifrices, subsurface lesions also responded significantly to fluoride. The lesion depth (LD) of enamel specimens treated with both PreviDent® and Clinpro® dentifrices were found to be relatively smaller than the lesions treated with Tom's of Maine fluoride-free dentifrice. Because Clinpro® 5000 produced essentially the same LD; it appears that, in general, the subsurface lesion size is affected by 5000 ppm fluoride regardless of the presence of pyrophosphate or fTCP. In fact, the application of either 5000 ppm F formulation convoluted the distinction between surface and subsurface layers in PLM examinations; hence, we were only able to report total distance (TD) for this analysis. (We note that specimens treated with Tom's of Maine fluoride-free dentifrice produced visibly distinct layers via PLM.) Although the LD values were indifferent for specimens treated with either of the two 1.1% NaF formulations, the higher mineral density (ΔZ) for enamel lesions treated with Clinpro® 5000 suggests fTCP plays an important role in subsurface remineralization as well: the ΔZ values in Table 2 show Clinpro® 5000 produces approximately 30% denser subsurface lesions relative to PreviDent® Booster 5000. Visually, this is observed in Figure 1 where
the definition of the lesion in C appears less pronounced than that in B.

In summary, the combination of surface and subsurface measurements collected in this study demonstrate that 1.1% NaF imparts significant remineralization at the enamel surface and within the subsurface lesion relative to a fluoride-free system. Between the two 1.1% NaF systems, Clinpro® 5000, which contains a fluoride-compatible functionalized calcium phosphate ingredient, imparts superior remineralization at both the enamel surface and within the subsurface lesion relative to PreviDent® Booster 5000. These results suggest that the synergistic combination of fluoride plus fTCP may provide superior dental health benefits over a dentifrice system designed to promote faster dispersion and therefore fluoride uptake, into enamel white-spot lesions.

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## Disclosure statement

The authors claim no conflicts of interest that would affect the outcome or quality of this work. Throughout the entire study, 3M was blinded to the study details, execution, and data collection and analysis. After performing the cycling and surface microhardness measurements (which was performed by Nanotech personnel blinded to the study groups evaluated in the present dental model), Nanotech provided blinded specimens (coded only by numbers) to the University of Texas Health Science Center at San Antonio for TMR and PLM analysis.

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