Journal of Dentistry and Oral Hygiene Vol. 1(4), pp. 052-058, October, 2009
Available online at http://www.academicjournals.org/jdoh

Full Length Research Paper

In vitro evaluation of eroded enamel treated with fluoride and a prospective tricalcium phosphate agent

Robert L. Karlinsey[1]*, Allen C. Mackey[1], Emily R. Walker[1], Katherine E. Frederick[1] and Christabel X. Fowler[2]

[1]Indiana Nanotech, Indianapolis, IN, United States of America.
[2]GlaxoSmithKline, Weybridge, United Kingdom.

Accepted 29 September 2009

The anti-erosion effects of 225 ppm fluoride plus an innovative form of tricalcium phosphate (TCP-Si-Ur) relative to 225 ppm fluoride were investigated using a pH cycling model comprising treatment, saliva and acid challenge periods. Polished bovine enamel specimens were initially softened in 1% citric acid (pH = 3.8) and stratified (N = 10) into the following groups according to their Vickers microhardness: water, 225 ppm fluoride and 225 ppm fluoride containing 20, 40, or 80 ppm TCP-Si-Ur. The cycling regimen consisted of three two-minute treatment periods and five two-minute acid challenges (1% citric acid, pH = 3.8) per day for a total of 20 days. Surface microhardness measurements were made at 10 and 20 days. After 10 days, statistical differences (t-tests, $p < 0.05$) only existed between water and each of the four fluoride-containing groups. After 20 days, significant differences were observed among the fluoride-containing groups, with fluoride plus 20 ppm TCP-Si-Ur providing significant surface strengthening relative to fluoride alone. Cross-sectional microhardness measurements revealed distinctly different strength profiles and infrared spectroscopy was employed to probe possible changes in enamel microstructure. Collectively, our results indicate a synergistic effect can be produced when TCP-Si-Ur is combined with fluoride and administered to eroded enamel.

Key words: Dental erosion, fluoride, tricalcium phosphate, TCP-Si-Ur, microhardness, IR spectroscopy, pH cycling, remineralization

INTRODUCTION

Dental erosion is a form of dental decay that is commonly produced through exposure to acids or mechanical wear of the teeth (Lussi et al., 1993; Bartlett, 2005; Lussi, 2006). For example, acids present in fruits or in soft drink beverages contribute to the erosive processes that degrade the quality and quantity of enamel. Regardless of the erosion mechanism, the end result is a bulk loss of tissue on the order of several microns (West et al., 2000; Barbour and Rees, 2004). If not regulated, dental erosion could lead to increased sensitivity and loss of tooth if left unchecked. For these reasons, dental erosion is rapidly gaining attention as a leading form of dental decay.

Although dental researchers have been aware of erosion for decades, advances in the repair of eroded enamel remain elusive. Fluoride, with its renowned anticaries benefits, has been considered as a possible solution to regulated dental erosion. However, while some clinical and laboratory studies demonstrate fluoride may be able to help manage dental erosion (Fowler et al., 2006; Zero et al., 2006; Hove et al., 2007; Vieira et al., 2007), other studies have shown fluoride efficacy is insufficient (Larsen et al., 2002; Wang et al., 2008). Thus, there is a need to develop innovative materials that may be able to enhance the protective/strengthening benefits of fluoride to better address dental erosion (Fowler et al., 2006). One approach may be the inclusion of other minerals, such as calcium and phosphate, with fluoride in order to elucidate a synergistic mineralization response (LeGeros, 1999; Jensdottir et al., 2007).

In the present work, we explored the anti-erosion potential of fluoride plus an innovative form of tricalcium phosphate. This form of tricalcium phosphate is comprised of beta-tricalcium phosphate (β-TCP), silica

*Corresponding author. E-mail: rkarlins@gmail.com. Tel: 317-278-7892. Fax: 317-278-4102.

(Si) and urea (Ur) that has been synthesized using a solid-state mechanochemical grinding process. A major benefit of this TCP-Si-Ur hybrid calcium phosphate material is that it maintains fluoride compatibility when introduced in an aqueous environment (such as a sodium fluoride, NaF, solution) and may act synergistically with fluoride to provide enhanced anti-erosion benefits relative to fluoride alone (Karlinsey et al., 2008a,b).

In order to better understand the anti-erosion impact of fluoride (F) and fluoride plus TCP-Si-Ur, we executed a 20-day pH cycling model involving human saliva (pH = 7), treatment periods and acid challenges (pH 3.8). Study design logistics and characterization of enamel specimens using micro-hardness and infrared (IR) measurements are discussed herein.

MATERIALS AND METHODS

Specimen preparation

Specimen preparation for the remineralization/demineralization pH cycling was performed as follows. 3 mm enamel cores were drilled from clean bovine teeth and mounted in acrylic rods. The cores were then serially ground with 600, 1000 and 1500 grit sandpaper, then polished with 9, 3 and 1 µm polycrystalline diamond suspension (Buehler).

Specimens were removed from the solution, rinsed with distilled (DI) water and measured for baseline Vickers microhardness (200 gf, 15 s dwell time) using a microhardness indenter (Leco, model 247AT) interfaced with Confident software (Leco). Specimens exhibiting mean Vickers hardness numbers (VHN) between 300 and 350 were selected for the study. An initial erosion period was then performed by immersing each enamel specimen in 10 ml of 1% citric acid (pH = 3.8) for 30 min. Subsequently the surface microhardness was again measured (200 gf, 15 s dwell time) and those specimens exhibiting VHN between 200 and 230 were accepted.

Study Groups

Specimens were then stratified into five groups (N = 10) having an overall mean VHN of about 214 VHN. The five groups evaluated in this study are as follows:

Group 1: Distilled water.
Group 2: 225 ppm F aqueous solution.
Group 3: 225 ppm F + 20 ppm TCP-Si-Ur aqueous suspension.
Group 4: 225 ppm F + 40 ppm TCP-Si-Ur aqueous suspension.
Group 5: 225 ppm F + 80 ppm TCP-Si-Ur aqueous suspension.

The fluoride source used in Groups 2 through 5 was NaF.

TCP-Si-Ur preparation

The TCP-Si-Ur agent was prepared using a solid-state mechanochemical grinding process described as follows. β-TCP (Budenheim), silica (Degussa-Evonik) and urea (Spectrum Chemical) were loaded into 500 ml stainless steel grinding jars lined with yttrium-stabilized zirconium oxide in the weight fractions of 93, 5 and 2 wt. %, respectively. Added to the jars were ten 20 mm diameter yttrium-stabilized zirconium oxide balls and 200 ml of pentane (> 99%, Sigma Aldrich), which served as a lubricant during grinding. Lids lined with zirconium oxide were then clamped tightly to each grinding jar and the grinding jar assembly was placed into a rotatable station mounted on the turntable of a PM400 planetary ball mill (Retsch), where it was secured with a quick-action locking spider clamp. Two jars were secured in the PM400 to ensure necessary counter-balancing. The turntable speed was set to 375 rpm (jar speed was 750 rpm in the direction opposite the turntable rotation) and the grinding process lasted for a period of two hours, with less than 5 rpm in speed variation as monitored with a CDT-100 hand-held tachometer (Check-Line). After the grinding process was completed, each jar system was carefully opened in a fume hood with the resultant moist powders deposited into a collection pan fitted with a sieve. The pan was then placed into a vacuum oven at ~ 45ºC (Fisher Scientific) and gently evacuated to a pressure of -29 in Hg (Ashcroft). This pressure was maintained until the pressure inside the oven did not increase when the oven valve to the vacuum pump was closed for at least 30 s. The resultant TCP-Si-Ur powder was off-white and did not stick to the jar wall or balls. Suspensions of 225 ppm fluoride plus TCP-Si-Ur were then made for the *in vitro* pH cycling evaluation described below.

Table 1. Outline of daily events and duration employed in the dental model evaluating repair of eroded enamel.

Event	Duration
Treatment #1*	2 min
Saliva, pH =7.0	2 h
Acid Challenge #1, pH = 3.8	2 min
Saliva, pH =7.0	1 h
Acid Challenge #2, pH = 3.8	2 min
Saliva, pH =7.0	1 h
Treatment #2	2 min
Saliva, pH =7.0	1 h
Acid Challenge #3, pH = 3.8	2 min
Saliva,** pH =7.0	1 h
Acid Challenge #4, pH = 3.8	2 min
Saliva, pH = 7.0	1 h
Acid Challenge #5, pH = 3.8	2 min
Saliva, pH = 7.0	1.5 h
Treatment #3	2 min
Saliva, pH = 7.0	Overnight

*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.

pH cycling model

The five groups of enamel specimens were then subjected to a remineralization/demineralization pH cycling model as described in Table 1. This model includes three two-minute treatment periods and five two-minute acid challenge periods. In between these events, the specimens are immersed in a 50:50 mixture of pooled human saliva and artificial saliva (Ten Cate et al., 1988). The pooled human saliva was collected from two healthy (that is, neither was on antibiotics) Caucasian women ages 21 and 24. 0.03% sodium azide (Alfa Aesar) was added to the saliva and it was stored at 4°C until use. The cycling regimen in Table 1 was repeated for a total of 20 days, with interim microhardness measurements made after 10 days of cycling. The treatment and saliva systems were magnetically agitated at 300 rpm, while the acid challenge was static. After each treatment and acid challenge, the specimens were

Table 2. Summary of surface microhardness results.

Group	VHN^0	VHN^{10}	VHN^{20}	Depth of Indent (µm)
1	214.7 ± 2.7[a]	237.0 ± 3.2[a]	233.1 ± 3.3[a]	5.7 ± 0.1[a]
5	214.3 ± 2.4[a]	261.4 ± 5.3[b]	269.4 ± 3.7[b]	5.3 ± 0.1[b]
2	214.7 ± 2.6[a]	265.6 ± 3.3[b]	270.6 ± 3.9[b]	5.3 ± 0.1[b]
4	214.4 ± 2.5[a]	261.3 ± 3.7[b]	281.8 ± 5.1[b,c]	5.2 ± 0.1[b,c]
3	214.6 ± 2.6[a]	265.5 ± 3.4[b]	286.7 ± 4.5[c]	5.1 ± 0.1[c]

VHN^0 = Mean baseline Vickers Hardness Number (VHN) ± SEM (N = 10).
VHN^{10} = Mean VHN ± SEM (N = 10) after 10 days of cycling.
VHN^{20} = Mean VHN ± SEM (N = 10) after 20 days of cycling.
Depth of Indent = Mean depth of Vickers indenter ± Std. Dev. (N = 10) for VHN^{20}.
Superscripts (a - c) indicate significant differences (*t*-test comparisons, $p < 0.05$).
Data is presented in order of increasing VHN^{20}.

rinsed with DI water prior to placement into the saliva mixture, which was changed once daily after the third acid challenge.

Surface and cross-sectional microhardness measurements

After 10 and 20 days of cycling, the enamel specimens were examined for Vickers surface hardness (200 gf, 15 s dwell time). The change in Vickers hardness number (ΔVHN) was determined as the difference between the post and baseline values ($\Delta VHN = VHN_{post} - VHN_{base}$).

Upon completion of surface measurements after 20 days of cycling, nine of the enamel specimens from each group were examined for longitudinal microhardness. This procedure is described as follows. A Lapcraft Lil' Trimmer circular saw was used to section the enamel specimens. The sections where then mounted with ClaroCit methylmethacrylate-based cold mounting resin (Struers) with the freshly cut surfaces exposed. The mounted specimens were serially ground with 180, 1000 and 1500 grit sandpaper (3M) and then serially polished with 9, 3 and 1 µm polycrystalline diamond suspension (Buehler). Longitudinal microhardness was performed with the Knoops indenter fitted on the microhardness tester. A series of three indentations per specimen were made under a 10 gf load at 12.5 µm, a 25 gf load at 25 µm and a 50 gf load at 37.5, 50, 75 and 100 µm below the specimen surface, resulting in a total of 18 indents per specimen. The Knoops indentation lengths were then converted to Knoops Hardness Numbers (KHN).

Infrared spectroscopy

Infrared (IR) spectroscopy was also performed from biopsies extracted from sound and initially eroded bovine enamel and also from one specimen from each of the five groups. Biopsies were extracted from the enamel cores by drilling under water-cooled conditions using a Power Glide 5-speed bench top drill press with a 7/64" bit. The resultant enamel powder was collected and dried in a Fisher Scientific Isotemp Model 280A vacuum oven at 80°C under normal pressure. The enamel powder was mixed with dried KBr powder (Buck Scientific) in a ratio of 1:100 and then finely ground with an agate mortar and pestle. 55 mg of this mixture was added to the barrel of a KBr pellet press (Buck Scientific). The press was secured in a vise, tightened to 40 ft.-lbs. using a torque wrench and then left under pressure for ten minutes. The bolts were carefully removed and the pellet was scanned from 4000 to 600 cm^{-1} using a Buck Scientific Model 500 Infrared Spectrophotometer (2 cm^{-1} resolution, 3 min scan time, 0 scan gain, 0.8 pen response). Acceptable pellet windows had percent transmission between 70 and 75% at 4000 cm^{-1}. A background scan was subtracted from the sample scan and the spectrum was analyzed using graphing software (Microcal Origin 6.0). The pellet preparation method was standardized so that the absorbance values of different samples could be quantitatively compared. Reproducibility (the error in absorbance intensity units (A.U.) was about ± 0.03 at 1035 cm^{-1}) was confirmed in triplicate for sound and eroded enamel.

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

Surface microhardness

Table 2 summarizes the surface Vickers microhardness data (mean ± SEM) for the five groups at baseline (VHN^0), after 10 days of cycling (VHN^{10}) and after 20 days of cycling (VHN^{20}). Statistical differences were determined at each endpoint. Among the NaF systems at this 10-day endpoint, all fluoride-containing groups provided significant surface rehardening relative to the water control. At the 20-day endpoint, 225 ppm F plus 20 ppm TCP-Si-Ur provided the greatest surface rehardening benefit and was statistically superior relative to the 225 ppm F control and the distilled water. A clear dose response of TCP-Si-Ur was not observed in this study: 225 ppm F plus 20 and 40 ppm TCP-Si-Ur were found to be statistically equivalent while 80 ppm TCP-Si-Ur plus 225 ppm F did not enhance benefits over fluoride alone. The penetration depth of the Vickers indenter into the enamel specimens was computed based on the Vickers microhardness value and loading conditions. The weakest specimens (that is, those experiencing the highest penetration) were treated with water (Group 1). The strongest specimens (that is, those experiencing the least penetration) belonged to the 225 ppm F plus 20 ppm

Table 3. Summary of longitudinal microhardness results.

Group	~ 5.5 µm	12.5 µm	25 µm	37.5 µm	50 µm	75 µm	100 µm
1	233.1 ± 3.3[a]	330.8 ± 13.0[c]	326.3 ± 8.9[c]	303.6 ± 4.9[b]	307.7 ± 5.7[b,c]	309.4 ± 5.3[a,b]	303.9 ± 6.0[c]
2	270.6 ± 3.9[b]	260.1 ± 25.1[a,b]	275.2 ± 18.4[a]	286.8 ± 15.4[a,b]	301.8 ± 7.4[a,b]	330.7 ± 5.3[c]	287.1 ± 11.9[a,b]
3	286.7 ± 4.5[c]	264.5 ± 22.1[b]	289.2 ± 14.5[a,b]	280.7 ± 14.5[a]	321.2 ± 6.2[c]	331.6 ± 11.7[c]	321.0 ± 7.1[d]
4	281.8 ± 5.1[b,c]	326.2 ± 14.6[c]	351.2 ± 8.9[d]	313.0 ± 7.3[b,c]	327.8 ± 5.8[c]	320.2 ± 5.1[b,c]	307.4 ± 6.9[c,d]
5	269.4 ± 3.7[b]	250.4 ± 18.1[a]	295.0 ± 15.2[b]	281.4 ± 6.6[a]	292.7 ± 9.8[a]	306.0 ± 9.1[a]	296.6 ± 9.6[b,c]

Mean Hardness Number (HN) (error bars represent the standard error of the mean) of enamel specimens after 20 days of remineralization/demineralization cycling. HN data at 5.5 µm correspond to Vickers Hardness Numbers (VHN) from Table 2. HN data between 12.5 µm and 100 µm correspond to Knoops Hardness Numbers (KHN). Superscripts (a - d) indicate significant differences (*t*-test comparisons, $p < 0.05$) in increasing order from the smallest to largest HN.

TCP-Si-Ur group (Group 3).

Longitudinal microhardness

The cross-sectional microhardness for all five groups is summarized in Table 3. Below the outer enamel surface (that is, below 5.5 µm) the remineralization effect due to each treatment group produces distinct trends. The strengths of the specimens receiving water (Group 1) are practically unchanged between 12.5 and 100 µm. In contrast, the specimens receiving only fluoride (Group 2) are weak at 12.5 µm and gradually display microhardness strengths characteristic of sound enamel at 80 µm. Even further, for specimens receiving fluoride plus TCP-Si-Ur, alternating strength trends are observed throughout. Collectively, these results suggest distinct protective and/or rehardening mechanisms are generated for water, fluoride and fluoride plus TCP-Si-Ur. For all groups between 80 and 100 µm, however, the data trends likely indicate the proximity of the dentin-enamel junction (DEJ).

Infrared spectroscopy

IR spectra for sound and eroded enamel are shown in Figure 1a. This qualitative and quantitative comparison clearly shows the initial 30 min erosion treatment produces a bulk mineral change in enamel structure with significant reductions in carbonate (between 1400 and 1500 cm^{-1}) and orthophosphate constituents (between 800 and 1200 cm^{-1}). The IR spectra of enamel biopsies collected after 20 days of pH cycling from each of the five groups are shown in Figure 1b. Statistically, the water and 225 ppm F treatment groups produce equivalent IR quantitative absorbance despite some qualitative differences in the spectra. A statistically lower absorbance is observed in the enamel biopsy treated with 225 ppm F + 20 ppm TCP-Si-Ur, while the remaining two groups with 40 and 80 ppm TCP-Si-Ur are statistically equivalent. These latter two treatment groups (Groups 4 and 5) produce relatively wider bandwidths compared to the other three groups. The progressive decrease in IR absorbance appears to indicate water, fluoride and fluoride plus TCP-Si-Ur impart distinct protective/rehardening effects on eroded enamel.

DISCUSSION

Application of pH cycling models to evaluate the effects of various agents on tissue repair should be consistent with clinical observations (White, 1992). In the present model, the surface microhardness data reveal statistical differences between water (the negative control) and fluoride (the positive control). Although studies reveal fluoride may not be considered the ultimate anti-erosion agent (Larsen et al., 2002; Wang et al., 2008), fluoride has shown significant anti-erosion promise in clinical (Zero, 2006; Vieira et al., 2007) and laboratory (Fowler et al., 2006; Hove et al., 2007) studies; thus, we selected fluoride as an appropriate control agent. The cycling model employed is slanted towards remineralization due in part to the relatively short acid challenge periods relative to the longer salivary exposure periods.

Microhardness measurements made after 10 days of cycling provide initial repair/protection metrics for each group. The microhardness measurements observed for the water group at 10 and 20 days of cycling lead to the probable conclusion that the saliva mixture is responsible for the enamel strengthening enjoyed by the water control group. Alternately, fluoride does appear to offer strengthening benefits and this effect is readily observed after 10 and 20 days of cycling. Addition of TCP-Si-Ur to fluoride does not promote significant strengthening effects above fluoride alone at 10 days of cycling; however, marked strengthening effects are observed after 20 days of cycling for groups containing 20 and 40 ppm TCP-Si-Ur.

Further insights into the effect from each treatment can be learned using the VHN^{20} values in Table 2 along with the cross-sectional microhardness results in Table 3. With respect to the water control group, there appears significant softening near the outer enamel layers, at least down to about 6 µm. At depths near and extending beyond 12.5 µm, the microhardness measurements reveal KHN values consistent with sound enamel. Bulk tissue loss near the enamel surface is indicative of erosion

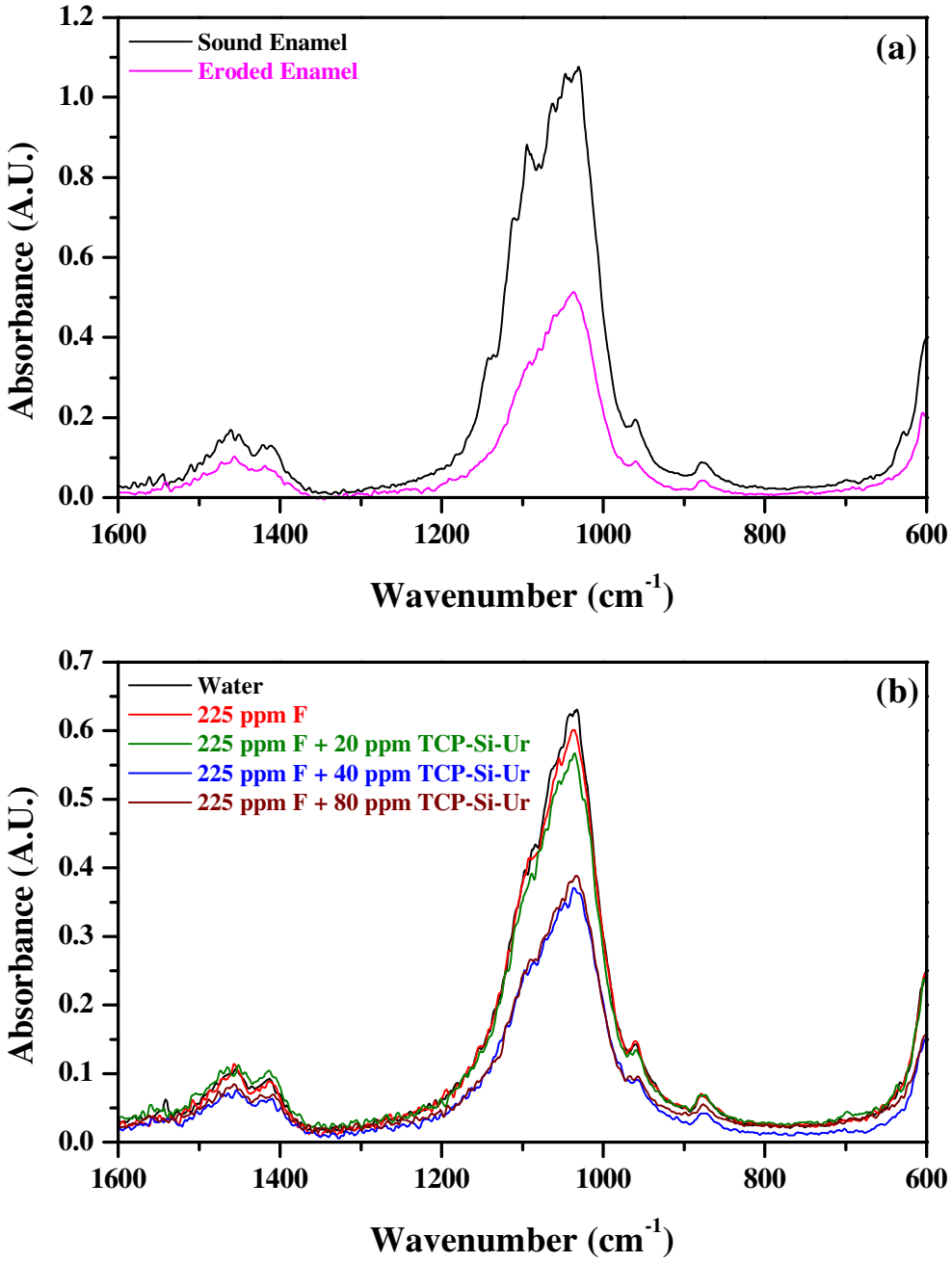

Figure 1. Histograms of infrared (IR) spectra collected from enamel biopsies of sound and initially eroded bovine enamel with 1% citric acid (pH = 3.8) for 30 min (a) and after 20 days of remineralization/demineralization pH cycling with the five different treatment groups (b).

rather than caries formation and has been observed previously via scanning electron microscopy (Eisenburger et al., 2004). In fact, if as little as 1.5 µm (West et al., 2000) of enamel are lost per two-minute acid etch over a period of 20 days, this would equate to 60 µm of mineral lost.

This also means saliva does not provide ample protection against bulk mineral loss; as a result, enamel layers are repeatedly worn away over the course of the 20-day cycling model. With respect to the fluoride-only group, the VHN^{20} value is similar to the KHN value at 12.5 µm and gradually increases up to 80 µm. The repetition of citric acid challenges will leach soluble enamel constituents, such as hydroxyl and carbonate groups. Since fluoride can readily replace hydroxyl and carbonate groups, it is reasonable to expect fluoride incorporation and reaction into the bulk and surface of enamel tissue.

As a result, the effect of bulk tissue loss (as experienced

in the water control group) is attenuated somewhat, even though the strength of the retained eroded lesion may still be weaker than sound enamel. Profilometry and other experiments have been previously used to assess eroded enamel and the effect of different fluoride concentrations on size of eroded enamel lesions (Barbour and Rees, 2004; Ren et al., 2009).

In Table 3, the alternating KHN trends for 225 ppm F plus 20, 40 and 80 ppm TCP-Si-Ur differ markedly from the water and fluoride-only groups. While enamel treated with water did little to protect enamel layers from eroding and fluoride provided some strengthening benefit to eroded enamel, the addition of TCP-Si-Ur appears to provide distinct anti-erosion behavior. The constant rise and fall of KHN values in Table 3 for all three TCP-Si-Ur groups indicates a mechanistic shift from both water and fluoride. At depths less than 6 µm, the VHN^{20} values demonstrate the potential benefits from 225 ppm F plus 20 and 40 ppm TCP-Si-Ur. Both the surface and longitudinal microhardness assessments indicate that 80 ppm TCP-Si-Ur may not provided substantial benefits relative to fluoride alone.

Alternately, 20 ppm TCP-Si-Ur provides significant surface benefits and some longitudinal benefits as well. Overall, 40 ppm TCP-Si-Ur appears to provide substantially harder mineral at depths between 12.5 µm and 37.5 µm relative to either 20 ppm or 80 ppm TCP-Si-Ur or 225 ppm F alone. It is possible that the mineral seeding due to 225 ppm F plus TCP-Si-Ur produces more acid-resistant mineral during growth periods in saliva and we have previously reported on the relatively higher acid-resistance of enamel treated with fluoride plus TCP-Si-Ur compared to fluoride alone (Karlinsey et al., 2008a).

In addition to providing bulk measurement observations, we also characterized the enamel microstructure with IR spectroscopy. Figure 1a reveals significant modification of the carbonate and phosphate framework when enamel was subjected to an initial 30 min citric acid challenge (1%, pH = 3.8). Recently we reported on the ability of using IR spectroscopy to qualitatively and quantitatively describe changes in enamel structure due to mineral seeding events (Karlinsey et al., 2009).

In the present study, we performed biopsies on one specimen from each group after 20 days of cycling and evaluated the enamel structure. The histogram in Figure 1b summarizes these results. Keeping in mind the initially eroded specimen generates an absorbance around 0.5 (Figure 1a), there is an increase in absorbance for the water, 225 ppm F and 225 ppm F + 20 ppm TCP-Si-Ur group. For the water group, the relative increase in intensity is likely attributed to the loss of eroded enamel layers due to the cycling regimen. The combination of the absence of an effective mineral nucleating agent and the elimination of porous eroded enamel layers appears to render the biopsy to be comprised of mostly sound enamel.

The longitudinal microhardness data support this conclusion. With respect to 225 ppm F, the inclusion of fluoride provides some protection against dental erosion and this is especially true near the surface as shown in Table 2. The gradual increase in microhardness as shown in Table 3 reveals the cycling model enables eroded enamel to be somewhat preserved during the acid challenges; therefore, the loss of tissue may account for the similarity in IR signatures collected on biopsies extracted from water-treated and fluoride-treated specimens.

There is a significant reduction in orthophosphate intensity for groups containing TCP-Si-Ur, beginning with 20 ppm TCP-Si-Ur. One possibility for the relatively attenuated IR response is that distinct mineral phases are produced in the eroded enamel. Such mineral phases may be devoid of substantial carbonate and hydroxyl groups, but are comprised of fluoride and TCP-Si-Ur constituents instead. Although the resulting mineral phases may increase the strength of eroded enamel, these mineral phases do not necessarily emulate the natural mineral structure of sound enamel and are not expected to due the replacement of carbonate and hydroxyl enamel constituents with fluoride, calcium and phosphate. The IR spectra generated from biopsies of specimens treated with 225 ppm F plus 40 ppm and 80 ppm TCP-Si-Ur are essentially overlapping, indicating substantial fluoride-TCP-Si-Ur mineral formation may be developing within the eroded enamel; ultimately, mineral formation appears to be limited to the amount of TCP-Si-Ur present. In consideration of the surface and longitudinal microhardness measurements, it appears that the combination of 225 pm F plus 80 ppm TCP-Si-Ur does not provide adequate acid-resistant mineral; hence, too much TCP-Si-Ur may interfere with mineral integration processes. As such, the ratio of fluoride and TCP-Si-Ur appears to impart a significant role in the strengthening/protection of eroded enamel.

Conclusion

In summary, the results described in this paper demonstrate a synergistic effect is produced when TCP-Si-Ur is combined with fluoride and administered to eroded enamel. The pH cycling model design produced significant differences among the water-only, fluoride-only and fluoride plus TCP-Si-Ur groups. Longitudinal measurements demonstrated the protection/strengthening of eroded enamel is distinct for water, fluoride and fluoride plus TCP-Si-Ur. Importantly, we observed TCP-Si-Ur can be combined with fluoride to produce anti-erosion benefits greater than those achieved with fluoride alone. While IR spectroscopy provided useful insight into the changes in enamel structure due to erosion and various treatments, further investigation into the mineral seeding behavior of TCP-Si-Ur and its effect on eroded enamel is

reserved for another study. These results therefore support the continued development and investigations of anti-erosion formulations containing fluoride and a promising TCP-Si-Ur agent.

ACKNOWLEDGEMENT

This work was sponsored through a research grant by GlaxoSmithKline. The authors claim no conflicts of interest in pursuit of this research.

REFERENCES

Barbour ME, Rees JS (2004). The laboratory assessment of enamel erosion: A review. J. Dent 32: 591-602.

Bartlett DW (2005). The role of erosion in tooth wear: aetiology, prevention and management. Int. Dent. J. 55: 277-284.

Eisenburger M, Shellis RP, Addy M (2004). Scanning electron microscopy of softened enamel. Caries. Res. 38(67-74).

Fowler C, Willson R, Rees GD (2006). *In vitro* microhardness studies on new anti-erosion desensitizing toothpaste. J. Clin. Dent. 17(Spec Iss): 100-105.

Hove LH, Young A, Tveit AB (2007). An *in vitro* study on the effect of TiF_4 treatment against erosion by hydroxychloric acid on pellicle-covered enamel. Caries Res. 41: 80-84.

Jensdottir T, Nauntofte B, Buchwald C, Bardow A (2007). Effect of calcium on the erosive potential of acidic candies in saliva. Caries Res. 41: 68-73.

Karlinsey RL, Mackey AC, Stookey GK (2008a). *In vitro* anti-erosion efficacy of a modified fluoride dentifrice. J Dent. Res. 87(Spec Iss B): Abstract 2593.

Karlinsey RL, Mackey AC, Stookey GK (2008b). *In vitro* study evaluating the anti-erosion potential of multi-mineral suspensions. J. Dent. Res. 87(Spec Iss B): Abstract 2595.

Karlinsey RL, Mackey AC, Walker ER, Frederick KE (2009). Spectroscopic evaluation of native, milled and functionalized β-TCP seeding into dental enamel lesions. J. Mater. Sci. 44: 5013-5016.

Larsen MJ, Richards A (2002). Fluoride is unable to reduce dental erosion from soft drinks. Caries Res. 36: 75-80.

LeGeros RZ (1999). Calcium phosphates in demineralization/ remineralization processes. J. Clin. Dent. 10: 65-73.

Lussi A (2006). Erosive Tooth Wear - A Multifactorial Condition of Growing Concern and Increasing Knowledge. Dental Erosion. A. Lussi. Basel, Karger pp.1-8.

Lussi A, Jaggi T, Scharer S (1993). The influence of different factors on *in vitro* enamel erosion. Caries Res. 27: 387-393.

Ren Y-F, Zhao Q, Malmstrom H, Barnes V, Xu T (2009). Assessing fluoride treatment and resistance of dental enamel to soft drink erosion *in vitro*: Applications of focus variation 3D scanning microscopy and stylus profilometry. J. Dent. 37: 167-176.

Ten Cate JM, Timmer K, Shariati M, Featherstone JDB (1988). Effect of timing of fluoride treatment on enamel de- and remineralization *in vitro*: a pH-cycling study. Caries Res. 22: 20-6.

Vieira A, Jager DHJ, Ruben JL, Huysmans MCDNJM (2007). Inhibition of erosive wear by fluoride varnish. Caries Res. 41: 61-67.

Wang W, Klocke A, Mihailova B, Tosheva L, Bismayer U (2008). New insights into structural alteration of enamel apatite induced by citric acid and sodium fluoride solutions. J. Phys. Chem. B 112: 8840-8848.

West NX, Hughes JA, Addy M (2000). Erosion of dentine and enamel *in vitro* by dietary acids: the effect of temperature, acid character, concentration and exposure time. J. Oral. Rehab. 27: 875-880.

White DJ (1992). The comparative sensitivity of intra-oral, in vitro and animal models in the 'profile' evaluation of topical fluorides. J. Dent. Res. 71(Spec Iss): 884.

Zero DT, Hara AT, Kelly SA, Gonzalez-Cabezas C, Eckert GJ, Barlow AP, Mason SC (2006). Evaluation of a desensitizing test dentifrice using an in situ erosion remineralization model. J. Clin. Dent.17(Spec Iss): 112-116.