Comparison of the Incipient Lesion Enamel Fluoride Uptake from Various Prescription and OTC Fluoride Toothpastes and Gels

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Abstract

- **Objective:** The objective of this *in vitro* study was to compare the fluoride uptake into incipient enamel lesions of a novel 970 ppm F^- ion SnF_2 over-the-counter (OTC) gel (Enamelon® Preventive Treatment Gel) and a novel 1150 ppm F^- ion OTC toothpaste (Enamelon®), each delivering amorphous calcium phosphate (ACP), to the uptake from two different prescription strength, 5000 ppm F^- ion dentifrices containing tri-calcium phosphate (TCP) and a prescription 900 ppm F^- ion paste containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).
- **Methods:** The test procedure followed method #40 in the US-FDA Anticaries Drug Products for OTC Human Use, Final Monograph testing procedures. Eight sets of twelve incisor enamel cores were mounted in Plexiglas rods and the exposed surfaces were polished. The indigenous fluoride levels of each specimen were determined prior to treatment. The treatments were performed using slurries of a negative control (water) and the following products applied to a set of sound enamel cores: 5000 ppm F^- ion, sodium fluoride (NaF) prescription (Rx) dentifrice "A" containing TCP; 5000 ppm F^- ion, NaF Rx dentifrice "B" containing TCP; 900 ppm F^- ion, NaF Rx paste with CPP-ACP; 1150 ppm F^- ion, NaF OTC toothpaste; 1150 ppm F^- ion, stannous fluoride (SnF_2) OTC toothpaste delivering ACP (Enamelon®); 1100 ppm F^- ion, SnF_2 OTC toothpaste; and 970 ppm F^- ion, SnF_2 OTC gel delivering ACP (Enamelon® Preventive Treatment Gel). The twelve specimens of each group were immersed into 25 ml of their assigned slurry with constant stirring (350 rpm) for 30 minutes. Following treatment, one layer of enamel was removed from each specimen and analyzed for fluoride and calcium. The pre-treatment fluoride (indigenous) level of each specimen was subtracted from the post-treatment value to determine the change in enamel fluoride due to the test treatment.
- **Results:** The increase in the average fluoride uptake for treated enamel cores was: 10,263 ± 295 ppm for the 970 ppm F^- ion, Enamelon Preventive Treatment Gel; 7,016 ± 353 ppm for the 1150 ppm F^- ion Enamelon Toothpaste; 4,138 ± 120 ppm for the 5000 ppm F^- ion, NaF prescription dentifrice "A" with TCP; 3801 ± 121 ppm for the 5000 ppm F^- ion, NaF prescription dentifrice "B" with TCP; 2,647 ± 57 ppm for the 1100 ppm F^- ion, SnF_2 OTC toothpaste; 1470 ± 40 ppm for the 1150 ppm F^- ion, NaF OTC toothpaste; and 316 ± 9 ppm for the 900 ppm F^- ion, NaF paste with CPP-ACP. The differences among all the products tested were statistically significant ($p < 0.05$), except for the two 5000 ppm F^- ion products with TCP that were not statistically different from one another, and the 900 ppm F^- ion, NaF paste with CPP-ACP that was not statistically different from the negative water control.
- **Conclusion:** The Enamelon products (970 ppm and 1150 ppm F^- ion, SnF_2 OTC dentifrices) delivering ACP provide statistically significantly more fluoride to incipient enamel lesions than two prescription strength 5000 ppm F^- ion toothpastes containing TCP, the 900 ppm F^- ion prescription paste containing CPP-ACP, and the other OTC toothpastes compared in this study.

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Introduction

Use of fluoride toothpaste has been recommended for more than 50 years to prevent and control dental caries.[1] Fluoride has long been recognized to provide an anticaries benefit.[2,3] Despite the use of fluoride, tooth decay remains a prevalent chronic disease in both children and adults, even though it is highly preventable.[4] Although many ingredients have been introduced to help fight tooth decay, the most universally used active ingredient in dentifrices for caries control is still fluoride.[5]

The evidence for the anticaries efficacy of daily fluoride dentifrice use is strong;[6] however, many OTC and prescription toothpastes and gels have sought to include additional ingredients in an effort to safely help make fluoride more effective. The influx, popularity, and availability of new fluoride technologies to fight dental caries provides consumers with many benefits, but also can raise concerns. Along with the decline in caries, an increase in the prevalence of dental fluorosis, has been recognized.[7]

Researchers in oral health are constantly searching for ways to improve caries prevention while minimizing systemic patient risk. In 1991, ADA Foundation's Paffenbarger Research Center chemist and researcher Ming S. Tung, PhD demonstrated the value of amorphous calcium phosphate (ACP) in remineralizing teeth. The ADA Foundation funded the research on the use of this unstructured form of calcium phosphate molecules for use in dental products, and thus holds the patents. Since 1991, ACP

has been proven to be the first non-fluoride therapy to remineralize enamel and dentin.[8]

There is a growing body of literature showing how calcium and phosphate supplementation of fluoride treatments can enhance fluoride uptake.[9] In several studies, products designed to deliver ACP have been shown to contribute to greater fluoride uptake.[10-13] A review of the research on this topic shows that ACP incorporated into fluoride toothpaste has been found to provide greater fluoride uptake compared to a fluoride toothpaste without the addition of calcium and phosphate technology.[14] Another *in vitro* study demonstrates greater fluoride uptake by the ACP-containing varnish than the varnish containing tri-calcium phosphate (TCP).[9]

Since calcium and phosphate ions are the primary natural constituents of tooth structure, adequate quantities of these ions must be present in the delivery system for remineralization to occur. Human saliva naturally contains varying amounts of these ions, but may not be sufficient to aid in remineralization when the normal physiological conditions are disrupted. More advanced fluoride products with different calcium and phosphate ion sources have been developed to supplement the level of these ions in saliva in the hopes of enhancing remineralization.[9]

The design philosophy behind a pair of new dentifrices was to create products that utilize the adjuvant properties of these remineralizing ions to provide greater fluoride uptake into lesions in enamel using less fluoride ion. Research seemed to also support that inclusion of highly substantive carriers which will help to retain these remineralizing ions on tissue for longer periods of time may further enhance fluoride uptake. This research has extensively influenced the design of the new products.

One way to determine if the Enamelon® products achieve these objectives is to compare them against other prescription and OTC fluoride toothpastes and gels, which may also contain different sources of calcium and phosphate (TCP, CPP-ACP), for their ability to deliver fluoride into tooth enamel lesions.

The two new products of interest are Enamelon Preventive Treatment Gel (970 ppm F^- ion, SnF_2 gel with ACP technology) and Enamelon Toothpaste (1150 ppm F^- ion, SnF_2 toothpaste with ACP technology); both by Premier Dental Products, Plymouth Meeting, PA, USA. The addition of two highly substantive polymer complexes have been incorporated with the calcium and phosphate salts to promote long-term bioavailability of these remineralizing ions. This combination of substantive agents and remineralizing ions are intended to form the backbone of an enhanced fluoride delivery system without having to increase the fluoride ion concentration.

The study reported here was designed to determine if the Enamelon 970 or 1150 ppm F^- ion products containing calcium and phosphate salts which combine in an aqueous media to form ACP, can be optimized with the help of the two copolymers, to provide greater fluoride uptake than products containing up to five times the amount of F^- ion.

Materials and Methods

Sound, upper central bovine incisors were selected and cleaned of all adhering soft tissue. A core of enamel 3 mm in diameter was prepared from each tooth by cutting perpendicularly to the labial surface with a hollow-core diamond drill bit. This was performed under water to prevent overheating of the specimen. Each specimen was embedded in the end of a Plexiglas rod (1/4" diameter x 2" long) using methyl methacrylate. The excess acrylic was cut away exposing the enamel surface. The enamel specimens were polished with 600 grit wet/dry paper and then with micro-fine gamma alumina. The resulting specimen was a 3 mm disk of enamel with all but the exposed surface covered with acrylic. Twelve specimens per group were prepared.

Each enamel specimen was etched by immersion into 0.5 ml of 1M HCl04 for 15 seconds. Throughout the etch period, the etch solutions were continuously agitated. A sample of each solution was buffered with TISAB to a pH of 5.2 (0.25 ml sample, 0.5 ml TISAB, and 0.25 ml 1N NaOH) and the fluoride content determined by comparison to a similarly prepared standard curve (1 ml std + 1 ml TISAB). For use in depth of etch calculation, the Ca content of the etch solution was determined by taking 50 µl and analyzing for Ca by atomic absorption (0.05 ml qs to 5 ml). This data was the indigenous fluoride level of each specimen prior to treatment.

The specimens were again ground and polished as described above. An artificial incipient lesion was formed in each enamel specimen by immersion into a 0.1M lactic acid/0.2% Carbopol 907 solution for 24 hours at room temperature. The specimens were then rinsed well with distilled water and stored in a humid environment until used.

The treatments were performed using slurries of the dentifrice. The slurries consisted of 1 part dentifrice and 3 parts distilled water (9 g:27 ml). The products tested were: two 5000 ppm F^- ion, NaF dentifrices containing TCP; a 900 ppm F^- ion, NaF paste containing CPP-ACP; a 1150 ppm F^- ion, NaF toothpaste; a 1100 ppm F^- ion, SnF_2 toothpaste; Enamelon Preventive Treatment Gel (970 ppm F^- ion, SnF_2 delivering ACP); and Enamelon Toothpaste (1150 ppm F^- ion, SnF_2 toothpaste delivering ACP).

Each slurry was mixed for exactly one minute after adding the water. The 12 specimens of each group were then immersed into 25 ml of their assigned slurry with constant stirring (350 rpm) for 30 minutes. Following treatment, the specimens were rinsed with distilled water. One layer of enamel was then removed from each specimen and analyzed for fluoride and calcium as outlined above (*i.e.*, 15-second etch). The pre-treatment fluoride (indigenous) level of each specimen was then subtracted from the post-treatment value to determine the change in enamel fluoride due to the test treatment.

The mean, SD, and SEM for each group were calculated. Statistical analysis comparing the groups was performed by a one-way analysis of variance model using Sigma Stat Software (3.1). Since significant differences were indicated, the individual means of each group were compared by the Student Newman Keuls test.

Results

The increase of fluoride into the incipient lesion in enamel results are shown in Table I. The 900 ppm F^- ion NaF paste with CPP-ACP result was not statistically different from the negative control. The 1150 ppm F^- ion OTC NaF toothpaste was statistically significantly more effective than the 900 ppm F^- ion NaF paste with CPP-ACP. That was followed by the OTC SnF_2

Table I
Change in Incipient Lesions Enamel Fluoride Content

Treatment	Ppm F^- ion	Enamel Fluoride Concentration (ppm) Pre-Treatment		Post-Treatment	Increase
Negative Control	0	37 ± 2*	**	45 ± 3	8 ± 2
Rx NaF Paste with CCP-ACP	900	43 ± 3		359 ± 10	316 ± 9
OTC NaF Toothpaste	1150	41 ± 2		1511 ± 39	1470 ± 40
OTC SnF_2 Toothpaste	1100	40 ± 3		2686 ± 55	2647 ± 57
Rx NaF Toothpaste "B" with TCP	5000	37 ± 3		3838 ± 121	3801 ± 121
Rx NaF Toothpaste "A" with TCP	5000	41 ± 3		4178 ± 121	4138 ± 120
Enamelon OTC SnF_2 Toothpaste Delivering ACP	1150	38 ± 2		7055 ± 353	7016 ± 353
Enamelon OTC SnF_2 Treatment Gel Delivering ACP	970	38 ± 3		10300 ± 295	10263 ± 295

*Mean ± SEM (N = 12)
**Values connected within brackets do not differ significantly ($p > 0.05$) as determined by Newman-Keuls analysis.

toothpaste which was statistically significantly more effective than the OTC NaF toothpaste. The two prescription strength 5000 ppm F^- ion NaF toothpastes with TCP were statistically significantly more effective than all the previously mentioned products, but not statistically different from each other. The Enamelon toothpaste was statistically significantly different than all the previously mentioned products. Enamelon Preventive Treatment Gel with 970 ppm F^- ion was the most effective in promoting enamel fluoride uptake into the incipient lesions and it was significantly superior to all other products tested.

Discussion

In this study, the novel Enamelon toothpaste and gel delivered more fluoride into the lesioned enamel than the prescription strength and OTC products tested. It is important to note that the 970 ppm F^- ion Enamelon Preventive Treatment Gel with 80% less F^- ion provided nearly two-and-a-half times greater fluoride uptake than two prescription strength toothpastes containing 5000 ppm F^- ion and TCP.

The results also indicate the ability of Enamelon Preventive Treatment Gel to provide 30 times greater increase in fluoride uptake into the incipient lesions in enamel than the 900 ppm F^- ion prescription paste containing CPP-ACP tested. Therefore, this study seems to have demonstrated that the OTC Enamelon treatment gel and toothpaste do in fact provide a significantly greater fluoride increase into lesions in enamel with considerably less fluoride ion than the prescription strength 5000 ppm F^- ion products with TCP. The greater bioavailability of calcium and phosphate ions in these ACP products may be the reason for the enhanced fluoride uptake.

A separate study produced similar results demonstrating that a fluoride toothpaste, simultaneously delivering calcium and phosphate salts, released about two-and-a-half times as much fluoride into the lesioned enamel than the reference toothpaste containing a similar content of fluoride but without ACP.[14] In another study, a 1100 ppm fluoride dentifrice delivering soluble calcium and phosphate ions as ACP was shown to significantly remineralize and prevent root caries in head and neck radiation patients.[15] The results from prior studies seem to be consistent with the results of this study; products with bio-available ACP may produce greater fluoride uptake into the lesioned enamel than products with similar ingredients without ACP.

Calcium and phosphate technologies currently incorporated into dental products tested in this study include ACP, CPP-ACP, and TCP. The purpose of adding these technologies is to increase the amount of available calcium and phosphate which work together with fluoride to enhance product performance. The results of this study seem to indicate that the various forms of calcium and phosphate salts are not equally bio-available and thus, not equally efficacious. The greater bio-availability of the calcium and phosphate salts in ACP could be the reason why the Enamelon products significantly outperformed the prescription strength and OTC products in this study containing less bio-available forms of calcium and phosphate salts (TCP, CPP-ACP)

TCP is a fairly insoluble crystalline form of calcium phosphate.[9] ACP is non-crystalline and has no systematic structure. As a result, ACP is more soluble than TCP and, therefore, is more bio-available (reactive) than other crystalline calcium phosphates.[16] Amorphous calcium phosphate compounds have the highest rates of formation and dissolution among all the calcium phosphate forms, and rapidly hydrolyze *in situ* to apatite (the tooth mineral).[17] Calcium fluoride globule reservoirs are believed to release calcium and fluoride under an acid challenge, which can help to buffer acidic pH and promote remineralization. The research presented shows that ACP has been found to increase fluoride release and uptake, which supports the superior results associated with the ACP delivering Enamelon products in this study.

Additionally, the Enamelon products utilize stannous fluoride and contain two highly substantive polymer carriers which may aid in decreasing the ability of biofilm to adhere to enamel and help to retain the remineralizing agents on the tooth surface considerably longer. This novel Enamelon combination and the growing body of ACP research in conjunction with the results of this study seem to demonstrate that the Enamelon products can provide greater fluoride uptake into enamel lesions with significantly lower doses of fluoride than prescription strength dentifrices.

The results of this study indicate that SnF_2 Enamelon Preventive Treatment Gel with ACP technology and the SnF_2 Enamelon Toothpaste with ACP technology were statistically significantly more effective in promoting enamel fluoride uptake into incipient enamel lesions than two prescription strength 5000 ppm F^- ion dentifrices with TCP, a NaF paste with CPP-ACP, and OTC toothpastes. It is likely that this outcome results from the greater bio-availability of the calcium and phosphate ions and the substantivity enhancing polymers in the Enamelon formulations.

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Abstract

- **Objective:** The purpose of this *in vitro* study was to determine if a novel 970 ppm F^- ion SnF_2 OTC gel (Enamelon® Preventive Treatment Gel) and a 1150 ppm F^- ion SnF_2 OTC Enamelon® Toothpaste, each delivering amorphous calcium phosphate (ACP), can significantly reduce the effect of an acid challenge to enamel as compared to two prescription (Rx) strength 5000 ppm F^- ion (NaF) dentifrices containing tri-calcium phosphate (TCP), and an Rx 900 ppm F^- ion (NaF) paste with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). The effect will be determined by measuring the resistance of enamel specimens to an acid challenge before and after treatment with the test dentifrices.
- **Methods:** The procedure used in this study was the FDA Test Method #33 for the determination of the effect of different test dentifrices on enamel solubility reduction. Twelve sets of three extracted human teeth were unprotected and etched prior to treatment with 0.1M lactic acid buffer solution. The amount of phosphate dissolved from the teeth was quantified via measuring the phosphate in the retained lactate buffer solution with phosphorous analysis (pre-treatment phosphorous levels). The teeth sets were then exposed to the following treatments (diluted 1:3 parts in preheated [37ºC] distilled water): 5000 ppm F^- ion, sodium fluoride (NaF) Rx dentifrice containing TCP; 5000 ppm F^- ion, NaF Rx dentifrice; 900 ppm F^- ion, NaF Rx paste with CPP-ACP; 1150 ppm F^- ion, stannous fluoride (SnF_2) OTC toothpaste delivering ACP Enamelon Toothpaste; and 970 ppm F^- ion, SnF_2 OTC gel delivering ACP (Enamelon Preventive Treatment Gel). The teeth sets were rinsed with distilled water and then exposed to 0.1M buffered lactic acid solution. The amount of phosphate in the lactic acid buffer was determined for a second time (post-treatment phosphorous levels). The percent of enamel solubility reduction was then computed as the difference between the amount of phosphorous in the pre- and post-treatment lactic acid solutions divided by the amount of phosphorous in the pre-treatment solution, and multiplied by 100.
- **Results:** The percent reduction in enamel solubility recorded in this study was as follows: 60.14 ± 0.79 for the Enamelon Toothpaste; 56.91 ± 1.05 for the Enamelon Preventive Treatment Gel; 18.78 ± 3.20 for the 5000 ppm F^- ion, NaF prescription dentifrice "A" with TCP; 6.84 ± 1.20 for the 900 ppm F^- ion, NaF paste with CPP-ACP; 5.82 ± 3.10 for the 5000 ppm F^- ion, NaF prescription dentifrice "B" with TCP; and -5.45 ± 1.86 for the negative control. The differences between all the products tested were statistically significant ($p < 0.05$), except for the Enamelon products that were not statistically different. The 900 ppm F^- ion, NaF paste with CPP-ACP and the 5000 ppm F^- ion, NaF toothpaste results were also not statistically different.
- **Conclusion**: The Enamelon products (970 ppm and 1150 ppm F^- ion, SnF_2 OTC dentifrices) delivering ACP were statistically significantly more effective in reducing enamel solubility than two Rx strength 5000 ppm F^- ion NaF toothpastes containing TCP and the Rx 900 ppm F^- ion NaF paste containing CPP-ACP.

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Introduction

The U.S. Food and Drug Administration (FDA) has mandated the use of various bioavailability tests to demonstrate the effectiveness of over-the-counter (OTC) anticaries dentifrices. An OTC fluoride dentifrice product shall meet the biological test requirements for animal caries reduction and one of the following tests: Enamel Solubility Reduction (ESR) Method #33 or Enamel Fluoride Uptake (EFU) Method #40.[1]

These tests are designed to demonstrate that fluoride is chemically available from the products. The enamel solubility test demonstrates whether the fluoride contained in the product is available to be taken up by tooth mineral and whether, once taken up, it is in a useful form such that it reduces the rate of dissolution of tooth mineral when exposed to an acidic solution.[2]

The purpose of this study was to evaluate the bioavailability of the fluoride provided from the Enamelon® products to effectively reduce the percentage of enamel solubility using the Method #33 enamel solubility reduction (ESR) protocol from the FDA. Distilled water was used as a negative control. This *in vitro* test was designed to determine if Premier's 970 and 1150 ppm F^- ion SnF_2 products containing calcium and phosphate salts, which combine in an aqueous medium to form ACP, along with two substantivity promoting copolymers, can provide greater resist-

ance to enamel solubility than prescription (Rx) strength products containing up to five times more fluoride ion.

Materials and Methods

Tooth Preparation

Three sound human molars were placed in a disc of red boxing wax so that only the enamel surfaces were exposed. Twelve sets of three teeth each were prepared for this study. All specimens were cleaned and polished with a flour of pumice slurry and a rag wheel to remove any deposits or stains.

Lactate Buffer Preparation

Two moles (203.58 g) of 88.5% pure lactic acid were diluted with approximately 500 ml of distilled water. To this a solution of 84 g NaOH dissolved in about 600 ml of distilled water was added. The total volume was then adjusted to 2000 ml. This was the buffered 1.0 M lactic acid solution.

Another 1.0 M lactic acid solution was prepared by diluting two moles lactic acid to 2000 ml with distilled water. The solution of lactic acid and sodium hydroxide was placed in a 4000 ml beaker and pH electrodes placed in the solution. The 1.0 M lactic acid solution was used to adjust the pH of the buffered solution to 4.5. To obtain a 0.1 M working concentration (for all decalcifications), the 1.0 M buffer was diluted by a factor of 10 with distilled water.

Unprotection

Before every use, any residual antisolubility protection afforded by the previous treatment was eliminated. Unprotecting these specimens was accomplished by etching the teeth in the above prepared 0.1 M lactate buffer solution for a two-hour period. Each disc of three specimens was agitated (450 rpm) in about 50 ml of lactate buffer at room temperature during the unprotecting period. The teeth were rinsed well with distilled water immediately following unprotecting.

Pre-Treatment Etch

The test was performed using preheated (37°C) tooth sets and lactate buffer in an incubator. The now unprotected tooth sets were mounted on 1/4 inch diameter acrylic rods with molten red boxing wax. Multiplaced stirrers were used for treatments and etches. All slurries and solutions were pre-heated to 37°C. The actual treatments and etches were done on the bench top with the preheated solutions. Plastic specimen containers (120 ml) were used for the etching procedure. A 1/4 inch hole was drilled in each container lid to accommodate the plastic rod to which the tooth sets were mounted. A 40 ml portion of 0.1 M lactic acid buffer was placed in each container along with a one-inch magnetic stirring bar. The rod of the first tooth set was pushed through the hole in the lid, placed in the first container, and adjusted so that all enamel surfaces were immersed into the buffer solution. The container was then placed on the first magnetic stirrer and stirring began. The timer was started at that time. At 30-second intervals, the other tooth sets were started in the same manner. After 15 minutes of exposure to the buffered lactate solution, the first set was stopped and the lid and tooth set immediately removed from the container and placed in a tray of distilled water to terminate etching. The other sets were similarly removed at 30-second intervals in the same order that they were initiated and the 0.1 M lactate buffer solutions were retained for phosphorous analysis. The tooth sets were placed back in the 37°C water bath in preparation for the treatment step.

Treatment

The treatments were performed using slurries of the dentifrice. The slurries consisted of 1 part dentifrice and 3 parts preheated (37°C) distilled water (9 g:27 ml). Each slurry was mixed for exactly one minute after adding the water. The slurries were not prepared ahead of time, and they were not centrifuged**.** All tooth sets were treated at the same time (one for each product). The treatment procedure was similar to the etching procedure, with the exception of the dentifrice slurry in place of the acid. A 30 ml portion of the preheated dentifrice slurry was added to the first tooth set, the teeth were immersed in the slurry, and the container placed on the first stirrer. The stirrer and timer were started. At 90-second intervals (to allow time for stirring), the other tooth sets were started in the same manner. At the end of the five minutes of treatment, the first set was stopped, the tooth set removed and rinsed well with distilled water. The other sets were removed at 90-second intervals and rinsed well. The treatment slurries were then discarded.

Post-Treatment

A second acid exposure was then performed by the same method as the pre-treatment etch and the lactate buffer solutions were again retained for phosphorous analysis. The pre- and post-treatment solutions were analyzed using a calibrated Klett-Summerson Photelectric Colorimeter (Bel-Art Products, Wayne, NJ, USA).

Repeat Analyses

The tooth sets were unprotected and the procedure repeated additional times so that each dentifrice was treated and assayed on each tooth set. The treatment design was a Latin Square design so that no treatment followed another treatment consistently. The Latin Square for this six group design is outlined in Figure 1.

	Tooth Set											
Run	1	2	3	4	5	6	7	8	9	10	11	12
1	A	B	C	D	E	F	A	B	C	D	E	F
2	B	A	F	E	D	C	B	A	F	E	D	C
3	C	D	A	B	F	E	C	D	A	B	F	E
4	D	F	E	A	C	B	D	F	E	A	C	B
5	E	C	B	F	A	D	E	C	B	F	A	D
6	F	E	D	C	B	A	F	E	D	C	B	A

Figure 1. *Latin Square treatment design.*

Calculation of ESR

The percent of enamel solubility reduction was computed as the difference between the amount of phosphorous in the pre- and post-acidic solutions, divided by the amount of phosphorous in the pre-solution and multiplied by 100.

Statistical Analyses

Statistical analyses were performed using a one-way ANOVA model with Sigma Stat software (3.1). If a significant "F" value

was found, the Student Newman-Keuls test was used to determine statistically significant differences among the individual means.

Results

The enamel solubility reduction results are presented in Table I. In analyzing the percent reduction results, the DI water negative control was not effective in reducing enamel solubility. The 900 ppm F^- ion, NaF paste with CPP-ACP, and the Rx 5000 ppm F^- ion NaF toothpaste "B" with TCP were more effective than the DI water, but significantly less effective than all the other fluoride-containing products in reducing enamel solubility. The Rx 5000 ppm F^- ion NaF toothpaste "A" with TCP was significantly more effective in reducing enamel dissolution than the negative control, the 900 ppm F^- ion NaF paste with CPP-ACP, and the Rx 5000 ppm F^- ion, NaF toothpaste "B" with TCP, but was less effective than the two Enamelon products in reducing enamel solubility. The Enamelon Preventive Treatment Gel and Toothpaste were statistically significantly more effective in reducing enamel solubility than all the other products tested yet statistically similar.

Discussion

The results in Table I of this *in vitro* study show that the Enamelon Gel and Toothpaste (which contain 1/5th the amount of F^- ion as the 5000 ppm F^- ion products) provided three to nine times more resistance to enamel solubility than the prescription strength 5000 ppm F^- ion dentifrices with TCP. A previous study using the same FDA Method #33 testing seems to support these results. The study reported that a toothpaste designed to deliver ACP provided significantly greater reduction in enamel solubility than the reference toothpaste without ACP.[2]

Enamelon products provided over 2½ times more fluoride uptake into lesioned enamel than the prescription strength 5000 ppm F^- ion dentifrices tested.[3] There is a mounting body of research showing the ability of calcium and phosphate ions enhancing fluoride uptake.[4] Other studies evaluating products designed to specifically deliver ACP have also been shown to demonstrate the ability of these ions in promoting greater fluoride uptake.[4-6] Additionally, earlier studies have also indicated that a toothpaste delivering ACP increases remineralization and strengthens tooth enamel,[7] which again seem to support the aforementioned studies recently completed.

Research has shown efficacy of a dentifrice containing the ACP technology and fluoride over a standard fluoride-alone dentifrice.[8,9] Additionally, a clinical study on head and neck cancer patients demonstrated the superior efficacy of a 1100 ppm fluoride dentifrice delivering soluble calcium and phosphate ions significantly remineralizing and preventing root caries when compared to a fluoride dentifrice without the addition of calcium and phosphate salts.[10]

Although these *in vitro* test results are clearly insufficient to assume superior performance in preventing caries *in vivo*, it is rather encouraging to note that the fluoride uptake in the Enamelon products does result in the treated enamel becoming less soluble under low pH conditions, which is relevant to a cariogenic challenge.[2]

Without further testing, it is difficult to determine the mechanism by which the Enamelon products enhance fluoride uptake and provide greater resistance to enamel solubility. The research seems to support that the Enamelon products provide a highly bio-available, supersaturated solution of calcium, phosphate, stannous and fluoride ions. Under these conditions, fluorapatite, calcium fluoride, calcium hydroxyapatite, amorphous calcium phosphate fluoride, or various other calcium phosphates

Table I
Enamel Solubility Reduction

Treatment	ppm Fluoride Ion	Pre-Etch uP	**	Post-Etch uP	Delta uP	Percent Reduction
Negative Control	0	703 ± 24*	**	739 ± 22	-35 ± 11	-5.45 ± 1.86
Rx NaF Paste with CCP-ACP	900	762 ± 10		710 ± 13	52 ± 9	6.84 ± 1.20
Rx NaF Toothpaste "B" with TCP	5000	659 ± 33		611 ± 18	48 ± 20	5.82 ± 3.10
Rx NaF Toothpaste "A" with TCP	5000	755 ± 44		601 ± 21	155 ± 28	18.78 ± 3.20
Enamelon OTC SnF_2 Preventive Treatment Gel Delivering ACP	970	709 ± 28		304 ± 10	405 ± 21	56.91 ± 1.05
Enamelon OTC SnF_2 Toothpaste Gel Delivering ACP	1150	740 ± 23		295 ± 10	446 ± 17	60.14 ± 0.79

*Mean ± SEM (N = 12)
**Values connected within brackets do not differ significantly ($p > 0.05$) as determined by Student-Newman-Keuls.

The other FDA-approved OTC monograph study, for the determination of the incipient enamel fluoride uptake (Method #40), appears to provide a positive correlation with this enamel solubility reduction study (Method #33). If a product is shown to provide significant fluoride uptake in the Method #40 study, then further testing of the product using the Method #33 protocol may show if the greater fluoride uptake does indeed provide greater resistance to enamel solubility.

A recent fluoride uptake study concluded that the same

and mixtures of these salts could precipitate out and form on the available surfaces presented by the tooth enamel.[11,12]

The most stable form of calcium phosphate in the mouth is hydroxyapatite. The conversion of ACPs to crystalline calcium phosphates is an important part of the proposed surface-enhancement process. It is believed that after deposition onto the tooth surface, precipitated ACPs convert *in situ* into apatite, filling microporosities and microscopic surface defects. The conversion of ACPs is complex and depends on the composition of

the ACPs and the solution's conditions. The hydrolysis of pure ACP to apatite at the physiological pH of 7.4 and 37°C is fast, occurring within 6 minutes, and goes through an intermediate octacalcium phosphate-like pathway.[13] It is interesting to presume that the formation of ACP by the use of Enamelon could be converting to a more insoluble form of fluoride containing hydroxyapatite on the surface which may be contributing to the higher resistance to acid solubility.

In addition to the delivery of ACP, the Enamelon products utilize stannous fluoride and contain two highly substantive polymer carriers that aid in decreasing the ability of biofilm to adhere to enamel and help to retain the remineralizing agents on the tooth surface. This novel Enamelon formulation, and the growing body of ACP research in conjunction with the results of this study, seem to demonstrate that the Enamelon products can provide greater resistance to enamel solubility with significantly lower, safer doses of fluoride than the prescription strength dentifrices tested in this study.

The results of this study indicate that the OTC SnF_2 Enamelon Preventive Treatment Gel with ACP technology, delivering 970 ppm F^- ion, and the OTC SnF_2 Enamelon Toothpaste with ACP technology, delivering 1150 ppm F^- ion, were significantly more effective in reducing enamel solubility than the two prescription strength 5000 ppm F^- ion dentifrices with TCP and the 900 ppm F^- ion NaF prescription paste with CPP-ACP tested. It is likely that this outcome results from the greater bioavailability of the calcium and phosphate ions in ACP and the substantivity enhancing polymers in the Enamelon formulations.

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