

Remineralization Effect of a Low-Concentration Fluoride Rinse in an Intraoral Model

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Key Words

Calcium fluoride · Enamel · Fluoride · Fluoride rinse ·
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Sodium hexafluorosilicate

Abstract

A previous study showed that a sodium hexafluorosilicate-calcium chloride-based two-solution fluoride (F) rinse containing 6 mmol/l of F was more effective than a 12 mmol/l F sodium fluoride rinse in depositing F on tooth surfaces and increasing oral F levels. The present study compared the remineralization effects of these two rinses in an intraoral de- and remineralization model. The results showed that the 6 mmol/l F two-solution rinse produced greater remineralization in increasing lesion mineral contents and reducing lesion depths. The results demonstrated that the effectiveness of an F regimen depends less on the F dose and more on the ability of the treatment to utilize F efficiently for remineralization.

Dentifrices that contain 13.2 and 52.6 mmol/l of fluoride (F) (approximately equal to 250 and 1,000 µg/g or ppm of F, respectively) as sodium fluoride (NaF) are considered as 'gold standards' because of their well-documented, clinically observed dose-response anticaries effects [Proskin et al., 1992]. Current caries models would suggest that the greater anticaries effects of the 1,000-ppm F dentifrice is derived primarily from its ability to produce a greater F deposition in the mouth. The precipitation of calcium fluoride (CaF₂) or 'CaF₂-like' deposits has been suggested to be a major form of this loosely bound F after an NaF rinse [Rølla, 1998; Lagerlöf et al., 1988; Rølla and Saxegaard, 1990]. F deposition in plaque may additionally be attributed to the formation of bacteria-Ca-F complexes [Rose et al., 1996].

Recent studies showed that a two-solution F rinse produced a larger F deposition on teeth [Chow and Takagi, 1991] and in plaque and saliva [Vogel et al., 1992, 1997] than a conventional NaF rinse with the same F concentration of 12 mmol/l (228 ppm F). This two-solution rinse was also found to be more effective than the NaF rinse in remineralizing enamel and root lesions in vitro [Chow et al., 1992; Takagi et al., 1997] and in vivo [Chow et al., 2000]. The greater F deposition by the two-solution F

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rinse was attributed to a reaction mechanism that precipitates F from the rinse solution during application. One solution of this rinse contained calcium chloride (20 mmol/l) and an acetate buffer (50 mmol/l). The other solution contained sodium hexafluorosilicate, Na₂SiF₆ (4 mmol/l), a complex F salt. When the two solutions were combined, a reaction between calcium and free F ions released by the hydrolysis of fluorosilicate caused continued precipitation of CaF₂ during the 1-min application time [Takagi et al., 2001]. This is in contrast to the conventional F rinses in which deposition of F relies primarily on adsorption [White et al., 1994] or other reactions of F ions with oral substrates. Thus, two-solution rinses have the potential to deposit a greater amount of F without requiring the oral tissues to have a good affinity for F. Because CaF₂ precipitation is driven by both F⁻ and Ca²⁺ ion activities, F deposition by a two-solution rinse with a lower F content can be enhanced by raising the Ca concentration. Thus, these rinses offer the possibility of lowering the F content of a rinse or dentifrice without sacrificing the amount of F deposition and the potential anticaries effect. Indeed, a recent *in vitro* study [Takagi et al., 2001] showed that a 6 mmol/l F two-solution rinse was able to produce greater F deposition on teeth than a 12 mmol/l F NaF rinse. In the present study, we sought to test the hypothesis that this 6 mmol/l F two-solution rinse can produce a greater remineralization effect than the 12 mmol/l F NaF rinse *in vivo*.

Materials and Methods

Test Oral Rinses

Reagent grade chemicals were used in preparing the rinses. An NaF rinse (12 mmol/l F) and a placebo rinse (no F) served as the positive and negative controls, respectively. The NaF rinse was prepared by dissolving an appropriate amount of NaF in distilled water. The placebo rinse was distilled water. The test rinse consisted of the following two solutions that were combined immediately before use: solution A contained 100 mmol/l CaCl₂ and 50 mmol/l sodium acetate; solution B contained 2 mmol/l Na₂SiF₆. When equal volumes of solutions A and B are combined, the total F concentration is 6 mmol/l; the pH of the combined solution was 5.4.

Selection of Panelists and Compliance of Study Requirements

The intraoral procedures were conducted with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. Twelve subjects were recruited from staff members of the National Institute of Standards and Technology (NIST). The inclusion criteria were good general health, good dental health, normal salivary flow (unstimulated whole saliva flow rate 0.2 ml/min or greater) and willingness to comply with the study requirements. Exclusion criteria were signs of gross dental neglect and medication that affects salivary function. The subjects

were instructed to maintain their normal dietary habits and were given compliance check-off sheets on which they would record the completion of the required procedures daily.

Choice of Tooth Substrate and Method for Preparing Single-Section Specimens

A single-section method utilizing thin (110–140 μm) human enamel sections was used. This method allowed the same lesion to be assessed before and after the treatment regimen, which should lead to lower random error effects between specimens and a smaller number of samples required [Stookey et al., 1992]. The method for preparing the thin sections, which was modified from that of Mellberg et al. [1992], is outlined as follows. *Tooth substrate*: enamel slabs consisted of the lingual or buccal surfaces of human premolars and molars that were extracted for orthodontic reasons. The slabs were screened to select those that were free from apparent caries, macroscopic cracks, abrasions, and extensive staining. *Thin sections*: the enamel slabs were sectioned longitudinally with a diamond blade (Isomet, Buehler Ltd., Lake Bluff, Ill., USA) and ground by hand on wet 400- and then 600-grit abrasive papers (Buehler Ltd.) to a thickness of approximately 110–140 μm. *Attachment of the markers*: nickel TEM grids (Ted Pella Inc., Redding, Calif., USA) were used as markers. The marker allowed the specimen to be accurately referenced in the horizontal and vertical directions during image analysis. The circular TEM grids were first embedded in a thin layer (approximately the same thickness as the grid) of a polyester resin casting compound (Castin Graft casting resin No. 00175, E.T.I. Co., Fields Landing, Calif., USA) and then cut into strips parallel to the grids. A strip was then attached to each enamel section using a minimum amount of the casting compound. The markers were attached at a position 400–500 μm from the tooth surface of the enamel section with the grids in one direction being approximately parallel to the surface. *Embedding the thin sections*: an acetate sheet was placed on top of a Plexiglas plate for support. Several plastic microscope cover slides were placed on the Plexiglas plate just outside the edges of the acetate sheet to serve as spacers. A thin layer of the casting compound was poured and spread uniformly over the acetate sheet and was allowed to set until it reached a gel stage. The thin tooth sections with the markers attached were pressed onto the film of partially hardened casting. Additional casting compound was poured to cover the specimens, a second acetate sheet was placed on top of the resin film and another Plexiglas plate was placed on top of the acetate sheet. The two Plexiglas plates were then clamped together, and the resin film was allowed to harden fully. After hardening, the individual thin sections, which were completely surrounded in the resin, were cut from the sheet using a scalpel. *Exposure of enamel surface*: the surface of the tooth specimen was exposed by grinding off the excess resin with wet 400- and then 600-grit sand papers parallel to the grid and no closer than 300 μm from it. *Lesion formation*: cast tooth sections were placed in a pH-4.3 solution containing 75 mmol/l acetate, 2 mmol/l Ca, 2 mmol/l P [White and Featherstone, 1987] for 2 days. The lesions produced under these conditions had a depth of 100–150 μm and a well-defined mineral-dense surface layer. The mineral contents of the lesions were characterized by quantitative microradiography, as described later. To reduce the experimental variance, specimens were assigned to the various treatment groups in such a way that all groups had lesions with similar parameters [Schafer et al., 1992].

Type of Oral Appliance and Mounting of the Specimens

A removable mandibular appliance was used. The appliance could easily be removed during periods in which a standardized cariogenic challenge was applied to the specimens (see below). Three single-section specimens, mounted with sticky wax (Corning Rubber Co., Brooklyn, N.Y., USA) on either side of the lingual walls of the appliance, were placed in such a way that the tooth surfaces were 0.5–1 mm below the surface of the appliance to facilitate accumulation of plaque [Dijkman et al., 1986; Featherstone and Zero, 1992]. The specimen-mounting arrangement allowed plaque to cover an area beyond the boundaries of the tooth sections in order to minimize the possible differences in demineralization/remineralization patterns in the central and peripheral part of the lesion [ten Cate et al., 1992]. The completed assembly was sterilized in 1% ethylene oxide [Zimmermann et al., 1985] prior to use.

Assessment of Mineral Content

Quantitative microradiography was used to assess the mineral content of the lesions. This method utilized a 20-step aluminum step wedge as the thickness standard and a digital image analysis technique that captured the gray levels (brightness) of a 400 μm high \times 400 μm wide area of the sample with 256 levels of intensity resolution and 1.33 and 1.37 μm of spatial resolution in the horizontal and vertical directions, respectively. A mineral content profile across the lesion was computed and two parameters that describe the mineral content distribution of the lesion, ΔZ [Chow et al., 1992] and lesion depth, D [Dijkman et al., 1986], were calculated from the profile. The mineral contents of enamel lesions measured with this method were found to have a standard uncertainty of <5% [Chow et al., 1991]. The mineral content profiles and lesion parameters, ΔZ and D , were determined for 2–3 windows on each single section specimen. These values were averaged to give the mean ΔZ and D for the tooth specimen. The mean ΔZ and D from each of the 3 tooth sections from a given subject were again averaged to give the mean ΔZ and D for the subject. Finally, the mean ΔZ and D from each subject in the treatment group were averaged to give the mean ΔZ and D for the group. These values determined before the treatment regimens are referred to as the mean ΔZ_b and D_b , and the values determined after the treatment as the mean ΔZ_a and D_a . The change in ΔZ , i.e., $\Delta(\Delta Z)$, for a given window before and after treatment was calculated as follows:

$$\Delta(\Delta Z) = \Delta Z_a - \Delta Z_b,$$

where ΔZ_a and ΔZ_b refer to the ΔZ values of the same window area of a single-section specimen after and before the treatment regimen, respectively. The change in lesion depth, $\Delta(D)$, for a given window was similarly calculated.

Experimental Design and Treatment Protocol

The study employed a randomized, double-blind, crossover design with 12 subjects. Each of the 3 legs of the experiments lasted for 30 days during which the subjects brushed with a supplied silica-based NaF (1,100 $\mu\text{g/g}$ F) dentifrice twice daily. The subjects were instructed to brush as usual and, in addition, to rinse twice with 20 ml of water for 10 s each at the end. Duckworth et al. [1991] reported that rinsing with water after brushing caused salivary F to decrease to 0.02 $\mu\text{g/g}$ within 15 min. Thus, the use of a 1,100 $\mu\text{g/g}$ F dentifrice in the prescribed way should produce no significant effects on the tooth substrate or plaque on the appliance, which was removed during brushing. Day 1–14 constituted the washout period

before each treatment regimen. The subject wore the appliance on day 15, 2 days prior to the beginning of the treatment regimen, to allow plaque to accumulate. Each subject wore his/her appliance continuously except when eating, drinking, or tooth brushing. During these periods, the appliance was protected from dehydration by placing it in a plastic box with moist paper liners. There was a 15-min wait between the end of eating, drinking or brushing and the time the appliance was returned to the mouth. Twice daily (after breakfast and before bedtime) during the 14-day treatment period (days 17–30), the subjects received a 1-min rinse with 20 ml of either (1) 6 mmol/l F two-solution rinse, (2) 12 mmol/l F NaF rinse, or (3) placebo rinse (0 mmol/l F). For each leg of the study, each subject received two 500-ml metered plastic dispensing bottles (Nalgene, Rochester, N.Y., USA), labeled solutions A and B. In the cases of the NaF and placebo rinses, the contents of the two dispensers were the same. The subjects were asked to dispense 10 ml each of solutions A and B into a plastic cup and rinse immediately for 1 min. No food or drink was allowed during the 30 min following the rinse applications. Standardized cariogenic challenges were applied to the specimens during the 14-day treatment period by 10-min extra-oral immersions of the appliance in a 10% w/w sucrose solution [Featherstone and Zero, 1992] twice daily (10 a.m. and 4 p.m.). Since the subjects were staff members of NIST, the sucrose rinses were administered in the lab except during weekends. Individually bottled (for each application) and pasteurized sucrose solutions that require no refrigeration were provided to the subjects for home administration during weekends. At the end of the experimental period, the sections were retrieved, and the ΔZ and D values were again determined.

Statistical Analysis of the Results

Commercial software (Winks, TexaSoft, Cedar Hill, Tex., USA) was used to conduct the statistical analysis of the data. Independent group one-way ANOVA was performed on the mean ΔZ_b and D_b of the three treatment groups to ascertain that these values were equivalent, as would be expected by design. After the treatment regimen, independent group one-way ANOVA was performed on the mean ΔZ_a and D_a of the three treatment groups, and Tukey's test was done if a difference existed. Paired-measurement one-way ANOVA was performed on the mean $\Delta(\Delta Z)$ and mean $\Delta(D)$ of the three treatment groups, and Tukey's test was done if a difference existed. Single-variable t test was also performed on the mean $\Delta(\Delta Z)$ and mean $\Delta(D)$ values to determine if the values were different from 0.

Results

Of the 12 subjects, 2 dropped out before the end of the first leg of the study, and 1 was able to complete only 1 leg of the study. Thus, the sample numbers were 9, 10, and 9 for the 6 mmol/l F two-solution rinse, the 12 mmol/l F NaF rinse, and the 0 mmol/l F placebo rinse groups, respectively. Examination of the compliance check-off sheets suggest that all subjects carried out the required procedures as instructed.

Table 1 lists the mean ΔZ_b , ΔZ_a and $\Delta(\Delta Z)$ values for the three treatment groups. The mean ΔZ_b values of

Table 1. Mean \pm SD ΔZ values of the samples in the three study groups before and after treatment and mean delta(ΔZ) values of the same window before and after the treatment

Group	Independent-groups ANOVA		Paired-difference ANOVA delta(ΔZ), μm
	before treatment ΔZ_b , μm	after treatment ΔZ_a , μm	
6 mmol/l F two-solution rinse (n = 9)	44.2 \pm 4.5 ¹	23.9 \pm 5.6	-20.2 \pm 6.0 ²
12 mmol/l F NaF rinse (n = 10)	42.9 \pm 8.3	28.6 \pm 8.6	-14.4 \pm 5.5 ²
F-free placebo rinse (n = 9)	43.1 \pm 6.9	35.3 \pm 10.3	-7.7 \pm 5.5 ²

¹ The means of any two groups connected by a vertical line are not significantly different ($p > 0.05$).

² Delta(ΔZ) different from 0 ($p < 0.05$); paired difference t test.

Table 2. Mean \pm SD D values of the samples in the three study groups before and after treatment and mean delta(D) values of the same window before and after treatment

Group	Independent-groups ANOVA		Paired-difference ANOVA delta(D), μm
	before treatment D_b , μm	after treatment D_a , μm	
6 mmol/l F two-solution rinse (n = 9)	105 \pm 10 ¹	78 \pm 23	-37 \pm 17 ²
12 mmol/l F NaF rinse (n = 10)	104 \pm 17	87 \pm 25	-17 \pm 16 ²
0 ppm F placebo rinse (n = 9)	115 \pm 23	101 \pm 22	-14 \pm 8 ²

¹ The means of any two groups connected by a vertical line are not significantly different ($p > 0.05$).

² Delta(D) different from 0 ($p < 0.05$); paired difference t test.

the three groups before treatment were found to be equivalent as expected. ANOVA and multiple-comparison tests of the ΔZ_a values showed that the mean ΔZ_a fell into two groups, with the 6 mmol/l F two-solution rinse and the placebo rinse being significantly different, while the 12 mmol/l F NaF rinse and the placebo rinse were nondistinguishable. Paired-measurement ANOVA and Tukey's test showed that the delta(ΔZ) values were significantly different ($p < 0.05$) between each of the three treatment groups, indicating that the remineralization effectiveness of the three rinses was in the order: 6 mmol/l F two-solution rinse $>$ 12 mmol/l F NaF rinse $>$ placebo rinse. Single-variable t test showed that all the delta(ΔZ) values were different from 0, indicating that there was remineralization in all groups.

Table 2 lists the mean lesion depths before and after the treatment for each group, as well as the mean paired difference in lesion depth measured from the same window area before and after the treatment. ANOVA results of the independent groups showed that the mean lesion depths of the three groups were not significantly different either before ($p = 0.34$) or after ($p = 0.13$) the treatment regimen. However, paired-measurement ANOVA on the

delta(D) values showed that the groups fell into two populations: one for the 6 mmol/l F two-solution rinse, and the other for the 12 mmol/l F NaF and the placebo rinse groups, indicating that the two-solution rinse was more effective in reducing lesion depth than the other two rinses. Single-variable t test results showed that the mean delta(D) values were different from 0 ($p < 0.05$) in each of the three treatment groups, indicating that there were significant reductions in lesion depths in all groups.

Discussion

Because of the relatively small sample, independent-groups ANOVA was unable to separate the 6 mmol/l F two-solution rinse from the 12 mol/l F NaF rinse in terms of ΔZ_a or D_a values. In contrast, paired-difference ANOVA results indicate that the two-solution rinse produced greater remineralization than the NaF rinse in terms of both lesion mineral content and depth. These results confirmed the previous observation that the ability to measure mineral contents of the same lesion area before and after the treatment regimen reduced errors due

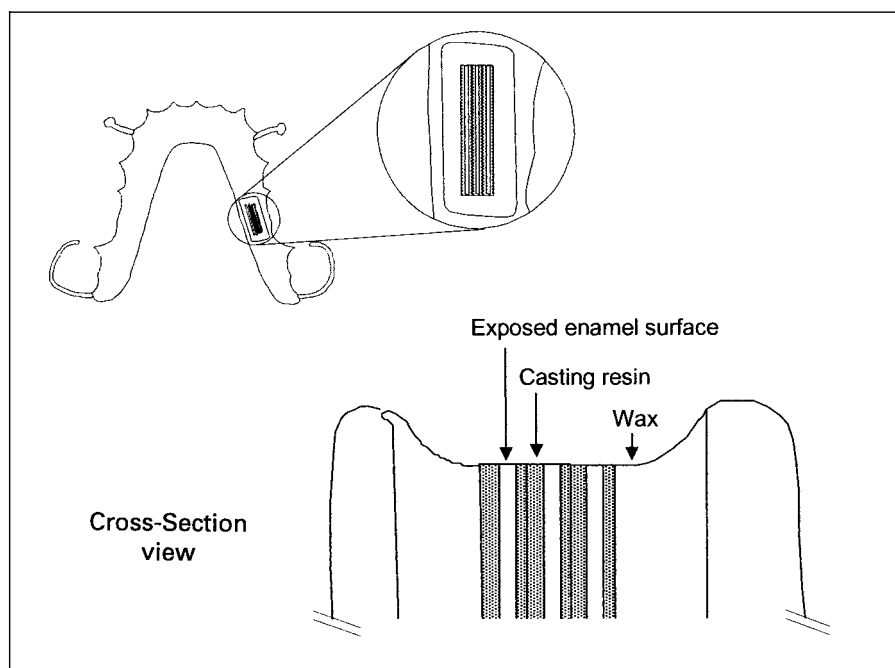


Fig. 1. Schematic drawing of a removable appliance showing the location of specimens, the exposed tooth surfaces being recessed approximately 1 mm below the surface of the appliance.

to specimens and, consequently, sample size required [Stookey et al., 1992].

A recent *in vitro* study [Takagi et al., 2001] showed that a two-solution rinse with an even lower F concentration of 3 mmol/l also produced significantly greater F deposition on teeth and greater remineralization of enamel lesions than did a 12 mmol/l F NaF rinse. However, a pilot *in vivo* plaque study [Vogel, unpubl. obs.] showed that only the 6 mmol/l F and not the 3 mmol/l F two-solution rinse produced higher plaque fluid and salivary F levels than the 12 mmol/l F NaF rinse. A possible reason was that, in order to produce a sufficiently high supersaturation with respect to CaF_2 , it was necessary to raise the levels of calcium chloride in the 3 mmol/l F and 6 mmol/l F two-solution rinses to 200 and 50 mmol/l, respectively, as compared with 10 mmol/l in the 12 mmol/l F two-solution rinse. As a result, most of the F deposition formed by the 3 mmol/l F two-solution rinse was in the nonlabile form, i.e., not soluble except in strong acids. Because there was no basis to expect the 3 mmol/l F rinse to be more effective than the NaF rinse from the plaque chemistry standpoint, this rinse was not tested in the present study.

Recent studies [Schemehorn et al., 1999; Sullivan et al., 2001] showed that, with the use of various two-component systems, the remineralization effects of F rinses and dentifrices were enhanced without increasing the F content. The present study showed, for the first time, that a

rinse with lower F content produced greater remineralization than a rinse with higher F content and demonstrated that the effectiveness of an F treatment depends less on F dose than more on whether the F can be used efficiently to promote remineralization, probably by producing a greater F deposition on teeth and in plaque. The reduced F content in the two-solution rinse should be advantageous in minimizing excessive systemic F intake in children.

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