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ABSTRACT

Results from previous studies show that a two-solution fluoride (F) rinse is significantly more effective than a NaF rinse of the same F content of 250 $\mu\text{g/g}$ (ppm) in remineralizing enamel and root lesions in an *in vitro* cyclic de- and remineralization model. In the present study, the two-solution rinse and two NaF rinses with F contents of 250 ppm and 1000 ppm were evaluated in an intra-oral remineralization model. Caries-like lesions were formed in the enamel of extracted human molars with the use of a pH 4 demineralizing solution. Thin sections of the enamel ($\approx 120 \mu\text{m}$) containing lesions were prepared, and the mineral contents of the lesions were assessed by quantitative microradiography. With the cut surfaces protected by nail varnish, 3 enamel specimens were mounted with wax in the lingual areas of a removable mandibular appliance. The study used a randomized, crossover design with seven subjects. In each of the 3 legs of the study, subjects wore the appliances continuously except when eating, drinking, and brushing their teeth. Twice daily (after breakfast and before bedtime), the subjects received a one-minute rinse with 20 mL of (1) 250-ppm-F NaF rinse, (2) 1000-ppm-F NaF rinse, or (3) 228-ppm-F two-solution F rinse. At the end of the 14-day experimental period, the sections were retrieved, and the mineral contents of the lesions were again assessed quantitatively. The results show that both the 1000-ppm-F NaF and 228-ppm-F two-solution rinses produced a greater ($p < 0.05$) remineralization than did the 250-ppm-F NaF rinse. The remineralization produced by the two-solution rinse was not statistically different ($p > 0.05$) from that produced by the NaF rinse with 4x the F content (1000 ppm F).

KEY WORDS: fluoride rinse, fluoride, enamel, remineralization, calcium fluoride, sodium hexafluorosilicate, *in situ* model.

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Remineralization Effects of a Two-solution Fluoride Mouthrinse: An *in situ* Study

INTRODUCTION

Previous *in vitro* studies showed that an 'active' two-solution fluoride (F) rinse deposited significantly more loosely bound F on enamel than did a sodium fluoride (NaF) rinse with the same F concentration (12 mmol/L or 228 ppm) (Chow and Takagi, 1991). The greater F deposition by the two-solution F rinse was attributed to a reaction mechanism that precipitates F from the rinse solution during application. One solution of this rinse system contained calcium chloride and an acetate buffer. The other solution contained sodium hexafluorosilicate (Na_2SiF_6), a complex F salt. When the two solutions were combined, a reaction between calcium and the free F ions released by the hydrolysis of fluorosilicate caused continued precipitation of calcium fluoride (CaF_2) during the one-minute application time. The F deposition on enamel by the two-solution rinse was $4.36 \mu\text{g}/\text{cm}^2 \pm 0.16 \mu\text{g}/\text{cm}^2$ (mean \pm standard uncertainty, $n = 3$), which was many times higher than that ($0.23 \mu\text{g}/\text{cm}^2 \pm 0.07 \mu\text{g}/\text{cm}^2$) produced by the NaF rinse (Chow and Takagi, 1991). Similarly, this two-solution rinse produced a significantly greater loosely bound F deposition on the tooth-root surface than did the NaF solution of the same F concentration (Takagi *et al.*, 1997).

The two-solution rinse was also found to be significantly more effective than the NaF rinse in remineralizing human enamel lesions (Chow *et al.*, 1992) and root lesions (Takagi *et al.*, 1997) in an *in vitro* pH-cycling model. The greater remineralization is believed to be a result of the higher ambient F produced by the greater 'loosely bound' F deposition, presumably in the form of CaF_2 or 'CaF₂-like' deposits (Øgaard *et al.*, 1983; Lagerlöf *et al.*, 1988; Rølla and Saxegaard, 1990). The objective of the present study was to determine the remineralization effect of the two-solution F rinse in an *in situ* model and to test the hypotheses that the two-solution rinse will produce a greater remineralization effect than a NaF rinse with similar F content under *in vivo* conditions.

MATERIALS & METHODS

Tooth Substrate and Formation of Caries-like Lesions

The experimental procedures used in this study were done with the informed consent of the subjects, following protocols reviewed and approved by the appropriate institutional review boards. A single-section method utilizing thin ($120 \mu\text{m}$ to $150 \mu\text{m}$) human enamel sections was used. This method, which allows the same lesion area to be assessed before and after the treatment regimen, should lead to a lower random-error effect due to specimen variation (Stookey *et al.*, 1992). Human premolars and molars extracted for orthodontic reasons were examined visually, and those that were free from apparent caries, macroscopic cracks, abrasions, and extensive staining on the lingual or buccal surfaces were selected for the study. Enamel blocks containing the lingual or buccal tooth surface were covered with wax except for the natural tooth surfaces. The slabs were placed in a pH 4 demineralizing solution containing 100 mmol/L lactate, 3

mmol/L calcium, 1.8 mmol/L phosphate, and a mass fraction of 1% carboxymethyl cellulose (Teranaka and Koulourides, 1987) for 3 to 4 days. The demineralized enamel slabs were then sectioned longitudinally with a diamond blade (Isomet, Buehler Ltd., Lake Bluff, IL) and ground by hand on wet 400- and then 600-grit sandpapers (Buehler Ltd.) to a thickness of approximately 120 μm to 150 μm . The thin sections were then coated with a water-resistant nail varnish on all sides except for the natural enamel surfaces. The mineral contents of the lesions were characterized by quantitative microradiography as described below. The lesions selected for use in this study typically had a depth of 100 μm to 120 μm and a ΔZ value (Chow *et al.*, 1992) in the range of 24 μm to 32 μm . To reduce the experimental variance (Schafer *et al.*, 1992), we assigned the enamel sections to the 3 treatment groups in such a way that the average ΔZ values for the 3 groups were approximately the same before treatment.

Intra-oral Appliance and Mounting of the Specimens

A removable mandibular appliance (Fig.), which could be worn by dentulous subjects for several weeks without excessive discomfort, was used in this study. Three single-section enamel specimens were mounted in either the left or right side of the lingual flange of the appliance (additional dentin sections were mounted in the side not occupied by the enamel sections, but were not used in this study). The specimens were mounted in such a way that the natural tooth surfaces were recessed 0.5 mm to 1 mm below the surface of the appliance, to facilitate the accumulation of plaque (Dijkman *et al.*, 1986; Featherstone and Zero, 1992). The assembly was sterilized in a volume fraction of 1% ethylene oxide (Zimmermann *et al.*, 1985) prior to use.

Quantitative Assessment of Mineral Content

The mineral contents of each partially demineralized enamel section were measured by quantitative microradiography following previously described methods (Chow *et al.*, 1991, 1992; Takagi *et al.*, 1997). Contact microradiographs were made of the sections both before and after the treatment regimen with CuK_α radiation in a Faxitron (Model 43855A, Hewlett-Packard, McMinnville, OR,

USA) operating at 40 kV and 3 mA. The x-ray source-to-film distance was 30.5 cm, and the exposure time was 15 min. A fine-grain film (Kodak Professional film SO343, Eastman Kodak Co., Rochester, NY, USA) designed for contact microradiography was used. Each radiograph contained images of enamel sections and an aluminum stepwedge that served as a thickness standard (Chow *et al.*, 1991).

Digital image analysis was conducted with the use of a commercial digital image-analysis system (Bioquant System IV, R & M Biometrics, Inc., Nashville, TN, USA) interfaced with a microscope (Leitz Ortholux, Wetzlar, Germany), operating at 250x optical magnification, and a desk-top computer. The system digitized the microradiographic image of the enamel section in a window that measured 277 μm (H) x 357 μm (L). Digitized data from the central [102 μm (H) by 220 μm (L)] parts of 4 such windows were obtained from each enamel section (Chow *et al.*, 1992). The data collected in this manner covered the natural enamel surface, the entire body of the lesion, and the sound enamel beyond the lesion.

The mineral loss, ΔZ , for each window was calculated from the mineral content profiles, which were obtained from the same area of the specimen before and after the treatment regimen (Chow *et al.*, 1991, 1992). In accordance with the International System of Units, micrometer (μm) instead of mass% $\cdot\mu\text{m}$ is used as the unit for ΔZ (Takagi *et al.*, 1997). The relationship between the two units is: 1 μm = 100 mass% $\cdot\mu\text{m}$. A ΔZ value of 1 μm reflects that the mineral loss is equivalent to losing a 1- μm -thick layer of sound enamel. For each window, the difference in ΔZ values 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 specimen after and before the treatment regimen, respectively. Lesion depth, D , in the unit of μm for each specimen was determined from the mineral profile as the point where the lesion mineral content reached a value of 95% of the sound enamel, as previously defined by Dijkman *et al.* (1986). For each window, the difference in D values before and after the treatment regimen was calculated as follows:

$$\Delta(D) = D_a - D_b$$

where D_a and D_b refer to D values of the same window area of the specimen after and before the treatment regimen, respectively.

Experimental Design

The study used a randomized, crossover design with seven subjects. Each of the 3 legs of the experiments lasted for 14 days. The study compared the remineralization effects of 3 F rinses: the experimental two-solution rinse (228 ppm F) and two NaF rinses with F contents of 250 and 1000 ppm. The two NaF rinses have known clinical effectiveness and served as positive controls for determination of whether the

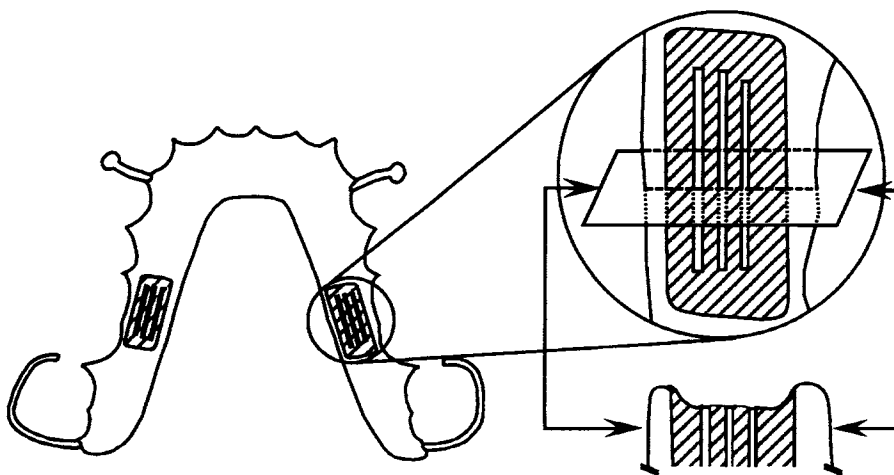


Figure. Schematic drawing of a removable appliance showing the location of the enamel specimens. The natural tooth surfaces are recessed 0.5 mm to 1 mm below the surface of the appliance. Additional dentin sections were mounted in the side not occupied by the enamel sections, but were not used in this study.

study had the sensitivity needed for evaluation of the test rinse (Proskin *et al.*, 1992). The two-solution F rinse consisted of the following 2 solutions that were combined just before use: Solution A contained 20 mmol/L CaCl₂ and 25 mmol/L sodium acetate; Solution B contained 4 mmol/L Na₂SiF₆, 1 mmol/L NaH₂PO₄, and 1 mmol/L Na₂HPO₄. When equal volumes of solutions A and B were combined, the total F concentration was 12 mmol/L (228 ppm), and the pH was about 5.1. The subjects brushed with a F-free dentifrice from day 1 to day 14. They started wearing the appliance on day 8, 2 days prior to the beginning of the treatment regimen, to allow plaque to accumulate. Each subject wore his/her appliance continuously except during eating, drinking, or carrying out oral hygiene procedures. During these periods, the appliance was protected from dehydration by being placed in a plastic box with moist paper liners. During the five-day treatment regimen period (days 10 to 14), twice daily (after breakfast and before bedtime), the subjects rinsed for 1 min with 20 mL of (1) the 250-ppm-F NaF rinse, (2) the 1000-ppm-F NaF rinse, or (3) the 228-ppm-F two-solution F rinse. For the two-solution rinse, 10 mL each of solutions A and B were mixed just before use. The subjects were instructed not to eat or drink during the 30 min following the rinse applications. At the end of the experimental period, the sections were retrieved, and the mineral contents of the lesions were again assessed quantitatively.

Statistical Analysis of the Data

Two kinds of statistical analyses were conducted:

One-way independent group ANOVA (analysis of variance): For each treatment group, the mean mineral loss (ΔZ) and lesion depth (D) values of 12 (4 windows/section \times 3 sections) windows from each subject both before and after the treatment regimen were calculated. We performed ANOVA tests on the ΔZ and D values to determine whether there were significant differences among the treatment groups before or after the treatment regimens.

Paired difference ANOVA: The delta(ΔZ), *i.e.*, difference in ΔZ for a given window before and after the treatment, was computed. The mean delta(ΔZ) value of 12 windows from each subject was then calculated, and the mean delta(ΔZ) value of the seven subjects for each treatment group was computed. We also made similar calculations to obtain the mean delta(D) for each treatment group. We performed single-variable *t* tests and ANOVA tests on the mean delta(ΔZ) and delta(D) values to determine whether the values were statistically different from zero and whether there were significant differences among the groups.

RESULTS

Table 1 lists the mean ΔZ_b , ΔZ_a , and delta(ΔZ) values for the three treatment groups. As described above, the mean ΔZ_b values of the three groups before treatment were approximately the same by design. After the treatment, the mean ΔZ_a value of the 250-ppm-F NaF rinse group ($31.8 \mu\text{m} \pm 5.0 \mu\text{m}$; $n = 7$) was significantly ($p < 0.05$) larger than the mean ΔZ_a values for the 1000-ppm-F NaF group ($25.4 \mu\text{m} \pm 4.9 \mu\text{m}$) and the two-solution rinse group ($22.6 \mu\text{m} \pm 1.5 \mu\text{m}$).

The mean ΔZ_a values of the 228-ppm-F two-solution rinse and the 1000-ppm-F NaF groups were not significantly different ($p > 0.05$). Single-variable *t* tests performed on the delta(ΔZ) values showed that the delta(ΔZ) value for the 1000-ppm-F NaF group ($-2.9 \mu\text{m} \pm 3.6 \mu\text{m}$) was not significantly different from zero ($p > 0.05$), whereas the delta(ΔZ) values for the 250-ppm-F NaF group ($3.1 \mu\text{m} \pm 2.7 \mu\text{m}$) and the two-solution group ($-6.2 \mu\text{m} \pm 2.9 \mu\text{m}$) were different from zero. Thus, the samples in the 250-ppm-F NaF group experienced significant demineralization, and those in the two-solution rinse groups experienced significant remineralization. ANOVA tests showed that the mean delta(ΔZ) values for the 1000-ppm-F NaF and the two-solution rinse groups were not significantly different, and that both are significantly more negative than that of the 250-ppm-F NaF group.

Independent group ANOVA results showed that the mean lesion depths of the three groups were not significantly different ($p > 0.05$) before treatment regimen (Table 2). After the treatment, the mean D_a value of the three treatment groups fell into two populations: one for the 250-ppm-F NaF ($110 \mu\text{m} \pm 12 \mu\text{m}$) and the 1000-ppm-F NaF ($105 \mu\text{m} \pm 7 \mu\text{m}$) groups, and the other for the 1000-ppm-F NaF and the two-solution rinse ($97 \mu\text{m} \pm 8 \mu\text{m}$) groups. ANOVA analysis on the delta(D) values produced the same conclusion. Single-variable *t* test results showed that only in the two-solution group was the mean delta(D) value not different from zero ($p < 0.05$), indicating that there was a significant reduction in lesion depth in this group.

DISCUSSION

Previous *in vitro* studies showed that the two-solution F rinse produced a significantly greater F deposition on enamel (Chow and Takagi, 1991) and root (Takagi *et al.*, 1997) lesions than did the 250-ppm-F NaF rinse. The two-solution rinse was also shown to produce a greater remineralization effect on enamel (Chow *et al.*, 1992) and root (Takagi *et al.*, 1997) lesions than did the 250-ppm-F NaF rinse in an *in vitro* pH-cycling model. Since dental plaque was not present in these models, the greater remineralization effect of the two-solution rinse was believed to be a result of the greater amount of F deposited directly onto teeth and in the lesions. The enhanced remineralization observed with the two-solution rinse in the present *in vivo* study is partially attributable to this greater F deposition.

Two *in vivo* studies (Vogel *et al.*, 1992, 1997) also found

Table 1. Mean Values of Mineral Loss, ΔZ (μm), of the Samples in the Three Study Groups Before and After the Treatment Regimen and Mean Values of the Delta(ΔZ) of the Same Window Before and After the Treatment Regimen

Group	Before	After	Paired Difference
	Mean \pm std dev (n = 7)	Mean \pm std dev (n = 7)	delta(ΔZ) Mean \pm std dev (n = 7)
250 ppm F NaF rinse	28.7 \pm 2.7	31.8 \pm 5.0	3.1 \pm 2.7 ^b
1000 ppm F NaF rinse	28.3 \pm 3.2	25.4 \pm 4.9	-2.9 \pm 3.6
228 ppm F two-solution rinse	28.8 \pm 2.7	22.6 \pm 1.5	-6.2 \pm 2.9 ^b

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

^b Delta(ΔZ) different from zero ($p < 0.05$); paired-difference *t* test.

Table 2. Mean lesion depth, D, of the samples in the three study groups before and after the treatment regimen

Group	Before	After	Paired Difference
	Mean \pm std dev (n = 7) D ₆ (μ m)	Mean \pm std dev (n = 7) D ₆ (μ m)	delta(D) (μ m) Mean \pm std dev (n = 7)
250 ppm F NaF rinse	107 \pm 8 ^a	110 \pm 12	2.8 \pm 5.4
1000 ppm F NaF rinse	108 \pm 8	105 \pm 7	-2.3 \pm 9.9
228 ppm F two-solution rinse	105 \pm 7	97 \pm 8	-7.9 \pm 7.6 ^b

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

^b Delta(D) different from zero ($p < 0.05$); paired-difference *t* test.

greater oral F levels after a similar two-solution rinse compared with a NaF rinse of the same F content (228 ppm). Specifically, 2 hrs after a one-minute application of the two-solution rinse, the salivary, plaque-fluid, and whole-plaque F concentrations were significantly higher by factors of about 4, 2, and 6, respectively, compared with the NaF rinse (Vogel *et al.*, 1992). For F levels measured the morning after a rinse given the night before, the corresponding factors were 2, 1.2, and 3 (Vogel *et al.*, 1997). The greater F deposition in whole plaque and the elevation of salivary and plaque-fluid F levels by the two-solution rinse most likely also contribute to the greater remineralization effect observed here.

For the two-solution rinse, the extent of remineralization observed in the present study was less than that observed in the *in vitro* remineralization study (Chow *et al.*, 1992) cited above. The 250-ppm-F NaF rinse produced a slight demineralization effect in the present study in contrast to a remineralization effect observed in the *in vitro* study. These differences are probably a result of the significantly different experimental environment in intra-oral and *in vitro* studies and the different treatment regimens used. Examination of the mean mineral profiles of samples in the various treatment groups before and after the treatment regimen indicated that, in both the two-solution-rinse and 1000-ppm-F-rinse groups, remineralization occurred throughout the entire depth of the lesion, although the bulk of remineralization was at about the outer two-thirds of the lesion. In contrast to the finding of the previous *in vitro* study (Chow *et al.*, 1992), we did not observe a surface coating being formed by the two-solution rinse treatment.

The release of F from labile stores of 'loosely bound' F increases the mineral saturation of oral fluid, and hence can promote the repair of lesions (Margolis and Moreno, 1990). The precipitation of CaF₂ (or 'CaF₂-like' deposits) has been suggested as a major source of this 'loosely bound' F after a NaF rinse (Lagerlöf *et al.*, 1988; Rølla, 1988; Rølla and Saxegaard, 1990). Recent x-ray diffraction analysis and plaque extraction studies have shown that CaF₂ is also the major product from the two-solution rinse (unpublished data). An advantage of the two-solution rinse with regard to formation of CaF₂ deposits is that it contains a high concentration of calcium (20 mmol/L in solution A), while a NaF rinse must scavenge this ion from the oral environment. Because free F ions in the two-solution rinse are released gradually by Na₂SiF₆ hydrolysis, the formation of CaF₂

can occur continuously during the one-minute application time. It is likely that some undissociated SiF₆²⁻ may penetrate enamel and dentin pores, plaque, etc., where it releases free F ions to react with Ca²⁺ ions to form CaF₂ (Chow and Takagi, 1991; Chow *et al.*, 1992; Vogel *et al.*, 1992, 1997). With the NaF rinse, the reaction to form CaF₂ is limited by the availability of Ca, and only a very small fraction of the F in the rinse is fixed by the CaF₂ formation and retained in the mouth. It should be noted that bacterial binding of F in plaque (Rølla and Bowen, 1977; Rose *et al.*, 1996), perhaps mediated by calcium bridging, and enamel binding of F (White *et al.*, 1994) have been proposed as alternatives

to CaF₂ formation for the fixation of 'loosely bound' F. It would seem that some of the F deposition from the two-solution rinse may be produced through these mechanisms.

The enhanced F deposition from the 228-ppm-F two-solution rinse produced an increase in mineral density and a decrease in lesion depth that were not statistically different from those produced by the 1000-ppm-F NaF rinse ($p = 0.094$). Numerically, these changes in lesion parameters produced by the two-solution rinse (Tables 1 and 2) were about twice those from the 1000-ppm-F NaF rinse. Based on the assumption that the variance remained the same, calculations showed that the differences in the effects may become significant had the study involved a larger number (> 10) of subjects. The findings support the possibility of achieving a greater anticaries effect than the NaF regimen with the use of a two-solution rinse having the same F dose or, alternatively, achieving the same anticaries effect with a significantly lower F dose.

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Certain commercial equipment, instruments, or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or the American Dental Association Health Foundation, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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