Short Communication
Cariology

# Fluoride rinse effect on retention of $CaF_2$ formed on enamel/dentine by fluoride application

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**Abstract:** Calcium fluoride-like materials ("$CaF_2$") formed on dental surfaces after professional fluoride application are unstable in the oral environment but can be retained longer with a daily NaF mouthrinse. We tested the effect of twice daily 0.05% NaF rinses on the retention of "$CaF_2$" formed on enamel and dentine after applying acidulated phosphate fluoride (APF). "$CaF_2$" formed on enamel/dentine by APF application significantly decreased after exposure to artificial saliva and the 0.05% NaF rinse was ineffective to avoid this reduction. These findings suggest that the combination of APF and 0.05% NaF is not clinically relevant, either for caries or dental hypersensitivity.

**Keywords:** Calcium Fluoride; Dentin; Fluorides, Topical.

## Introduction

The anticaries benefit of acidulated phosphate fluoride (APF) application is well established.[1] It results in the formation of a high concentration of calcium fluoride-like materials ("$CaF_2$") on enamel and dentine surfaces, which has been considered responsible for the anticaries effect of professionally applied topical fluoride treatments.[2]

Nevertheless, 80%-90% of "$CaF_2$" formed is dissolved in the oral environment during the first week because saliva is undersaturated with respect to "$CaF_2$".[3] Therefore, retaining "$CaF_2$" on enamel or root dentine for a prolonged period of time is desirable, mainly for patients with a high risk for caries.[4]

The dissolution of "$CaF_2$" formed on enamel and dentine after applying APF could be decreased by the daily use of fluoride products, such as a 0.05% NaF mouthrinse. A 0.05% NaF mouthrinse may compensate for the dissolution of "$CaF_2$" because it contains 225 ppm F, which forms a small amount of "$CaF_2$" on enamel-dentine; however, this subject has not been explored.

Therefore, we evaluated if twice daily exposure to 0.05% NaF solution *in vitro* (simulating F mouthrinse) would prolong the retention of "$CaF_2$" formed on enamel and dentine after applying APF.

## Methodology

### Experimental design

An *in vitro* 2 × 3 factorial study was conducted, including the following factors: 1) APF application at two levels, *i.e.*, applied ($APF^+$) or not ($APF^-$)

and 2) 0.05% NaF treatment at three levels, *i.e.*, not treated (NT), treated with NaF during saliva immersion ($NaF^+$), or treated with purified water ($NaF^-$) during saliva immersion.

Enamel (48) and dentine (48) slabs were randomly divided into six groups of eight slabs each, according to the factorial design: $APF^-$/NT; $APF^-$/$NaF^-$; $APF^-$/$NaF^+$; $APF^+$/NT; $APF^+$/$NaF^-$, and $APF^+$/$NaF^+$. The $APF^+$ and $APF^-$ groups were pre-treated with 0.5 M NaF in 0.1 M $H_3PO_4$, pH 3.5 ($APF^+$) or 0.1 M $H_3PO_4$, pH 3.5 ($APF^-$) solutions, respectively. The alkali-soluble fluoride ("$CaF_2$") concentration formed in groups $APF^-$/NT and $APF^+$/NT was immediately determined after treatment. Slabs from the $NaF^+$ and $NaF^-$ groups were individually immersed in artificial saliva following pre-treatment and treated twice daily with 0.05% NaF solution ($NaF^+$) or purified water ($NaF^-$), respectively. "$CaF_2$" concentration was determined in the slabs after 7 days.

### Preparation of enamel and dentine slabs

The enamel and dentine slabs (3 × 3 × 2 mm) were obtained from sound bovine incisors, and their external surfaces were polished flat. Then, the slabs were isolated with wax; thus, only the external surface of the enamel or dentine was exposed to the treatment.

### Treatments

The enamel and dentine slabs were immersed into either the control or APF solution for 4 min under agitation (120 rpm). The slabs were washed for 30 s with a stream of purified water and gently dried with absorbent paper. The slabs in the $APF^-$/NT and $APF^+$/NT groups were immediately analyzed for alkali-soluble F concentration. The remaining slabs were individually immersed in artificial saliva[5] (1.7 mL/mm$^2$) and maintained at 37°C for 7 days. The slabs from both groups were exposed to purified water or a 0.05% NaF solution for 1 min under agitation (120 rpm) twice daily at 7 am and 5 pm. Following each treatment, the slabs were flushed with purified water (10 s), dried, and immersed again in artificial saliva. The artificial saliva was changed every morning after treatment.

### Determination of "$CaF_2$"

Slabs were individually immersed in 1 M KOH (0.4 mL/block) for 24 h under agitation. An additional 6-h extraction with 1 M KOH (0.3 mL/block) was performed to deplete all alkali-soluble fluoride. The extraction solution was buffered with TISAB II containing 1 M HCl. Fluoride was measured with an ion-selective electrode (Orion 96-09; Thermo Scientific, Inc.) and an ion analyzer (Orion EA-940, Thermo Scientific) against standards prepared as the samples, and the alkali-soluble fluoride concentration (sum of the two alkali extractions) was expressed as µg F/cm$^2$.

### Statistical analysis

A factorial 2 × 3 analysis was conducted. Enamel and dentine data were independently analyzed. The alkali-soluble fluoride concentrations in the enamel and dentine slabs were transformed to square root and log10, respectively. Tukey test was used for the post-analysis of variance comparisons. The data were analyzed using SAS software version 8.01 (SAS Institute, Cary, USA), and a p-value of $< 0.05$ was considered significant.

## Results

APF resulted in significantly higher "$CaF_2$" concentrations in the enamel (Table 1) and dentine (Table 2) slabs ($p < 0.05$) than the control. "$CaF_2$" concentrations significantly decreased by 85 and 90% in the enamel and dentine after exposure to saliva and purified water, respectively ($p < 0.05$). The "$CaF_2$" concentration retained in the enamel and dentine after treatment with 0.05% NaF did not differ from the control group treated with water ($p > 0.05$).

## Discussion

Our results confirmed that loosely-bound fluoride ("$CaF_2$") was formed after APF was applied to enamel,[2] but also showed that the same occurs with dentine. The "$CaF_2$" concentration formed on dentine was 7-fold higher than that on enamel, which may be explained by the smaller hydroxyapatite crystals in dentine resulting in a larger surface area to crystallite volume ratio

**Table 1.** Alkali-soluble fluoride ("$CaF_2$") ($\mu$g F/cm$^2$) formed on enamel by APF or control pre-treatments and retained after 7 days under saliva exposure and daily treatment with 0.05% NaF or the control (mean ± SD; n = 8).

| Pre-treatment groups | Formed (not treated) | Retained after saliva exposure | |
|---|---|---|---|
| | | $NaF^-$ (purified water) | $NaF^+$ (0.05% NaF) |
| $APF^-$ (0.1 M $H_3PO_4$, pH 3.5) | 0.50 ± 0.25 A,a | 0.25 ± 0.04 A,b | 0.44 ± 0.12 A,a |
| $APF^+$ (0.5 M NaF in 0.1 M $H_3PO_4$, pH 3.5) | 17.82 ± 11.56 B,a | 3.21 ± 1.91 B,b | 2.57 ± 1.13 B,b |

APF: acidulated phosphate fluoride.

Differences between rows are indicated by capital letters, and among columns by lower-case letters.

**Table 2.** Alkali-soluble fluoride ("$CaF_2$") ($\mu$g F/cm$^2$) formed on dentine by APF or control pre-treatments and retained after 7 days under saliva exposure and daily treatment with 0.05% NaF or the control (mean ± SD; n = 8).

| Pre-treatment groups | Formed (not treated) | Retained after saliva exposure | |
|---|---|---|---|
| | | $NaF^-$ (purified water) | $NaF^+$ (0.05% NaF) |
| $APF^-$ (0.1 M $H_3PO_4$ , pH 3.5) | 0.46 ± 0.24 A,a | 0.56 ± 0.17 A,a | 1.31 ± 0.77 A,b |
| $APF^+$ (0.5 M NaF in 0.1 M $H_3PO_4$, pH 3.5) | 127.19 ± 20.11 B,a | 13.34 ± 5.56 B,b | 10.27 ± 2.82 B,b |

APF: acidulated phosphate fluoride.

Differences between rows are indicated by capital letters, and among columns by lower-case letters.

and therefore a more reactive mineral phase.[6] Additionally, dentine is more acid-soluble than enamel,[7] resulting in more calcium being released by the APF treatment, which reacts with fluoride and precipitates as "$CaF_2$". Nevertheless, our data confirmed that "$CaF_2$" formed on the enamel and dentine was not stable in a saliva-like solution.[3] "$CaF_2$" concentration decreased similarly (85%-90%) in enamel and dentine after 7 days of exposure to artificial saliva, and the 0.05% NaF twice daily treatment did not prevent this decrease. However, the amount of "$CaF_2$" remaining was higher than the concentration found in slabs pre-treated with a non-fluoridated control solution ($APF^-$). The findings for enamel show that these reservoirs may last for a significant period of time, as previously shown *in vitro*[3] and *in situ*[8] and the same is expected for dentine.

The finding that the 0.05% NaF rinse did not prevent the dissolution of "$CaF_2$" formed by APF may be explained by the very small amount of fluoride deposited on sound dental tissues from a mouthrinse.[9] In fact, the twice daily NaF treatment significantly increased alkali-soluble fluoride concentrations on the dentine slabs that were not pre-treated with fluoride, but the concentration after 7 days was only approximately 1% of that resulting from the APF treatment (Table 2).

The present results are also relevant when APF is recommended as a dentine hypersensitivity treatment.[10] Although "$CaF_2$" may block dentinal tubules and decrease sensitivity for some time, the daily use of a fluoride mouthrinse may not be suitable to prolong this effect.

## Conclusion

Our findings suggest that 0.05% NaF treatment twice daily does not prevent oral dissolution of "$CaF_2$" formed on enamel or dentine by APF application.

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