Fluoride rinse effect on retention of CaF$_2$ formed on enamel/dentine by fluoride application

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. 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.

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$_2$". 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.

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 $2 \times 3$ factorial study was conducted, including the following factors: 1) APF application at two levels, i.e., applied (APF) or not (APF)
Fluoride rinse effect on retention of CaF₂ formed on enamel/dentine by fluoride application

and 2) 0.05% NaF treatment at three levels, i.e., not treated (NT), treated with NaF during saliva immersion (NaF⁰), 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⁻/NaF⁰; APF⁰/NT; APF⁰/NaF⁻, and APF⁰/NaF⁰. The APF⁰ and APF⁻ groups were pre-treated with 0.5 M NaF in 0.1 M H₃PO₄, pH 3.5 (APF⁰) or 0.1 M H₃PO₄, pH 3.5 (APF⁻) solutions, respectively. The alkali-soluble fluoride ("CaF₂") concentration formed in groups APF⁻/NT and APF⁰/NT was immediately determined after treatment. Slabs from the NaF⁻ and NaF⁰ groups were individually immersed in artificial saliva following pre-treatment and treated twice daily with 0.05% NaF solution (NaF⁰) or purified water (NaF⁻), respectively. "CaF₂" 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 APF⁰/NT groups were immediately analyzed for alkali-soluble F concentration. The remaining slabs were individually immersed in artificial saliva following pre-treatment and treated twice daily with 0.05% NaF solution (NaF⁰) or purified water (NaF⁻), respectively. "CaF₂" concentration was determined in the slabs after 7 days.

Determination of "CaF₂"

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/cm².

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 "CaF₂" concentrations in the enamel (Table 1) and dentine (Table 2) slabs (p < 0.05) than the control. "CaF₂" concentrations significantly decreased by 85 and 90% in the enamel and dentine after exposure to saliva and purified water, respectively (p < 0.05). The "CaF₂" 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 ("CaF₂") was formed after APF was applied to enamel,² but also showed that the same occurs with dentine. The "CaF₂" 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.
and therefore a more reactive mineral phase.\textsuperscript{6} Additionally, dentine is more acid-soluble than enamel,\textsuperscript{7} resulting in more calcium being released by the APF treatment, which reacts with fluoride and precipitates as “CaF\textsubscript{2}”. Nevertheless, our data confirmed that “CaF\textsubscript{2}” formed on the enamel and dentine was not stable in a saliva-like solution.\textsuperscript{3} “CaF\textsubscript{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\textsubscript{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 \textit{in vitro}\textsuperscript{3} and \textit{in situ}\textsuperscript{8} and the same is expected for dentine.

The finding that the 0.05% NaF rinse did not prevent the dissolution of “CaF\textsubscript{2}” formed by APF may be explained by the very small amount of fluoride deposited on sound dental tissues from a mouthrinse.\textsuperscript{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.\textsuperscript{10} Although “CaF\textsubscript{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\textsubscript{2}” formed on enamel or dentine by APF application.

**Acknowledgements**

This study was developed during the discipline of “\textit{In vitro} models in Cariology” and the authors thank the graduate students Alhethea Ratti, Karla E. Cook and Lívia H. Terra e Souza for their collaboration during data acquisition.

---

### Table 1. Alkali-soluble fluoride ("CaF\textsubscript{2}") (µg F/cm\textsuperscript{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).

<table>
<thead>
<tr>
<th>Pre-treatment groups</th>
<th>Formed (not treated)</th>
<th>Retained after saliva exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaF− (purified water)</td>
<td>NaF+ (0.05% NaF)</td>
</tr>
<tr>
<td>APF− (0.1 M H\textsubscript{3}PO\textsubscript{4}, pH 3.5)</td>
<td>0.50 ± 0.25 A,a</td>
<td>0.25 ± 0.04 A,b</td>
</tr>
<tr>
<td>APF+ (0.5 M NaF in 0.1 M H\textsubscript{3}PO\textsubscript{4}, pH 3.5)</td>
<td>17.82 ± 11.56 B,a</td>
<td>3.21 ± 1.91 B,b</td>
</tr>
</tbody>
</table>

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\textsubscript{2}") (µg F/cm\textsuperscript{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).

<table>
<thead>
<tr>
<th>Pre-treatment groups</th>
<th>Formed (not treated)</th>
<th>Retained after saliva exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaF− (purified water)</td>
<td>NaF+ (0.05% NaF)</td>
</tr>
<tr>
<td>APF− (0.1 M H\textsubscript{3}PO\textsubscript{4}, pH 3.5)</td>
<td>0.46 ± 0.24 A,a</td>
<td>0.56 ± 0.17 A,a</td>
</tr>
<tr>
<td>APF+ (0.5 M NaF in 0.1 M H\textsubscript{3}PO\textsubscript{4}, pH 3.5)</td>
<td>127.19 ± 20.11 B,a</td>
<td>13.34 ± 5.56 B,b</td>
</tr>
</tbody>
</table>

APF: acidulated phosphate fluoride.
Differences between rows are indicated by capital letters, and among columns by lower-case letters.
Fluoride rinse effect on retention of CaF₂ formed on enamel/dentine by fluoride application

References