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The effect of fluoride toothpaste on root dentine demineralization progression: a pilot study*

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Abstract: The anticaries effect of fluoride (F) toothpaste containing 1100 µg F/g in reducing enamel demineralization is well established, but its effect on dentine has not been extensively studied. Furthermore, it has been shown that toothpaste containing a high F concentration is necessary to remineralize root dentine lesions, suggesting that a 1100 µg F/g concentration might not be high enough to reduce root dentine demineralization, particularly when dentine is subjected to a high cariogenic challenge. Thus, the aim of this pilot study was to evaluate *in situ* the effect of F toothpaste, at a concentration of 1100 µg F/g, on dentine demineralization. In a crossover and double-blind study, conducted in two phases of 14 days, six volunteers wore a palatal appliance containing four slabs of bovine root dentine whose surface hardness (SH) was previously determined and to which a 10% sucrose solution was applied extra-orally 8×/day. Volunteers used a non-F toothpaste (negative control) or F toothpaste (1100 µg F/g, NaF/SiO₂) three times a day. On the 10th and 14th days of each phase, two slabs were collected and SH was determined again. Dentine demineralization was assessed as percentage of SH loss (%SHL). The effect of toothpaste was significant, showing lower %SHL for the F toothpaste group (42.0 ± 9.7) compared to the non-F group (62.0 ± 6.4; $p < 0.0001$), but the effect of time was not significant ($p > 0.05$). This pilot study suggests that F toothpaste at 1100 µg F/g is able to decrease dentine caries even under a high cariogenic challenge of biofilm accumulation and sugar exposure.

Descriptors: Dentin; Sucrose; Fluorides; Biofilms; Dental Caries.

Introduction

Over the last decades, the significant increase in longevity and the reduction in the prevalence of enamel caries around the world have raised the concern about caries prevention in the elderly, who will have a large amount of root dentine exposed in their mouths. Therefore, since dentine is more susceptible to caries than enamel,^{1,2} the concern about prevention of root caries is increasing.³

Fluoride (F) toothpaste is widely used and it has been considered responsible for the caries decline that occurred in developed countries in the 1980s⁴ and in developing ones in the 1990s.⁵ The anticaries effect of F toothpaste containing 1100 µg F/g (ppm F) in reducing enamel caries is well established,^{6,7} but its effect on dentine has not been extensively

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studied.^{8,9}

Furthermore, it has been shown that toothpaste containing a high F concentration is necessary to remineralize root dentine lesions,¹⁰⁻¹³ and the effect of F in decreasing demineralization has been little explored.¹⁴⁻¹⁶ Considering that the critical pH for dentine dissolution is about 6.8 to 6.0, a slight decrease in the pH of the biofilm fluid will lead to demineralization.¹⁷ However, if F is present in biofilm fluid and the pH is not lower than 4.5, fluorapatite will be formed as long as hydroxyapatite is dissolved.¹⁸ This reduction in demineralization is not able to avoid loss of part of the minerals, but a higher availability of F might have a better effect. Thus, a concentration of 1100 µg F/g might not be high enough to reduce dentine demineralization if this dental tissue is exposed to a high cariogenic challenge by biofilm accumulation and sugar exposure over time.

The aim of this pilot study was thus to evaluate *in situ* the effect of 1100 µg F/g toothpaste on the progression of root dentine demineralization.

Methodology

Ethical aspects and volunteers

This pilot study was approved by the Research and Ethics Committee of the Piracicaba Dental School (protocol no. 104/2011), and volunteers signed an informed, written consent to participate (Resolution no. 196 of the *Conselho Nacional de Saúde* - CNS, *Ministério da Saúde* - MS, Brasília, Brazil, 10/03/1996).

Six volunteers (21–35 years old), who fulfilled inclusion criteria (normal salivary flow rate, good general and oral health with no active caries lesions or periodontal treatment needs, ability to comply with the experimental protocol, not having used antibiotics during the 2 months prior to the study and not using fixed or removable orthodontic devices) were selected to participate in the study. The volunteers selected had a mean decayed, missing and filled tooth surfaces index (DMFS) of 9.5 ± 10.03 .

Experimental design

The study used a double-blind, crossover design, and was conducted in two phases of 14 days each, during which six volunteers wore palatal appliances

containing four slabs of bovine root dentine with known surface hardness (SH).¹⁹ A 10% sucrose solution, prepared by the researchers, was provided for the volunteers. This solution was applied extra-orally to the slabs eight times per day as a cariogenic challenge. The volunteers brushed their teeth and the appliance with a non-F toothpaste (negative control) or F toothpaste (1100 µg F/g, NaF/silica-based) three times a day (the dentifrice formulations were prepared by Colgate/Palmolive, São Bernardo do Campo, Brazil). On the 10th and 14th days of each phase, two slabs were collected and evaluated for mineral loss by SH. The sequence of toothpaste used by each volunteer was randomly assigned and, after the two phases, all volunteers had undergone the two treatments. For all determinations, the volunteer was considered the experimental unit.

Dentine slabs and palatal appliance preparation

The root dentine slabs (4 × 4 × 2 mm) were prepared from bovine incisor teeth as previously described.¹⁹ The artificial saliva, prepared by the researchers, contained 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.1 M Tris, pH 7.0.²⁰ The slabs were immersed in this solution for 24 h to allow dentine mineral gain and minimize further ionic changes when exposed to saliva *in situ*.²¹ The SH of the dentine slabs was determined by making 3 indentations, spaced 100 µm apart, using a Knoop indenter with a 5 g load for 5 s and a microhardness tester coupled to FM-ARS 900 software (Future-Tech Corp., Kawasaki, Japan). Before performing the dentine hardness measurements, the slabs were allowed to dry for at least 30 minutes to minimize the interference of dentine dehydration with the measurements.⁸ Forty-eight slabs with a mean hardness of 43.2 kg/mm² (SD 4.4) were selected and were randomly divided into two groups of 24 specimens each, according to the toothpaste treatments. Acrylic palatal appliances were made for each volunteer with four positions (two on each side of the appliance) for slabs. Plastic meshes were fixed over the cavities to protect the dentine slab surfaces from mechanical attrition, leaving a 1 mm space for biofilm accumulation (see Hara *et al.*¹⁹ for details).

Treatments

During the lead-in and washout periods of 7 days each, the volunteers brushed their teeth with non-F toothpaste. Volunteers lived in an optimally fluoridated city (0.6–0.8 mg F/L for the region) and received instructions as previously described.²² Considering the crossover design of this study, no restriction was made with regards to the volunteers' diet, except that they were instructed to avoid F-rich foods containing bioavailable F, such as tea. They also received oral and written information to refrain from using any antibacterial substance.

To provide a cariogenic challenge during the 14 days of each experimental phase, 8 times per day (8:00, 9:00, 10:00, 11:00, 14:00, 15:30, 17:00, 19:00 h), volunteers removed the device from the oral cavity and applied one drop of the 10% sucrose solution on each dental slab. After 5 minutes, the device was re-inserted in the mouth.

Throughout the experiment, volunteers brushed their teeth and the device after the main mealtimes, 3 times a day (7:30, 12:30, 20:00 h), with the toothpaste assigned for each phase. The device was removed and volunteers brushed their teeth and the appliance with the assigned toothpaste, taking care to only apply the slurry formed on the slab area of the device. The device was then washed gently in tap water and re-inserted in the mouth. The volunteers were instructed to wear the intraoral devices throughout the 14 experimental days, removing them only for oral hygiene and during meals.

Dentine analysis

On the 10th and 14th days of each phase, two slabs were removed from the devices and the SH was measured again. One row of three adjacent indentations spaced 100 µm apart was made 100 µm from the three baseline measurements. The mean values of the three baseline indentations and the three measurements after treatments were then averaged, and the percentage of SH loss (%SHL) was calculated according to the following equation:

$$\%SH = (baseline\ SH - SH\ after\ in\ situ\ test) \times 100 / baseline\ SH$$

SH loss was used as an indicator of dentine demineralization.^{8,22} The results of the two dentine slabs for each volunteer subjected to each treatment were averaged and analyzed statistically ($n = 6$). The SH was used to estimate demineralization of root dentine because there is a high correlation with mineral loss assessed by transverse microradiography.⁸

Statistical analysis

A 2×2 factorial was considered for the statistical analyses, and the factors evaluated were:

- toothpaste at 2 levels (fluoridated and non-fluoridated) and
- time at 2 levels (10th and 14th days).

Volunteers were considered statistical blocks. The assumption of equality of variances and normal distribution of errors was checked. The variables were analyzed by two-way analysis of variance (ANOVA). The analysis was conducted with SAS 9.0 software (SAS Institute, Cary, USA), with a significance level fixed at $p < 0.05$.

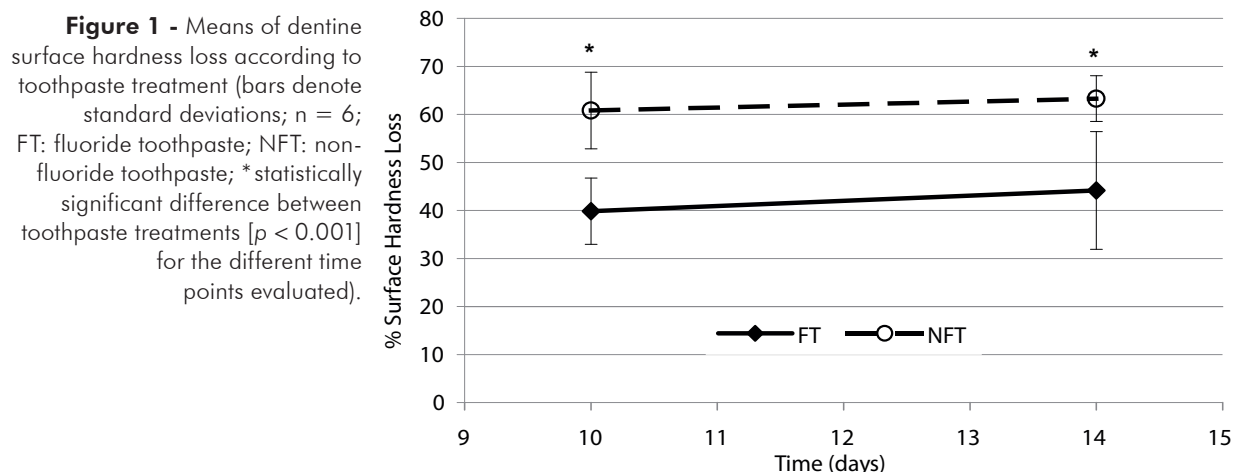
Results

The effect of the dentifrice factor was significant ($p < 0.001$, Figure 1), showing lower %SHL for the group treated with F toothpaste (42.0 ± 9.7) compared with the control (62.0 ± 6.4) group. However, the effect of time was not significant ($p > 0.05$).

Discussion

The effect of F toothpaste in reducing enamel caries, provided the concentration is 1000 µg F/g or higher, is based on evidence.⁷ Considering that dentine is more caries-susceptible and demineralizes 2.5 times faster than enamel,²³ it is possible that a concentration of 1100 µg F/g might not be high enough to control root dentine caries. In fact, there is some evidence that a toothpaste containing 5000 µg F/g is more effective in repairing root dentine caries than one containing 1100 µg F/g.^{11,12}

In the present *in situ* study, simulating *in vivo* conditions of biofilm accumulation and high exposure to sucrose, the toothpaste containing 1100 µg F/g significantly reduced ($p < 0.05$) root dentine demineralization compared with the placebo denti-



frice. The 32% of reduction found is in agreement with *in vitro* findings,²⁴ but was lower than that found previously *in situ* (47%),⁸ which might be explained by differences in the duration of both studies.

The effect of the 1100 µg F/g toothpaste in reducing root dentine demineralization by 32% was accomplished by using toothpaste 3 times/day. This value is very close to that found by Kusano *et al.*,⁹ when a 1100 µg F/g toothpaste was used only once a day at night. The data suggest that when brushing at night is performed daily, the other two brushing episodes (in the morning and after lunch) are less important. The percentage of reduction of dentine demineralization found in the present study was lower than the 67% reduction in root caries found *in vivo* by Jensen and Kohout²⁵ with elderly people living in a non-fluoridated community. The high cariogenic challenge of our *in situ* study and the effect of optimally fluoridated water, attenuating the strength of F-toothpaste use, may partially explain the smaller effect observed.

We also evaluated the effect of time and tested whether the experimental phases lasting for 10 or 14 days would demonstrate caries progression. Nevertheless, our findings showed that the %SHL was similar in both periods for the F toothpaste and control groups. This may be explained by the fact that dentine demineralizes faster than enamel, and that demineralization is very fast in the first week and progresses slower after that, as a result of the presence of a demineralized organic matrix acting as a barrier between biofilm and the dentine surface.²³

In this context, the period between the two evaluations might not be long enough to show the possible differences in SH or, perhaps, progression of the lesion could not be measured because the outer surface layer may have reached equilibrium with the biofilm fluids and progression would be observed in the body of the lesion.

These results showed that although the 1100 µg F/g toothpaste could not avoid dentine demineralization, it was able to reduce it even under a high cariogenic challenge of biofilm accumulation and sugar exposure. Moreover, this is a pilot study with six volunteers, and other *in situ* studies with more volunteers may be necessary, followed by clinical trials with elderly populations to assess the initiation and progression of dentine caries lesions while using toothpastes with different F concentrations.

Conclusion

In summary, this pilot *in situ* study suggests that a 1100 µg F/g toothpaste is able to decrease root dentine caries.

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