Combination effect of fluoride dentifrices and varnish on deciduous enamel demineralization

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Abstract: The aim of this study was to evaluate the anticaries potential of 500 or 1100 ppm F dentifrices combined with fluoride varnish using a pH-cycling regimen. Seventy primary canines were covered with nail polish, leaving a 4×4 mm window on their buccal surface, and randomly assigned into 7 groups (n = 10): S: sound enamel not submitted to the pH-cycling regimen or treatment; N: negative control, submitted to the pH-cycling regimen without any treatment; D1 and D2: subjected to the pH-cycling regimen and treated twice daily with 1100 or 500 ppm F dentifrice, respectively; VF: fluoride varnish (subjected to F-varnish before and in the middle of the pH-cycling regimen); and VF+D1 and VF+D2. After 10 days, the teeth were sectioned, and enamel demineralization was assessed by cross-sectional hardness at different distances from the dental surface. Data were analyzed using a two-way ANOVA followed by Tukey's test. Dentifrice with 1100 ppm F and the combination of F-varnish with the dentifrices significantly reduced enamel demineralization compared with the negative control ($p < 0.05$), but the isolated effects of F-varnish and dentifrice with low concentration were not significant ($p > 0.05$). The effect of combining F-varnish with the dentifrices was not greater than the effect of the dentifrices alone ($p < 0.05$). The data suggest that the combination of F-varnish with dentifrices containing 500 and 1100 ppm F is not more effective in reducing demineralization in primary teeth than the isolated effect of dentifrice containing 1100 ppm F.

Descriptors: Demineralization; Dental Enamel; Dentifrices; Tooth, Deciduous.

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Introduction

Topical fluoride treatments have frequently been used to prevent dental caries for over three decades.[1,2] The decline in the prevalence of caries is greatly due to fluoridated water supplies and professional topical application but is primarily due to the widespread use of fluoride toothpaste.[3]

The regular use of fluoridated dentifrice may be a beneficial preventative measure, independent of the occurrence of caries[3]. However, Ögaard *et al.*[4] recommended that when the risk of caries is high, this measure should be conducted with high concentration methods. The benefit of combining fluoridated dentifrice with professional applications has not been clearly established, and more studies are necessary.[5]

Considering the relationship between the prevalence of dental fluo-

rosis and the use of fluoride dentifrice, and because young children have not fully developed their swallow reflex and may therefore ingest large amounts of dentifrice during tooth brushing,[6] several preventative measures have been suggested.[7] Some recommendations have been made for tooth brushing in children younger than six years old:

- non-fluoridated dentifrice,
- a small amount of dentifrice[8] and
- dentifrice with reduced fluoride concentrations.[9]

Therefore, it is necessary to assess if dentifrices with lower fluoride concentrations (500 ppm F) that are targeted to children younger than six years old are effective against demineralization, and if this preventive effect is the same as those for 1100 ppm F dentifrices.

The present study aimed to assess the preventive effect of 500 ppm F and 1100 ppm F fluoridated children's dentifrices and fluoride varnish *in vitro*, either applied in combination or alone, on primary teeth enamel after a caries challenge.

Methodology

This research was approved by an Ethical Board. Seventy primary canines from the Human Tooth Bank were used. The samples were assessed by visual inspection and were free of dental caries or enamel defects.

The root portion of all teeth was sealed with epoxy resin (Araldite®, Brascola, Florianópolis, Brazil) and then covered with red nail polish (Colorama, São Paulo, Brazil). A 4×4 mm window area on the buccal surface was left free of nail varnish. All teeth were previously cleaned with a detergent solution (Tergencal®, Biodinâmica, Curitiba, Brazil) and pumice powder using a low speed motor. The teeth were then left under running water for one minute to eliminate debris.

Teeth were submitted to the formation of artificial caries by pH cycling,[10] keeping the teeth in demineralizing solution ($CaCl_2$ 2.2 mM, NaH_2PO_4 2.2 mM and acetic acid 0.05 M; pH of 4.5, adjusted with KOH 1M;[13] 15 mL per tooth) for 3 hours and in remineralizing solution ($CaCl_2$ 1.5 mM, $NaHPO_4$ 0.9 mM and KCl 0.15 mM; pH of 7.0; 15 mL per tooth) for 21 hours. A total of 10 cycles were conducted. The teeth were briefly washed in deionized water between solutions and placed in artificial saliva for 30 minutes ($CaCl_2$ [15 mg], $MgCl_2$ [5 mg], KCl [0.1 g], KSCN [10 mg], Na_2HPO_4 [40 mg], sodium carboxymethylcellulose [1.0 g], methylparaben [0.1 g] and water [1 L]; pH of 7.0). The demineralizing solutions were changed daily, and the artificial saliva was changed at every treatment.

One of the groups was comprised of sound enamel (S), was not submitted to pH cycling and had no treatment. Group S was kept in deionized water for a later hardness assessment.

The negative control (N) group had no treatment but was submitted to pH cycling. The remaining five groups (VF, VF+D1, VF+D2, D1, D2) received topical fluoride treatment. Groups VF, VF+D1 and VF+D2 were treated with the fluoridated varnish Duraphat® (Colgate-Palmolive, São Paulo, Brazil) (22600 ppm F NaF and pH of 7.0) on the 5th and 10th day of the pH cycling. Duraphat® was applied to the delimited area (4×4 mm). The teeth were then stored in the remineralizing solution for 5 hours. The varnish was then carefully removed with acetone, and the teeth were washed with deionized water for one minute[11] and again immersed in the remineralizing solution. The varnish application was conducted within the 21 hours that the teeth were kept in the remineralizing solution.

During the pH cycling, the VF+D1 and VF+D2 groups were treated with the varnish as well as fluoridated dentifrice, while the D1 and D2 groups were only treated with fluoridated dentifrice. The VF+D1 and D1 groups were treated with the dentifrice Tandy® (Colgate-Palmolive, São Paulo, Brazil) (1100 ppm F in NaF, pH of 7.0), and the VF+D2 and D2 groups were treated with Colgate Baby Barney® (Colgate-Palmolive, São Paulo, Brazil) (500 ppm F in NaF, pH of 6.9). The dentifrices were applied twice daily on the enamel at 10 a.m. and 4 p.m. by manually brushing the surface for 1 minute before changing solutions. After every brushing period, the teeth were briefly washed in deionized water.

To standardize the minimum amount of dentifrice used in the experiment, the lids of the Tandy® (Colgate-Palmolive, São Paulo, Brazil) and Col-

gate Baby Barney® (Colgate-Palmolive, São Paulo, Brazil) were used. These lids close under pressure, and both have similar compartments for dentifrice dosage. According to Chedid and Cury,[10] the dentifrice quantity accumulated in the lids is 0.16 g on average. The standardized technique is to press the brush once on the top of the lid. The penetration of the brush into the lid is limited by the depth of the lid.

The teeth were then immersed in orthophtalic resin and cut along the crown's longitudinal axis through the middle of the window area to assess the hardness. The cut surface was polished in a rotating machine (Arotec Aropol 2V, São Paulo, Brazil) using sand paper with grits of 320, 600, and 1200. The 320 grit paper was used for 30 seconds, and the remaining 2 grit papers were used for 60 seconds each under running water. The polished surface was verified by visual inspection before using the next grit paper. For this procedure, the samples were held on top of the sandpaper by hand, with minimum pressure. The final polish was conducted by felt disks and diamond paste (Diamond Excel, FGM, Florianópolis, Brazil) at low speed. The samples were later washed and placed in the ultra-sound bath for 12 minutes to remove debris.

The cross sectional hardness measurements were made using a hardness tester (Pantec-Digital Microhardness tester HVS-1000, Panambra Ind. e Técnica S/A, São Paulo, Brazil) with a Knoop indentor and static load of 25 g and with 5 seconds of dwell time.[10]

Three rows of 5 indentations each, separated by 100 μm, were made at 20, 40, 60, 80 and 100 μm from the outer dental surface of the exposed area. The mean hardness values (kg/mm²) of the 3 rows at each distance from the surface were then averaged and statistically analyzed within and between treatments. A Kolmogorov-Smirnov test was used to verify the sample distribution, and a two-way ANOVA with repeated measures (for distance) and a *post-hoc* Tukey test were used to verify differences in hardness between treatments and distances from the surface. The statistical analyses were conducted with the statistical software SPSS (IBM Corp, New York, USA) version 13.0, with a 5% significance level.

Results

Results reached statistical significance for the group and distance factors and the interaction between group and distance, which indicates that the effect of the treatments was different, depending on the depth of the enamel surface.

The demineralization data according to the distance from the dental surface for the negative control (N) group showed that the produced lesion was narrow because the statistical difference was only

Table 1 - The means and standard deviations (n = 10) of microhardness (kg/mm²) according to the treatments and distance from the surface.

Group	Distance from dental surface (μm)			
	20	40	60	80
S (Sound)	260.4 (85.4)$^{C\,a}$	288.5 (66.9)$^{B,C,D\,a}$	302.8 (76.0)$^{B\,a}$	282.8 (62.6)$^{A,B\,a}$
N (negative control)	148.7 (71.5)$^{A\,a}$	238.0 (44.7)$^{A\,b}$	252.6 (43.1)$^{A\,b}$	255.0 (45.1)$^{A\,b}$
VF (fluoride varnish treatment)	174.9 (58.9)$^{A\,a}$	240.3 (46.6)$^{A\,b}$	254.1 (60.6)$^{A\,b}$	249.6 (64.8)$^{A\,b}$
D1(1100 ppm dentifrice treatment)	258.7 (54.5)$^{C\,a}$	275.9 (62.9)$^{A,B,C\,a}$	284.9 (56.3)$^{A,B\,a}$	282.9 (78.1)$^{A,B\,a}$
D2 (500 ppm dentifrice treatment)	186.4 (75.7)$^{A,B\,a}$	254.3 (63.3)$^{A,B\,b}$	285.3 (57.9)$^{A,B\,b}$	284.4 (56.5)$^{A,B\,b}$
VF + D1	249.6 (65.3)$^{B,C\,a}$	306.9 (50.3)$^{B,C,D\,a,b}$	318.3 (43.2)$^{B,C\,b}$	303.7 (43.7)$^{B\,b}$
VF + D2	243.8 (42.4)$^{B,C\,a}$	328.3 (84.9)$^{B,C,D\,b}$	361.0 (73.5)$^{C\,b}$	361.1 (63.3)$^{C\,b}$

Distinct capital letters show differences between groups at each distance (within columns), while distinct small letters show significant differences among distances for each group (in the lines) after a two-way ANOVA and Tukey's test ($p < 0.05$). S = not subjected to the pH-cycling regimen or any treatment; N = subjected to the pH-cycling regimen without any treatment; VF, D1, D2, V+D1 and V+D2 = subjected to the pH-cycling regimen and respective treatments.

observed at a distance of 20 μm from the enamel surface (Table 1).

When the effect of the treatments was compared with the negative control group at each distance from the surface, the treatments with F-varnish (V) and 500 ppm F dentifrice (D2) were not effective ($p > 0.05$) in reducing enamel demineralization in all depths analyzed (Table 1). At 20 μm of depth, the dentifrice with 1100 ppm F (group D1) significantly reduced demineralization compared with group N ($p > 0.05$), but did not differ in the other distances ($p > 0.05$). The groups VF+D1 and VF+D2 did not significantly differ at any distance from the surface ($p > 0.05$). The combination of dentifrice and varnish (groups VF+D1 and VF+D2) significantly reduced demineralization compared with the N group at all distances evaluated ($p < 0.05$), but the combination did not differ from the dentifrice groups at most distances from the surface ($p > 0.05$).

Discussion

The present study used the pH cycling suggested by Chedid and Cury,[10] which is specifically indicated for primary teeth. The daily 3-hour demineralization cycle is related to effects that result when the patient ingests cariogenic products and does not remove the biofilm. In relation to this *in vitro* study, it was observed that there was a general enhanced preventative effect from combining fluoridated varnish with dentifrices (VF+D1 and VF+D2) compared with other groups. The preventative effect of low-fluoride dentifrice is still a concern in the literature. Winter *et al.*[12] and Vilhena *et al.*[9] demonstrated good performance in preventing new caries lesions in clinical trials using 550 ppm F and 500 ppm F toothpaste, respectively. However, Lima *et al.*[13] showed that in active caries in children, the 1100 ppm F performed better than the 500 ppm F. The low anticaries efficacy of 500 ppm F dentifrice compared with 1000-1100 ppm F dentifrice has been shown experimentally,[14] and it has also been supported by evidence.[15]

Our results suggest that the fluoridated varnish alone was not effective in avoiding mineral loss. The results are consistent with the *in vitro* study conducted by Maia *et al.*[11] Grodzka *et al.*[16] found that the best preventative effect may be achieved when fluoridated varnish is combined with other forms of fluoride supplements. Similarly, the present study demonstrated that lower mineral loss values were obtained in the VF+D2 and VF+D1 groups. However, other studies[2,11] have suggested that the combination of low (fluoridated dentifrice) and high fluoride concentration methods (fluoridated varnish) do not have additional benefits in remineralization and fluoride incorporation.

The professional cleaning was conducted to simulate the effects of the oral environment. Although the acquired pellicle is permeable to small ions, it tends to act as a barrier to diffusion, and it reduces the possible mineral transportation between the tooth and the oral environment, thus promoting or interfering with the remineralization process. However, Hellwig *et al.*[17] reported that applying fluoridated varnish on demineralized enamel covered by young biofilm only interfered in the formation of soluble fluoride (CaF_2). It did not impair the fluoride ionic exchange or its incorporation into the enamel[17] and reduced caries lesion progression if the fluoride availability in the biofilm was high.[14]

Considering that only the varnish group had enamel cleaned with acetone to remove resin residue, this procedure might cause damage to the fluoride product formed. Bruun and Givskov[18] tested this hypothesis and showed that acetone could not dissolve the CaF_2 from the enamel treated with varnish.

A comparison of the present study with other research is difficult to conduct due to differences in experimental design, pH-cycling models, the type of enamel used (primary, permanent or bovine), the trademark of the products used and the different concentrations of fluoride. However, based on our results, some considerations can be extrapolated.

Independent of the distance from the surface, the VF group always showed the lowest values for hardness. For the most superficial layers of the enamel, such as within 20 μm, where the teeth initially suffer cariogenic action, a decrease in hardness was observed for all treatments compared with the control group (N). At this distance, the effect of the 500 ppm F dentifrice (D2) was significantly different

from the 1100 ppm F (D1) dentifrice, which showed greater hardness. The combination of fluoride varnish with 500 ppm F (VF+D2) and 1100 ppm F (VF+D1) dentifrices also did not show an advantage compared with the 1100 ppm F dentifrice (D1) used alone.

Delbem *et al.* (2006)[19] verified the pH influence (4.0, 5.0, 6.0 and 7.0) and the anti-cariogenic effect of 0.02%, 0.05% and 0.1% NaF solutions using a pH-cycle model in bovine enamel. The results show that the pH influenced the percentage of surface microhardness loss (% SMH) in the 0.02% and 0.05% NaF at a pH of 4.0. In such solutions, the mineral loss was lower when compared with a pH of 7.0 ($p < 0.05$). The authors concluded that the acidification of solutions with low fluoride concentrations reduced mineral loss. The availability of saliva was also determined in the low-fluoride dentifrices with lower pH values.[20]

However, it should be stated that these results were obtained in an *in vitro* study, without diluting the dentifrice after application and biofilm formation, among other factors, and the results can therefore be different *in vivo*. More research, especially clinical trials, is necessary to confirm if the association of children's fluoride dentifrices and fluoride varnish can provide an adequate preventative effect in primary enamel.

Nevertheless, our data using this pH-cycling model are consistent with two systematic reviews: one showing that there is evidence for an anticaries effect if the dentifrice concentration is higher than 1100 ppm F,[15] and another concluding that the preventive effect of combining topical fluorides (dentifrice, mouth rinse or professional application) is modest compared with the isolated effect of fluoride dentifrice.[21]

Conclusion

The findings suggest that the combination of F-varnish with dentifrices containing 500 or 1100 ppm F is not more effective in reducing demineralization in primary teeth than the isolated effect of dentifrice containing 1100 ppm.

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