

## Effect of 0.02% NaF solution on enamel demineralization and fluoride uptake by deciduous teeth *in vitro*

### *Efeito da solução de NaF a 0,02% na desmineralização e incorporação de fluoreto pelo esmalte de dentes decíduos in vitro*

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**ABSTRACT:** The application of 0.02% NaF solution on teeth with a cotton swab instead of brushing with fluoride dentifrice has been suggested for young children to reduce the risk of dental fluorosis, but its anticariogenic effect has not been evaluated. Thus, we studied the *in vitro* effect of 0.02% NaF solution on enamel demineralization and fluoride uptake in deciduous teeth; non-fluoride dentifrice and fluoride dentifrice (1,100 µg F/g) were used, respectively, as negative and positive controls. The treatment with fluoride dentifrice was more effective in reducing enamel demineralization ( $p < 0.05$ ) and on fluoride uptake by the enamel ( $p < 0.05$ ) than the non-fluoride dentifrice and the 0.02% NaF solution. Data suggest that the alternative use of 0.02% NaF solution instead of fluoride dentifrice should be reevaluated especially if dental caries are to be controlled.

**DESCRIPTORS:** Fluorides; Dentifrices; Dental enamel; Tooth, deciduous; Fluorosis, dental.

**RESUMO:** A aplicação da solução de NaF a 0,02%, no lugar de dentifricio fluoretado, tem sido sugerida para ser aplicada com cotonete nos dentes de bebês para reduzir o risco de fluorose dental. Como o efeito anticariogênico dessa recomendação não tem sido estudado, avaliou-se *in vitro* seu efeito na redução da desmineralização e incorporação de fluoreto no esmalte de dentes decíduos; dentifricio não fluoretado e fluoretado (1.100 µg F/g) foram utilizados como controles negativo e positivo, respectivamente. O dentifricio fluoretado foi mais efetivo que a solução de NaF a 0,02% na redução de desmineralização e na incorporação de fluoreto no esmalte ( $p < 0,05$ ). Os dados sugerem que uso alternativo de NaF a 0,02% ao invés de dentifricio fluoretado para reduzir o risco de fluorose dental deve ser reavaliado, especialmente se a cárie dental precisa ser controlada.

**DESCRIPTORIOS:** Fluoretos; Dentifricios; Esmalte dentário; Dente decíduo; Fluorose dentária.

## INTRODUCTION

Fluoride dentifrice is considered an important factor in explaining the decline in dental caries observed either in developed or in developing countries at the end of the last century<sup>3,14</sup>. However, fluoride dentifrice is also a risk factor with respect to dental fluorosis<sup>13</sup> because young children ingest a large amount of toothpaste during brushing<sup>16</sup>. In addition, there is evidence that dental fluorosis is increasing in developed countries irrespective of whether the areas concerned have water fluoridation or not<sup>12</sup>. These findings in developed countries are an alert to developing countries that have caries control programs based on fluoride use<sup>17</sup>.

In order to reduce the risk of dental fluorosis some recommendations have been made for young children: (i) do not use dentifrice, or use a non-fluoride dentifrice<sup>7</sup>; however, the presence of fluoride during toothbrushing is considered in-

dispensable<sup>21</sup>; (ii) use a dentifrice with low fluoride concentration<sup>9</sup>; however, the anticaries effect of dentifrice with less than 1,100 µg F/g is not well-established<sup>15,19</sup>; (iii) use a small amount of dentifrice<sup>18</sup>; however, the anticaries effect of this procedure has not yet been established.

In addition to these international recommendations, the use of 0.02% NaF solution for children under 3 years of age in Brazil has been suggested, instead of fluoride dentifrice<sup>23</sup>. Parents or guardians should apply this solution to the children's teeth daily, using a cotton swab. This alternative is safer than the use of fluoride dentifrice with regard to dental fluorosis because the child is subjected daily to a low amount of fluoride (approximately 0.01 mg F/cotton swab). However, its anti-caries potential is unknown.

Thus, the aim of this study was to evaluate

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the *in vitro* effect of 0.02% NaF solution on enamel demineralization and fluoride uptake in deciduous teeth.

## MATERIAL AND METHODS

### Experimental design

Seventy-five enamel blocks (3 x 3 mm) were prepared from human upper incisor deciduous teeth provided by the Human Tooth Bank, School of Dentistry, University of São Paulo (USP). The enamel surface of the blocks was then sequentially polished and those with a hardness of  $43 \pm 3$  KHN (knoop hardness number) units were randomized into three groups of 15 specimens each. The groups of blocks, presenting known baseline enamel surface microhardness, were submitted to a pH-cycling model simulating a high cariogenic challenge. During pH-cycling, one group was treated with a non-fluoride dentifrice (negative control); the experimental group was treated with 0.02% NaF solution and the positive control with fluoride dentifrice (1,100  $\mu\text{g F/g}$ , w/w). After pH-cycling, surface enamel microhardness was again determined in the blocks to evaluate its loss. Enamel cross-sectional microhardness was also determined to evaluate the caries lesion. In addition, fluoride in enamel was assessed to evaluate fluoride uptake in each treatment. The use of human teeth was ethically conducted according to the Brazilian guidelines (Resolution No. 196 of the National Health Council, Health Ministry, Brasília, DF, 10/03/1996).

### pH-cycling

The enamel blocks were submitted to a pH-cycling model for 10 days, simulating a high caries challenge, essentially according to Featherstone *et al.*<sup>5</sup>. The blocks were kept in a demineralizing solution (2.0 mM calcium, 2.0 mM phosphate in 0.075 M acetate buffer, pH 4.3) at 37°C for 3 h (60 ml per block), and in a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, pH 7.0) for 21 h (30 ml per block). During the weekend, the enamel blocks were stored in the remineralizing solution and both solutions were changed before starting another cycle of 5 days. During pH-cycling, twice a day, the enamel blocks were treated with the dentifrices or the 0.02% NaF solution.

### Fluoride treatment

The treatments were applied to the enamel blocks before and after the time that these were im-

mersed in the demineralizing solution. The negative control group was treated with a non-fluoride dentifrice (placebo) and the positive one with fluoride dentifrice (Tandy, Colgate-Palmolive Ind. Com. Ltda., São Bernardo do Campo, SP, Brazil) containing 1,100  $\mu\text{g F/g}$  (w/w as NaF). Both dentifrices were silica-based. The dentifrices were applied to the enamel surface for 20 s, using a toothbrush, but without pressure. The amount of dentifrice applied was  $0.1 \pm 0.02$  g and it was standardized by collecting the dentifrice set in the tip-top cap of the dentifrice tube. In the experimental group of enamel blocks, 0.02% NaF solution (w/v) was applied to the enamel surface with a cotton swab for 20 s. After the treatments the blocks were immersed in artificial saliva<sup>2</sup> (1.5 mM Ca, 3.0 mM P, 20.0 mM NaHCO<sub>3</sub>, pH 7.0, 1.0 ml per block). The blocks were briefly washed with deionized water and the artificial saliva was changed for a fresh amount every time treatment was applied. After the pH-cycling, the enamel of the blocks was evaluated.

### Microhardness determination

After pH-cycling, the surface microhardness of the enamel block was measured again. Five indentations spaced 100  $\mu\text{m}$  from each other and from the baseline ones were made. A Shimadzu HMV-2000 (Shimadzu Corp., Kyoto, Japan) microhardness tester with a Knoop diamond with 50 g load was used for 5 s. The percentage of surface microhardness change (%SMC) was calculated (%SMHC = hardness after pH-cycling – baseline  $\times$  100/baseline). Surface microhardness (SMH) was evaluated because this method is highly sensitive and reproducible to evaluate enamel demineralization<sup>24</sup>. After surface microhardness analysis, the blocks were longitudinally sectioned in the centre. Half of each block was used for cross-sectional microhardness determination and the other half for fluoride enamel analysis.

To perform cross-sectional microhardness (CSMH) tests, one of the halves of each block was embedded in acrylic resin so that the cut section was exposed and could be polished. The indentations were made at 20  $\mu\text{m}$  from the outer enamel surface up to 100  $\mu\text{m}$ . Three lanes of indentations were made, one in the central region of the dental block and the other two 100  $\mu\text{m}$  below and above it, using Knoop diamond with a 25 g load for 5 s. The mean values at all measuring points, at each distance from the surface were then averaged. CSMH was used to evaluate demineralization con-

sidering that there is a high correlation ( $r = 0.91$ ) between enamel microhardness and percentage of mineral in caries lesions, and the values of Knoop hardness number (KHN) were converted to mineral contents (%vol) using the relation: mineral content =  $4.3 (\sqrt{\text{KHN}}) + 11.3$ , according to Featherstone *et al.*<sup>6</sup>.

### Fluoride concentration in enamel

Five enamel layers were sequentially removed from half of each block by immersion in 0.25 ml of 0.5 M HCl for 30, 30, 30, 60 and 60 s under agitation<sup>10</sup>. An equal volume of TISAB II pH 5.0 modified with 20 g NaOH/l was added to each solution containing the dissolved enamel layer. Fluoride measurements were performed using an ion-selective electrode Orion 96-09 and an ion analyzer Orion EA-940 (Orion Research Inc., Boston, MA, USA), previously calibrated with various standard fluoride solutions from 0.1 to 5.0  $\mu\text{g F/ml}$ . The thickness of enamel layer removed during the acid biopsy was calculated from the inorganic phosphorus concentration, determined colorimetrically<sup>8</sup>. Phosphorus content in enamel of 17.4% was estimated to calculate the amount of enamel removed and enamel density of 2.92 was considered to calculate the depth of each enamel layer<sup>11</sup>.

### Statistical analysis

The SMH and fluoride uptake data were analyzed by ANOVA, after transformation by exponential and square root respectively. The difference among the treatments was analyzed by Newman-Keuls test. The results of cross-sectional microhardness were analyzed by the non-parametric Kruskal-Wallis test. The software BioEstat 2.0 was used<sup>1</sup> for all the analyses, and the significance level was established at 5%.

## RESULTS

None of the treatments were able to prevent

the reduction of surface microhardness (Table 1), because the difference (baseline values *vs.* after treatment) was statistically significant ( $p < 0.0001$ ). However, the effect of the treatments was found ( $p < 0.0001$ ) by ANOVA regarding the percentage of SMH change (%SMC). The treatment with fluoride dentifrice was more effective than those with placebo dentifrice and with 0.02% NaF regarding reduction of the percentage of SMC ( $p < 0.01$ ). The effect of 0.02% NaF treatment was not statistically significant in comparison with placebo dentifrice ( $p > 0.05$ ).

Graph 1 shows that only the treatment with fluoride dentifrice (FD) was statistically more effective than the placebo (PD) in reducing the loss of mineral at all the distances from the enamel surface ( $p < 0.05$ ). The results observed with the 0.02% NaF solution were not statistically different to those observed with the non-fluoride dentifrice treatment (PD) regarding caries lesions.

With regard to fluoride uptake (Graph 2), data collected from the first layer of enamel analyzed show that the 0.02% NaF solution was able to increase concentrations of F in enamel in comparison with the non-fluoride dentifrice (PD). However, the fluoride dentifrice (FD) was more effective than the other two treatments ( $p < 0.05$ ).

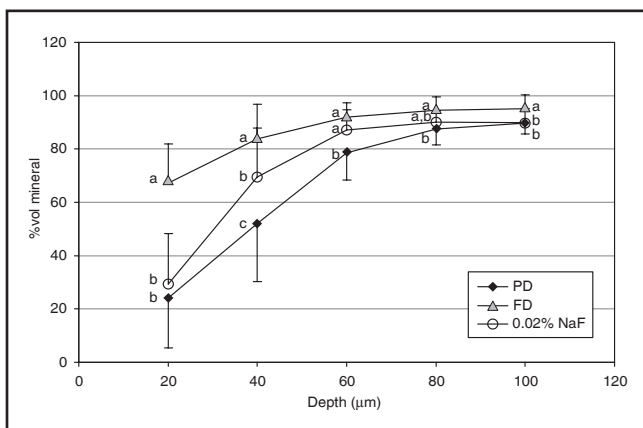
## DISCUSSION AND CONCLUSION

Some recommendations have been made to reduce the risks of dental fluorosis, considering that pre-school children involuntarily swallow a considerable amount of dentifrice during toothbrushing. However, the effect of these recommendations on the anticariogenic properties of fluoride has not been evaluated. In the present study we evaluated the *in vitro* use of 0.02% NaF solution, in terms of its effect on the anticariogenic potential of fluoride. In this *in vitro* study we tried to simulate clinical situations regarding toothbrushing by children and caries: (i) as substrate we used deciduous

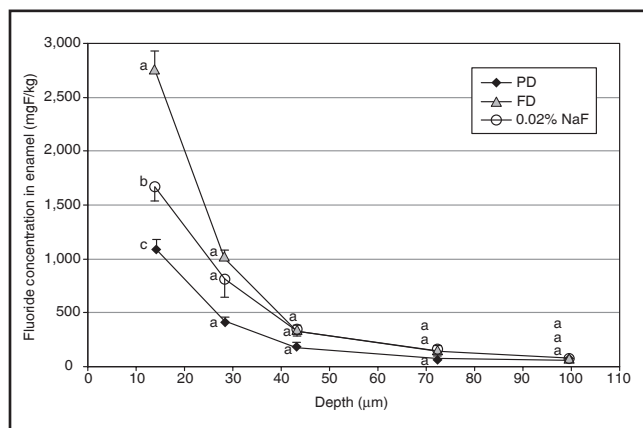
**TABLE 1** - Surface microhardness analysis of enamel blocks according to the treatments (means  $\pm$  SD; n = 15).

Treatments	SMH		%SMC
	Baseline	After treatment	
Placebo dentifrice	355.4 $\pm$ 17.7 <sup>A</sup>	64.8 $\pm$ 59.7 <sup>B</sup>	-82.0 $\pm$ 16.3 <sup>a</sup>
0.02% NaF	357.7 $\pm$ 13.2 <sup>A</sup>	63.2 $\pm$ 27.8 <sup>B</sup>	-82.4 $\pm$ 7.4 <sup>a</sup>
Fluoride dentifrice	368.9 $\pm$ 18.1 <sup>A</sup>	151.7 $\pm$ 50.2 <sup>B</sup>	-54.1 $\pm$ 13.2 <sup>b</sup>

Means followed by different letters are statistically significant ( $p < 0.05$ ). Capital letters show difference between baseline and after treatment, SMH for each treatment and lower case among the treatments. SMH = surface microhardness; SMC = surface microhardness change. SD = standard deviation.



**GRAPH 1** - Enamel volume mineral percentage according to the treatments and the distance (µm) from the surface. Different letters show significant difference ( $p < 0.05$ ) among treatments at each distance from the enamel surface; bars denote standard errors ( $n = 15$ ). PD: non-fluoride dentifrice; FD: fluoride dentifrice.



**GRAPH 2** - Fluoride concentration in enamel (mg/kg) according to the groups/treatments and the distance (µm) from the surface. Different letters show significant difference ( $p < 0.05$ ) among groups/treatments at each distance from the enamel surface; bars denote standard errors ( $n = 15$ ). PD: non-fluoride dentifrice; FD: fluoride dentifrice.

teeth, (ii) a way to use a small amount of dentifrice was idealized, (iii) a pH-cycling model was used to mimic caries lesion development.

The results clearly showed that the use of a small amount of fluoride dentifrice was as effective in reducing enamel demineralization (Table 1 and Graph 1) as in forming fluoride in enamel (Graph 2). However, the findings with the use of 0.02% NaF solution were not conclusive because it did not show better effects than the negative control used (non-fluoride dentifrice) for all the evaluations made.

The positive effect of fluoride dentifrice and the lack of effect of 0.02% NaF solution are comprehensible because the concentration of fluoride in dentifrice (1,100 µg F/g) is 12 times higher than that in the 0.02% NaF solution (90 µg F/ml). It is recognized that loosely-bound fluoride, like  $\text{CaF}_2$ , is the product formed in the enamel, which is responsible for the anticariogenic effect of topical fluoride<sup>22</sup>. Considering that the formation of a  $\text{CaF}_2$ -like product is dependent on the concentration of fluoride applied to the enamel<sup>20</sup>, the result found is not surprising. However, some effects of the 0.02% NaF solution were expected, because 90 µg F/ml of fluoride is reacting on the enamel surface twice a day. We did not find any effect of this fluoride solution in reducing enamel surface demineralization (Table 1), and a very small effect was observed in the reduction of mineral loss in the caries lesion (Graph 1) and in fluoride uptake (Graph 2). These findings suggest that 0.02% NaF

solution applied with a cotton swab in enamel has doubtful anticariogenic potential. Thus, this alternative to fluoride dentifrice can be safer with regard to dental fluorosis risk, but it must not be chosen, especially for a child with caries risk or activity. The use of a small amount of dentifrice would be a better solution considering the benefits and risks of fluoride use<sup>17</sup>.

With regard to the effect of fluoride dentifrice, during toothbrushing we did not simulate the dilution of the dentifrice by saliva, which is approximately 3-4 times. We tried to simulate the dilution after toothbrushing, by immersing the enamel blocks in artificial saliva for 30 min. The dilution of the dentifrice by saliva is important, as shown by DenBesten, Ko<sup>4</sup> (1996). The authors suggest that the reduction in the amount of fluoride toothpaste to a pea-sized amount should be limited only to young children, who are at risk of ingesting toothpaste. However, these authors did the study with 4-5 year old children and the risk of dental fluorosis occurs at an earlier age.

In conclusion, the results of this laboratory study suggest that the use of 0.02% NaF solution in a clinical basis has very little anticariogenic potential, and the recommendation to use it instead of fluoride dentifrice should be reevaluated.

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