

# In vitro evaluation of professional fluoride therapies for improving softening resistance against dental erosion

*Avaliação in vitro de terapias de aplicação profissional de flúor para aumento da resistência à desmineralização erosiva*

Heitor Marques Honório<sup>1</sup>  
Daniela Rios<sup>2</sup>  
Ana Carolina Magalhães<sup>3</sup>  
Fernanda Herrera Stancari<sup>4</sup>  
Marília Afonso Rabelo Buzalaf<sup>5</sup>

1 - PhD, Assistant Professor, Department of Pediatric Dentistry, Alfenas Federal University.

2 - PhD; Assistant Professor, Department of Pediatric Dentistry, Bauru School of Dentistry, University of São Paulo

3 - PhD; Assistant Professor, Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo

4 - Under Graduate Student, Bauru School of Dentistry, University of São Paulo

5 - PhD, Full Professor; Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo;

Correspondence:

Heitor Marques Honório  
Rua Gabriel Monteiro da Silva, 714;  
Alfenas/MG 37130-000, Brazil  
Telephone: 55 35 32991424  
e-mail: heitorhonorio@yahoo.com.br

## ABSTRACT

This *in vitro* study evaluated the effect of professional fluoride therapies against enamel erosion. Ninety bovine enamel blocks were randomly allocated to each group: GI-Duraphat® (NaF, 2.26%F), GII-Duofluorid® (NaF, 2.71% F), GIII-TiF<sub>4</sub> varnish (2.45% F), GIV-TiF<sub>4</sub> solution (2.45% F), GV-no fluoride varnish and GVI-water (control). The varnishes, the solution and water were applied onto the enamel. The blocks were subjected to 6 sequential erosive cycles (cola drink for 10 min and artificial saliva for 50 min, each) per day, during 4 days. After the erosive cycles, the blocks were maintained in artificial saliva for 18 h. Enamel alterations were determined in the 2<sup>nd</sup> and 4<sup>th</sup> days, by microhardness test (%SMHC). Data were tested using ANOVA and Tukey's tests ( $p < 0.05$ ). The mean %SMHC ( $\pm$ SD) in the 2<sup>nd</sup> and 4<sup>th</sup> days was, respectively: GI (-78.13 $\pm$ 4.08<sup>a</sup> and -90.01 $\pm$ 2.83<sup>A</sup>), GII (-77.70 $\pm$ 5.26<sup>a</sup> and -89.63 $\pm$ 6.08<sup>A</sup>), GIII (-88.93 $\pm$ 2.65<sup>b</sup> and -91.90 $\pm$ 2.4<sup>A,B</sup>), GIV (-92.23 $\pm$ 2.21<sup>b</sup> and -97.16 $\pm$ 1.43<sup>C</sup>), GV (-87.72 $\pm$ 2.04<sup>b</sup> and -94.16 $\pm$ 1.14<sup>B,C</sup>) and GVI (-88.10 $\pm$ 1.51<sup>b</sup> and -93.12 $\pm$ 1.93<sup>B</sup>). The data from this *in vitro* study show that NaF varnishes were able to partially prevent softening from dental erosion until the 4<sup>th</sup> day of erosive cycle.

**Key words:** Erosion; Prevention; Dental enamel; Fluoride; Microhardness

## RESUMO

O presente estudo *in vitro* avaliou o efeito de terapias de aplicação profissional de flúor para aumento da resistência à desmineralização erosiva. Noventa blocos de esmalte bovino foram aleatoriamente alocados nos seguintes grupos: GI-Duraphat® (NaF, 2.26%F), GII-Duofluorid® (NaF, 2.71% F), GIII-verniz de TiF<sub>4</sub> (2.45% F), GIV-solução de TiF<sub>4</sub> (2.45% F), GV-verniz sem flúor e GVI-água (controle). Os vernizes, a solução e a água foram aplicados sobre o esmalte dentário. Os blocos foram submetidos a 6 ciclos seqüenciais de erosão (bebida a base de cola por 10min e saliva artificial por 50min, cada) por dia, durante 4 dias. Após as ciclagens erosiva os blocos foram mantidos em saliva artificial por 18h. As alterações do esmalte foram avaliadas no 2º e 4º dias, por testes de microdureza superficial (%PDS). Os dados foram analisados por ANOVA e teste de Tukey ( $p < 0.05$ ). A média da %PDS ( $\pm$ DP) no 2º e 4º dias foi respectivamente: GI (-78,13 $\pm$ 4,08<sup>a</sup> e -90,01 $\pm$ 2,83<sup>A</sup>), GII (-77,70 $\pm$ 5,26<sup>a</sup> e -89,63 $\pm$ 6,08<sup>A</sup>), GIII (-88,93 $\pm$ 2,65<sup>b</sup> e -91,90 $\pm$ 2,4<sup>A,B</sup>), GIV (-92,23 $\pm$ 2,21<sup>b</sup> e -97,16 $\pm$ 1,43<sup>C</sup>), GV (-87,72 $\pm$ 2,04<sup>b</sup> e -94,16 $\pm$ 1,14<sup>B,C</sup>) e GVI (-88,10 $\pm$ 1,51<sup>b</sup> e -93,12 $\pm$ 1,93<sup>B</sup>). Os resultados deste estudo mostram que o verniz de NaF foi capaz de prevenir parcialmente o amolecimento do esmalte pela erosão até o 4º dia de ciclagem erosiva.

**Palavras-chave:** Erosão; Prevenção; Esmalte dentário; Flúor; Microdureza

## INTRODUCTION

Dental erosion is a well recognized dental problem that is apparently increasing among the younger population in the last decades<sup>1</sup>. Erosion involves a chemical removal of superficial hard tissue from the tooth surface by acids from soft drinks and

fruit juices or from eating disorders and gastric reflux<sup>2</sup>.

The best solution for erosion is its primary prevention by the treatment and elimination of the cause before the lesions on teeth occur<sup>3</sup>. However, until the cause is not eliminated, several preventive measures have been proposed, including topical

fluoride applications<sup>3-6</sup>. Although the preventive action of fluoride on dental caries is well known<sup>7</sup>, its role in erosion is still controversial<sup>4</sup>, since the deposited calcium fluoride-like material from topical fluoride application is supposed to dissolve readily in most acidic drinks<sup>4</sup>. However, high-concentrated fluoride applications, like oral rinses, acidic gels or varnishes, have been demonstrated, in some cases, to decrease the development of erosion in enamel<sup>5,6,8,9</sup>. More acidic and concentrated fluoride preparations, which form thicker calcium fluoride-like layer, might offer additional minerals to be dissolved during the erosive challenge, before the subjacent enamel is attacked.

Titanium tetrafluoride (TiF<sub>4</sub>) solution have also been investigated as a concentrated fluoride product for erosion prevention<sup>10-13</sup>. The literature describes that after TiF<sub>4</sub> application there is a glaze like layer formation that could have an inhibitory effect on erosion<sup>10,12</sup>. On the other hand, other experiments did not demonstrate promising results in increasing enamel resistance and decreasing the rate of dental erosion development<sup>13,14</sup>. Similarly to the solution, the titanium tetrafluoride varnish also showed controversial results, with limited<sup>15</sup> and significant effect against erosion<sup>16</sup>.

In view of the above considerations, the purpose of this *in vitro* study was to test the capacity of different concentrated professional fluoride therapies in improving softening resistance against enamel erosion.

## METHODS

### Experimental design

Ninety enamel blocks were obtained from bovine teeth, polished and subjected to initial surface microhardness analysis, for selection. Enamel with microhardness ranging from 332 to 377 KHN was randomly distributed into 6 groups: GI- Duraphat<sup>®</sup> (NaF, 2.26%F, pH 4.5; Colgate, São Bernardo, São Paulo, Brazil, n=15), GII- Duofluorid<sup>®</sup> (2.71%F as NaF, 2.92%F as CaF<sub>2</sub>, pH 8.0; FGM, Joinville, Santa Catarina, Brasil, n=15), GIII- TiF<sub>4</sub> varnish (2.45% F, pH 1.0; FGM, Joinville, Santa Catarina, Brasil, n=15), GIV- TiF<sub>4</sub> solution (2.45% F, pH 1.0), GV- no-fluoride varnish (FGM, Joinville, Santa Catarina, Brasil, n=15) and GVI- water (control). The varnishes used in GIII and GV had a composition identical to

GII, except for the presence of TiF<sub>4</sub> instead of NaF and the absence of fluoride, respectively. The varnishes and the solutions were applied onto the enamel surfaces. Thus, the blocks were subjected to 6 erosive cycles per day, in an oven at 25° C, during 4 days. In each cycle, demineralization and remineralization were performed by immersion in cola drink (10 min) and artificial saliva (50 min), respectively. Each day, the 6 cycles were conducted sequentially (totalizing 6 h) and the blocks were then immersed in artificial saliva for 18 h. Enamel softening was determined in the 2<sup>nd</sup> and 4<sup>th</sup> days, using microhardness tests (%SMHC).

### Enamel blocks preparation

Enamel blocks (4X4X2.5mm) were prepared from incisor bovine teeth, freshly extracted, sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. The enamel surface of the blocks was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al<sub>2</sub>O<sub>3</sub> papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 µm; Buehler), resulting in removal of about 100 µm depth of the enamel which was controlled with a micrometer. The surface microhardness determination was performed by five indentations (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). The enamel blocks with microhardness ranging from 332 to 377 KHN were randomly distributed into 6 groups.

### Treatment and erosive cycling

A thin layer of the varnishes was applied with microbrush on enamel surface. After 6 hours, the varnishes were removed carefully using a surgical blade. Removal was completed with cotton swabs with soaked in acetone. For preparing the 4% (2.45% F) TiF<sub>4</sub> solution, solid TiF<sub>4</sub> (Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in deionized water. The pH of solution was 1.2. The application was made in drops with cotton roll, during 1 min. The drop was left undisturbed until the surface appeared dry. Additional drops were applied in the same manner until 1 min had elapsed. The water was applied in the same manner.

The blocks were subjected to a erosive cycling model, during 4 days. The 6 erosive cyclings were performed by day. In separate

containers, the blocks were immersed in cola drink (Spal, Porto Real, RJ, Brazil) at room temperature, for 10 min (30 mL per block) and in artificial saliva [ $1.5 \text{ mmolL}^{-1} \text{ Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $0.9 \text{ mmolL}^{-1} \text{ NaH}_2\text{PO}_4 \cdot 2 \text{ H}_2\text{O}$ ,  $150 \text{ mmolL}^{-1} \text{ KCl}$ ,  $0.1 \text{ molL}^{-1} \text{ Tris}$  buffer,  $0.03 \text{ ppm F}$ , pH 7.0] for 50 min (15 mL per block).<sup>17</sup> Each day, the 6 cycles were conducted sequentially (totalizing 6 h) and the blocks were then maintained in artificial saliva for 18 h.

#### Microhardness determination

At 2<sup>nd</sup> and 4<sup>th</sup> days, the final microhardness test (SMH1) was made as described earlier (Knoop diamond, 25 g, 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Five indentations, at distances of 200  $\mu\text{m}$  from each other, were made in the center of enamel blocks (SMH). The percentage of surface microhardness change was calculated for both days:  $100(\text{SMH}_1 - \text{SMH})/\text{SMH}$ .

#### Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked. Since the assumptions were satisfied, ANOVA and Tukey's test were carried out for statistical comparisons and the significance limit was set at 5%.

## RESULTS

The data showed that at the 2<sup>nd</sup> experimental day, GI (Duraphat) and GII (Duofluorid) significantly reduced softening when compared to the other groups ( $p < 0.001$ ). These, did not differ significantly from each other (Table 1).

At the 4<sup>th</sup> experimental day, the softening increased for all the groups. GI (Duraphat) and GII (Duofluorid) significantly reduced softening when compared to GIV (TiF<sub>4</sub> solution), GV (no F varnish) and GVI (control water) ( $p < 0.001$ ). The 4% titanium tetrafluoride varnish (GIII) showed an effect similar to the other commercial fluoride varnishes (GI and GII). However, this varnish (GIII) also showed no significant difference when compared to the no-fluoride varnish (GV) and the control water (GVI) (Table 1).

## DISCUSSION

This *in vitro* experimental study included an intensive erosive attack to simulate what occurs in the clinical situation with patients that show eating disorders, gastric reflux or excessive acid diet. In these cases the high-concentrated fluoride applications have been demonstrated to decrease the development of erosion in enamel<sup>5,6</sup>. However these applications must be done by the professional and are not suitable for daily home usage. Considering these aspects, only one application of the test products was employed along with a high erosive challenge.

Table 1 – Mean and standard deviation of %SMHC for each experimental group.

Day	%SMHC mean* $\pm$ SD						
	GI	GII	GIII	GIV	GV	GVI	
2 <sup>nd</sup>	-78.13 <sup>a</sup> $\pm$ 4.08	-77.70 <sup>a</sup> $\pm$ 5.26	-88.93 <sup>b</sup> $\pm$ 2.65	- 92.23 <sup>b</sup> $\pm$ 2.21	-87.72 <sup>b</sup> $\pm$ 2.04	-88.10 <sup>b</sup> $\pm$ 1.51	
4 <sup>th</sup>	-90.01 <sup>a</sup> $\pm$ 2.83	-89.63 <sup>a</sup> $\pm$ 6.08	-91.90 <sup>a,b</sup> $\pm$ 2.4	-97.16 <sup>c</sup> $\pm$ 1.43	-94.16 <sup>b,c</sup> $\pm$ 1.14	-93.12 <sup>b</sup> $\pm$ 1.93	

\*Values in the same line followed by distinct superscripts indicate statistical significance ( $p < 0.05$ )  
GI-Duraphat®, GII-Duofluorid®, GIII-TiF<sub>4</sub> varnish, GIV-TiF<sub>4</sub> solution, GV-no F varnish, GVI-water

The single fluoride application and the high erosive challenge could explain the limited softening prevention shown by GI and GII when compared to other studies in the literature, which tested the effect of commercial fluoride varnishes and found a better reduction of erosion *in vitro*<sup>8,9,13,18</sup>. Another important aspect to consider is the removal of the varnish, which was done six

hours after the pH cycling had been initiated, in order to mimic the clinical situation when there is a natural removal of the varnish by tooth brushing, mastication and tongue friction<sup>15,16,18</sup>. Vieira et al.<sup>14</sup> found that the mechanical barrier formed by the varnish played a fundamental role in the protection mechanism against erosion of the product, but in this case a less pronounced erosive attack was accomplished (three 10

min demineralization cycles followed by 2 h of remineralization) and during the experiment the varnish remained on the blocks. Opposite results were found in the present study, because the mechanical protection conferred by the varnish was not present. This rendered GV not different from the control water (GVI) on the 2<sup>nd</sup> and 4<sup>th</sup> days.

According to the literature, the beneficial action of TiF<sub>4</sub> solution on dental erosion has been attributed to its low pH (around 1.2), favoring the linking between titanium and oxygen of the group phosphate, thus leading to the formation of a titanium dioxide glaze-like layer on the surface<sup>10,12</sup>. In the present study, the formation of this layer was not able to prevent softening of enamel surface. This result is in agreement with Vieira *et al.*<sup>14</sup> that showed *in vitro* that the 4% TiF<sub>4</sub> solution did not have a significant protective effect against erosion.

The varnish allows a higher contact time of fluoride with enamel, thus increasing the formation of calcium fluoride-like deposits.<sup>19</sup> It was expected that the incorporation of TiF<sub>4</sub> into the varnish would allow the formation of a more resistant compound which in turn would enhance the prevention of erosion, but this was not the case. In fact, both forms of use of TiF<sub>4</sub> did not show a significant protective effect against erosion, which is in agreement to two other studies<sup>13,14,15</sup>. However, other studies evaluating the application of a TiF<sub>4</sub> solution or varnish showed better results<sup>10,16,20,21</sup>, but the methodology was different, with multiple applications or with mild erosive conditions.

The extrapolation of our data to the *in vivo* condition must be done carefully. For convenience, bovine enamel was used. Although bovine enamel has been widely used in dental research as a substitute for human enamel, morphological differences such as higher porosity exist when compared to human enamel, which result in higher rates of erosion formation<sup>22</sup>. An enhancement of erosion formation rate may also have been expected because polished surfaces were used and they are more susceptible to acid challenges than natural surfaces.<sup>22</sup> In addition, the salivary protection cannot be completely reproduced in the *in vitro* condition<sup>23</sup>. Furthermore, it must not be forgotten that in the mouth mechanical factors such as abrasion and attrition may act synergistically with erosion.

## CONCLUSION

In conclusion, using this *in vitro* protocol with a high erosive challenge, the NaF varnishes were able to partially prevent softening from dental erosion until the 4<sup>th</sup> day of erosive cycle.

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