

IN VITRO ASSESSMENT OF AN EXPERIMENTAL COAT APPLIED OVER FLUORIDE VARNISHES

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ABSTRACT

Objective: The time of contact between the product and enamel surface is important in ensuring the efficacy of fluoride varnishes. Thus, some alternatives could avoid fluoride loss to saliva and improve the anticariogenic action of the product. This study evaluated the effect of an experimental coat on the anticariogenic action of fluoride varnishes. Material and Methods: Enamel bovine blocks were selected by evaluating surface microhardness and randomized into five groups (n=24): placebo, Duraphat™, Duraphat™ with coat, Duofluorid™ and Duofluorid™ with coat. Twelve blocks from each group were used to analyze calcium fluoride (CaF₂) formed on enamel after treatment. The other 12 blocks were subjected to pH cycling for 7 days. The varnishes were kept on enamel for 6 h. Next, the percentage change of surface microhardness (%SMHC) and mineral loss (ΔZ) were calculated. CaF₂ retained and fluoride present in the pH-cycled solutions were also measured. Results: The use of the coat did not decrease %SMHC and ΔZ, but all fluoride varnishes had better results when compared to the placebo (ANOVA and Kruskal-Wallis, respectively). The values from CaF₂ formed were higher compared to the values of CaF₂ retained (non-paired t test, p<0.05). There was a trend to decrease the amount of F in the solutions at the end of pH cycling (Kruskal-Wallis, p<0.05). Conclusions: Although the experimental coat increased the formation of CaF₂ on the enamel surface, it did not significantly improve the anticariogenic action of fluoride varnishes.

Key words: Dental caries, prevention & control. Topical fluorides.

INTRODUCTION

The effectiveness of fluoride (F) varnishes has been well established in caries prevention studies involving permanent teeth¹. Clinical studies have demonstrated a decline of caries incidence from 18 up to 70%¹⁵. F varnishes are easy to apply, safe, and well accepted by patients³. Therefore, the use of F varnishes has been recommended for children at high or moderate risk to develop dental caries²¹. Moreover, it is the only product recommended for children up to 6 years of age who have special health care needs¹⁷.

Fluoride varnishes modify the physiochemical properties of teeth, enhancing remineralization and inhibiting demineralization¹⁰. Recently, the decrease in *S. mutans* counts after F varnish application has also been reported¹². Fluoride varnishes are viscous products that harden in contact to saliva. They adhere to the teeth and enhance F release to the enamel surface, dental plaque and saliva. This adhesion also enables the application in small amounts, decreasing the risk of F over-ingestion²³.

A previous work from our research group showed

differences on anticariogenic action and calcium fluoride (CaF₂) formation in seven marketed varnishes available in Brazil as a result of variations on physical properties of F varnishes¹⁴.

Physical properties can influence the activity of F varnishes on enamel surface. Considering that the contact time between the fluoride product and the enamel surface is very important to the efficacy of this preventive measure²³, the use of some materials could avoid F loss to saliva and, consequently, improve its anticariogenic effect. The aim of this study was to evaluate the effect of an experimental coat on the anticariogenic action of fluoride varnishes.

MATERIALS AND METHODS

Experimental Groups

Two market varnishes were used: Duraphat™ (“Dura”; 5% NaF, A. Natterman & Cie. GmbH., Colony, Germany) and Duofluorid XII™ (“Duo”; 6% NaF and 6% CaF₂, Dentscare Ltda, Joinville, SC, Brazil). The experimental coat

was supplied by FGM Dental Products. The coat has the same composition as that of Duofluorid XII™ (Dentscare Ltda, Joinville, SC, Brazil) but does not contain fluoride. The coat was applied over the fluoride varnishes, as a second layer, and was also used in the placebo group (negative control).

pH-cycling and Treatment with F varnishes

Enamel blocks (4x4 mm) were obtained from bovine incisors previously stored in a 2% formaldehyde solution at pH 7.0 for 30 days at room temperature²⁴. The enamel surfaces were serially polished and the blocks were selected based on the surface microhardness (SMH) (324.8 up to 357.0 KHN) and were randomized into five groups (n=12): a) coat only ("placebo"; varnish without fluoride), b) Dura, c) Dura with the experimental coat (Dura + coat), d) Duo and e) Duo with the experimental coat (Duo + coat).

The blocks were subjected to seven days of a pH-cycling model based on Vieira, et al.²⁴. Initially, the enamel was treated with the varnishes and coat, if applicable, and placed in a demineralizing solution ("DE": 2.0 mmol L⁻¹ calcium and phosphate in 75 mmol L⁻¹ acetate buffer, pH 4.7; 0.04 µg F/mL, 2.2 mL/mm²). After 6 h, the varnishes were removed with a blade and acetone⁵ and the blocks were transferred to a remineralizing solution ("RE": 1.5 mmol L⁻¹ calcium, 0.9 mmol L⁻¹ phosphate, 150 mmol L⁻¹ KCl in 0.1 mol L⁻¹ cacodylic buffer, pH 7.0; 0.05 mg F/mL, 1.1 mL/mm²) for 18 h. On the second day, both solutions were changed due to F release from the varnishes. These solutions were kept until the 5th day. During the 6th and the 7th day, the enamel blocks were kept in the remineralizing solution.

After pH cycling, aliquots of the solutions were mixed with TISAB II (total ionic strength adjustment buffer) at 1:1 TISAB II:sample ratio (pH 5.2) and the amount of F in the solutions was analyzed, using an ion-selective electrode (Orion 96-09; Orion Research Inc., Beverly, MA, USA) and a digital ion analyzer (Orion 720A; Orion Research Inc.),

Microhardness Analysis

Surface (SMH) and cross-sectional (CSMH) microhardness measurements were made on enamel surface as described before²⁴, except for the fact that the load used was 25 g. The %SMHC was calculated. Next, the blocks were bisected and CSMH was performed. The CSMH values were converted to mineral content by volume, according to Featherstone, et al.⁹. The integrated mineral loss (ΔZ) after pH cycling was calculated^{1,18}.

Calcium Fluoride (CaF₂) Analysis

The loosely-bound fluoride (CaF₂) was analyzed to evaluate the amount of CaF₂ present after fluoride varnish application (CaF₂ formed) or to analyze the amount of CaF₂ that was still adsorbed to the enamel after pH-cycling (CaF₂ retained). In order to assess CaF₂ formed, 12 blocks from each group were treated as described above, but were subjected to only one 24 h cycle. The analysis of CaF₂ retained was done in the pH-cycled blocks. The enamel blocks were immersed in 0.5 mL KOH 1mol L⁻¹ for 24 h

under agitation. Next, the same volume of TISAB II with HCl (8.2 mL/L) was added⁶. The final pH of the solutions was 5.2.

Fluoride measurement was performed with an ion-selective electrode Orion 96-09 (Orion Research Inc.) and an ion analyzer Orion 720 A+ (Orion Research Inc.), calibrated with standards containing 0.0625 up to 2.0 µg F/mL. The results were expressed as µg F/cm².

Statistical Analysis

The null hypothesis tested was that the use of the experimental coat does not improve the anticariogenic action of fluoride varnishes. GMC software was used for statistical analysis. First, normality and homogeneity of data was determined. Data from ΔZ , CaF₂ formed, CaF₂ retained and F present in solutions were not normal and had a non-homogeneous distribution. Thus, Kruskal-Wallis test was used at each data point. For %SMHC, each data point was tested with one-way ANOVA. The difference between CaF₂ formed and CaF₂ retained was evaluated with the non-paired *t* test. The significance level was set at 5% for all analyses.

RESULTS

Table 1 shows that the application of the coat was not able to improve either %SMHC or ΔZ . Moreover, when comparing the varnishes, there were no differences on surface demineralization (%SMHC) but the lesion depth (ΔZ) for the groups treated with Duo was shallower when compared to the groups treated with Dura (Table 1).

In addition, the presence of the coat allowed greater formation of CaF₂-like material for Duo+coat, but not for Dura+coat, when compared to the respective commercial varnishes without the coat. Greater retention of CaF₂ was observed for both varnishes associated with the coat. For all treatment groups, except for placebo, there were significantly lower CaF₂ retained than CaF₂ formed (Table 1).

F release to the solutions DE1, RE1 and DE2 was different in all treatment groups. Furthermore, the groups treated with Duo showed higher amount of F in the pH-cycling solutions. The presence of the coat decreased F release in DE1. On the other hand, after the coat was removed, the groups Duo+coat and Dura+coat presented the highest amount of F in the solutions RE1 and DE2. In the last 2 days of pH-cycling, the groups Duo and Duo+coat showed higher amount of F in RE2 when compared to Dura or Dura+coat, regardless of whether the coat was applied or not (Table 2).

DISCUSSION

Calcium fluoride is the main product formed after the application of a topical fluoride, especially with highly concentrated F products. The amount of CaF₂ formed depends on the pH, the duration of exposure and the F

TABLE 1- Results of percentage change of surface microhardness (%SMHC), mineral loss (ΔZ), CaF_2 formed and CaF_2 retained ($\mu g F/cm^2$). Mean \pm standard error, n = 12

Group	%SMHC* [†]	ΔZ * [§]	CaF_2 formed* ^{†,§}	CaF_2 retained* ^{†,§}
Placebo	-74.6 \pm 9.6 ^a	1535.6 \pm 210.6 ^a	^A 0.3 \pm 0.0 ^a	^B 0.6 \pm 0.0 ^a
Dura	-36.1 \pm 4.8 ^b	887.0 \pm 660.8 ^b	^A 2.9 \pm 0.2 ^b	^B 0.9 \pm 0.1 ^b
Dura+coat	-35.6 \pm 6.3 ^b	1007.8 \pm 540.7 ^b	^A 3.4 \pm 0.3 ^b	^B 1.2 \pm 0.1 ^c
Duo	-36.3 \pm 3.7 ^b	470.0 \pm 189.8 ^c	^A 11.8 \pm 1.0 ^c	^B 2.4 \pm 0.3 ^d
Duo+coat	-37.5 \pm 7.4 ^b	377.9 \pm 160.6 ^c	^A 26.1 \pm 1.5 ^d	^B 4.4 \pm 0.5 ^e

*Different lowercase letters indicate statistically significant differences among the groups. † Different uppercase letters indicate statistically significant differences between CaF_2 formed or CaF_2 retained (non paired *t* test, p<0.05). ‡ ANOVA, p<0.05. § Kruskal-Wallis, p<0.05.

TABLE 2- Amount of F (μg) detected in the solutions after pH cycling (mean \pm standard error)

Group	DE1	RE1	DE2	RE2
Placebo	1.51 \pm 0.03 ^a	1.05 \pm 0.01 ^a	1.37 \pm 0.01 ^a	0.61 \pm 0.01 ^a
Dura	5.69 \pm 0.24 ^b	2.16 \pm 0.04 ^b	2.26 \pm 0.03 ^b	0.69 \pm 0.01 ^b
Dura+coat	3.41 \pm 0.05 ^c	2.47 \pm 0.08 ^c	2.48 \pm 0.03 ^c	0.72 \pm 0.02 ^b
Duo	56.42 \pm 1.66 ^d	3.96 \pm 0.17 ^d	3.29 \pm 0.17 ^d	0.93 \pm 0.01 ^c
Duo+coat	17.93 \pm 2.10 ^e	6.70 \pm 0.04 ^e	4.26 \pm 0.21 ^e	1.01 \pm 0.03 ^c

Means followed by different letters indicate statistically significant difference between the groups (Kruskal-Wallis, p<0.05).

concentration in the products¹⁹, and it is very important for the efficacy of fluoride varnishes.

The fact that Duofluorid XII™ has twice the F concentration of Duraphat™ certainly contributed to the highest amounts of CaF_2 -like material observed in Duo and Duo+coat groups, which is probably originated from the pure CaF_2 present in Duofluorid XII™. This may also explain the shallower the lesion depth (ΔZ) and the higher amount of F in all solutions analyzed for the groups Duo and Duo+coat when compared to Dura and Dura + coat. Another reason for these results is the fact that the synthetic resin base of Duofluorid XII™ makes the varnish less viscous and dense, which leads to a higher ability to release F in their matrixes^{7,20}.

Analyzing the F released in the solutions and the CaF_2 in enamel, an overall F kinetics during the pH cycling is observed. The F that was not released to the solutions during the first 6 h of pH-cycling (when the coat was present) contributed to the formation of CaF_2 .

When the varnishes were removed the groups that received the coat treatment showed a higher amount of F released in “RE 1” because more CaF_2 was formed on the enamel. With this observation, it may be assumed that CaF_2 was readily loss to the medium as soon as the coat was removed in spite of the higher amount of CaF_2 formed in the presence of the coat. This event may have contributed to the lack of efficacy of the coat regarding %SMHC and ΔZ . This observation is also confirmed by the high release

of F for the coat groups in “DE 2”. Still, the coat did not influence the amount of F released in the last two days of pH-cycling (“RE2”).

The loss of CaF_2 after treatment is in agreement with other studies. Attin, et al.² found that F is leached away within the experimental period after a topical application of Bifluorid 12™ (Voco GmbH, Cuxhaven, Germany) or Duraphat™. Another interesting finding of the present study was that CaF_2 retained was higher than CaF_2 formed only in the placebo groups. This can be attributed to the pH cycling model used in the present study, which had a high acid challenge. The dental enamel becomes more reactive as enamel demineralization increases, which could have facilitated the deposition of F from the solutions, even if it was present at low concentrations.

Moreover, the presence of the coat was not able to improve either %SMHC or ΔZ possibly because the amount of CaF_2 was not high enough to induce a sensible change in enamel demineralization. It is probably necessary very high differences of CaF_2 , such as that observed between Duraphat and Duofluorid to detect changes in lesion depth or even higher amounts to show changes in surface microhardness.

F varnish can be clinically applied to prevent proximal caries²², enamel demineralization during orthodontic treatment⁸, early childhood caries¹³. Prevention of proximal caries by F varnishes showed better results compared to fluoride mouthrinsing and the cost-effectiveness ratio observed was 1.8²². Due to the wide variety of clinical

applications and the effectiveness of F varnishes, new products are constantly developed. Recently, two new F varnishes, including a bioerodible material, has been evaluated^{4,16} and showed good results.

CONCLUSION

It may be concluded that the application of the experimental coat reduced the amount of F released and increased the formation of CaF₂, but it was not able to enhance the anticariogenic action of the F varnishes.

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