

EFFECT OF ACIDULATED PHOSPHATE FLUORIDE – GEL AND FOAM – ON ENAMEL CARIES-LIKE LESION OF PRIMARY TEETH: AN IN VITRO STUDY

ABSTRACT

AIM: The purpose of this study was to evaluate in vitro the effect of APF, gel and foam, for 1 to 4 minutes, on artificial enamel caries-like lesion of primary teeth submitted to the pH cycling. **MATERIALS AND METHODS:** The specimens with medium values of initial superficial enamel micro-hardness between 272 and 331KHN were selected for the formation of the artificial caries lesion. Later, they were randomly divided into 6 groups (n=15): no pH cycling control, cycled control, gel 1min, gel 4min, foam 1min and foam 4min. The no pH cycling control group was maintained in an humid environment, while the application of APF and the pH cycling were accomplished. After, the specimens were sectioned at the center for the enamel cross-sectional micro-hardness test. The values of micro-hardness of the depths from 20 to 180 μ m were converted into percentile of mineral volume (%Vol) and the area of mineral recovery (Δ ZR) was calculated. **RESULTS:** In spite of there is no statistically significant differences, it was observed through the analysis of the Δ ZR a difference in the values for the groups gel 1min and foam 4min (p>0.05). The evaluation of %Vol indicated a significant difference only at 60 μ m depth (p=0.005). **CONCLUSIONS:** This study concluded that a single application of APF gel or foam, for 1 or 4 minutes, do not remineralize artificial enamel caries-like lesion of primary teeth.

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KEYWORDS

Dental enamel. Deciduous tooth. Dental caries.
Acidulated phosphate fluoride. Hardness.

INTRODUCTION

The action of fluoride in the prevention and therapeutics during the process of development of the caries lesion has been proven as a result of the use of fluoridated products in their several forms of presentation, different concentrations and frequency of application, promoting its constantly presence in mouth.¹⁻³

The use of fluoride in low concentration and high frequency, through the use of toothpastes and drinking water, has been considered as the main reason for the decline of caries disease in the world.³ On the other hand, the prevalence of that disease is still considered high in some individuals or groups.² In specific cases, individual factors should be considered, such as the risk or caries activity, where the topical application of fluoridated products with high concentration such as gel, foam and varnish is indicated,⁴⁻⁶ which promotes an increase in the formation of calcium fluoride, a compound that acts as a reservoir of fluoride available to act directly on the dynamics of the process of de- and remineralization, thus interfering with the progression of the caries lesion.⁷

However, there is a concern about the use of acidulated phosphate fluoride (APF) in the gel form, because of the risk of intoxication due to the high fluoride retention in the oral cavity after its use, as well as the intake of a great amount of it during its application, which

may incite gastric irritations, nauseas and vomiting.⁸⁻¹⁰

APF foam is an alternative with a more attractive consistence for children.¹¹ Besides, it provides the same intake of fluoride as the gel,⁸⁻¹¹ and it is advantageous, since its application requires a smaller amount,⁸⁻⁹ considering that there is a reduction of 80% in the amount of product required for the total teeth coverage,¹⁰ and, yet, a smaller amount of fluoride is retained in the oral cavity after the application, which reduces the risk of ingestion, thus minimizing the risk of intoxication.¹⁰

The effectiveness of APF foam in reducing caries increment in primary and permanent teeth is showed in clinical studies.¹²⁻¹³ However, the time of application of APF is still under discussion as many studies have demonstrated no significant differences either in the inhibition¹⁴ or in the reduction of the caries lesion depth¹⁵ or else in the enamel fluoride uptake¹⁵⁻¹⁶ with the application of APF gel for 1 or 4 minutes. Delbem and Cury (2002)¹⁴ found that there is a larger enamel fluoride uptake after 4 minutes. Regarding the foam form, Brown et al.⁹ (1994) also observed a larger enamel fluoride uptake after a 4 minute application.

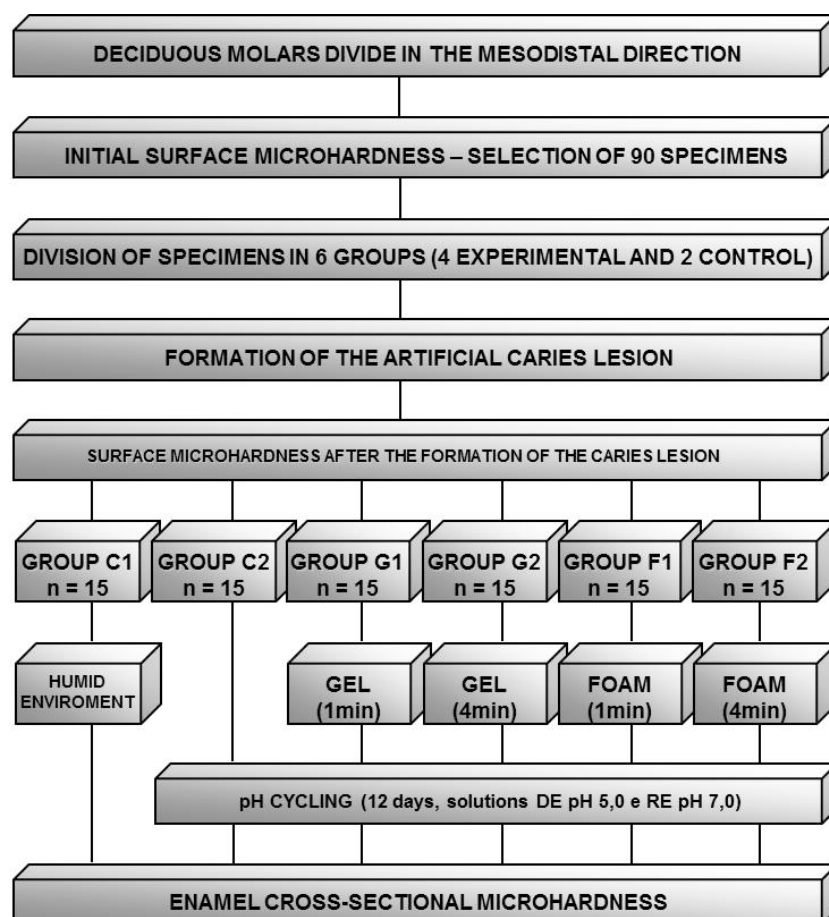
Thus the purpose of this in vitro study was to evaluate the isolated effect of APF gel and foam, applied for 1 and 4 minutes on artificial caries-like lesion in enamel of primary teeth submitted to the pH cycling, analyzing its

capacity of interfering on the mineral recovery, through the enamel cross-sectional microhardness.

MATERIAL AND METHODS

The experimental steps of this study are schematically shown in figure 1.

Figure 1 - Flowchart representing the experimental stages accomplished during the study: C1: Uncycled Control; C2: Cycled Control ; G1: Gel 1min; G2: Gel 4min; F1: Foam 1min and F2: Foam 4min.



Preparation of the specimens:

First and second sound primary molars stored in 0.1% thymol in 0.9% saline solution (pH=7.0) for no longer than 6 months at room temperature were utilized. This research was approved by the Ethical Committee for Research with Human Beings of the Federal University of Santa Catarina, Brazil (Process 281/04).

The teeth were cut mesio-distally utilizing an electric cutter (Isomet 1000; Buehler) and a

diamond disc (Buehler). The buccal and lingual surfaces were fastened to a double-faced ribbon (3M) and built-in in polyester resin, using PVC scouring pads as molds. The surface containing the specimens was smoothed on a polishing machine (Panambra DP-10; Struers) with water sandpaper with grits of 600 and 1200 (3M), for 10s each, at a speed of 300rpm running water. A metallic support was used to standardize the force exercised by the researcher at the moment of smoothing, so as to obtain a flat surface for carrying on the micro-

hardness test removing the minimum possible amount of enamel. In the sequence, the specimens were polished on the polisher with felt cloth (super texture; Arotec) and alumina pastes (1 μ , 0.3 μ and 0.05 μ grits; Arotec), at a speed of 600rpm. At each sandpaper and polishing pastes change, the specimens were washed with deionized water for 5min in ultrasound equipment (Ultrasonic Cleaner 1440D; Odontobrás) for removal of residues from the surface. After the use of the last polishing paste, the specimens were washed in ultrasound for 20min and stored in humid environment.

Determination of the initial surface micro-hardness (SMH):

The initial surface micro-hardness was verified with a Knoop diamond indenter, using a micro-hardness tester (HMV Micro Hardness Tester; Shimadzu), to select the specimens with values between 272 and 440KHN (Knoop Hardness Number), normal for the permanent dental enamel¹⁷.

Three indentations were accomplished with a load of 50g, for 5s, at intervals of 100 μ m. The average values of the surface micro-hardness of the selected specimens varied between 272 and 331KHN, presenting an average of 290.8KHN. The 90 specimens were randomly divided into 6 groups with 15 specimens each (four experimental and two control groups, with no statistical difference among them) ($p=0.3673$).

Formation of the artificial caries lesion:

The specimens were individually immersed in 14mL of lactic acid solution with formulation

similar as that described by White¹⁸ (1987), pH 5.0, modified by the use of Carbopol 980 (0.2%). The lactic acid was used, for being this the acid produced in larger amount by the bacterial plaque on initial carious lesion formation (90%). Carbopol, a polymer from acrylic acid was used to simulate the action of the acquire pellicle in the carious process in vivo, for having the thickening action giving to the solution a gel consistency.¹⁸ The specimens were then stored at 37°C for 12 hours, and the time of exposure was determined through a pilot study. After the exposure to the caries-inducing solution, three new indentations were made at 100 μ m below the previous ones with the same equipment, same configurations, and by the same tester. The average value of the surface micro-hardness after the formation of the lesion was 173.1KHN. The percentile of surface micro-hardness loss (%SMHL) regarding to initial micro-hardness was 40.5%.

Application of acidulated phosphate fluoride (APF):

The specimens of the groups G1 and G2 were submitted to the topical application of 1.23% APF gel at pH 3.6 – 3.9 (Flutop - SSWhite Dental Goods Ltd., Rio de Janeiro - Brazil), for 1 and 4 minutes, respectively (the time indicated by the manufacturer is 1 min). In the specimens of F1 and F2 groups, a 1.23% APF foam at pH 3.5 was applied, (Topical Fluoride Foam, Laclede, Inc. USA) for 1 and 4 minutes, respectively (the time indicated by the manufacturer is 4 minutes). The treatment was carried out using disposable applicators (KG Sorensen), and then the specimens were washed with deionized water for 10s and dried with absorbent paper.

pH Cycling:

The specimens of C2, G1, G2, F1, and F2 groups were submitted to the pH cycling method, while the specimens of C1 group were maintained in a moisture environment for subsequent analysis. In order to evaluate the enamel remineralization, pH cycling was carried out using the model proposed by White¹⁸ (1987).

Initially, the samples were immersed in artificial saliva (14mL for specimen) that was used as remineralizing solution (RE).⁷ Samples were then replaced twice a day to avoid alteration in the pH. Furthermore, to avoid a superficial erosion of the enamel, 0.05ppm F per liter was added to this solution.¹⁹ The specimens were immersed in a demineralizing solution for 2 hours (DE) to simulate the cariogenic challenge. The demineralizing solution was replaced on the sixth day of cycling (14mL per specimen with the same composition of the solution used for the formation of an artificial caries lesion). The specimens were washed with deionized water at each change of the solution. The duration of the continuous cycles of DE/RE was 12 days, and the specimens were stored at 37°C. As the pH cycling finished, the specimens were washed with deionized water for 10s, dried with absorbent paper, and stored in a humid environment.

Preparation of the specimens for determination of cross-sectional micro-hardness (CSMH):

After the pH cycling, the specimens were sectioned throughout the center by using an electric cutter and a diamond disposable, followed by smoothing with water sandpaper of 1200 grit, at a speed of 300rpm. Then, the specimens were

washed with deionized water by ultrasound for 5min, and polished in the same way as previously described. Thereafter, they were stored in a moisturized environment.

Determination of the enamel cross-sectional micro-hardness:

The specimens were positioned at the base of the micro-hardness meter, so that the larger diagonal of the indentation was positioned parallel to the external surface of the enamel. For that, 27 indentations were made applying a load of 25g for 5s, and organized into 3 columns in a 100µm distance among them. The indentations were started from 20µm from the enamel external surface up to the depth of 180µm, with a 20µm space among them. Later, the average of the 3 Knoop values of micro-hardness obtained from each depth were converted into mineral volume percentile (%Vol).²⁰

The enamel cross-sectional micro-hardness was determined to evaluate the mineral recovery, since according to Featherstone et al.²⁰ (1983) there is a good relationship (0.91) between enamel micro-hardness and %Vol in caries lesions obtained through microradiograph. The area under the curve (%Vol x µm) was calculated and the mineral content determined. The calculation of the enamel mineral profile which was considered sound was obtained through the projection of the average %Vol encountered in the depths from 100 to 180µm,²¹ of all of the specimens of all groups and the value obtained was 80.1% of mineral volume. The difference between the sound mineral profile and the mineral profile of the other groups resulted in the Delta Z (ΔZ), which is considered an area of mineral loss when its demineralization is

studied. In this study, indicated the area of mineral recovery (ΔZR) that was used for comparison of treatments.¹¹

Statistical analysis:

The ΔZR values presented normal distribution and homogeneity of variances, and being submitted to ANOVA (2x2), variables of treatment and time were considered. For the multiple comparisons of averages, Tukey's test was chosen at a 5% level of significance.

Furthermore, the ΔZR values of the experimental groups were compared with the control groups through the Dunnet test ($p < 0.05$), to verify if treatments could alter the ΔZR only in relation with the decayed group (C1), as well as corroborate if treatments could alter ΔZR in relation with the decayed group that was submitted to the pH cycling (C2).

Later, the comparison was carried out among the groups in accordance with the average of %Vol in each of the depths. The data of the depths 20, 40, 60, 100, 120, and 140 μ m presented a

normal distribution and homogeneity of variances and were then submitted to one-way ANOVA ($p < 0.05$). However, the data of the depths 80, 160, and 180 μ m were compared through the nonparametric test of Kruskal-Wallis ($p < 0.05$).

RESULTS

The results of the enamel cross-sectional micro-hardness (CSMH) analysis through the evaluation of the ΔZR of the experimental groups (Table 1 and Figure 2) demonstrated that G1 and F2 groups presented a better performance, despite the non-existence of significant statistical differences either among the treatments or the times, or else in the interaction of the variables (Figure 3).

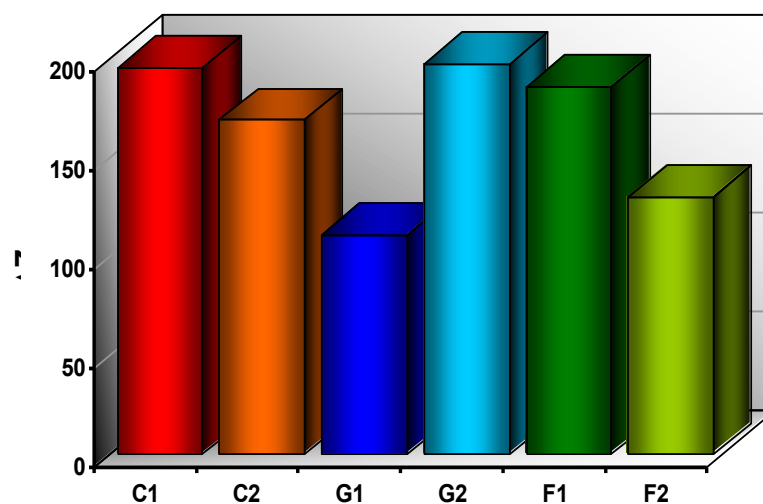
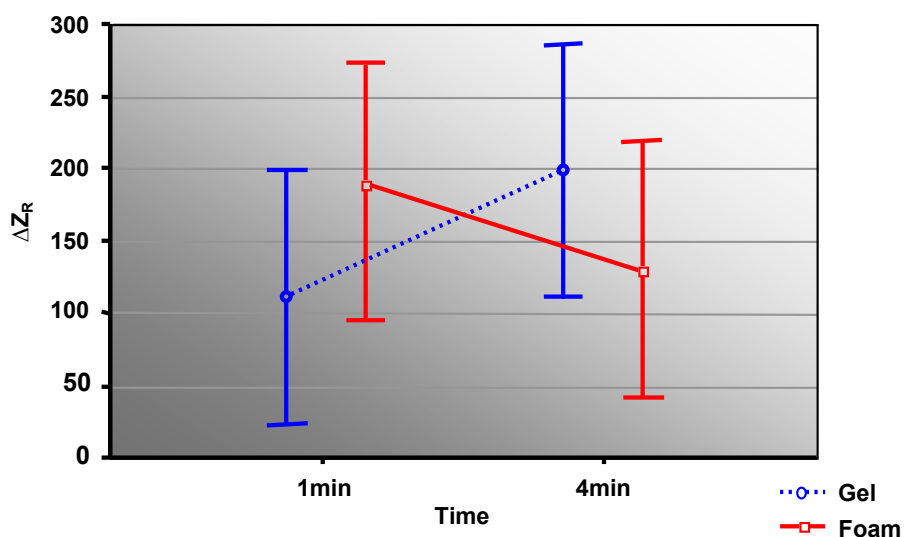
The results of the comparison of the ΔZR of the experimental groups with the control groups indicated that there are not significant statistical differences (Table 1 and Figure 2).

The evaluation of %Vol in each depth identified a significant statistical difference ($p = 0.005$) only at 60 μ m depth between F1 and F2 (Table 2 and Figure 4).

Table 1 - Averages +/- standard-deviation of the area of mineral recovery (ΔZR) in accordance with the treatment procedures.

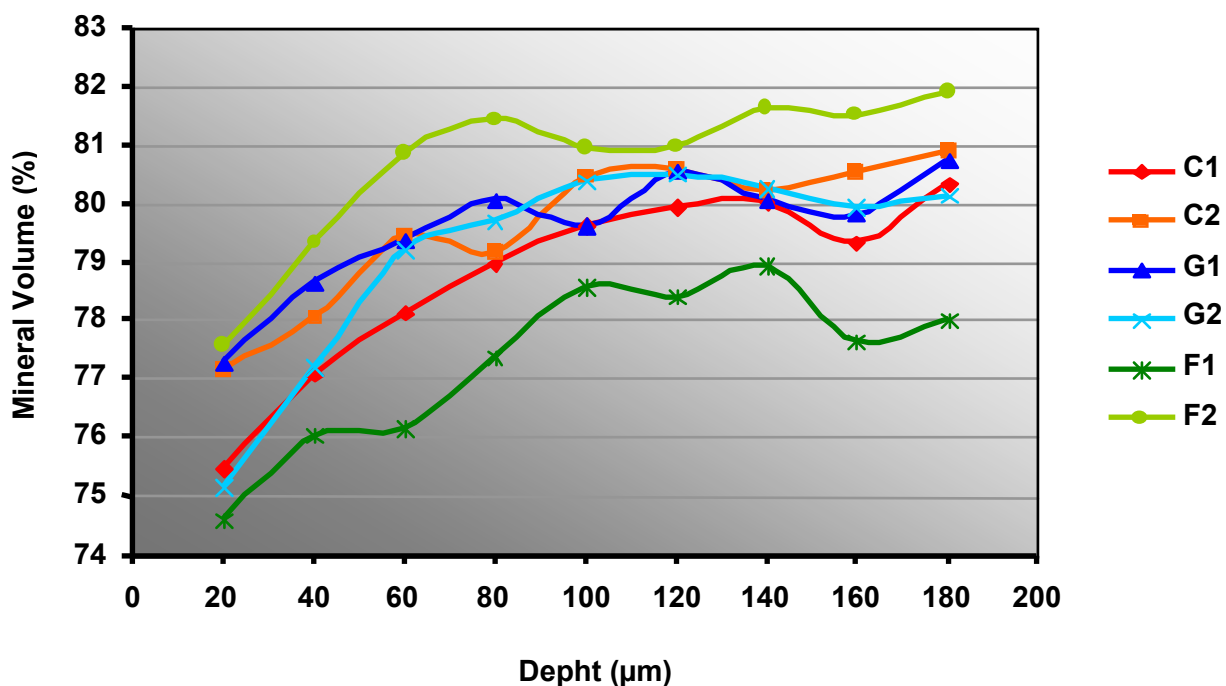
Treatments/Groups	N	ΔZR
Uncycled Control	15	195.73 \pm 160.96
Cycled Control	15	169.27 \pm 189.45
Gel 1min	15	110.76 \pm 201.76
Gel 4min	15	197.44 \pm 167.45
Foam 1min	15	185.78 \pm 158.16
Foam 4min	15	130.09 \pm 173.31

N: Number of samples. ANOVA (2x2) and Tukey's test ($p < 0.05$) demonstrated that there are not differences statistically significant among the experimental groups. Dunnet's Test ($p < 0.05$) evidenced that there are not differences statistically significant between the experimental and the control groups.

Figure 2 - Graphic representation of the area of average mineral recovery (ΔZR) of different groups.Figure 3 - Averages and Interval of Trust of the area of average mineral recovery (ΔZR) in the interaction treatment*time ($p=0.123$). The vertical bars denote a Trust Interval of 0.95.Table 2 - Average values of % Vol (percentile of mineral volume) in each depth (μm), in accordance with the groups.

Group	20	40	60	80	100	120	140	160	180
C1	75.478	77.096	78.137	79.002	79.609	79.941	80.023	79.341	80.344
C2	77.186	78.067	79.473	79.164	80.472	80.600	80.241	80.558	80.921
G1	77.275	78.654	79.391	80.072	79.638	80.555	80.050	79.809	80.745
G2	75.146	77.212	79.205	79.713	80.395	80.513	80.273	79.961	80.133
F1	74.620	76.062	76.175	77.382	78.588	78.404	78.951	77.668	78.007
F2	77.575	79.361	80.871	81.413	80.941	80.999	81.634	81.497	81.907
Total	76.213	77.742	78.875	79.458	79.940	80.168	80.195	79.806	80.343
Valor p	0.167	0.182	0.005	0.075	0.546	0.411	0.515	0.099	0.227

One-way ANOVA ($p < 0.05$) in 20, 40, 60, 100, 120 and 140 μm depths: difference statistically significant only in the 60 μm depth between F1 and F2 ($p = 0.005$). Non-parametric Test by Kruskal-Wallis ($p < 0.05$) in the 80, 160, and 180 μm depths: did not depict difference statistically significance.

Figure 4 - Percentile of mineral volume (%Vol) of the different groups versus depth (μm).

DISCUSSION

The use of products with high fluoride concentration is indicated in the treatment of patients, who present a high risk or with an active decay disease.⁴⁻⁶ APF as gel and foam has been used mainly in pediatric dentistry. However, there are still some issues to be discussed, such as time of application and presentation form of these products, and their capacity to promote the remineralization of early caries lesion in the enamel of primary teeth.

In this research, a micro-hardness essay was carried out in the enamel cross-sectional to evaluate the mineral recovery, on account of previous studies that demonstrated that this test can be used for the analysis of the mineral

profile in the enamel and dentine, either with a caries lesion or remineralized.^{18,20,22} This analysis allows a quantitative evaluation and the comparison among treatment groups in the progression or reversibility of the caries process.²⁰

The results obtained with the analysis of ΔZR among the experimental groups (Table 1 and Figure 2) indicated a tendency of better results with the application of gel for 1 minute and foam for 4 minutes, which are the suitable application periods indicated by the product manufacturers. However, the results did not present significant statistical differences between the treatments - gel and foam, or else, between the recommended times - 1 and 4 minutes, and not even in the interaction of the

variables (Figure 3). Nevertheless, in accordance with the results of %Vol (Table 2 and Figure 4), it was found that there were significant statistical differences concerning the depth of 60µm between the groups F1 and F2, showing a better result when APF foam was applied for 4 minutes. In an in vitro study, Brown et al.⁹ (1994) found greater fluoride incorporation in sound enamel after 4 minutes of application of an APF foam compared with the 1-minute time. Other studies also demonstrated the enamel fluoride uptake with the application of APF gel^{4,8,10-11,14-15,23-24} and APF foam.⁸⁻¹¹ These results may suggest that the effect of APF does not happen immediately after the application, however, the enamel fluoride uptake may occur and perhaps continue giving continuity to the process of mineral recovery.

The closest depths to the surface of the enamel presented lower %Vol values, a characteristic that confirms the cariogenic challenge to which the specimens were submitted. However, the analysis of the ΔZR values of the treated groups in relation with the control groups also demonstrated that there are not significant statistical differences. Therefore, the results indicate that C1 group presented the same behavior as experimental and control groups, which were submitted only to pH cycling. It was then found that a single application of APF, whether in gel or in foam, for 1 or 4 minutes, did not result in the

recovery of the mineral loss and was unsuitable to activate the remineralization of the early decay lesion. This result suggests that the mechanism of APF action may be mainly related to the reduction of enamel demineralization,²⁵ and not to the remineralization of early caries lesions.¹¹

On the other hand, some studies observed remineralization of decay lesions with the application of high concentration fluoridated products, as the research carried out by Tagliaferro²¹ (2007), who, in an in vitro study, observed that treatment procedures, either isolated or combined with APF gel and CO2 laser were effective in the inhibition of the decay progression in enamel of primary teeth. Also, Buchalla et al.²⁶ (2002), in an in situ study, found that there is an increase of remineralization of the bovine enamel previously demineralized, after a single application of a solution with high fluoride concentration.

However, despite those studies have found different results from the present research, data found here are in agreement with the results showed by other studies^{4,11,23} which evaluated the APF, either in the gel or in the foam form, capacity of remineralization. Paes Leme et al.⁴ (2003) did not find any repair of caries lesions in bovine tooth enamel in the groups treated with a single application of APF gel combined with the use of placebo

dentifrice or fluoridated dentifrice, when compared with the daily treatment with fluoridated dentifrice. In an in situ evaluation of tooth decay lesions in bovine enamel, Jardim²³ (2008) verified an increase in the values of superficial micro-hardness and fluoride content only in the blocks that received 3 or 4 applications of APF, and found that the cross-sectional micro-hardness values did not show differences among the groups. Monte Alto¹¹ (2006), in an in vitro study, concluded that although fluoridated products (gel, foam, varnish and centrifuged varnish) have been effective to form and keep fluoride into the decayed permanent enamel, they did not activate the remineralization of the lesion.

Consequently, the results of the present study together with those of the literature demonstrated that a single application of APF was not sufficient for the remineralization of the artificial caries lesion. However, it seems to have promoted a larger fluoride uptake, 4,8-11,14-15,23 making it available to participate in the reduction of the demineralization^{14,16,24-25} and possibly keep giving continuity to the remineralization process.

It is important to assume that new researches with the appropriate models are necessary for a better understanding of the effect of the fluoride in high concentration in the mineral recovery of the decayed enamel of primary teeth, once these have their

particulars, such as a smaller enamel thickness and a larger crown convexity, characteristics that may make them more sensitive to the preparation techniques, as well as to the exposure to the products with low pH.

CONCLUSION

1. Applications of APF gel for 1 minute and foam for 4 minutes show a better performance, although their results do not present significant statistical differences;
2. In accordance to the studied data, a single application of APF, in gel and in foam, for 1 or 4 minutes, is not sufficient to promote the remineralization of the enamel caries-like lesion of primary teeth.

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