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ADRIANA DE CÁSSIA ORTIZ

POTENCIAL ANTICÁRIE DOS DENTIFRÍCIOS DE BAIXA CONCENTRAÇÃO DE FLUORETO ENCONTRADOS NO MERCADO BRASILEIRO

ANTICARIES POTENTIAL OF LOW FLUORIDE DENTIFRICES FOUND IN THE BRAZILIAN MARKET

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Odontologia, na Área de Cariologia.

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Orientadora: Profa. Dra. Lívia Maria Andaló Tenuta

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RESUMO

Apesar de não haver evidência de que dentifrícios de baixa concentração de fluoreto (< 600 μg F/g) sejam efetivos para controlar cárie ou reduzir o risco de fluorose, eles estão disponíveis em vários países do mundo. Assim, o objetivo deste estudo foi avaliar o potencial anticárie de dentifrícios de baixa concentração de fluoreto encontrados no mercado brasileiro, utilizando um modelo validado de ciclagens de pH. Blocos de esmalte bovinos foram selecionados pela dureza de superfície e randomizados em quatro grupos (n=12): dentifrício sem fluoreto (controle negativo), dentifrício de baixa concentração de fluoreto (500 μg F/g, formulação convencional), dentifrício acidulado de baixa concentração de fluoreto (550 μg F/g, formulação modificada com o objetivo de melhorar o efeito anticárie) e dentifrício de 1100 μg F/g (controle positivo, dentifrício convencional). Os blocos foram submetidos ao regime de ciclagem de pH por 8 dias e tratados com os dentifrícios 2 x/dia. Após a ciclagem de pH, a dureza de superfície foi novamente determinada e a porcentagem de perda de dureza foi calculada como indicador de demineralização. As concentrações de fluoreto fracamente e firmemente ligado ao esmalte também foram determinadas. O dentifrício de 1.100 μg F/g foi mais efetivo do que os de baixa concentração na redução da desmineralização do esmalte e foi o único que diferiu significativamente do não fluoretado ($p<0,05$). Todos os dentifrícios fluoretados foram capazes de formar maiores concentrações de fluoreto fracamente ligado ao esmalte do que o não fluoretado ($p<0,05$), mas o de 1.100 μg F/g foi o único que diferiu do não fluoretado na capacidade de formar fluoreto firmemente ligado ao esmalte. Os resultados sugerem que dentifrícios de baixa concentração de fluoreto disponíveis no mercado brasileiro, independentemente da formulação, não têm potencial anticárie, o que está de acordo com a melhor evidência disponível nesse assunto.

Palavras-chave: Cremes Dentais. Fluoretos. Cárie Dental. Desmineralização.

ABSTRACT

Although there is no evidence that low fluoride (F) dentifrices (< 600 µg F/g) are effective either to control caries or to avoid fluorosis, they are found in most markets worldwide. Therefore, the aim of this study was to evaluate the anticaries potential of low-F dentifrices found in Brazilian market, using a validated and tested pH-cycling model. Bovine enamel blocks were selected by surface hardness (SH) and randomized into four treatment groups (n=12): non-F dentifrice (negative control), low-F dentifrice (500 µg F/g; conventional formulation), low-F acidulated dentifrice (550 µg F/g; modified formulation trying to improve its anticaries efficacy) and 1,100 µg F/g dentifrice (positive control; conventional formulation). The blocks were subjected to pH-cycling regimen for 8 days and were treated with the dentifrices 2x/day. After the pH-cycling, SH was again determined and the percentage of surface hardness loss was calculated as indicator of demineralization. Loosely- and firmly-bound F concentrations in enamel were also determined. The 1,100 µg F/g dentifrice was more effective than the low-F ones to reduce enamel demineralization and was the only one that differed from the non-F ($p<0.05$). All F dentifrices formed higher concentration of loosely-bound F on enamel than the non-F ($p<0.05$) but the 1,100 µg F/g was the only one that differed from the non-F in the ability to form firmly-bound F. The findings suggest that the low-F dentifrices available in the Brazilian market, irrespective of their formulation, do not have anticaries potential, in agreement with the best evidence on this subject.

Keywords: Toothpastes. Fluorides. Dental Caries. Demineralization.

SUMÁRIO

1 INTRODUÇÃO

O uso de dentifrícios fluoretados constitui um dos principais fatores responsáveis pelo declínio nos índices de cárie ocorrido nas últimas décadas (Bratthall et al., 1996; Cury et al., 2004), sendo considerado o método mais racional de uso de fluoreto. O efeito anticárie do uso de dentifrício fluoretado deve-se à associação do efeito mecânico da escovação, que remove ou desorganiza o biofilme, e da ação físico-química do fluoreto disponibilizado para o meio bucal (Tenuta e Cury, 2013). O fluoreto disponibilizado para o biofilme residual, atua reduzindo a desmineralização durante as quedas de pH e ativando a remineralização durante os períodos em que o pH estiver neutro. Nas superfícies dentais livres de biofilme, o fluoreto disponibilizado para a saliva potencializa a remineralização de lesões pré-existentes. Também pode ocorrer reação do fluoreto com a estrutura dental limpa, formando fluoreto de cálcio (Cruz et al., 1992), embora este não seja um mecanismo de ação importante do dentifrício fluoretado (Tenuta et al., 2009).

Apesar do benefício anticárie relacionado ao uso de dentifrício fluoretado (Marinho et al., 2003), este método preventivo constitui um fator de risco para a ocorrência de fluorose dental devido à ingestão crônica de dentifrícios pelas crianças que pode ocorrer durante a escovação (Mascarenhas, 2000). Assim sendo, uma das alternativas sugeridas para minimizar o risco de fluorose dental é a utilização de dentifrícios contendo baixa concentração de fluoreto (inferior a 600 μg F/g). No entanto, considerando o binômio benefício anticárie com menor risco de fluorose dental, não há evidência que justifique o uso de dentifrícios contendo baixa concentração de fluoreto. Santos et al. (2013b) realizaram uma revisão sistemática para avaliar o efeito de dentifrícios de baixa concentração de fluoreto (inferior a 600 μg F/g) e de dentifrícios de concentração convencional (1000 a 1500 μg F/g) na prevenção à cárie na dentição decídua e a ocorrência de fluorose na dentição permanente e os resultados mostraram que crianças que utilizaram dentifrício de baixa concentração de fluoreto tiveram um risco aumentado de cárie na dentição decídua em relação às que utilizaram dentifrícios de concentração convencional. Além disso, os resultados mostraram que o uso de dentifrícios contendo baixa concentração de fluoreto não diminuiu significativamente o risco de fluorose dental na dentição permanente em relação ao uso dos dentifrícios de concentração convencional. Em acréscimo, revisões sistemáticas da literatura (Walsh et al., 2010; Santos et al., 2013a) que estimaram o efeito preventivo de dentifrícios de diferentes concentrações de fluoreto, tanto na dentição permanente como na dentição decídua, concluíram que os dentifrícios devem conter, pelo menos, 1.000 µg F/g em sua formulação para que apresentem benefício anticárie, não havendo justificativa para a indicação de dentifrícios de baixa concentração de fluoreto considerando seu efeito preventivo.

Entretanto, os dentifrícios de baixa concentração de fluoreto estão disponíveis em muitos países. Nos Estados Unidos, dentifrícios contendo baixa concentração de fluoreto não podem ser vendidos, pois a legislação americana (FDA) exige que os dentifrícios contenham uma quantidade mínima de 650 ppm de fluoreto solúvel na forma de fluoreto de sódio (NaF) ou, no mínimo, 800 ppm de fluoreto solúvel na forma de monofluorofosfato (MFP). Por outro lado, a legislação européia (Regulamentação 1284, 2008) e os regulamentos do Mercosul (MERCOSUL/GMC/RES nº 48/02) indicam apenas a concentração máxima de fluoreto que um creme dental deve conter (1.500 µg F/g). Da mesma forma, a regulamentação brasileira (ANVISA, Regulamentação 79) exige apenas que um creme dental tenha no máximo 1.500 µg F/g, sem mencionar a concentração de fluoreto solúvel ou a eficácia das formulações baseadas na melhor evidência disponível (Cury et al., 2015)

Dessa forma, este trabalho teve como propósito avaliar o potencial anticárie de dentifrícios de baixa concentração de fluoreto (500-550 µg F/g) disponíveis no mercado brasileiro, utilizando um modelo de ciclagem de pH validado para o teste de dentifrícios de baixa concentração de fluoreto para avaliar a eficácia dos dentifrícios em reduzir a desmineralização do esmalte.

2 ARTIGO: Anticaries potential of low fluoride dentifrices found in the Brazilian market

Article submitted to the Brazilian Dental Journal (Anexo 1)

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ABSTRACT

Although there is no evidence that low fluoride (F) dentifrices (< 600 μg F/g) are effective either to control caries or to avoid fluorosis, they are found in most markets worldwide. Therefore, the aim of this study was to evaluate the anticaries potential of low-Fdentifrices found in Brazilian market, using a validated and tested pH-cycling model. Bovine enamel blocks were selected by surface hardness (SH) and randomized into four treatment groups (n=12): non-F dentifrice (negative control), low-F dentifrice (500 μg F/g; conventional formulation), low-F acidulated dentifrice (550 μg F/g; modified formulation trying to improve its anticaries efficacy) and 1,100 μg F/g dentifrice (positive control; conventional formulation). The blocks were subjected to pH-cycling regimen for 8 days and were treated with the dentifrices 2x/day. After the pH-cycling, SH was again determined and the percentage of surface hardness loss was calculated as indicator of demineralization. Loosely- and firmly-bound F concentrations in enamel were also determined. The 1,100 μg F/g dentifrice was more effective than the low-F ones to reduce enamel demineralization and was the only one that differed from the non-F (p<0.05). All F dentifrices formed higher concentration of loosely-bound F on enamel than the non-F (p<0.05) but the 1,100 μg F/g was the only one that differed from the non-F in the ability to form firmly-bound F. The findings suggest that the low-F dentifrices available in the Brazilian market,

irrespective of their formulation, do not have anticaries potential, in agreement with the best evidence on this subject.

Key Words: fluorides, toothpaste, dental caries, demineralization

INTRODUCTION

The importance of fluoride (F) dentifrices to the decline in dental caries which occurred in the last decades is clearly established (1,2). On the other hand, their use has been pointed out as a risk factor for dental fluorosis (3). As an alternative to reduce the risk of fluorosis from dentifrices, low-F formulations (< 600 µg F/g) have been suggested. However, considering the best evidence available, they are not effective to satisfy the binomial anticaries effect by the use of F dentifrice with a lower risk of fluorosis (4). Moreover, systematic reviews focusing on caries prevention in permanent and deciduous teeth (5,6), showed that a dentifrice should have at least 1000 µg F/g to provide significant anticaries effect.

Nevertheless, low-F dentifrices are available in many countries. Although in the United States they cannot be sold, because the American legislation (7) requires a minimum amount of soluble F of at least 650 ppm F for sodium fluoride (NaF) or 800 ppm F for monofluorophosphate (MFP) formulations, the European (8) and Mercosur (9) regulations only state the maximum F concentration that a dentifrice must contain (1500 µg F/g). Similarly, the Brazilian regulation (Anvisa) (10) only requires that a dentifrice has a maximum of 1500 µg F/g, without mentioning the concentration of soluble F or the effectiveness of the formulations based on the best available evidence (11). Given that there is no evidence that low-F dentifrices, when compared with the use of conventional ones, can reduce the risk of fluorosis (4), and with the current evidence that they can even increase caries risk (5,6), we aimed to assess the anticaries potential of low-F dentifrices available in the Brazilian market. Therefore, the inhibition of enamel demineralization of two low-F dentifrices (500-550 µg F/g), with conventional or modified formulations, available in the Brazilian market was compared with a standard dentifrice (1100 µg F/g) using a validated pH-cycling model.

MATERIALS AND METHODS

Experimental Design

An *in vitro* pH-cycling model validated for the test of low-F dentifrices was used (12). The experimental units were bovine enamel blocks selected by surface hardness (SH) values and randomized into four treatment groups (n = 12): non-F dentifrice (A - Cocoricó®, Bitufo) as a negative control, 500 µg F/g dentifrice (B - Oral-B Pro-Saúde Stages®, Procter & Gamble), 550 µg F/g acidulated dentifrice (C - Escovinha®, Oralls, Dentalprev) and 1100 µg F/g dentifrice (D - Tandy®, Colgate Palmolive), as a positive control. All the dentifrices were bought in the Brazilian market and they were within the expiration time. They were NaF/silica-based and F concentration was checked in all dentifrices through analysis with ion selective electrode (13). The enamel blocks were submitted to daily pH cycles for 8 days; treatments with dentifrices slurries were made twice a day, before and after the demineralization cycle. After pH-cycling, the percentage of SH loss (%SHL) was calculated. Fluoride concentration in enamel (µg F/cm^2) as loosely- and firmly-bound F was determined. F concentration in the de-remineralization solutions (µg F/mL) was also analyzed.

Enamel Block Preparation

Bovine enamel blocks (4 x 4 x 2 mm) were flattened, polished and baseline SH was determined using a Knoop diamond indenter under a 50-g load for 5 s. Three indentations, spaced 100 µm from each other, were made on the central area of each block and used to calculate SH, whose values were averaged. Forty-eight enamel blocks with hardness of 306.7 ± 7.9 kg/mm^2 were selected for this study.

Treatments and pH-cycling regimen

An adhesive tape was placed in the center of the enamel surface and the remaining surfaces of the block were coated with an acid-resistant varnish (Risqué®, Brazil). After removal of the tape, an area of enamel of 8.0 mm^2 (4 x 2 mm) was left to be exposed to the treatments.

The pH-cycling regimen (12) consisted of daily 4-h exposure to the demineralizing solution and approximately 20-h exposure to the remineralizing solution, at 37°C, for 8 days. Twice a day (before and after immersion in the demineralizing solution), the blocks were treated with a 1:3 (w/w) slurry of the dentifrices in purified water, for 5 min, under agitation (60 rpm), at room temperature, to simulate *in vivo* dentifrice exposure during toothbrushing (14). Before and after the treatments, the blocks were washed with purified water for 20 s and dried with soft paper. The demineralizing solution (pH 5.0) consisted of a 0.05 mol/L acetate buffer, containing 1.28 mmol/L Ca, 0.74 mmol/L P and 0.03 µg F/mL. The remineralizing solution (pH 7.0) was 0.1 mol/L TRIS buffer, containing 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl and 0.05 µg F/mL. The proportion of de-remineralizing solutions per area of exposed enamel surface was 6.25 and 3.12 mL/mm^2, respectively. After the 4^{th} day, the de-remineralizing solutions were replaced by fresh batches. After the 8^{th} day of the pH-cycling regimen, the blocks were kept on the remineralizing solutions for 24 h until analysis.

Soluble F concentration in dentifrices slurries was measured in triplicate, using an ion-selective electrode (Orion 96-09) and an ion analyzer (Orion EA-940), previously calibrated with F standards containing 0.5 to 32.0 µg F/mL according to a previously described method (13). The results were expressed as µg F/mL. pH of the slurries was checked in four different samples of each dentifrice, immediately after the preparation of the slurries for use in the pH-cycling.

Enamel demineralization assessment

After the pH-cycling, the SH of the enamel blocks was measured again, as described above, and the %SHL was calculated (% SHL = 100 x (sound enamel hardness – hardness after pH-cycling) /sound enamel hardness). SH is a validated technique to estimate mineral loss or gain by enamel because it reflects the demineralization degree of the enamel lesion (15).

Determination of Enamel Loosely- and Firmly-Bound Fluoride

After surface hardness analysis, the area of the blocks which were covered by acid resistant varnish was cut out and the remaining section was longitudinally sectioned through the center. The cut surfaces were isolated with wax leaving only a 4-mm^2 area (2 x 2 mm) of the enamel surface exposed for F enamel analysis.

Each enamel block was immersed in 0.15 mL of 1 M KOH for 24 h under agitation. After this period, the extract was buffered with an equal volume of TISAB II containing 1 M HCl. The concentration of loosely-bound F was determined in the extract using an ion-selective electrode (Orion 96-09) and an ion analyzer (Orion EA-940) previously calibrated with F standards containing 0.1 to 8.0 µg F/mL. The results were expressed as µg F/cm^2 of enamel area.

After loosely-bound fluoride extraction, the blocks were immersed in 0.25 mL of 0.5 M HCl for 30 s under agitation. After this period, the extracts containing the dissolved enamel layer were buffered with an equal volume of TISAB II modified with 20 g of NaOH/L. The concentration of firmly-bound fluoride was determined as described above, against standards containing 0.125 to 4.0 µg F/mL. The results were expressed as µg F/cm^2 of enamel area.

Determination of Fluoride Concentration in the De- and Remineralizing Solutions

Fluoride concentration in the de- and remineralizing solutions were checked immediately after preparation. After the pH-cycling, all the solutions in which the blocks were individually immersed were again measured for fluoride concentration. Solutions were buffered with TISAB III and F concentration determined using an ion-selective electrode (Orion 96-09) and an ion analyzer (Orion EA-940) previously calibrated with fluoride standards containing 0.025 to 0.4 µg F/mL.

Statistical Analysis

The equality of variances and a normal distribution of error were checked for all response variables. The %SHL, loosely- and firmly-bound F data were transformed to the log_{10}. ANOVA was used for all analysis, followed by Tukey test. The SAS System 9.0 software (SAS Institute Inc., Cary, NC, USA) was used and the significance level was set at 5%.

RESULTS

Fluoride concentration and pH of the dentifrices slurries are presented in table 1.

Table 1. Soluble fluoride and pH of the dentifrice slurries (1 part of dentifrice diluted in 3 parts of purified water) prepared for the study (mean ± SD).

Treatment/Groups	Soluble Fluoride (µg F/mL) n=3	pH n=4
A	1.6 ± 0.2	7.02 ± 0.02
B	144.8 ± 12.8	7.57 ± 0.07
C	143.3 ± 3.4	4.37 ± 0.08
D	314.7 ± 7.2	7.27 ± 0.09

A: non-fluoride dentifrice (Cocoricó®, Bitufo), B: low fluoride dentifrice 500 µg F/g (Oral B Stages®, Oral B), C: low fluoride acidulated dentifrice 550 µg F/g (Escovinha®, Oralls), D: standard dentifrice 1100 µg F/g (Tandy®, Colgate).

After the pH-cycling regimen (table 2), all groups presented a decrease of surface hardness, which was significantly lower for the positive control ($p<0.05$). Similarly, only blocks treated with the positive control presented significantly higher firmly-bound F concentration after the cycling than the negative control group ($p<0.05$). The low-F dentifrices did not significantly differ from the negative control regarding the %SHL and firmly-bound F concentration ($p>0.05$). The concentration of loosely-bound F was higher in all F groups when compared with the negative control ($p<0.05$), being the highest for the acidulated dentifrice.

Table 2. Enamel surface hardness loss (%SHL) and F concentration after pH-cycling (mean ± SD, n=12).

Treatment/ Groups	%SHL	Fluoride Concentration in Enamel (μg F/cm^2)	
		Loosely-Bound F	Firmly-Bound F
A	35.9 ± 8.9 a	0.3 ± 0.1 a	2.6 ± 0.8 a
B	30.8 ± 11.1 a	0.6 ± 0.2 b	2.8 ± 1.2 a
C	30.2 ± 6.6 a	1.1 ± 0.2 c	2.9 ± 1.1 ab
D	15.9 ± 5.2 b	0.6 ± 0.3 b	4.8 ± 2.4 b

A: non-fluoride dentifrice (Cocoricó®, Bitufo), B: low fluoride dentifrice 500 µg F/g (Oral B Stages®, Oral B), C: low fluoride acidulated dentifrice 550 µg F/g (Escovinha®, Oralls), D: standard dentifrice 1100 µg F/g (Tandy®, Colgate). Means followed by distinct letters differ significantly ($p<0.05$)

All F groups were able to significantly increase F concentration in the de- and remineralizing solutions when compared with the negative control ($p<0.05$) (table 3). This effect was higher for the group treated with the acidulated dentifrice (C).

Table 3. Fluoride concentration in de-remineralizing solutions after the pH cycling (mean ± SD, n=12).

Treatment/ Groups	Demineralizing Solution (µg F/mL)		Remineralizing Solution (µg F/mL)	
	1st cycling period	2nd cycling period	1st cycling period	2nd cycling period
A	0.035 ± 0.001 a	0.035 ± 0.001 a	0.040 ± 0.001 a	0.038 ± 0.001 a
B	0.036 ± 0.001 b	0.037 ± 0.001 b	0.049 ± 0.001 b	0.050 ± 0.002 b
C	0.042 ± 0.002 c	0.048 ± 0.002 c	0.061 ± 0.003 c	0.076 ± 0.008 c
D	0.036 ± 0.001 b	0.038 ± 0.001 b	0.049 ± 0.002 b	0.050 ± 0.002 b

A: non-fluoride dentifrice (Cocoricó®, Bitufo), B: low fluoride dentifrice 500 µg F/g (Oral B Stages®, Oral B), C: low fluoride acidulated dentifrice 550 µg F/g (Escovinha®, Oralls), D: standard dentifrice 1100 µg F/g (Tandy®, Colgate). Means followed by distinct letters differ significantly ($p<0.05$).

DISCUSSION

F present in dentifrices acts as a preventive-therapeutic agent (16), but the current available evidence suggests that they must have at least 1000 μg soluble F/g of to be able to significantly control caries in permanent (5) and deciduous teeth (6). However, the current Brazilian legislation (10) on fluoride dentifrices only determines the maximum F concentration that a dentifrice must contain, without specifying that it should be soluble or requiring a minimum concentration. This raises two concerns: 1. Depending on the toothpaste formulation, not all fluoride is soluble (11,13,17), specially as dentifrices ages (18), suggesting that the concentration of soluble F should be considered in the legislation (11); 2. If low-F toothpastes are used, the concentration of F used does not reach the recommended 1000 μg F/g. In addition, currently there is no evidence that using a low-F dentifrice reduces the risk of dental fluorosis (4). Therefore, considering the availability of low-F dentifrices in the Brazilian market, we aimed to test their anticaries potential.

The results showed that only the standard F dentifrice (1100 μg F/g) was able to significantly reduce mineral loss during the pH cycling. This result is confirmed by the firmly-bound F concentration found after the cycling, representing the F that was incorporated in enamel as a result of the caries process (16). This suggests that the highest concentration of F present in the standard toothpaste provided higher incorporation of fluorapatite in the enamel during the de-remineralization cycles. In this process, part of the dissolved minerals was replaced in the enamel in the form of a more stable mineral, slowing the mineral loss.

On the other hand, the loosely-bound F concentration found on enamel after the cycling showed that the reduction in pH in the low-F dentifrice was able to enhance the formation of this material (calcium-fluoride like) on enamel. This result is expected given that the formation of calcium fluoride reservoirs on the enamel increases with lowering the pH of the fluoridated agent (19). In fact, previous study (20) confirmed that lowering the pH of a low-F dentifrice may enhance its reactivity with enamel to values similar to a standard F dentifrice.

Taking into account the solubility of calcium fluoride deposits formed on the dental structure (21), the greater reactivity of the acidulated dentifrice resulted in higher F release for de-remineralizing solutions, according to the results obtained by

Brighenti et al. (22). However, the higher reactivity of acidulated dentifrice and the subsequent higher fluoride concentration released to the solutions, did not reduce the mineral loss. F released to the solutions was not sufficient to facilitate the incorporation of fluorapatite in enamel, limiting the anticaries potential of the acidulated low F dentifrice. These results differed from those obtained by Brighenti et al. (22) and Alves et al. (23), who showed that acidic formulations (550 μg F/g) had similar anticaries effect than neutral formulations (1100 μg F/g) in reducing enamel demineralization. One hypothesis that could explain the difference between our results and the previous ones is that in the mentioned studies, a lower proportion of de-remineralizing solutions per area of block was used. This may have impaired the differentiation of the treatments, because the F released from the loosely-bound reservoirs formed by the low F acidulated dentifrices may have accumulated in the pH cycling solutions. The volumes of de- and remineralizing solutions used in the present study may allow a better simulation of the mouth open system; in order to simulate the continuous dilution promoted by saliva in a closed *in vitro* design, the volume of the solutions must be high enough to avoid accumulation of the anticaries agents being tested and artifacts due to the closed *in vitro* model (24). In fact, the importance of loosely-bound F reservoirs formed by dentifrices has been questioned in a previous study designed to test their importance (25).

The results of the present study emphasize the importance of a minimum F concentration in dentifrices for a significant anticaries effect, since the higher reactivity of the low pH formulation was not able to reduce mineral loss when compared with the negative control. Besides, it is important to consider that the dentifrice slurries were prepared with water, which maintains the low pH of the acidulated dentifrice slurries and consequently promotes higher reactivity with dental structure. However, when the acidulated formulation slurry was prepared using artificial saliva, the pH was higher than 6 (data not shown), suggesting that *in vivo* it would be even less effective to form loosely-bound F reservoirs on enamel.

In conclusion, the present *in vitro* study confirmed that a dentifrice must have at least 1000 μg F/g to control caries, since none of the low-F dentifrices found in the Brazilian market were able to significantly reduce mineral loss.

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3 CONCLUSÃO

Os resultados mostraram que os dentifrícios de baixa concentração de fluoreto (500-550 µg F/g) disponíveis no mercado brasileiro não foram eficazes em reduzir a desmineralização do esmalte comparado ao efeito proporcionado pelo dentifrício de concentração convencional de fluoreto (1.100 µg F/g).

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ANEXO 1

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Submission Confirmation

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