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FACULDADE DE ODONTOLOGIA DE PIRACICABA

Celso Silva Queiroz

Modelos de estudos *in vitro* para avaliar o efeito do fluoreto na desmineralização e remineralização do esmalte e dentina

Tese apresentada à Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas para obtenção do título de Doutor em Odontologia – Área de concentração em Cariologia.

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SUMÁRIO

RESUMO

Os modelos de estudos *in vitro* são amplamente utilizados em pesquisas sobre cárie dental, principalmente na avaliação do efeito do fluoreto (F) na desmineralização (des-) e remineralização (re-) do esmalte ou dentina. Como não há consenso no tipo de substrato mineral usado nestas avaliações, o objetivo geral deste estudo foi desenvolver modelos, apresentando efeito dose-resposta, para avaliar o efeito do F na des- e remineralização do esmalte e dentina de origem bovina. O estudo foi conduzido em três etapas e apresentado na forma de três artigos científicos, com objetivos específicos para cada estudo. No estudo 1 foi desenvolvido um modelo para avaliar o efeito de dentifrícios com baixa concentração de F (500 μg F/mL) na des- e remineralização do esmalte. No estudo 2 foi desenvolvido um modelo para avaliar o efeito de dentifrício F na des- e re da dentina. No estudo 3 foi desenvolvido um modelo para desmineralizar a dentina e avaliar o efeito da resistência de união da resina composta em substratos com diferentes graus de mineralização. Os resultados desses estudos mostraram respectivamente que, o dentifrício com baixa concentração de F apresentou potencial anticárie, porém este não foi equivalente ao dentifrício convencional (1100 μg F/g), tanto na redução da des- como na ativação da re- do esmalte. No estudo 2 foi observado que um dentifrício comercial brasileiro (1100 μg F/g) apresentou potencial anticárie equivalente ao controle positivo (Crest - 1100 μg F/g) durante os processos de des- e re em dentina. Foi observado também que a dentina desmineralizada ou remineralizada pode alterar a união de um material restaurador. Dessa forma, os estudos sugerem que os modelos *in vitro* desenvolvidos foram capazes de avaliar o efeito do F na des- e remineralização do esmalte e da dentina bovina.

ABSTRACT

In vitro models are widely used in dental caries researches, mainly for assessing the effect of fluoride (F) on demineralization (de-) or remineralization (re) on enamel or dentin. Since there is no consensus about the type of mineral substrate used in these experiments, the general aim of this study was to develop models, presenting dose-response effect, to evaluate the effect of F on de- and remineralization of bovine enamel or dentin. The study was performed in three parts with specific objectives. In study 1, a model to evaluate the effect of low F dentifrices (500 μg F/g) on de- and remineralization in enamel was developed. In study 2, a model to evaluate the effect of F dentifrices on de- and remineralization in dentin was developed. In study 3, a model of demineralizing dentin was developed and the effect of the level of dentin mineralization on composite resin bond strength was evaluated. The results of study 2 showed that although the low F dentifrice presented anti-caries potential, inhibiting demineralization and enhancing enamel remineralization, its effect was not equivalent to that of conventional dentifrice (1100 μg F/g). In study 2, a Brazilian commercial dentifrice (1100 μg F/g) presented anti-caries potential equivalent to positive control (Crest - 1100 μg F/g) of de- and re of dentin. The results of study 3 showed that demineralized or remineralized dentin may interfere with composite resin bond. Thus, the studies suggest that the *in vitro* models developed were able to assess the effect of F on bovine enamel or dentin de- and remineralization.

1. INTRODUÇÃO

Experimentos *in vitro* são comumente usados em pesquisas sobre cárie dental, através deles os processos de desmineralização (des-) (Arends et al, 1992; Featherstone et al, 1993) e remineralização (re) (Damato et al, 1990; White, 1987) nos substratos dentais têm sido avaliados. Entre estes estudos, os modelos de ciclagens de pH (ten Cate & Duijsters, 1982, Featherstone et al 1986, ten Cate et al, 1988, White, 1987, 1995), têm sido usados para diversos propósitos, e a mais importante aplicação tem sido a avaliação do efeito do fluoreto (F) na inibição da des- e ativação da remineralização.

Os modelos *in vitro* de ciclagens de pH permitem a avaliação de perda ou ganho mineral do esmalte-dentina, simulando o processo natural de cárie dental, e também servem para avaliar o potencial anticárie de dentifrícios e bochechos fluoretados (White 1987, Featherstone et al, 1986). Eles podem se diferenciar sobre vários aspectos, um deles é o tipo de substrato empregado, como dentes humanos ou bovinos. Estudos *in vitro* têm mostrado que o esmalte humano e o bovino apresentam comportamento similar em condições de des- e re (Koulorides & Housch, 1986), e mais recentemente foi mostrado que a dentina bovina pode substituir a humana em modelos *in situ* que avaliam desafios cariogênicos e agentes anticariogênicos (Hara et al, 2003). A vantagem em usar dentes bovinos, ao invés de humanos, é que eles são mais fáceis de se obter e manipular [Mellberg, 1992]. Além disso, eles têm uma composição química relativamente mais homogênea, o que permitiria uma menor variação na resposta de tratamentos cariogênicos e anticariogênicos [Mellberg, 1992].

Diversos modelos foram desenvolvidos para avaliar *in vitro* o potencial anticárie de dentifrícios na des- e re dos esmaltes humano e bovino, no entanto eles não apresentaram efeito dose resposta do F para diferenciar o efeito de dentifrício de baixa concentração de F (500 μg F/mL) em comparação com o convencional de 1000 - 1100 μg F/g (Bloch-Zupan, 2001).

Modelos *in vitro* têm avaliado o efeito de dentifrício fluoretado na ativação da re em dentina bovina (ten Cate et al, 1995; Mukai et al, 2001). No entanto, estes modelos não apresentaram efeito dose-resposta do F. Assim, no estudo de Dunipace et al (1994), foi desenvolvido e um modelo para estudar o efeito de dentifrício fluoretado na resistência da des- na dentina. No entanto, não foi verificada a ativação da re-, e o substrato utilizado foi a dentina humana.

Desse modo, o objetivo desta tese foi desenvolver e validar modelos *in vitro* para avaliar o efeito do F na desmineralização e remineralização do esmalte e dentina bovina.

2. CAPÍTULOS

Esta tese está baseada na norma CCPG/001/98/UNICAMP que regulamenta o formato alternativo para tese de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato.

Dessa forma, esta tese é composta de três artigos; dois a serem submetidos e um publicado, em revistas científicas, conforme descrito abaixo:

2.1. ARTIGO 1 - pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- remineralization.

2.2. ARTIGO 2 - pH-cycling models to evaluate the effect of fluoride dentifrice on dentin de- remineralization.

2.3. ARTIGO 3 - Influence of the mineral content and morphological pattern of artificial root caries lesion on composite resin bond strength.

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2.1. ARTIGO 1

pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- remineralization.

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Key words**:** demineralization; remineralization; fluoride; pH-cycling

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ABSTRACT

Since the pH-cycling models available are not able to differentiate *in vitro* the anti-caries potential effect of dentifrices with low fluoride (F) concentration, this study was conducted. Two models of pH-cycling were developed, one to evaluate the effect of F on resistance to demineralization and the other one to assess the enhancement of remineralization of caries-like lesions. Blocks of bovine enamel were submitted to the pH-cycling models and treated with fluoride solutions containing 70, 140 and 280 µg F/mL to validate the models. These concentrations simulate the effect of F-dentifrice containing 275, 550 and 1100 µg F/g; distilled deionized water was used as a negative control. Furthermore, commercial fluoride dentifrices were evaluated: non-fluoridated dentifrice (negative control), Colgate Baby (500 µg F/g), Tandy (1,100 µg F/g) and Crest (1,100 µg F/g, positive control). The inhibition of demineralization (%SMC) and enhancement of remineralization (%SMR) were evaluated by surface and cross-sectional microhardness, and lesion depth by polarized light microscopy. The pH-cycling models showed F dose-response effect (r^2=0.97). The findings showed that low F commercial dentifrice presents anti-caries potential, but it is not equivalent to that of the conventional ones containing 1,100 µg F/g. The data suggest that the models developed are able to evaluate the anti-caries potential effect of low F dentifrices either on enamel resistance to demineralization or on the enhancement of remineralization.

INTRODUCTION

In spite of the progress in *in situ* and *in vivo* experimentation in caries research, *in vitro* tests are still widely used. Numerous *in vitro* studies have evaluated demineralization (Featherstone et al, 1986; ten Cate, 1990) and remineralization (White, 1987, Damato et **al**, 1990) processes on tooth substrates. Among these studies, the pH-cycling models (ten Cate & Duijsters, 1982, Featherstone et al 1986, ten Cate et al, 1988, White, 1987, 1995) have been frequently used for different purposes; the most important application has been the evaluation of fluoride (F) effect on caries.

The pH-cycling models were developed to evaluate mineral loss and gain simulating the natural caries process, and they can provide evaluations of the anti-caries potential of toothpastes and mouth rinses containing F (White 1987, Chow et al, 1992).

Fluoride toothpastes have made an important contribution to the reduction in caries prevalence in many industrialized countries (Bratthall et al, 1996). They contain F in varying concentrations, but there is no consensus about the anti-caries benefits of higher or lower concentrations than the conventional 1,000 –1,100 µg F/g most used.

Fluoride dentifrice has been considered a risk factor for dental fluorosis (Pendrys, 1995; Mascarenhas, 2000). Low F dentifrice is an alternative to reducing the risk of fluorosis (Horowitz, 1992). Furthermore, the anti-caries effect of low F dentifrice is not clearly established (Ammari et al, 2003). Additionally, the known *in vitro* models, which evaluate the anti-caries potential of fluoride dentifrice, are not able to differentiate between the effects of dentifrices containing 500 and 1,000 µg F/g (Bloch-Zupan, 2001).

Thus, the aim of this study was to develop and test pH-cycling models to evaluate the anti-caries potential of low F dentifrices on either inhibition of

demineralization or enhancement of remineralization. Evaluations were made of the dose-response effect of F, using solutions containing 70, 140 and 280 µg F/mL and of the anti-caries potential of commercial dentifrices with low F concentration.

MATERIAL AND METHODS

Experimental Design

The factors under study were two conditions: demineralizing and remineralizing pH-cycling models, respectively simulating the inhibition of caries progression and the enhancement of remineralization. The experimental units were bovine enamel blocks. In each of the pH-cycling conditions, the dose response effect of F solutions was assessed and the anti-caries potential of a commercial dentifrice with low F concentration was evaluated. The quantitative response variables for the demineralizing pH-cycling model were percentage of surface microhardness change (%SMC), mineral loss area (ΔZ) and lesion depth (LD); for the remineralizing pH-cycling model, percentage of surface microhardness recovery (%SMR), percentage of mineral area recovery (%ΔZ) and lesion depth (LD).

Demineralizing Solution preparation (ANEXO 1)

This solution was used either in demineralizing or in remineralizing pH-cycling models and to induce caries-like lesions on enamel blocks. It contained 0.05 M acetate buffer, pH 5.0, 50% saturated with respect to enamel. According to Moreno & Zahradnik (1974), an acid buffer at 50% saturation with respect to enamel would induce a typical enamel subsurface demineralization without erosion. Bovine incisors were used to prepare this solution. Enamel powder (particles of 74 - 105 µm) was obtained from the

crowns (Asgar, 1956) and added to 0.05 M acetate buffer pH 5.0 (0.5 g/L), which were kept under agitation for 96 h at 37°C to create 100% saturated solution. The filtered solution was diluted with an equal volume of the same buffer obtaining a 50% saturated solution with respect to the solubility of enamel. Fluoride (F), inorganic phosphorus (Pi) and calcium (Ca) concentrations were determined in these solutions by ion selective electrode, colorimetrically (Fiske & Subbarow, 1925) and by atomic absorption (Spectra A 50 Varian), respectively. A mean of 1.28±0.058 mM Ca, 0.74±0.005 mM Pi and 0.023±0.006 µg F/mL was found.

From these results, a demineralizing solution containing 0.05 M 50% saturated acetate buffer of Ca, Pi and F with respect to enamel was prepared, using the salts $Ca(NO_3)_2.4\ H_2O$, KH_2PO_4 and NaF respectively.

Preparation of Tooth Specimens (ANEXO 2)

Enamel blocks (4x4x3 mm) were obtained from bovine incisor teeth that were stored in 2% formaldehyde solution, pH 7.0 (White, 1987). The block surfaces were flattened and polished (Hara et al, 2003). An adhesive strip of 2.0 x 4.0 mm was placed over the surface of enamel blocks, which were coated with acid-resistant varnish; after it was removed; only a surface area of 8.0 mm^2 was left exposed. The baseline enamel surface microhardness was determined using a Future-Tech FM-ARS microhardness tester with a Knoop diamond under 25 g for 5 s. Five indentations were made at the center of the surface and the enamel blocks with hardness of 353.4 ± 12.2 Kg/mm^2 were selected for this study.

pH-Cycling Model to evaluate resistance to demineralization

Dose-response effect to fluoride (ANEXO 3)

Fifty-two blocks were randomly distributed into 4 groups (n=13) and submitted to one of the following treatments: distilled deionized water (negative control), 70, 140 and 280 µg F/mL. These concentrations of fluoride were chosen because they simulate the dilution (1:3 w/w) that occurs in the oral cavity when dentifrices containing 275, 550 and 1100 µg F/g respectively are used (Duke & Forward, 1982); all fluoridated solutions were prepared with NaF.

The pH-cycling regimen took 8 days, and the blocks were kept for 4 h in demineralizing and for approximately 20 h in the remineralizing solution. Twice a day (before and after immersion in the demineralizing solution) the blocks were washed with deionized water and submitted to the groups of treatments under agitation for 5 min. After the treatment the blocks were washed, and then individually kept in a demineralizing solution containing 1.28 mM Ca, 0.74 mM P, 0.03 µg F/mL, pH 5.0. The remineralizing solution used contained 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, 0.1 M Tris buffer, pH 7.0 (ten Cate & Duijsters, 1982). The proportion of demineralizing and remineralizing solution volume per area of blocks was 6.25 mL/mm^2 and 3.12 mL/mm^2, respectively. On the 4th day, the de- or remineralizing solutions were changed. The experiment was carried out at 37°C. After the 8th cycle the blocks remained in the remineralizing solution for 24 h until analysis.

F-dentifrice evaluation (ANEXO 3)

The effect of F dentifrice on inhibiting demineralization was tested using the same conditions described before. Forty blocks were randomly distributed into 4 groups

(n=10) and submitted to one of the following treatments: non-fluoridated dentifrice (negative control), Colgate Baby (500 µg F/g), Tandy (1100 µg F/g, active F dentifrice) and Crest (1100 µg F/g, positive control). The blocks were treated twice a day for 5 min with dentifrice/water slurries (1:3 w/w); all the dentifrices were silica-based.

pH-Cycling Model to evaluate remineralization enhancement

Caries-Like Lesion preparation (ANEXO 4)

A preliminary study to induce caries-like lesions on bovine enamel blocks was conducted. Forty blocks were immersed individually in demineralizing solution (2 mL/mm^2) for 8, 16, 32 and 64 h and mineral loss was evaluated. The period of 32 h (Table 1) was chosen for inducing caries-like lesion on enamel (Fig. 1), since the enamel blocks presented caries-like subsurface lesions without surface loss and allowed surface microhardness to be determined.

Effect of dose-response to fluoride (ANEXO 5)

Sixty-five enamel blocks were randomly distributed into 5 groups (n=13). Four groups were submitted to one of the following treatments: distilled deionized water (negative control), 70, 140 and 280 µg F/mL; the extra group was not submitted to any treatment and was kept for analysis of the caries-like lesion.

The pH cycling regimen took 8 days. This number of days was determined by prior study (Fig. 2). The blocks were kept for 2 h in demineralizing and for approximately 22 h in the remineralizing solution, at 37°C. Three times a day (9:00, 14:00 and 17:00 h) the blocks were washed with deionized water and submitted for 1 min (White, 1987) to the groups of treatments under agitation, to simulate exposure to tooth brushing. After the

treatments the blocks were washed again. On the fourth day the de- or remineralizing solutions were changed.

The blocks were individually kept in a demineralizing solution containing 1.28 mM Ca, 0.74 mM P, 0.03 µg F/mL, pH 5.0. The remineralizing solution used contained 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, 0.1 M Tris buffer, pH 7.0 (ten Cate & Duijsters, 1982). The proportion of demineralizing and remineralizing solution volume per area of blocks was 6.25 ml/mm^2 and 3.12 mL/mm^2, respectively.

F-dentifrice evaluation (ANEXO 5)

The effect of F dentifrices on enamel remineralization was tested using the same conditions described before. Forty blocks of enamel were randomly distributed into 4 groups (n=10) and submitted to one of the following treatments: non-fluoridated dentifrice (negative control), Colgate Baby (500 µg F/mL), Tandy (1100 µg F/mL) and Crest (1100 µg F/mL, positive control). The blocks were treated 3x/day for 1 min with dentifrice/water slurries (1:3 w/w), as described for the treatments with F solutions.

Microhardness Analysis (ANEXOS 6 – 7)

Surface microhardness was determined again; two rows of five adjacent indentations with 25 g load for 5 s (Argenta et al, 2003), spaced at 100 µm were made on both sides of the five baseline measurements. The mean values of the five baseline indentations and the ten measurements after treatments were then averaged within a treatment group and the percentage surface microhardness change (%SMC) was calculated as: (microhardness after pH cycling – sound enamel microhardness x 100/ sound enamel microhardness). Percentage of enamel surface microhardness recovery (%SMR) was

calculated as: (microhardness after pH cycling – microhardness after demineralization x 100/sound enamel microhardness - microhardness after demineralization).

After surface microhardness analysis, all blocks were longitudinally sectioned through the center of the exposed enamel. To measure cross-sectional microhardness (CSM), one half of each block was embedded in acrylic resin and the cut surfaces were exposed and polished. Three rows of 14 indentations each were made at 10 μm up to 200 μm depths from the outer surface of enamel blocks. The mean values at all 3 measuring points at each distance from the surface were then averaged. CSM values were converted to volume % mineral (Featherstone et al, 1983) and the mineral loss (ΔZ) was obtained (Featherstone & Zero, 1992).

After the remineralization regimes, the mineral area values for each experimental group were calculated (ΔZ_2) and compared with the mineral loss area (ΔZ_1) of the extra group, which was submitted only to caries-like lesion formation and was kept out of the treatments. The difference between ΔZ_1 and ΔZ_2 was obtained and the data were converted to percentage of mineral area recovery (%ΔZ), using the following relation: $\%\Delta Z = (\Delta Z_1 - \Delta Z_2) \times 100 / \Delta Z_1$.

Polarized light microscopy analysis (ANEXO 8)

The other half each block was longitudinally sectioned, in order to obtain a section of 100 μm (±10). These sections were mounted for examination under a polarizing light microscope at x100 magnification (DMLSP, Leica) after imbibition in deionized water and in air. Digital images were taken and the lesion depths were measured at three sites

using an Image-Pro-Plus software (Media Cybernetics; at each site five measurements were made and averaged.

Statistical Analysis

The (ANEXOS 13 - 14) surface microhardness change data (%SMC), mineral loss area (ΔZ), percentage of surface microhardness recovery (%SMR), percentage of mineral area recovery (%ΔZ) and lesion depth (LD) were subjected to one-way analysis of variance (ANOVA), followed by the Tukey and Dunnett tests (α=0.05). To show the dose-response effect of fluoride, the data were analyzed by ANOVA and the regression rate coefficient was determined. The analyses were performed with the SAS System 8.01 software (SAS Institute Inc.).

RESULTS

With regard to demineralization inhibition, Table 2 shows that %SMC and LD were significantly different among the solutions tested. All groups were statistically more effective in reducing ΔZ in comparison with the control, but there was no difference between 140 and 280 µg F/mL. A dose-response effect was found among fluoride concentrations to %SMC (R^2 =0.9731; p<0.0001, quadratic correlation), ΔZ (R^2 =0.8811; p<0.0001, quadratic correlation), and LD (R^2 =0.9358; p<0.0001, quadratic correlation).

With respect to evaluating the dentifrices as regards demineralization inhibition, Table 2 shows that all dentifrices were statistically more effective in reducing %SMC, ΔZ and LD in relation to the placebo. The low F dentifrice was statistically less effective than active dentifrice and positive control, which were not statistically different from each other.

With regard to enamel remineralization, Table 3 shows that %SMR and %ΔZ were statistically different among F solutions in comparison with the control, but there was no statistically significant difference between 140 and 280 µg F/mL for %ΔZ. The analysis of LD presented statistical difference among the F solutions ($p<0.05$). A dose-response effect was found among F concentrations for %SMR (R^2 =0.8870; p=0.0006, cubical correlation), %ΔZ (R^2 =0.8901; $p<0.0001$, quadratic correlation) and LD (R^2 =0.9292; $p<0.0001$, quadratic correlation).

With respect to the evaluation of the dentifrices, Table 3 shows that all dentifrices were statistically more effective for increasing %SMR, %ΔZ and LD in relation to the placebo. The low F dentifrice was statistically less effective than active dentifrice and positive control, which were not statistically different from each other.

DISCUSSION

The surface microhardness measurement has been widely used to study enamel demineralization and remineralization (Iijima & Koulorides, 1988; White, 1995; Zhang et al, 1995), allowing changes in the outermost layer of enamel to be evaluated (Zero, 1995). The time of 32 h for inducing caries-like lesions in enamel allowed surface microhardness to be evaluated before and after the treatments, which is an important fact, since fluoride reacts with dental tissues at the most superficial layers (Iijima & Koulorides, 1989). The result of %SMC and %SMR showed that low F commercial dentifrice presents anti-caries potential, but it was not equivalent to that of the conventional ones containing 1,100 µg F/g. The data of this study are in agreement with Reed (1973) who, in clinical trials, evaluated dentifrices containing 250, 500 and 1,000 µg F/g and found greater anti-caries potential for

dentifrices with 1,000 μg F/g. On the other hand, the results of this study do not agree with Winter et al (1989), who found the same anti-caries efficacy between dentifrices with 550 and 1,050 μg F/g.

According to Featherstone et al (1990), the aqueous fluid among the crystals in a forming caries lesion can present higher concentrations of Ca and Pi, and be saturated in relation to the adjacent apatite crystal. As the local pH falls, F in this aqueous phase among the crystals, together with Ca and Pi, contribute to the degree of saturation with respect to F apatite, inhibiting higher dissolution of enamel crystals. Therefore, the best performance of dentifrices with 1,000 μg F/g in relation to others with low F can be explained in this way.

Damato et al (1990) compared enamel remineralization using different F solutions (1, 250, 500, 1,000, 1,750 and 2,500 μg F/mL) and F solutions containing more than 500 μg F/mL were not significantly different. The cycling models developed in this study showed significant different between F solutions with 140 and 280 μg F/mL, this concentration was based on the dilution (1:3 w/w) of dentifrices in the oral cavity (Duke & Forward, 1982). Damato et al (1990) did not consider this dilution, and this could mask the effect of fluoride.

Fluoride dentifrice has been considered a risk factor for dental fluorosis (Pendrys, 1995) and the low F dentifrice is an alternative for reducing the risk of fluorosis (Horowitz, 1992). Therefore, Crest (1,100 μg F/g, positive control) and Tandy (1,100 μg F/g) dentifrices were more effective than Baby (500 μg F/g) dentifrice. In spite of the limitations of *in vitro* studies, these results show that the use of low F dentifrice could mean

a reduction to below the risk threshold for dental fluorosis in children that ingest dentifrice, but the anti-caries effect of dentifrice would be compromised.

In order to validate these pH-cycling models, a dose-response evaluation was made. Data showed a statistically significant correlation between the concentration of F in the solutions tested and the variables for the demineralizing and remineralizing pH-cycling regimens. This suggests that the models proposed are adequate for studying *in vitro* fluoride treatments by surface or cross-sectional microhardness.

In conclusion, the data suggest that the models developed are able to evaluate the anti-caries potential effect of low F dentifrices either on enamel resistance to demineralization or on the enhancing of remineralization.

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Table 1. Percentage of enamel surface microhardness change (%SMC), area of lesion (ΔZ) and lesion depth (LD) on enamel, according to the time (h) in the demineralizing solution (Mean ± SD; n=10)

Time (h)	% SMC	ΔZ	LD
8	-51.7 ± 0.5a	270.2 ± 30.1a	32.2 ± 0.9a
16	-73.0 ± 2.5b	348.4 ± 70.1b	45.3 ± 0.9b
32	-83.9 ± 6.6c	621.1 ± 134.4c	72.2 ± 1.7c
64	-97.1 ± 7.1d	1015.8 ± 151.6d	132.7 ± 2.9d

Treatments whose means are followed by different letters differ statistically (5%)

Table 2. Analysis of enamel submitted to the demineralization model, according to treatments groups with fluoride solutions or dentifrices (Mean ± SD)

Groups	Analyses		
Fluoride solutions (n=13)	% SMC	ΔZ	LD
0	-72.9 ± 0.9a	1314.5 ± 228.9a	64.0 ± 1.8a
70	-53.7 ± 1.7b	615.4 ± 242.6b	44.1 ± 5.9b
140	-42.2 ± 1.6c	356.4 ± 72.7c	35.0 ± 1.0c
280	-38.4 ± 1.1d	292.5 ± 97.3c	28.1 ± 6.5d
Dentifrices (n=10)			
Negative control	-74.8 ± 8.2A	1569.5 ± 215.1A	84.2 ± 2.8A
Low fluoride	-47.7 ± 10.1B	789.1 ± 122.1B	50.6 ± 4.6B
Active dentifrice	-33.8 ± 4.3C	399.8 ± 65.7C	30.1 ± 1.4C
Positive control	-35.4 ± 6.5C	376.4 ± 125.1C	32.9 ± 1.8C

Treatments whose means are followed by different letters differ statistically (5%), lower case among F solutions and capital letters among dentifrices.

Table 3. Analysis of enamel submitted to the remineralization model, according to treatments groups of fluoride solutions or dentifrices (Mean ± SD)

Groups	Analyses		
Fluoride solutions (n=13)	% SMR	%ΔZ	LD
0	13.9 ± 2.7a	9.6 ± 5.5a	78.4 ± 5.2a
70	34.5 ± 4.7b	56.0 ± 6.7b	56.9 ± 1.4b
140	42.1 ± 6.2c	61.0 ± 4.0c	31.4 ± 2.7c
280	49.1 ± 7.0d	65.8 ± 8.8c	29.5 ± 6.9d
Dentifrices (n=10)			
Negative control	9.1 ± 3.2A	6.2 ± 1.4A	64.7 ± 5.2A
Low fluoride	26.2 ± 2.2B	33.2 ± 11.0B	46.1 ± 3.6B
Active dentifrice	38.8 ± 4.0C	56.2 ± 11.1C	18.9 ± 6.5C
Positive control	40.9 ± 3.2C	52.3 ± 5.6C	20.7 ± 9.4C

Treatments whose means are followed by different letters differ statistically (5%), lower case among F solutions and capital letters among dentifrices.

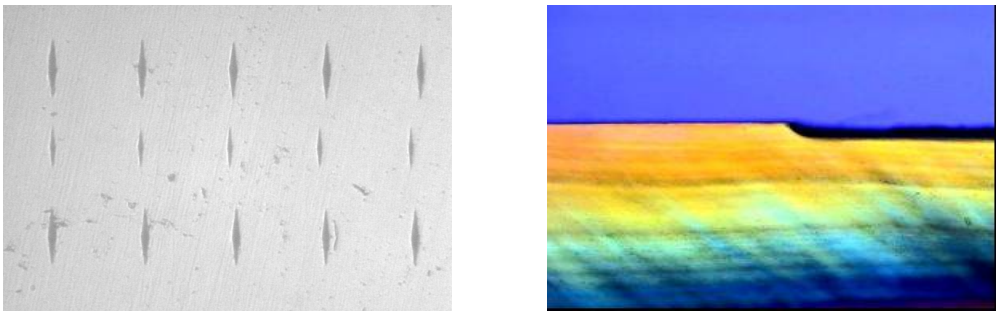

Fig. 1. Caries-like lesion on enamel, surface and lesion depth after 32h

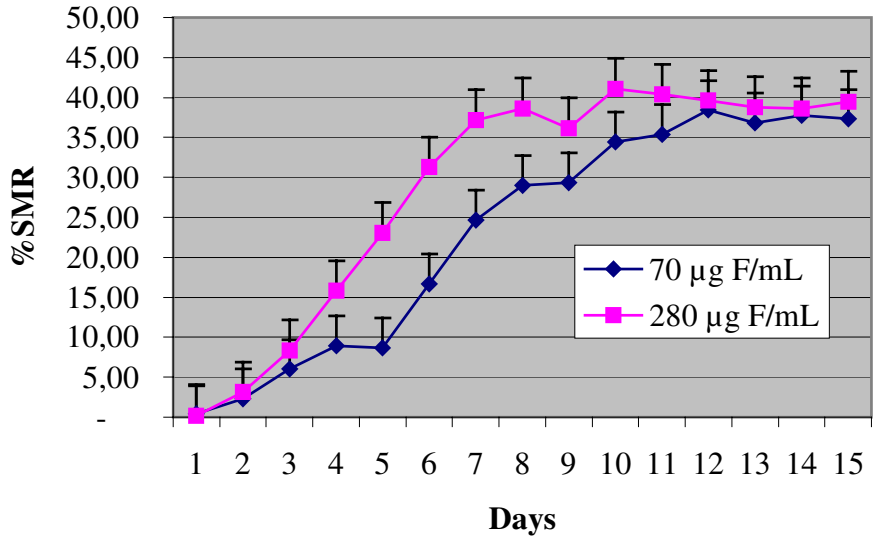

Fig. 2. Means (SD) of % surface microhardness recovery according to time and treatments with F solutions.

2.2. ARTIGO 2

pH-cycling models to evaluate the effect of fluoride dentifrice on dentin de- remineralization.

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Key words: demineralization; remineralization; fluoride; pH-cycling

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ABSTRACT

Since there are few pH-cycling models for evaluating the effect of fluoride (F) on bovine dentin, two models were developed: one to evaluate the effect of F on sound dentin resistance to demineralization and the other to evaluate the enhancement of remineralization of caries-like lesions. Blocks of bovine root dentin were submitted to the pH-cycling models and treated with F solutions containing 70, 140 and 280 µg F/mL to valid the models. These concentrations simulate the effect of F-dentifrice containing 275, 550 and 1100 µg F/g; distilled deionized water was used as a negative control. Furthermore, commercial fluoride dentifrices were evaluated: non-fluoridated dentifrice (negative control), Tandy (1,100 µg F/g, commercial Brazilian dentifrice) and Crest (1,100 µg F/g, positive control), all of them silica-based. Demineralization inhibition and remineralization enhancement were evaluated by cross-sectional microhardness, and lesion depth by polarized light microscopy. The pH-cycling models showed F dose-response effect ($p < 0.05$), but the effect of F between the solutions with 140 and 280 µg F/mL was not statistically different. The findings showed that the Brazilian F dentifrices present anti-caries potential equivalent to that of the positive control. The data suggest that the models developed are able to evaluate the anti-caries potential effect of F dentifrices containing 1,100 µg F/g on dentin resistance to demineralization or on remineralization enhancement.

INTRODUCTION

Root dentin caries is considered a dental health problem (Nyvad & Fejerskov, 1982; Burt et al, 1986). It has been documented that dentin is more susceptible to caries attacks than is enamel, with a critical pH more than one pH-unit higher than that for enamel (Hoppenbrouwers et al, 1986). On the other hand, according to clinical study (Jensen & Kohout, 1988), the use of fluoride (F) dentifrice has been effective in reducing root dentin caries.

pH-cycling models have been extensively used to evaluate the anti-caries potential of dentifrices in enamel, but few models using pH-cycling models have been developed for evaluating the effectiveness of F dentifrices in dentin. The effect of F dentifrice on resistance of human dentin to demineralization (Dunipace et al, 1994) and on enhancement of remineralization in bovine dentin (ten Cate et al, 1995; Mukai et al, 2001) was evaluated. However, a model to evaluate the resistance of bovine dentin to demineralization has not been conducted. In addition, the advantage of using bovine instead of human teeth is that they are easier to obtain and to manipulate (Mellberg, 1992). Moreover, they have a relatively more uniform chemical composition, which allows a lower variation in the experimental response of the cariogenic and anticariogenic treatments carried out on the substrate (Mellberg, 1992). Furthermore, *in situ* study showed that bovine dentin can be used instead of human dentin to evaluate caries progression and the effect of F dentifrice (Hara et al, 2003)

Thus, the aim of this study was to develop pH-cycling models and to evaluate the anti-caries potential of F dentifrice in either dentin demineralization inhibition or remineralization enhancement. The dose response effect of F was evaluated using solutions

containing 70, 140 and 280 µg F/mL, and the anti-caries potential of a Brazilian commercial dentifrice was also evaluated.

MATERIAL AND METHODS

Experimental Design

The factors under study were two conditions: demineralizing and remineralizing pH-cycling models, simulating the inhibition of caries progression, and the enhancement of remineralization, respectively. The experimental units were bovine dentin blocks. In each pH-cycling condition assessment was made of the dose response effect of F solutions and also the anti-caries potential of a Brazilian commercial F dentifrice. The quantitative response variables for the demineralizing pH-cycling model were mineral loss area (ΔZ) and lesion depth (LD); for the remineralizing pH-cycling model they were percentage of mineral area recovery (%ΔZ) and lesion depth (LD).

Demineralizing Solution preparation (ANEXO 1)

This solution was used either in demineralizing or in remineralizing pH-cycling models, and to induce caries-like lesions on the root dentin blocks. It contained 0.05 M acetate buffer, pH 5.0, 50% saturated with respect to dentin. Bovine incisors were used to prepare this solution. Dentin powder (particles of 74 - 105 µm) from ground root was added to 0.05 M acetate buffer pH 5.0 (0.5 g/L), which was kept under agitation for 96 h at 37°C to create 100% saturated solution. The filtered solution was diluted with an equal volume of the same buffer obtaining a 50% saturated solution with respect to the solubility of dentin. Fluoride (F), inorganic phosphorus (Pi) and calcium (Ca) concentrations were determined in these solutions by ion selective electrode, colorimetrically (Fiske &

Subbarow, 1925) and by atomic absorption (Spectra A 50 Varian), respectively. Means of 1.41±0.057 mM Ca, 0.91±0.017 mM Pi and 0.056±0.00078 µg F/mL were found in the 50% saturated demineralizing solution with respect to dentin.

From these results, a demineralizing solutions containing 0.05 M 50% saturated acetate buffer of Ca, Pi and F with respect to dentin were prepared, using the salts $Ca(NO_3)_2.4\ H_2O$, KH_2PO_4 and NaF respectively.

Preparation of Tooth Specimens (ANEXO 2)

Dentin blocks (4x4x2 mm) were obtained from bovine incisor teeth that were stored in 2% formaldehyde solution, pH 7.0 (White, 1987). The block surfaces were flattened and polished (Hara et al, 2003). A adhesive strip of 2.0 x 4.0 mm was placed over surface of enamel blocks, which were coated with acid-resistant varnish and after it was removed, it only left a surface area of 8.0 mm^2 exposed. The baseline dentin surface microhardness was determined using a Future-Tech FM-ARS microhardness tester with a Knoop diamond under 5 g load for 5 s. Five indentations were made at the center of the surface and blocks with hardness of 51.7 ± 17.2 Kg/mm^2, were selected for this study.

pH-Cycling Model to evaluate resistance to demineralization

Effect of dose-response to fluoride (ANEXO 3)

Fifty-two blocks were randomly distributed into 4 groups (n=13) and submitted to one of the follows treatments: distilled deionized water (negative control), 70, 140 and 280 µg F/mL. These concentrations of fluoride were chosen because they simulate the dilution (1:3 w/w) that occurs in the oral cavity when dentifrices containing 275, 550

and 1100 µg F/g respectively, are used (Duke & Forward, 1982); all fluoridated solutions were prepared from NaF.

The pH cycling regimen took 8 days, and the blocks were kept for 4 h in demineralizing and for approximately 20 h in the remineralizing solutions Twice a day (before and after the immersion in the demineralizing solutions) the blocks were washed with deionized water and submitted to the groups of treatments under agitation for 5 min. After the treatment the blocks were washed.

The blocks were kept individually in a demineralizing solution containing 1.4 mM, Ca, 0.91 mM P, 0.06 µg F/mL, pH 5.0. The remineralizing solution used contained 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, 0.1 M Tris buffer, pH 7.0 (ten Cate & Duijsters, 1982). The proportion of demineralizing and remineralizing solution volumes per area of blocks was 6.25 ml/mm^2 and 3.12 mL/mm^2, respectively. On the fourth day the de- or remineralizing solutions were changed. The experiment was carried out at 37°C. After the 8^{th} cycle the blocks remained in the remineralizing solution for 24 h until analyses.

F-dentifrice evaluation (ANEXO 3)

The effect of fluoride dentifrice on dentin demineralization inhibition was tested using the same conditions described before. Thirty blocks were randomly distributed into 3 groups (n=10) and submitted to one of the follows treatments: non-fluoridated dentifrice (negative control), Tandy (1100 µg F/g, commercial Brazilian dentifrice) and Crest (1100 µg F/g, positive control). The blocks were treated twice a day for 5 min with dentifrice/water slurries (1:3 w/w); all the dentifrices were silica-based.

pH-Cycling Model to evaluate remineralization enhancement

Caries-Like Lesion preparation (ANEXO 4)

A preliminary study to induce caries-like lesions in dentin blocks was conducted. Sixty blocks were immersed individually in demineralizing solution (2 mL/mm^2) for 8, 16, 32 and 64 h and mineral loss was evaluated. The time of 32 h (Table 1) was chosen to induce caries-like lesions in dentin (Fig 1) It was not possible to determine the surface microhardness of dentin, which suffered acid etching, but it was possible to evaluate the effect of solutions with different F concentrations.

Effect of dose-response to fluoride (ANEXO 5)

Fifty-two blocks were randomly distributed into 4 groups (n=13) and submitted to one of the following treatments: distilled deionized water (negative control), 70, 140 and 280 µg F/mL. An extra group was submitted only to caries-like induction without a pH-cycling regimen.

The pH cycling regimen took 8 days. The blocks were kept for 2 h in demineralizing and for approximately 22 h in the remineralizing solution. Three times a day (9:00, 14:00 and 17:00 h) the blocks were washed with deionized water and submitted to the groups of treatments for 1 min under agitation, to simulate exposure to tooth brushing. After the treatments the blocks were washed again.

The blocks were kept individually in a demineralizing solution containing 1.4 mM, Ca, 0.91 mM P, 0.06 µg F/mL, pH 5.0. The remineralizing solution used contained 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, 0.1 M Tris buffer, pH 7.0 (ten Cate & Duijsters, 1982). The proportion of demineralizing and remineralizing solution volumes per area of blocks was 6.25 ml/mm^2 and 3.12 mL/mm^2, respectively. On the 4^{th} day the de-or remineralizing solutions were changed. The experiment was carried out at 37°C.

F-dentifrice evaluation (ANEXO 5)

The effect of F dentifrices on dentin remineralization was tested using the same conditions described before. Thirty blocks were randomly distributed into 3 groups (n=10) and submitted to one of the following treatments: non-fluoridated dentifrice (negative control), Tandy (1100 µg F/mL, commercial Brazilian dentifrice) and Crest (1100 µg F/mL, positive control). The blocks were treated 3x/ day for 1 min with dentifrice/water slurries (1:3 w/w), as described for the treatments with F solutions.

Microhardness Analysis (ANEXOS 6 – 7)

The blocks were longitudinally sectioned in their central area and one half of each block was embedded in acrylic resin and the cut surfaces were exposed and polished. Three rows of 14 indentations each were made at 10 µm up to 200 µm depths from the outer surface of the blocks. The mean values at all 3 measuring points at each distance from the surface were then averaged. CSM values were converted to volume % mineral (Featherstone et al, 1983) and the mineral loss (ΔZ) was obtained (Featherstone & Zero, 1992).

After the remineralization regimes, the mineral area values for each experimental group were calculated (ΔZ_2) and compared with the mineral loss area (ΔZ_1) of the extra group, which was submitted only to caries lesion without pH-cycling regimen. The difference between ΔZ_1 and ΔZ_2 was obtained. Thus, the data were converted to percentage of mineral area recovery ($\%\Delta Z$) using the following relation: $\%\Delta Z = (\Delta Z_1 - \Delta Z_2) \times 100 / \Delta Z_1$.

Polarized light microscopy analysis (ANEXO 8)

The other half of the remaining halves of each block was longitudinally sectioned, in order to obtain a section of 100 μm (±10). These sections were mounted for examination under a polarizing light microscope at x100 magnification (DMLSP, Leica) after imbibition in deionized water and in air. Digital images were taken and the lesion depths were measured at three sites with an Image-Pro Plus software (Media Cybernetics), in each site five measurements were made and averaged.

Statistical Analysis

The (ANEXOS 15 - 16) mineral loss area data (ΔZ), percentage of mineral area recovery (%ΔZ) and lesion depth (LD) were subjected to one-way analysis of variance (ANOVA), followed by the Tukey and Dunnett tests (α=0.05). To show the dose-response effect of fluoride the data were analyzed by ANOVA and the regression rate coefficient was determined. The analyses were performed with the SAS System 8.01 software (SAS Institute Inc.).

RESULTS

With regard to demineralization inhibition Table 2 shows that ΔZ and LD decreased in all groups in comparison with the control, but there was no difference between 140 and 280 μg F/mL to ΔZ. A dose-response effect was found among fluoride concentrations with respect to ΔZ (R^2 =0.6548; p<0.0001) and LD (R^2 =0.8265; p=0.0017, quadratic correlation). With respect to the dentifrice evaluations, Table 2 also shows that the commercial Brazilian dentifrices were statistically more effective in reducing ΔZ and LD when compared to the placebo and were as effective as the positive control.

With regard to enamel remineralization Table 3 shows that %ΔZ increased in all groups in comparison with the control, but there was no difference between 140 and 280 µg F/mL. The results for LD were significantly different among the solutions tested. A dose-response effect was found among fluoride concentrations for %ΔZ (R^2 =0.7071; p<0.0001, quadratic correlation) and LD (R^2 =0.8819; p<0.0001, cubic correlation). With respect to the dentifrice evaluations, Table 3 also shows that F dentifrices were statistically more effective for increasing %ΔZ and LD than for the placebo. The commercial Brazilian dentifrice was as effective as the positive control.

DISCUSSION

The dose-response effect using solutions with different F concentrations was obtained. These results are according to Dunipace et al, (1994), who also obtained dose-response using F solutions (0, 250 and 1,100 µg F/mL). However, there are some considerations to be made: in the study conducted by Dunipace, the demineralizing solution used was 50% saturated with hydroxyapatite and the model was conducted at room temperature of 20-22^{o}C. In the present study, the demineralizing solution used, was 50% saturated with respect to dentin for caries-like formation, and the model was conducted at room temperature of 37^{o}C for it to be closer to oral conditions.

The time of 32h for inducing caries-like lesions in root dentin produced shallow lesions (95.4 ± 5.8 µm - Table 1), and it was not possible to measure the surface microhardness. The decreased surface layer thickness suggests rapid transport through the tubules, followed by a slower demineralization of the intertubular matrix (Melberg & Sanches, 1986).

For caries-like formation, F has been added to demineralizing solutions (Damem et al, 1998; ten Cate et al, 1998). In this study, the F concentration in the demineralizing solution (0.06 μg F/mL) corresponded to the concentration found in the 50% saturated demineralizing solution from dentin powder. The reagents used for preparing the demineralizing solution contained F (0.03 μg F/mL), which was checked with an ion specific electrode and another 0.03 μg F/mL was added. The low fluoride concentrations used (0.06 μg F/mL) did not interfere in the response of the treatments, as can be observed in Tables 2 and 3, and was relevant for avoiding erosive demineralization. The addition of F in the preparation of demineralizing or remineralizing solutions should be considered, so that no interference occurs in the results.

Experiments with dentin substrates have been conducted using high F concentrations (above 1100 pp F). Mukai et al (2001), observed that a solution containing 4,000 μg F/mL was more effective than F dentifrice (1,450 μg F/g) and Baysan et al (2001) observed that high-F toothpaste (5,000 μg F/g) was more effective that conventional toothpaste (1,100 μg F/g). It is probable that the model developed in this study could not evaluate high F dentifrices; the results (Table 3) show a maximum efficacy of 140 μg F/mL in solutions.

In conclusion, the data suggest that the models developed are able to evaluate the anti-caries potential of F dentifrices for dentin either in demineralization inhibition or remineralization enhancement.

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18. White DJ: Reactivity of fluoride dentifrices with artificial caries. **Caries Res.** 1987; 21:126-40.

Table 1. Mineral loss (ΔZ) and lesion depth (LD) in dentin, according to the time (h) in the demineralizing solution (Mean ± SD).

Time (h)	ΔZ	LD
8	314.2 ± 71.9a	48.5 ± 2.7a
16	312.1 ± 57.4a	57.9 ± 2.0b
32	643.3 ± 159.9b	95.4 ± 5.8c
64	1165.8 ± 150.7c	208.9 ± 3.9d

Treatments whose means are followed by different letters differ statistically (5%)

Table 2. Analysis of dentin submitted to the demineralization model, according to treatments groups of fluoride solutions or dentifrices (Mean ± SD).

Groups	Analyses	
Fluoride solutions n=13	ΔZ	LD
0	927.0 ± 641.4a	100.1 ± 2.2a
70	363.5 ± 134.1b	96.7 ± 3.1a
140	203.3 ± 50.9c	69.1 ± 2.6b
280	196.7 ± 39.2c	64.0 ± 1.8c
Dentifrices n=10		
Negative control	864.7 ± 385.9A	89.6 ± 5.9A
Active dentifrice	241.4 ± 68.4B	54.9 ± 1.8B
Positive control	246.3 ± 37.5B	51.6 ± 1.9B

Treatments whose means are followed by different letters differ statistically (5%), lower case among F solutions and capital letters among dentifrices

Table 3. Analysis of dentin submitted to the remineralization model, according to treatments groups of fluoride solutions or dentifrices (Mean ± SD).

Groups	Analyses	
Fluoride solutions n=13	%ΔZ	LD
0	11.3 ± 3.1a	81.0 ± 3.5a
70	20.6 ± 2.4b	64.1 ± 5.6b
140	29.9 ± 6.6c	31.6 ± 6.6c
280	32.2 ± 14.1c	28.8± 4.6d
Dentifrices n=10		
Negative control	19.9 ± 6.3A	73.5 ± 4.8A
Active dentifrice	48.1 ± 4.9B	39.1 ± 3.4B
Positive control	49.8 ± 10.4B	42.1 ± 1.4B

Treatments whose means are followed by different letters differ statistically (5%), lower case among F solutions and capital letters among dentifrices

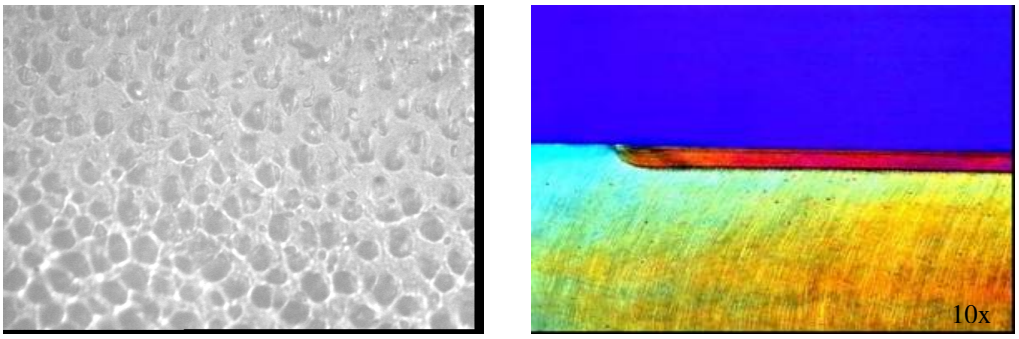

Fig. 1. Caries-like lesion on dentin, surface and lesion depth after 32h

2.3. ARTIGO 3

Influence of the mineral content and morphological pattern of artificial root caries lesion on composite resin bond strength

Anderson Takeo Hara

Celso Silva Queiroz

Marcelo Giannini

Jaime Aparecido Cury

Mônica Campos Serra

Eur J Oral Sci. 2004 Feb; 112(1):67-72.

3. CONCLUSÃO

Os resultados sugerem que os modelos *in vitro* são capazes de avaliar o efeito do F na desmineralização e remineralização do esmalte e dentina bovina.

Conclusões específicas:

1. O modelo *in vitro* desenvolvido é capaz de avaliar o efeito anticárie de dentifrícios com baixa concentração de F na resistência da des- e ativação da remineralização no esmalte bovino.

2. O modelo *in vitro* desenvolvido é capaz de avaliar o efeito anticárie de dentifrícios fluoretados na resistência da des- e ativação da remineralização na dentina bovina.

3. A morfologia da dentina pode ser mais relevante que o conteúdo mineral para explicar a resistência da união da resina composta à dentina.

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*De acordo com a norma utilizada na FOP/UNICAMP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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13. Mukai Y, Lagerweij MD, ten Cate JM: Effect of a solution with high fluoride concentration on remineralization of shallow and deep root surface caries in vitro**. Caries Res.** 2001; Sep-Oct;35(5):317-24

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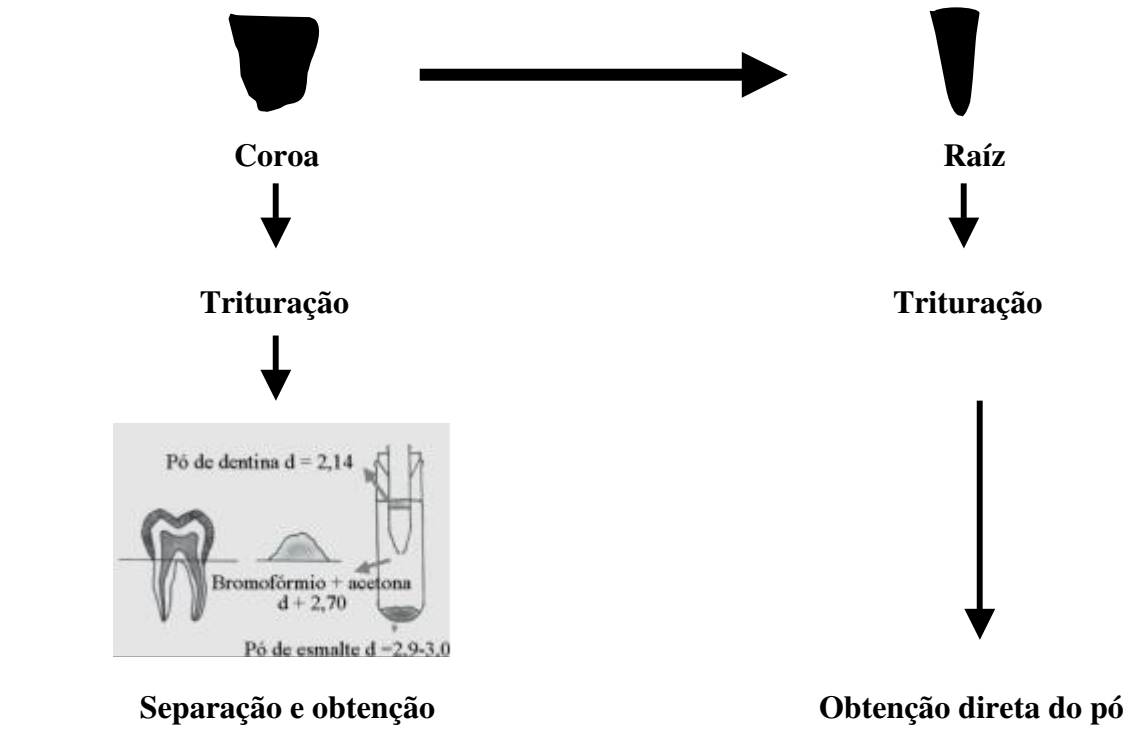
ANEXO 1

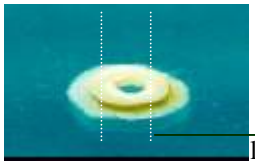
Preparo das soluções desmineralizantes para esmalte e para dentina bovina



ANEXO 2

Obtenção dos blocos de esmalte e de dentina bovina

Separação da coroa/raiz

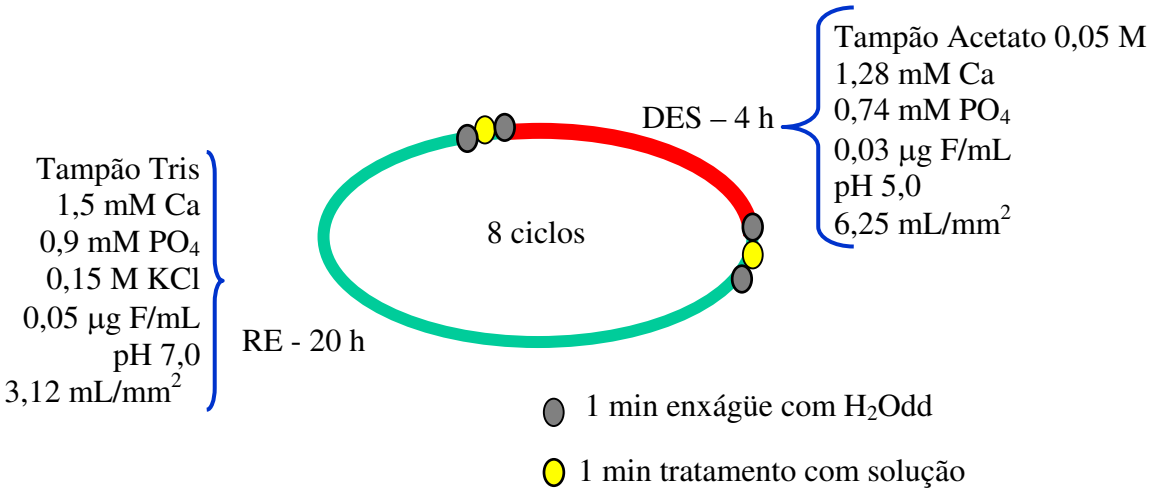


Blocos de Dentina (4x4x2 mm^2)

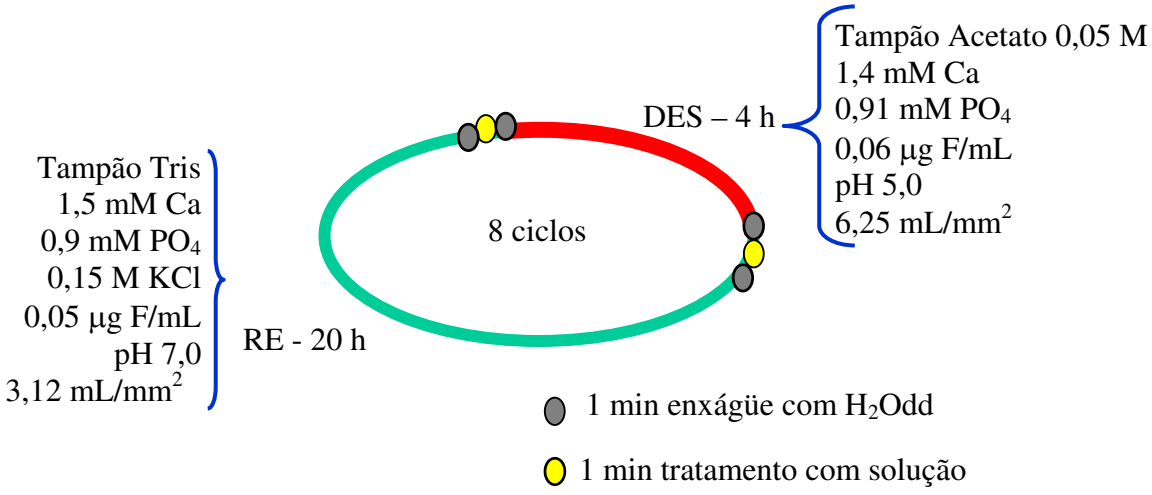
Blocos de Esmalte (4x4x3 mm^2)

ANEXO 3

Modelo de ciclagem para avaliar a resistência da desmineralização (Esmalte Bovino)

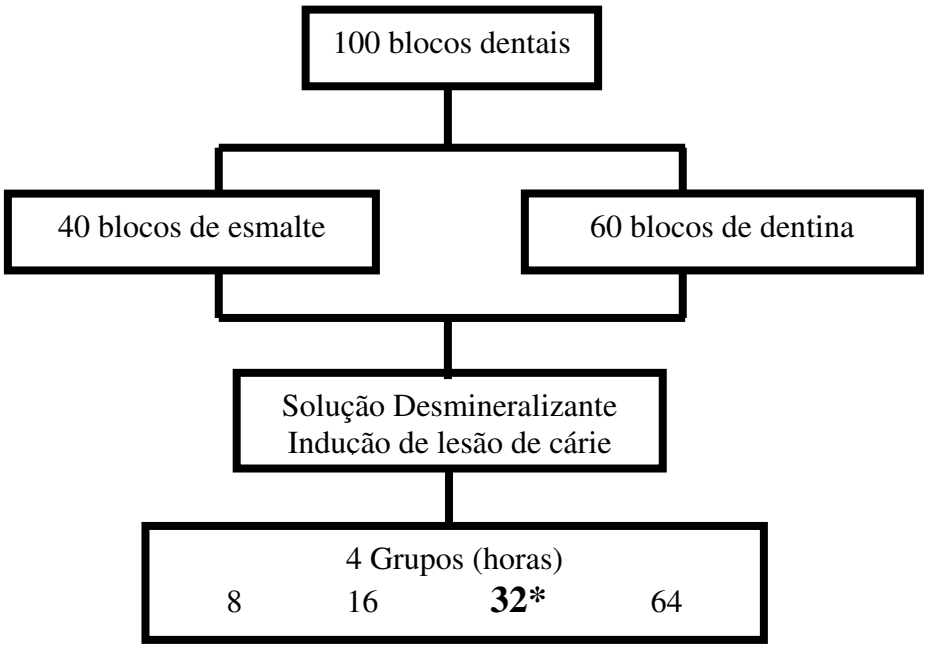


Modelo de ciclagem para avaliar a resistência da desmineralização (Dentina Bovina)



ANEXO 4

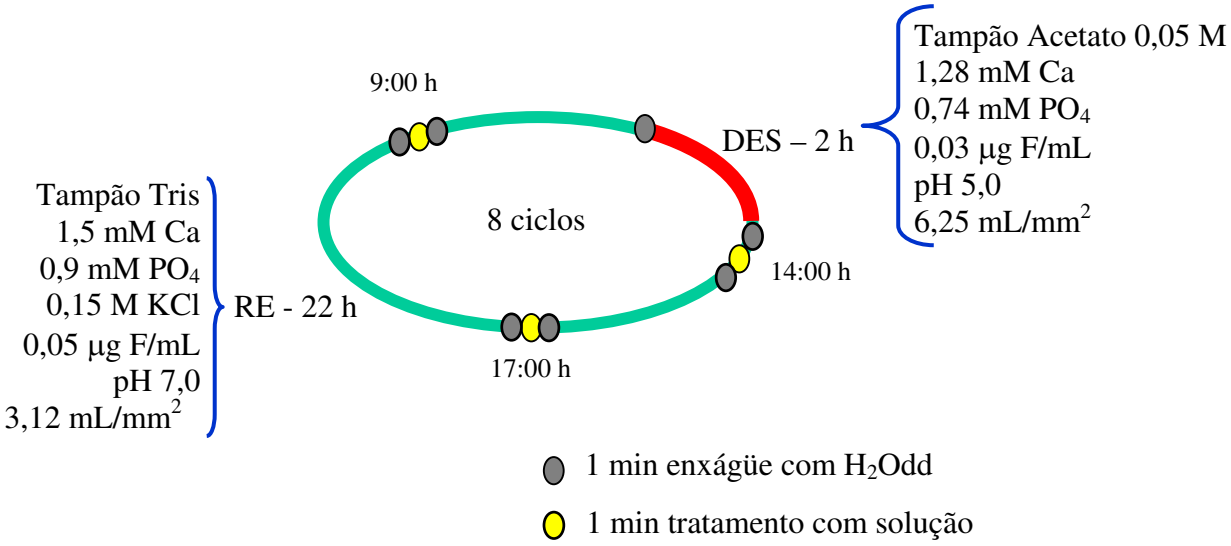
Determinação do tempo de indução de cárie artificial nos blocos de esmalte e dentina



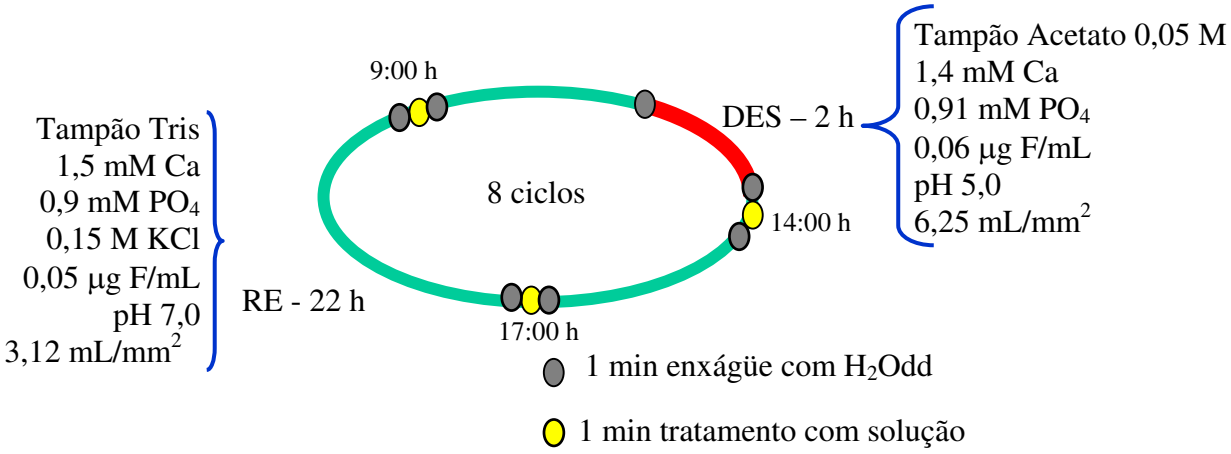
***tempo escolhido para induzir lesão em esmalte e dentina bovina.**

ANEXO 5

Modelo de ciclagem para avaliar a ativação da remineralização (Esmalte Bovino)



Modelo de ciclagem para avaliar a ativação da remineralização (Dentina Bovina)

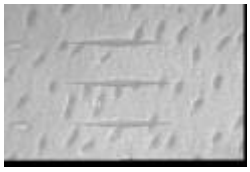


ANEXO 6

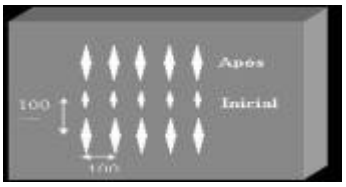
Análise de microdureza (Dureza de Superfície)

Microdurômetro

Superfície do bloco de esmalte hígido



Superfície do bloco de dentina hígida



Esquema das indentações iniciais (Baseline) e das posteriores aos tratamentos na superfície dos blocos

Fórmula para obter a % da perda da dureza de superfície:

% PDS = Dureza após ciclagem – Dureza inicial x 100
Dureza inicial

Fórmula para obter a % de recuperação da dureza de superfície:

% RDS = Dureza após o tratamento – Dureza após a formação d lesão x 100
Dureza inicial - Dureza após a formação d lesão

ANEXO 7

Análise de microdureza (Área da lesão de cárie)

Após as análises de microdureza de superfície, os blocos foram:

Secionados ao meio

Embutidos em acrílico

Lixados e polidos

microdureza longitudinal

Após a leitura da dureza nas diversa profundidades em cada bloco representamos os resultados em ΔZ (área da lesão) ou $\%\ \Delta Z$ (recuperação da área da lesão).

ANEXO 8

Análise da profundidade da lesão através da técnica de microscopia de Luz Polarizada

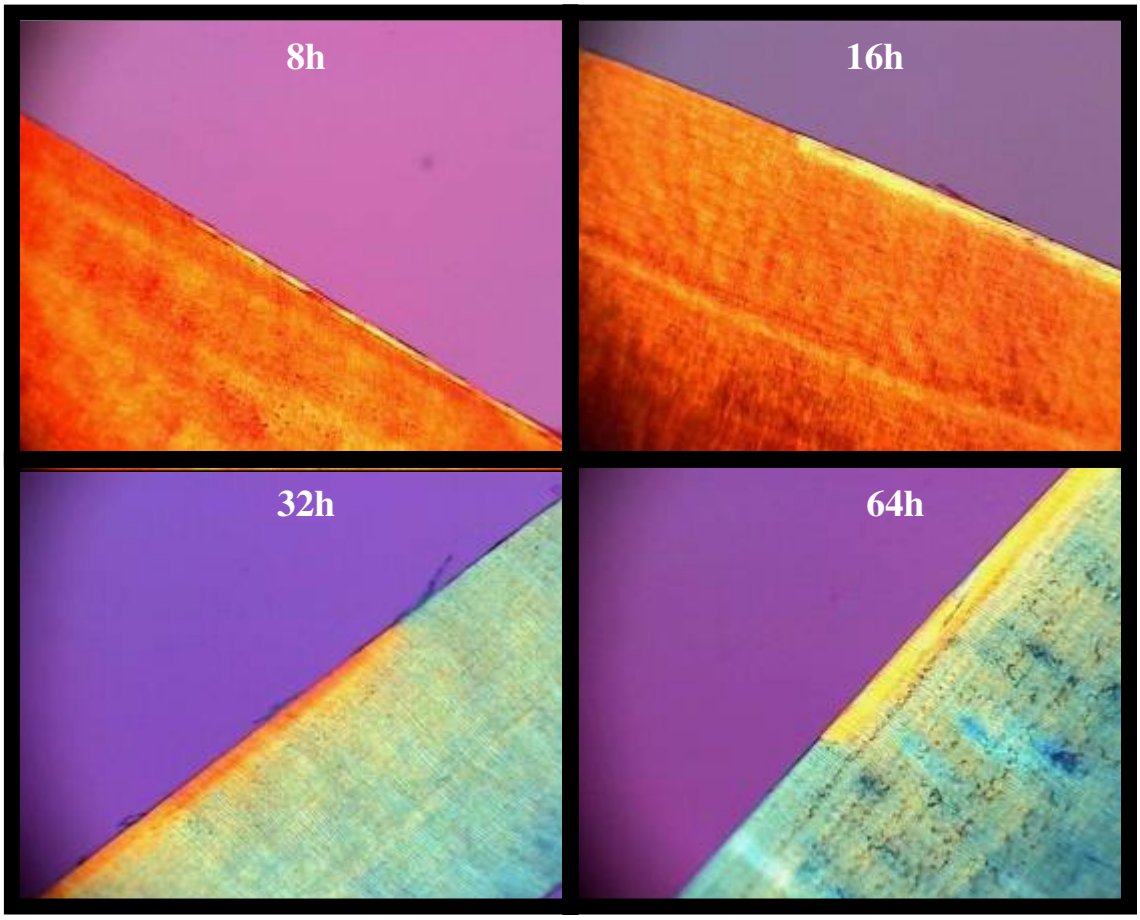
Quando os blocos de esmalte ou de dentina foram seccionados ao meio para a análise de microdureza longitudinal (anexo 7), as outras metades foram seccionadas no micrótomo de Silverstone, obtendo espécimes de 100± 10 μm e analisada no microscópio de luz polarizada (DMLSP - Leica)

Micrótomo

Microscópio de Luz Polarizada

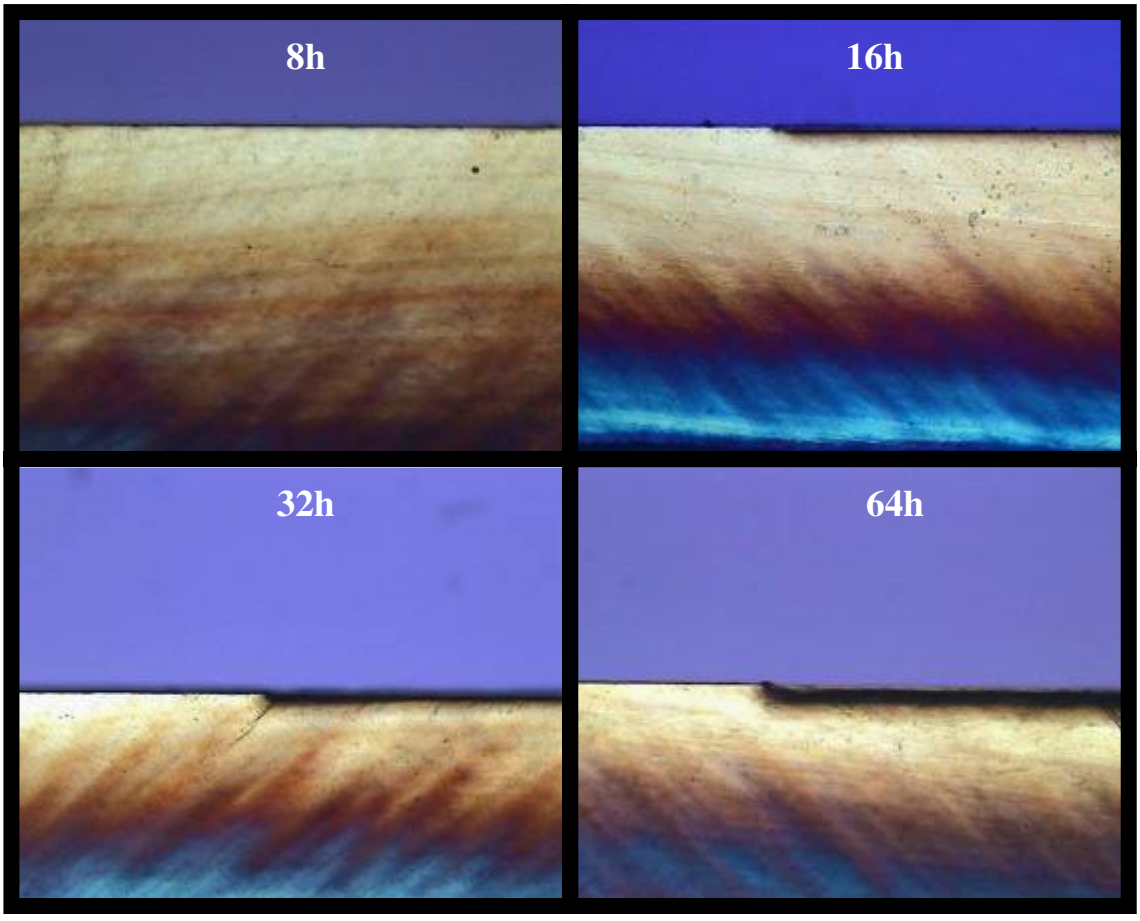
ANEXO 9

Imagens da profundidade da lesão da dentina analisada por microscopia de luz polarizada submetida à indução de cárie em diferentes tempos



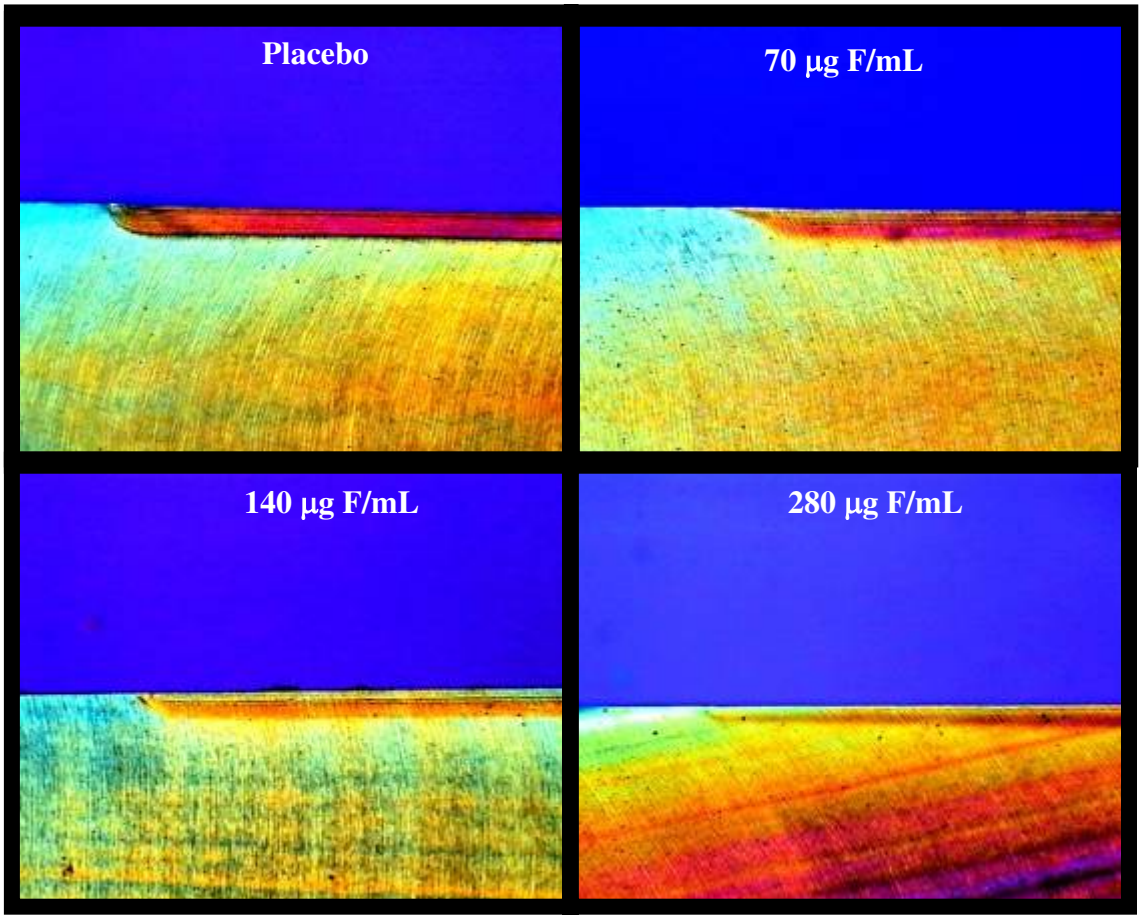
ANEXO 10

Imagens da profundidade da lesão do esmalte analisada por microscopia de luz polarizada submetido à indução de cáries em diferentes tempos



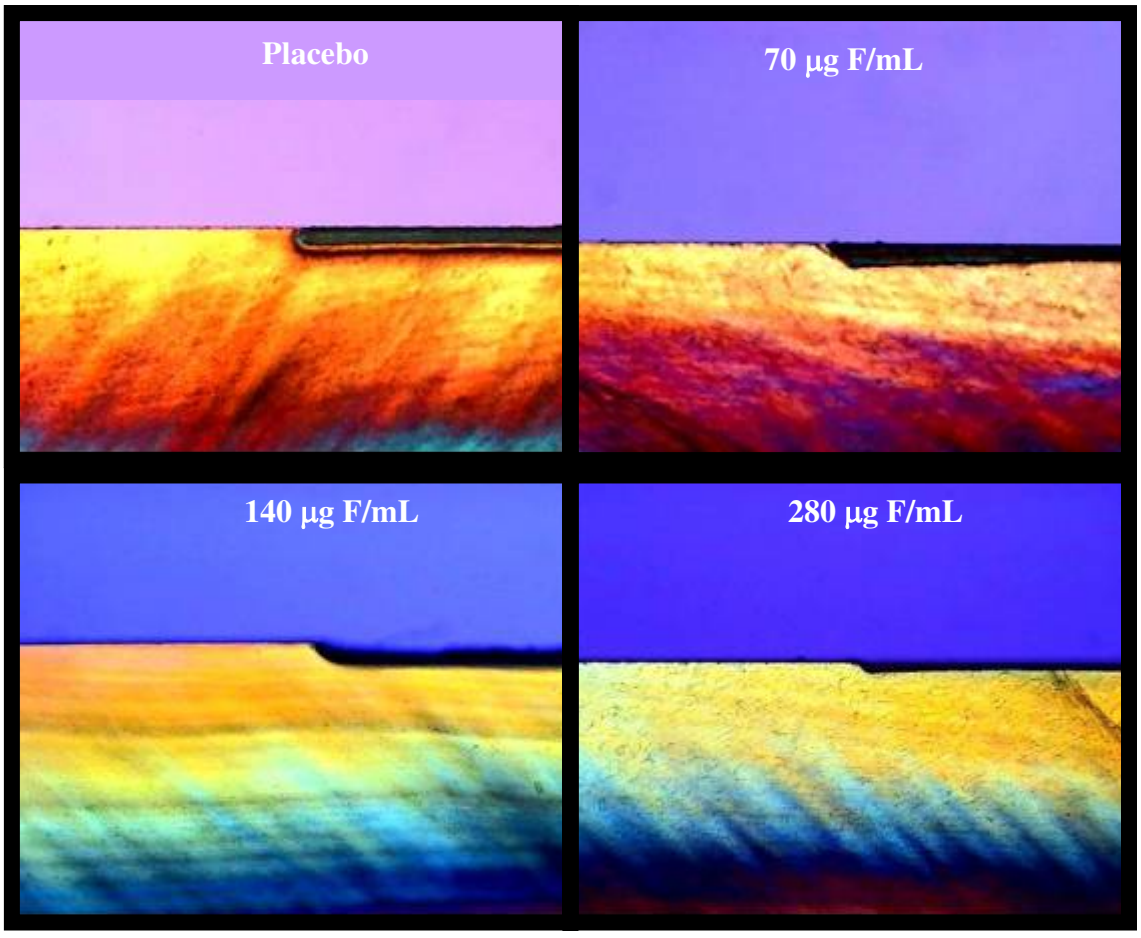
ANEXO 11

Imagens da profundidade da lesão da dentina analisada por microscopia de luz polarizada submetida à ciclagem de inibição da desmineralização



ANEXO 12

Imagens da profundidade da lesão do esmalte analisada por microscopia de luz polarizada submetida à ciclagem de inibição da desmineralização



ANEXO 13

Dados das análises de microdureza e profundidade da lesão submetidos à análise estatística – ciclagem de inibição da desmineralização em esmalte.

Cilagem Des Esmalte - Solução

% PDS				Deta Z				PL			
Placebo	70	140	280	Placebo	70	140	280	Placebo	70	140	280
73,5	51,8	41,7	40,4	1593,8	795,3	382,4	296,5	65,5	45,7	35,0	26,8
70,5	55,3	43,6	39,4	1018,0	702,2	451,4	258,9	61,3	46,0	34,4	23,6
75,4	56,5	44,9	38,2	1493,8	614,8	375,2	246,1	65,8	45,1	33,5	25,8
68,9	57,0	44,1	38,8	1036,6	439,0	389,4	277,6	65,7	45,9	34,6	25,7
74,5	56,7	41,1	35,3	1221,9	646,6	348,0	382,2	68,5	45,6	34,4	27,9
75,4	52,3	39,5	39,8	1430,6	587,7	300,0	443,6	63,7	45,1	33,6	25,7
73,8	50,5	41,1	32,0	1435,7	798,8	189,3	372,2	58,4	47,3	36,7	25,0
72,3	55,0	39,9	42,5	1292,8	626,0	439,2	107,7	67,4	45,7	35,7	37,4
73,8	52,6	43,8	38,0	1356,2	374,2	327,5	279,8	63,0	45,3	37,0	39,7
72,3	55,8	38,8	39,5	1094,4	782,7	390,4	316,0	63,1	44,1	33,6	26,5
72,2	49,1	42,4	39,4	1090,9	413,6	412,7	128,3	66,1	31,1	35,8	29,0
74,4	51,0	44,2	37,1	1774,6	682,5	261,2	296,2	62,3	42,9	35,5	25,7
70,8	55,2	43,7	38,5	1249,3	536,9	366,2	397,5	60,8	44,1	35,6	26,5

Cilagem Des Esmalte - dentifrício

% PDS				Deta Z				PL			
Placebo	Baby	Tandy	Crest	Placebo	Baby	Tandy	Crest	Placebo	Baby	Tandy	Crest
75,5	45,7	37,9	38,3	1457,0	606,0	321,7	402,3	85,5	45,7	37,0	30,3
74,6	46,0	33,6	34,4	1466,0	687,7	401,6	379,2	84,6	76,0	33,6	34,4
75,8	65,2	35,0	33,1	1835,3	887,5	483,5	484,1	85,8	65,2	34,4	33,5
75,7	45,9	33,5	39,7	1268,5	754,5	358,1	143,4	85,7	45,9	33,5	29,7
75,4	45,6	29,9	31,1	1844,1	639,5	286,6	209,4	85,1	45,6	27,9	31,1
73,7	45,3	35,7	38,2	1261,3	799,0	480,3	497,5	83,7	45,1	25,7	30,4
78,4	48,0	28,9	36,7	1490,8	942,9	392,3	278,5	78,4	47,3	25,0	36,7
72,8	45,7	37,4	32,4	1689,1	942,3	391,1	479,5	80,8	45,7	27,4	32,4
71,9	45,3	29,7	36,1	1616,0	880,6	464,5	477,1	89,7	45,3	29,7	37,0
73,9	44,1	36,5	33,6	1766,7	751,0	418,5	412,8	83,1	44,1	26,5	33,6

ANEXO 14

Dados das análises de microdureza e profundidade da lesão submetidos à análise estatística – ciclagem de ativação da remineralização em esmalte.

Cilagem Re Esmalte - Solução

% RDS			
Placebo	70	140	280
14,9	36,6	32,5	65,3
11,1	34,5	51,4	61,5
15,6	33,5	40,3	43,6
14,4	33,5	44,0	44,4
15,5	38,2	43,9	41,0
8,0	32,9	41,8	44,5
9,9	33,3	33,4	49,3
16,8	25,2	35,7	45,1
17,3	36,0	51,4	45,0
13,0	35,2	38,2	47,3
13,9	32,5	44,7	52,1
15,2	46,1	49,1	40,8
14,9	31,2	40,5	58,8

% Deta Z recup			
Placebo	70	140	280
2,8	54,5	64,8	66,3
8,3	50,3	61,6	73,4
6,0	54,0	56,3	56,1
14,7	68,5	53,0	73,1
2,4	60,8	61,1	68,3
5,0	55,2	61,5	69,9
6,0	56,6	56,4	72,8
14,3	44,3	65,7	56,8
9,8	60,3	67,8	73,9
17,3	54,0	60,3	64,9
11,6	65,4	60,2	64,5
7,2	58,8	61,8	48,1
19,6	45,3	62,1	66,8

PL			
Placebo	70	140	280
85,5	65,6	27,0	25,0
79,3	56,0	23,6	24,4
76,8	65,1	24,4	23,5
85,7	65,9	23,5	34,6
55,1	58,9	23,5	34,6
76,0	34,4	25,7	23,6
91,7	37,3	25,0	36,7
84,0	69,0	27,4	31,4
75,4	65,3	49,7	37,0
89,1	44,1	26,5	23,6
64,5	54,8	49,5	33,6
73,1	65,1	36,5	23,1
83,1	58,1	46,5	32,8

Cilagem Re Esmalte - dentifrício

% RDS			
Placebo	Baby	Tandy	Crest
6,7	25,7	32,5	37,6
7,4	24,5	39,2	38,5
5,5	28,0	40,3	43,6
14,4	24,4	44,0	44,4
7,5	28,4	43,9	41,0
5,3	24,3	41,8	44,5
9,9	25,9	33,4	37,3
8,6	25,2	35,7	37,8
12,3	30,9	39,2	45,0
13,0	25,2	38,2	39,0

% Deta Z recup			
Placebo	Baby	Tandy	Crest
7,4	26,4	59,0	57,2
5,1	20,1	37,3	53,3
6,2	49,4	46,5	46,8
5,7	25,2	67,2	42,8
3,1	30,1	61,4	52,6
6,1	26,2	63,3	53,1
6,2	43,6	66,9	46,9
7,6	50,9	47,5	58,2
6,4	25,6	68,2	60,8
7,9	34,1	45,1	51,7

PL			
Placebo	Baby	Tandy	Crest
66,9	45,7	17,0	15,0
62,6	46,4	13,6	14,4
66,1	45,1	14,4	13,2
66,7	55,9	13,2	14,6
65,9	45,6	13,2	13,4
66,0	45,1	19,0	33,2
62,5	43,9	15,0	36,7
74,1	42,3	27,4	32,4
63,0	45,6	29,7	17,0
53,1	44,8	26,5	17,0

ANEXO 15

Dados das análises de microdureza e profundidade da lesão submetidos à análise estatística – ciclagem de inibição da desmineralização em dentina.

Cilagem Des Dentina - Solução

Deta Z			
Placebo	**70**	**140**	**280**
767,3	734,8	227,2	255,5
1596,6	384,6	243,5	205,1
1856,6	312,8	254,4	153,6
1480,6	471,3	254,5	241,9
2022,9	407,8	242,0	190,0
1400,4	324,5	229,8	231,8
469,5	324,4	124,1	231,2
372,8	399,8	195,4	197,8
583,7	380,2	128,9	204,1
472,8	239,5	186,0	116,1
374,8	265,2	258,4	166,8
301,3	216,2	164,8	163,8
352,3	264,2	134,1	199,9

PL			
Placebo	**70**	**140**	**280**
103,7	95,7	65,0	65,5
101,6	94,7	67,7	61,3
99,7	95,1	70,2	65,8
94,9	95,9	71,2	65,7
94,0	95,6	67,8	68,5
110,9	98,4	63,6	63,7
107,6	100,6	73,3	58,4
104,1	95,7	75,7	67,4
98,6	95,3	70,3	63,0
91,9	100,8	73,6	63,1
99,3	95,1	65,8	66,1
96,3	96,6	65,5	62,3
98,2	97,5	68,9	60,8

Cilagem Des Dentina - dentifrício

Deta Z			
Placebo	**Baby**	**Tandy**	**Crest**
827,6	659,6	244,6	276,7
1178,4	384,6	366,5	224,9
1346,5	312,8	261,8	306,4
1070,5	468,8	283,5	234,3
1364,5	417,7	248,8	209,7
1018,7	331,9	291,2	239,4
466,9	367,2	235,9	242,6
315,0	397,3	147,3	306,4
588,5	380,4	136,6	204,3
470,2	247,1	198,1	218,4

PL			
Placebo	**Baby**	**Tandy**	**Crest**
83,7	65,8	65,0	45,5
101,6	52,2	67,7	41,3
72,3	58,9	35,5	65,8
94,9	66,1	61,6	65,7
94,0	62,3	64,3	25,1
84,2	50,8	33,6	63,7
84,3	100,6	65,8	58,4
90,8	95,7	65,1	24,1
98,6	95,3	67,0	63,0
91,9	54,1	23,6	63,1

ANEXO 16

Dados das análises de microdureza e profundidade da lesão submetidos à análise estatística – ciclagem de ativação da remineralização em dentina.

Cilagem Re Dentina - Solução

% Deta Z recup			
Placebo	**70**	**140**	**280**
10,7	18,0	24,3	35,4
12,8	12,171	24,1	21,8
14,5	19,218	35,9	37,2
9,8	23,0	35,3	34,3
14,3	20,8	33,1	38,3
9,8	25,2	41,5	29,9
7,3	26,6	26,4	22,8
11,6	24,3	25,7	36,8
10,6	19,3	27,8	23,9
12,2	20,0	20,3	48,9
10,6	15,4	30,2	24,5
10,9	18,8	41,8	18,1
11,6	25,3	22,1	46,8

PL			
Placebo	**70**	**140**	**280**
78,5	65,7	31,7	30,3
85,2	66,4	24,4	27,0
82,4	68,5	29,6	34,4
77,7	55,9	37,9	29,5
85,1	58,9	33,5	24,6
76,0	64,4	35,7	24,6
71,7	67,3	35,0	26,7
84,1	59,0	27,4	32,4
86,4	45,3	29,7	27,0
69,1	44,1	31,5	23,6
80,1	84,8	31,5	33,6
83,1	75,1	26,5	34,1
93,1	78,1	36,5	26,8

Cilagem Re Dentina - dentifrício

% Deta Z recup		
Placebo	**Tandy**	**Crest**
24,9	44,0	52,2
24,0	43,8	44,7
14,0	52,6	53,5
19,7	52,1	52,8
23,1	41,4	52,6
26,2	43,3	52,1
16,2	52,9	46,9
17,6	47,5	48,2
15,6	58,2	42,8
17,9	45,1	51,7

PL		
Placebo	**Tandy**	**Crest**
75,2	47,7	47,0
71,9	42,4	43,6
89,1	42,6	44,4
77,7	37,9	33,5
65,9	33,2	42,4
62,0	39,0	33,2
62,5	35,0	56,7
74,1	37,4	42,4
83,0	39,7	42,0
73,1	36,5	36,2

ANEX0 17

PRODUÇÃO DURANTE O PERÍODO DO CURSO DE DOUTORAMENTO:

ARTIGOS PUBLICADOS (ou Aceitos) EM REVISTAS INTERNACIONAIS

1. QUEIROZ CS; HAYACIBARA FM; TABCHOURY CPM; KLEIN FM; CURY JA.Relationship Among Stressfull Situation, Salivary Flow Rate And Oral Volatile Sulfur-Containing Compounds**. Eur J Oral Sci** 110: 337-340, 2002

2. HARA AT; QUEIROZ CS; PAES LEME AF; CURY JA; SERRA MC. Caries Progression And Inhibition In Human And Bovine Root Dentine In Situ **Caries Res**, 37(5):339-44, 2003.

3. HARA AT; QUEIROZ CS; GIANINI M; CURY JA; SERRA MC. Influence Of The Mineral Content And Morphological Pattern Of Artificial Root Caries Lesion On Composite Resin Bond Strength. **Eur J Oral Sci**, 111: 1-7, 2003

4. HAYACIBARA MF; QUEIROZ CS; TABCHOURY CPM; CURY JA. Fluoride And Aluminum In Teas And Tea-Based Beverages**. Rev Saúde Pública**, 38(1):100-5, 2004.

ARTIGOS PUBLICADOS (ou Aceitos) EM REVISTAS NACIONAIS

1. HAYACIBARA FM; QUEIROZ CS; LIMA YBO; GONÇALVES NCLAV; PAES LEME AF; GOMES MJ; KOZLOWSKI FC. Alkali-Soluble Fluoride Deposition On Enamel After Professional Application of Topical Fluoride In Vitro. **J Appl Oral Sci**, 12(1):18-21, 2004.