Nathália Viana de Souza Cruz

Efeito de dentifrícios com concentração reduzida de fluoreto associado ao trimetafosfato de sódio sobre a erosão do esmalte dentário

Araçatuba – SP

2013

Nathália Viana de Souza Cruz

Efeito de dentifrícios com concentração reduzida de fluoreto associado ao trimetafosfato de sódio sobre a erosão do esmalte dentário

Tese apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba para obtenção de título de Mestre em Ciência Odontológica- Área de Concentração: Saúde Bucal da Criança.

Orientador: Profº Drº Alberto Carlos Botazzo Delbem

Araçatuba – SP

2013

Catalogação na Publicação (CIP)

Serviço Técnico de Biblioteca e Documentação – FOA / UNESP

Cruz, Nathália Viana de Souza.

C955e Efeito de dentifrícios com concentração reduzida de fluoreto associada ao trimetafosfato de sódio sobre a erosão do esmalte dentário / Nathália Viana de Souza Cruz. - Araçatuba : [s.n.], 2013

63 f. : il. ; tab. + 1 CD-ROM

Mestrado (Dissertação) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba

Orientador: Prof. Dr. Alberto Carlos Botazzo Delbem

1. Fluoretos 2. Cremes dentais 3. Fosfatos

Black D27

CDD 617.645

Dados Curriculares

Nathália Viana de Souza Cruz

Nascimento	05.04.1988 – Dourados- MS
Filiação	Lértio Francisco da Cruz Magnória Viana de Souza Cruz
2007/2010	Curso de Graduação em Odontologia pela Faculdade de Odontologia de Araçatuba, FOA-UNESP.
2008-2010	Desenvolvimento de Projeto de Iniciação Científica.
2011/2012	Curso de Pós Graduação em Odontopediatria, nível de Mestrado, pela Faculdade de Odontologia de Araçatuba, FOA - UNESP, com auxílio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).
2011/2012	Curso de Pós Graduação em Odontopediatria, nível de Especialização, pela Faculdade de Odontologia de Araçatuba, FOA - UNESP.
Associações	CROSP - Conselho Regional de Odontologia de São Paulo SBPqO - Sociedade Brasileira de Pesquisa Odontológica

Dedicatória

Dedico este trabalho,

À minha família, que sempre me apoia e acredita na minha capacidade. Meu agradecimento pelas horas em que ficaram ao meu lado, não me deixando desistir e me mostrando que sou capaz de chegar onde desejo. Sem dúvida foram vocês quem me deram o maior incentivo para conseguir concluir mais esse trabalho.

Agradecimentos

À Deus, pela saúde, fé e perseverança que tem me dado.

Ao meu Orientador, Prof. Dr. Alberto Carlos Botazzo Delbem, agradeço por todos os ensinamentos e por me ajudar a concluir este trabalho.

Aos demais professores da Faculdade de Odontologia de Araçatuba – UNESP, em especial aos professores do Programa de Pós Graduação em Ciência Odontológica,
pela paciência, dedicação e ensinamentos disponibilizados nas aulas e clínicas, cada um de forma especial contribuiu para a conclusão desse trabalho e consequentemente para minha formação profissional.

Ao funcionário Mário Luís da Silva, pela paciência, amizade e companheirismo durante todo o curso.

À funcionária Maria dos Santos Ferreira Fernandes, pelo auxílio durante esse período.

Aos alunos de Mestrado, Doutorado, Pós-Doutorado e Iniciação Científica, pela convivência e momentos que passamos juntos, pela amizade que construímos ao longo desse tempo.

À Faculdade de Odontologia de Araçatuba, na pessoa dos professores Drª Ana Maria Pires Soubhia, digníssima Diretora e Dr. Wilson Roberto Poi, digníssimo Vice-Diretor, pela oportunidade de realizar o curso de Mestrado e Especialização nesta instituição.

Ao Curso de Pós-Graduação em Ciência Odontológica da Faculdade de Odontologia de Araçatuba-UNESP, na pessoa do coordenador Prof. Dr. Alberto Carlos Botazzo Delbem.

Aos funcionários da biblioteca e da Pós-Graduação da Faculdade de Odontologia de Araçatuba, pelos serviços prestados sempre com muita competência e paciência.

Ao Frigorífico FRIBOI, que permitiu a coleta dos dentes bovinos utilizados neste trabalho.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), pela concessão de recursos que possibilitou a realização deste Curso de Mestrado.

A todos aqueles que, de alguma forma contribuíram para a elaboração e conclusão deste trabalho,
minha eterna gratidão.

Epígrafe

Deus nos concede, a cada dia, uma página de vida nova no livro do tempo. Aquilo que colocamos nela corre por nossa conta.

Chico Xavier

Resumo

CRUZ, N.V.S. **Efeito de dentifrícios com concentração reduzida de fluoreto associado ao trimetafosfato de sódio sobre a erosão do esmalte dentário.** 2013. 58 f. Dissertação (Mestrado). Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2013.

Resumo

O objetivo deste estudo foi avaliar *in vitro* o efeito de dentifrícios com baixa concentração de fluoreto (250 ppm), combinado com trimetafosfato de sódio (TMP) sobre a erosão do esmalte. Cento e quarenta e quatro blocos de esmalte bovino foram selecionados pela dureza de superfície inicial e submetidos a desafios erosivos (2 e 5 dias). Os tratamentos foram realizados com slurry de dentifrício (5 mL / bloco por 15 s): Sem Fluoreto e TMP (controle negativo), 250 ppm de F, 250 ppm F com 0,25, 0,5 e 1,0% de TMP e controle positivo (1425 ppm F) . O desafio erosivo foi produzido por imersão em Sprite (pH 2,8) 4 vezes / dia por 5 minutos, seguido por 1 hora em saliva artificial. A dureza de superfície, dureza transversal e desgaste da superfície foram analisados como variáveis de resposta. Os dados foram submetidos à análise de variância (2-way), seguido pelo teste de Student-Newman-Keuls. O aumento do tempo do desafio erosivo resultou em maior desgaste do esmalte ($p < 0,001$). Grupos com TMP apresentaram menor desgaste do esmalte do que os grupos com apenas flúor e o controle negativo ($p < 0,001$). A presença de maior quantidade de fluoreto (controle positivo), levou a uma superfície mais dura do esmalte ($p < 0,001$) em comparação com o controle negativo e o dentifrício de 250 ppm F. A adição de TMP ao dentifrício com 250 ppm de F teve um efeito direto sobre a dureza. O trimetafosfato de sódio (0,25%) pode aumentar o efeito contra a erosão do dentifrício de baixa concentração de fluoreto (250 ppm). Este efeito foi melhor do que o observado com a pasta de dente comercial.

Palavras-chave: Fluoretos. Cremes Dentais. Fosfatos.

Abstract

CRUZ, N.V.S. **Effect of low-fluoride toothpaste combined with sodium trimetaphosphate on enamel erosion.** 2013. 58 f. Dissertation (Master"s degree) – Araçatuba School of Dentistry, UNESP—Univ. Estadual Paulista, 2013.

Abstract

The purpose of this study was to evaluate in vitro the effect of low-F toothpastes (250 ppm) combined with sodium trimetaphosphate (TMP) on enamel erosion, one hundred forty four bovine enamel blocks were selected by their initial surface hardness and subjected to a erosion challenges (2- and 5-days). Treatments were carried out using slurry toothpastes (5 mL/block for 15 s): non fluoride and TMP (negative control), 250 ppm F, 250 ppm F plus 0.25, 0.5 and 1.0% TMP toothpastes and positive control (1,425 ppm F). The erosive challenge was produced by immersion in soda (pH 2.8) 4 times/day, 5 minutes each time, followed by 1 hour in artificial saliva. The surface and cross-sectional hardness and surface wear were analyzed as response variables. The data were submitted to analysis of variance (2-way) followed by Student-Newman-Keuls's test.The rise in the time of erosive challenge resulted in a larger enamel wear ($p < 0.001$). TMP groups showed lower enamel wear than fluoride-only and negative control ($p < 0.001$). The presence of higher amounts of fluoride (positive control) led to a harder enamel surface ($p < 0.001$) compared to the negative control and 250 ppm F toothpaste. The addition of TMP to toothpaste with 250 ppm F had a direct effect on hardness.The results suggest that the sodium trimetaphosphate (0.25%) can enhance the effect against erosion of low fluoride toothpaste (250 ppm). This effect it was in higher level than the observed with the commercial toothpaste.

Keywords: Fluoride. Toothpastes. Phosphates.

Lista de tabelas

Lista de figuras

Lista de abreviaturas

% =	Porcentagem
µm =	Micrômetro
°C =	Graus Celsius
ΔKHN =	Perda integrada da dureza de subsuperfície
µg F/g =	Micrograma de fluoreto por grama
CSH =	Cross- sectional hardness
F =	Fluoreto
FI =	Flúor iônico
FT =	Flúor total
g =	Aceleração da gravidade
h =	Hora
HCl =	Ácido clorídrico
HA =	Hidroxiapatita
KNO_3=	Nitrato de Potássio
mL =	Mililitros
mm =	Milímetros
Mol/L =	Molaridade
NaF =	Fluoreto de Sódio
NaOH =	Hidróxido de Sódio
ppm =	Partes por milhão
pH =	Potencial hidrogeniônico
s =	Segundos
$SrCl_2$=	Cloreto de Estrôncio
TMP =	Trimetafosfato de Sódio
TISAB =	Tampão de ajustador de força iônica total

Sumário

Effect of low-fluoride toothpaste combined with sodium trimetaphosphate on enamel erosion

Nathália Viana de Souza Cruz1, Michele Mauricio Manarelli1, Ana Elisa Mello Vieira1, Maria Daniela Basso2, Alberto Carlos Botazzo Delbem1

1 Faculdade de Odontologia, Unesp – Univ Estadual Paulista, Araçatuba, SP, Brazil.
2 PhD student, Faculdade de Odontologia, UNESP—Univ. Estadual Paulista; Professor, Curso de Odontologia, UNIOESTE—Univ. Estadual do Oeste do Paraná, Cascavel-PR, Brasil

Word count: 3598

Running title: Fluoride and trimetaphosphate against erosion

Corresponding author:
Alberto Carlos Botazzo Delbem
Araçatuba School of Dentistry, UNESP—Univ. Estadual Paulista
Department of Pediatric Dentistry and Public Health
Rua José Bonifácio 1193Araçatuba, SP - Cep 16015-050 - Brazil
Tel: +55 18 3636 3235
Fax: +55 18 3636 3332
Email: adelbem@foa.unesp.br; adelbem@pq.cnpq.br

* De acordo com as instruções aos autores do periódico International Journal of Paediatric Dentistry (Anexo A).

Summary

Background: The effect on enamel demineralisation of low-F toothpastes containing sodium trimetaphosphate (TMP) has been studied. However, there is limited literature is still scarce in giving evidence on the effect of these formulations on dental erosion.

Aim: To evaluate the effect of low-F toothpastes (250 ppm) combined with TMP on enamel erosion in vitro.

Design: One hundred forty-four bovine enamel blocks were selected by initial surface hardness and subjected to erosion challenges (2 and 5 days). Treatments were carried out using toothpaste slurries (5 mL/block for 15 s): no fluoride or TMP (negative control); 250 ppm F; 250 ppm F plus 0.25, 0.5, or 1.0% TMP; and positive control (1,425 ppm F). The erosive challenge was produced by immersion in soda (pH 2.8) for 5 min four times/day, followed by 1 h in artificial saliva. The surface and cross-sectional hardnesses and surface wear were analysed as response variables. The data were submitted to analysis of variance (2-way) followed by Student–Newman–Keuls test.

Results: Increased duration of erosive challenge resulted in greater enamel wear ($p < 0.001$). TMP groups showed less enamel wear than fluoride-only and negative control groups ($p < 0.001$). Increased concentration of fluoride (positive control) led to a harder enamel surface ($p < 0.001$) compared with the negative control and 250 ppm F

toothpaste. The addition of TMP to toothpaste with 250 ppm F had a direct effect on hardness.

Conclusion: TMP (0.25%) enhances the anti-erosive effect of low-F toothpaste (250 ppm).

1 **Introduction**

Dental erosive wear has become more prevalent and its incidence have varied between 12.3% and 18% in 11 to 16-year-old children [1].Tooth erosion is a multifactorial condition (patient-related and nutritional factors) and over time, the interaction of all these factors may lead to either progression or indeed protection of the surface [2]. The terms erosion and erosive tooth wear have been suggested to refer to the chemical and chemical-mechanical processes [3].

As is the case in caries and periodontal disease, changes in oral health and dietary habits, and oral products and toothpastes, are used to prevent or reduce the progression of dental erosion [4]. Fluoride has been used as a supplementary preventative measure to decrease dental erosion, and displays superior effect when applied in high concentration [5]. Topical fluoride is most frequently applied as a toothpaste but has limited beneficial effects on erosion and abrasion [6-8].

The addition of some actives to improve the effectiveness against erosion has been studied in toothpastes with 1,000 to 1,450 ppm F. A positive association exists between 10% xylitol and fluoride at 1,030 ppm F on the effectiveness of the toothpaste on erosion. This may be related to the ability of xylitol to facilitate calcium movement and availability in the demineralised areas [9]. Some commercial toothpastes have both fluoride and desensitising agents (e.g., $SrCl_2$, potassium citrate, and KNO_3) and are claimed to prevent erosion [10]. However, the interaction between NaF and KNO_3 did not produce favourable results. Another possible combination is fluoride and inorganic phosphate, such as sodium trimetaphosphate (TMP) [11]; 3% TMP in a toothpaste with 500 ppm F showed greater erosion reduction than a toothpaste with 1,100 ppm F.

Utilisation of this TMP and F association has shown decreased enamel demineralisation [11-14] in caries and dental erosion. In addition, the fluoride concentration can be reduced in products functioning against

demineralisation. Considering the good results against dental caries (data not published - in vitro study) and which differs from erosion process, the present study evaluated, in vitro, the effect of low-F toothpastes (250 ppm) plus TMP on the erosion of tooth enamel.

2 Material and methods

Experimental design

One hundred forty-four bovine enamel blocks were assigned among the following toothpastes: no fluoride or TMP (negative control); 250 ppm F; and 250 ppm F plus 0.25, 0.5, or 1.0% TMP experimental toothpastes as well as a commercial toothpaste (positive control). The groups of blocks were additionally subdivided into 2 conditions according to duration of erosive challenge (2 or 5 days). A group size of 12 enamel blocks was calculated considering an α-error level of 5% and a β-error level of 10%, based in the results of Moretto et al. [11] and Manarelli et al. [14]. The erosive challenge was produced in all blocks by immersion in soda (Sprite Zero™, pH 2.8) for 5 min 4 times per day. The factors studied were type of dentifrice and duration of erosive challenge. The surface and cross-sectional hardnesses and surface wear were analysed as response variables.

Enamel sample preparation

Enamel blocks (4 × 4 mm) were harvested from bovine incisors kept in 2% formalin, pH 7.0, for 30 days prior to experimental procedures. The enamel surface of the blocks was ground flat with water-cooled silicon carbide paper discs (400, 600, and 1,200 grades; Buehler, Lake Bluff, Ill, USA) removing approximately 200 μm of the enamel surface. After polishing, the baseline hardness of the enamel surface was determined and blocks with 340.0 to 370.0 kgf/mm^2 were selected. To maintain a

reference surface for lesion-depth determination by profilometry, 2 layers of nail varnish were applied to half of the surface of each block [14].

Toothpaste formulation and fluoride and pH analysis

The experimental toothpastes were manufacture and comprised the same basic formula (except for F and the presence or lack of TMP) including the following ingredients: carboxymethyl cellulose, sodium methyl-*p*-hydroxybenzoate, sodium saccharin, peppermint oil, glycerol, hydrated silica, sodium lauryl sulphate, and water. To compare and validate the results, the following toothpastes were assembled: negative control (without F or TMP); 250 ppm F (NaF; Merck, Darmstadt, Germany); and 250 ppm F with TMP (TMP; Sigma-Aldrich Co., St Louis, MO, USA) added at concentrations of 0.25, 0.5, or 1.0%. A commercial toothpaste was utilised as positive control (Sensodyne Pro-Enamel, 1,425 ppm F as NaF, 5% KNO_3, pH 7.0; GlaxoSmithKline Brazil Ltd., Rio de Janeiro, Brazil).

Total fluoride (TF) and ionic fluoride (IF) were determined using a specific electrode for F (Orion 9609-BN; Orion Research Inc., Beverly, MA, USA) according to the methods described by Delbem et al. [15]. Approximately 0.1 g of each toothpaste was combined with 10.0 mL deionised water in a polypropylene tube (15 mL). For the TF measurement, 0.25 mL dentifrice suspension, to which was added 0.25 mL 2 mol/L HCl, was agitated for 1 h at 45°C, and then 0.5 mL 1.0 mol/L NaOH was added to the mixture. For IF, the dentifrice suspension was centrifuged at 906 × *g* for 20 min and 0.25 mL of the supernatant solution was treated with 0.25 mL 2 mol/L HCl and 0.5 mL 1.0 mol/L NaOH. TISAB II was added to the samples and the standards (total ionic strength adjustment buffer) and results were expressed in µg F/g.

The pH of the toothpaste slurries was analysed in triplicate with a pH electrode (FH 2A09E-Analyser; São Paulo, SP, Brazil) and ion analyser (Orion 720A^{plus}). The pH electrode was calibrated according to standard pH levels of 7.0 and 4.0.

Erosive challenge and toothpaste treatment

For 2 or 5 days, the enamel blocks were subjected to erosive challenges, which comprised immersion in soda (Sprite™, pH 2.8, 5 mL/block; Companhia de Bebidas Ipiranga, Ribeirão Preto, Brazil) during 5 min (unstirred), 4 times per day [16-19]. After each erosive immersion, the enamel blocks were washed with deionised water then exposed to one of the toothpaste slurries (toothpaste/deionised water, 1:3 w/w; 5 mL/block) for 15 s at room temperature. The enamel blocks were stored in artificial saliva (5 mL/block; unstirred; 37° C) for 1 h between acid challenges/treatments. The composition of the artificial saliva was as follows: 1.5 mmol/L $Ca(NO_3)_2$ $4H_2O$, 0.9 mmol/L NaH_2PO_4 $2H_2O$, 150 mmol/L KCl, 0.1 mol/L Tris buffer, pH 7.0, 0.03 ppm F [20]. Following all 4 challenges/treatments, blocks remained in artificial saliva until the beginning of the next day erosive challenge. The solutions were changed once a day.

Surface hardness and profilometric analysis

The surface hardness for selection of the enamel blocks (baseline) was determined using a Micromet 5114 hardness tester (Buehler, Lake Bluff, Ill, USA) and the software Buehler OmniMet (Buehler). Five indentations were placed 100 µm apart using a Knoop diamond (25 g, 10 s). After the experimental period, the surface hardness was again measured in the area exposed to the erosive challenge. Five indentations, as used for baseline hardness determination, were made in a different part of the surface.

Enamel loss was determined in relation to the reference surface by profilometry (Surftest SJ 401; Mitutoyo American Corp.). The profilometric trace (length = 2 mm) included the reference surface (baseline) and the exposed surface. Five readings were performed on each block and the average enamel loss was calculated (µm) [14].

Cross-sectional hardness determination

Blocks were sectioned at the centre, and half of each block was incorporated in acrylic resin and subsequently polished. Cross-sectional hardness (CSH) was determined under a 5-g load for 10 s using a Micromet 5114 hardness tester. Nine indentations at different distances (5, 10, 15, 20, 25, 30, 40, 50, and 70 μm) from the external surface of the enamel were made in the centre of the blocks, for both the control and the eroded areas. The integrated area of hardness (kgf/mm^2 × μm) of the demineralised and sound enamel was calculated using the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated area of hardness of sound enamel to yield integrated hardness (∆KHN) [21].

Statistical analysis

The values of enamel wear (μm), surface hardness, and ∆KHN were considered as outcome measures. The toothpastes and periods of erosive challenge were the variation factors. The data from wear and surface hardness were submitted to analysis of variance (2-way) followed by Student–Newman–Keuls method test for multiple comparisons, considering the toothpastes and periods of erosive challenge as variation factors. In each periods erosive (2 or 5 days), the results from □KHN were subjected to analysis of variance (2-way) followed by Student–Newman–Keuls method, considering the toothpastes and depth in enamel as variation factors. A correlation test (Spearman's test) was applied to verify relationships between the variables. SigmaPlot software version 12.0 was used for the statistical analyses, and the significance limit was set at 5%.

3 Results

The concentrations of fluoride in the experimental and commercial toothpastes were as expected and showed a variation of less than 10% (Table 1). The addition of TMP to toothpastes did not affect the pH of the

formulations and proved to be neutral. Data surface hardness of the enamel before the erosion challenge showed good standardisation of the preparation of enamel blocks. The mean values (342.5–350.9 kgf/mm^2) were similar among groups and the duration of the erosive challenge (p = 0.960).

Despite having more fluoride in the formulation, the positive control showed wear similar ($p \geq 0.682$) to those of the negative control and 250 ppm F toothpastes (Figure 1A). When TMP was combined with fluoride, the enamel wear was 2-fold lower than that of the negative control ($p < 0.001$). Increased duration of erosive challenge resulted in greater enamel wear ($p < 0.001$). Meanwhile, time did not influence the protective action of the toothpastes ($p = 0.224$). However, a reduction in the effect against erosion was observed by increasing the TMP concentration in the toothpaste.

The presence of a higher concentration of fluoride (positive control) led to a harder enamel surface ($p < 0.001$) compared with the negative control and 250 ppm F toothpastes (Figure 1B). Regards to positive control versus 250 ppm F toothpastes, this hardness was 40% greater after the 2-day challenge and 100% after the 5-day challenge. Because the toothpaste with 250 ppm F showed similar hardness values to the negative control ($p = 0.683$), the addition of TMP to toothpaste with 250 ppm F had a direct effect on hardness. By combining 0.5% and 1% TMP with 250 ppm F the surface hardness was similar to that of the positive control ($p \geq 0.100$). There was not correlation between surface hardness values and wear values (Spearman's r = -0.148; p = 0.077).

Regarding depth of demineralisation of remaining enamel (Figure 1C), the formulation with higher capacity of remineralisation than the negative control ($p < 0.001$). This analysis revealed that erosion time influenced the action of the toothpaste ($p < 0.001$). For a longer period of erosion, it is possible to observe a greater differences between: groups with and without fluoride, with lower and higher concentrations of fluoride and lower and higher percentage of TMP in the formulations ($p < 0.001$). This

is also evident in depth analysis in the enamel (Figure 2). The demineralisation at 5 days of the remaining enamel as a function of depth showed profile more different compared to 2 days. Nevertheless, the addition of TMP (0.5% and 1%) reduced demineralisation of the remaining enamel to a depth similar to that of the positive control toothpaste ($p \geq 0.473$). There was correlation between ΔKHN values and wear values (Spearman's $r = 0.566$; $p < 0.001$).

The demineralisation profile of the remaining enamel as a function of depth showed the mineral loss after 5 days of erosion was 15 µm greater than that after 2 days (Figure 2). At a depth of 5 µm after a 2-day erosion challenge, a concentration of 250 ppm F produced decreased softening of the remaining enamel when compared with the negative control.

4 Discussion

In recent decades, studies have focused on alternatives that may help improve effectiveness against enamel demineralisation (caries and erosion). However, there are limited reports on the association between phosphate and fluoride; this study aimed to evaluate the effectiveness of this combination in toothpaste formulations against enamel erosion. The choice of sodium trimetaphosphate (TMP) was due to its ability to reduce the solubility of hydroxyapatite (HA) in acidic medium and to remain bound to the HA longer than other polyphosphates [22]. In this study, formulations with TMP without fluoride were not tested because, despite its action against demineralisation, its effect is lower than when associated with fluoride [12].

The addition of TMP to the 250 ppm F formulation enabled verification of an effect against erosion not present with a concentration of 1,425 ppm F. Notably, increasing the TMP concentration from 0.25% to 1.0% did not improve the anti-erosive effect of the toothpaste, but the remaining enamel was less demineralised. The adsorption of TMP onto the enamel surface [22,23] facilitates the diffusion of calcium ions [13,23], as well as

fluoride ions, according to Takeshita et al. [12]. This may explain the lower demineralisation of remaining enamel with increasing TMP concentration in a low-fluoride formulation (250 ppm). However, as noted earlier, enamel with decreased softening does not have decreased wear. After a 5-day period of erosion, the enamel was less demineralised with increasing TMP concentration in the 250 ppm F formulation; however, there was no change in the effect against erosion. Thus, the lower wear in the presence of the TMP/F combination may be related to the ability of TMP to block acid diffusion into the deep layers of the enamel. In addition, TMP interacts with the enamel, which may produce a protective layer on the enamel surface thereby hindering acid diffusion [13,23]. Thus, the major effect of TMP during the erosive challenge is to prevent acid diffusion into the enamel.

Acid accumulation over 5 days increased enamel wear and affected the efficacy of the TMP/F combination against erosion. This effect was seen mainly at high TMP concentrations in association with fluoride. These results agree with those of Manarelli et al. [14], which revealed increased enamel wear with increased TMP concentration in the formulation, together with an increase in enamel hardness as in the present study. Because polyphosphates may be adsorbed in greater quantities and more rapidly at acid pH [24], TMP might have displayed a disaggregating effect [22]. TMP has a smaller disaggregating effect than other polyphosphates [22], but when applied to demineralised enamel at high concentrations, this effect seems more apparent thereby facilitating a greater loss of enamel. The following sequence of events is possible: (1) TMP reacts with demineralised enamel, especially at higher concentration, and (2) promotes disaggregation of outer enamel with resultant (3) increased enamel erosion, which exposes the inner enamel—which is harder. According to Takeshita et al. [13], TMP enhanced the reduction into the demineralisation area in the deeper layers.

As the blocks were kept stored in artificial saliva after the last erosive/treatment process would be more correct to state that the

hardness testing (superficial and in depth) analyzed the remineralizing ability of the formulations. Surface hardness may be related to enamel wear [11] but does not indicate how much structure was lost. In the present study, the surface hardness was not related to wear. Nevertheless, the cross-sectional hardness can provide great potential to explain the results obtained after erosive challenge mainly the data from 5 days. The comparison between 250 ppm F and positive control (1,425 ppm F) toothpastes clearly demonstrates this situation. With regard to the positive control toothpaste, the greater available fluoride in the formulation explains the lower enamel softening after a long period of remineralization. The erosive challenge is a process that occurs at a pH of <4.0 and a surface with less softening will not prevent demineralisation and acid diffusion into the enamel. The pH of the dissolution of calcium fluoride, fluorapatite or any precipitate that might be formed in the presence of fluoride is exceeded during the erosive challenge. The fluoride-rich precipitate inside the lesion does not necessarily block acid diffusion to deeper layers 10 and fluoride alone does not prevent enamel wear in a challenge of this level. Addition to pH, the erosive medium is not saturated with calcium and phosphate [25]. These data strengthen the effect of TMP against erosion and its mechanism of action described previously.

The in vitro studies can estimate the role of new anti-erosive compounds [26] and to screening tests [25]. However this is a chemical model and the data obtained using this model (or any other in vitro protocol) should be considered carefully due to limitations in reproducing the oral environment with all of the biological variations known to influence erosion [27].

5 Conclusion

Based on results and considering the limitations of this in vitro study, it was concluded that sodium trimetaphosphate (0.25%) can enhance the effect against erosion of low-fluoride toothpaste (250 ppm). This effect was greater than that observed with the commercial toothpaste claimed to prevent erosion. However, additional in situ and clinical studies must be conducted to confirm these in vitro results.

Agradecimentos

Agradecemos a CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela bolsa de estudos.

References

1 Huysmans MCDNJM, Chew HP, Ellwood RP. Clinical Studies of Dental Erosion and Erosive Wear. Caries Res 2011; 45: 60–68.

2 Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental Erosion – An Overview with Emphasis on Chemical and Histopathological Aspects. Caries Res 2011; 45: 2–12.

3 Shellis RP, Ganss C, Ren Y, Zero DT, Lussi A. Methodology and Models in Erosion Research: Discussion and Conclusions. Caries Res 2011; 45: 69–77.

4 Magalhães AC, Wiegand A, Rios D, Honorio HM, Buzalaf MA. Insights into preventive measures for dental erosion. J Appl Oral Sci 2009; 17: 75 – 86.

5 Sorvari R, Meurman JH, Alakuijala P, Frank RM. Effect of fluoride varnish and solution on enamel erosion in vitro. Caries Res 1994; 28: 227–232.

6 Ponduri S, Macdonald E, Addy M. A study in vitro of the combined effects of soft drinks and tooth brushing with fluoride toothpaste on the wear of dentine. Int J Dent Hyg 2005; 3: 7–12.

7 Magalhães AC, Rios D, Delbem ACB, Buzalaf MAR, Machado MAAM. Influence of fluoride dentifrice on brushing abrasion of eroded human enamel: an in situ/ex vivo study. Caries Res 2007; 41: 77–79.

8 Rios D, Magalhães AC, Polo ROB, Wiegand A, Attin T, Buzalaf MAR. The efficacy of a highly concentrated fluoride dentifrice on bovine enamel

subjected to erosion and abrasion. J Am Dent Assoc 2008; 139: 1652-1656.

9 Rochel ID, Souza JG, Silva TC, et al. Effect of experimental xylitol and fluoride-containing dentifrices on enamel erosion with or without abrasion in vitro. J Oral Sci 2011, 53: 163-168.

10 Kato MT, Lancia M, Sales-Peres SHC, Buzalaf MAR. Preventive effect of commercial desensitizing toothpastes on bovine enamel erosion in vitro. Caries Res 2010; 44: 85–89.

11 Moretto MJ, Magalhães AC, Sassaki KT, Delbem ACB, Martinhon CC. Effect of different fluoride concentrations of experimental dentifrices on enamel erosion and abrasion. Caries Res 2010; 44: 135–140.

12 Takeshita EM, Castro LP, Sassaki KT, Delbem AC. In vitro evaluation of dentifrice with low fluoride content supplemented with trimetaphosphate. Caries Res 2009; 43: 50– 56.

13 Takeshita EM, Exterkate RAM, Delbem AC, ten Cate JM. Evaluation of different fluoride concentrations supplemented with trimetaphosphate on enamel de- and remineralization in vitro. Caries Res 2011; 45: 494–497.

14 Manarelli MM, Vieira AEM, Matheus AA, Sassaki KT, Delbem AC. Effect of mouth rinses with fluoride and trimetaphosphate on enamel erosion: an in vitro study. Caries Res 2011; 45: 506–509.

15 Delbem AC, Bergamaschi M, Rodrigues E, Sassaki KT, Vieira AE, Missel EM. Anticaries effect of dentifrices with calcium citrate and sodium trimetaphosphate. J Appl Oral Sci 2012; 20:94-98.

16 West NX, Maxwell A, Hughes JA, Parker DM, Newcombe RG, Addy M. A method to measure clinical erosion: the effect of orange juice consumption on erosion of enamel. J Dent 1998; 26: 329–335.

17 Attin T, Knöfel S, Buchalla W, Tutunar R. In situ evaluation of different remineralization periods to decrease brushing abrasion of desmineralized enamel. Caries Res 2001; 35: 216–222.

18 Hughes JA, Jandt KD, Baker N, et al: Further modification to soft drinks to minimize erosion: a study in situ. Caries Res 2002; 36: 70– 74.

19 Hunter ML, Hughes JA, Parker DM, West NX, Newcombe RG, Addy M. Development of low erosive carbonated fruit drinks. 1. Evaluation of two experimental orange drinks in vitro and in situ. J Dent 2003; 31: 253–260.

20 Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res 2005; 39: 514–520.

21 Spiguel MH, Tovo MF, Kramer PF, Franco KS, Alves KMRP, Delbem AC. Evaluation of laser fluorescence in the monitoring of the initial stage of the de/remineralization process: an in vitro and in situ study. Caries Res 2009; 43: 302-307.

22 McGaughey C, Stowell EC. Effects of Polyphosphates on the solubility and mineral of HA: relevance of a rationale for anticarie activity. J Dent Res 1977; 56: 579-587.

23 Van Dijk JW, Borggreven JM, Driessens, FC. The effect of some phosphates and a phosphonate on the electrochemical properties of bovine enamel. Arch Oral Biol 1980; 25: 591-595.

24 Bartels T, Schuthof J, Arends J. The adsorption of two polyphosphonates on hydroxyapatite and their influence on the acid solubility of whole bovine enamel. J Dent 1979; 7: 221-229.

25 Barbour ME, Lussi A, Shellis RP. Screening and Prediction of Erosive Potential. Caries Res 2011; 45: 24–32.

26 Wiegand A, Attin T. Design of Erosion/Abrasion Studies – Insights and Rational Concepts. Caries Res 2011; 45: 53–59.

27 West NX, Davies M, Amaechi BT. In vitro and in situ Erosion Models for Evaluating Tooth Substance Loss. Caries Res 2011; 45: 43–52.

Table and figure legends

Table 1. Mean values (SD, n = 3) of total (T) and ionic (I) fluoride (F) and pH in the toothpastes

Figure 1. Mean values of wear, surface hardness, and ∆KHN after the erosive challenges (2 and 5 days). Distinct letters indicate statistic difference between treatments as well as period of challenge. Vertical bars denote standard deviation.

Figure 2. Depth profiles of mean hardness for each treatment according to time of erosion challenge

(A) Erosion period of 2 days

5 µm: 250 TMP 0.25% = 250 TMP 0.5% (p = 0.617); 250 TMP 0.5% = 250 ppm F (p = 0.223); and 250 TMP 1% = positive control (p = 0.591)

10 µm: negative control = 250 ppm F (p = 0.299); 250 TMP 0.25% = 250 TMP 0.5% (p = 0.627); and 250 TMP 1% = positive control (p = 0.796)

15 µm: negative control = 250 ppm F (p = 0.647); 250 ppm F = 250 TMP 0.5% (p = 0.507); and 250 TMP 0.25% = 250 TMP 1% = positive control (p = 0.623)

20 µm: sound = 250 TMP 0.25% = 250 TMP 1% = positive control (p = 0.896); negative control = 250 ppm F (p = 0.166); negative control = 250 TMP 0.5% (p = 0.234); and 250 TMP 0.25% = 250 TMP 0.5% (p = 0.245)

* no difference among groups (p = 0.169; Student–Newman–Keuls, $p < 0.001$)

(B) Erosion period of 5 days

5 µm: 250 TMP 1% = positive control (p = 0.217)

10 µm: 250 TMP 0.5% = positive control (p = 0.660)

15 µm: 250 TMP 0.5% = 250 TMP 1% = positive control (p = 0.659); 250 ppm F = 250 TMP 0.25% (p = 0.893)

20 µm: 250 TMP 0.5% = 250 TMP 1% = positive control (p = 0.569); 250 ppm F = 250 TMP 0.25% (p = 0.265)

25 µm: sound = 250 TMP 0.5% = 250 TMP 1% = positive control (p = 0.725); 250 ppm F = 250 TMP 0.25% (p = 0.754)

30 µm: sound = 250 ppm F = 250 TMP 0.25% = 250 TMP 0.5% = 250 TMP 1% = positive control (p = 0.725)

* no difference among groups (p = 0.754; Student–Newman–Keuls, $p < 0.001$)

Table 1. Mean values (SD, n = 3) of total (T) and ionic (I) fluoride (F) and pH in the toothpastes

Groups	TF (μg F/g)	IF (μg F/g)	pH
Negative control	11.1 (1.4)	11.5 (0.8)	7.7 (0.02)
250 ppm F	268.7 (3.1)	272.3 (1.1)	7.1 (0.02)
250 TMP 0.25%	282.0 (2.8)	273.4 (6.8)	7.5 (0.01)
250 TMP 0.5%	270.6 (3.5)	277.3 (2.1)	7.3 (0.01)
250 TMP 1%	271.2 (0.9)	270.3 (6.7)	7.0 (0.02)
Positive control	1,578.2 (14.0)	1,432.2 (230.3)	7.3 (0.04)

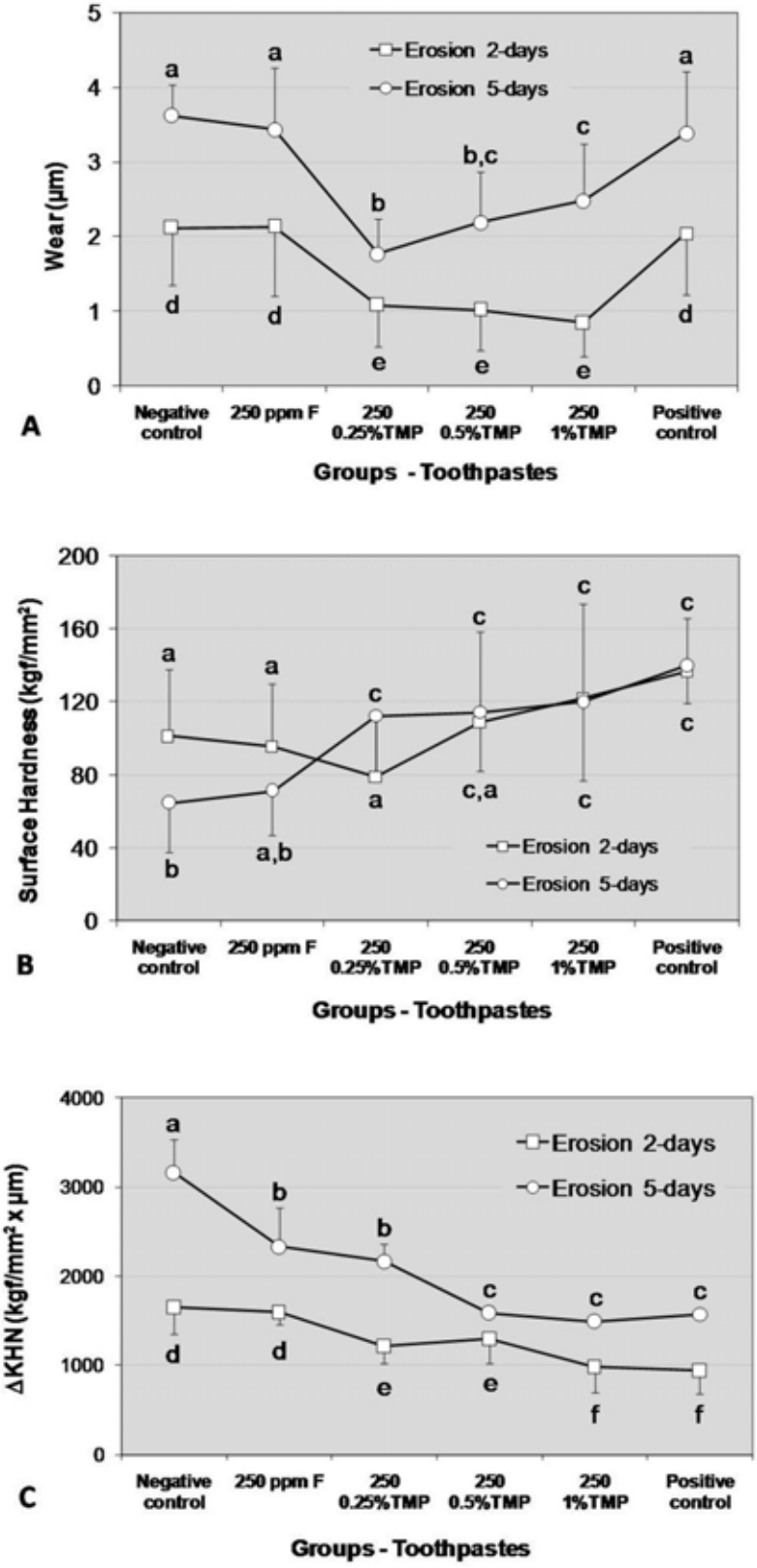


Figura 1. Mean values of wear, surface hardness, and ΔKHN after the erosive challenges (2 and 5 days). Distinct letters indicate statistic difference between treatments. Vertical bars denote standard deviation.

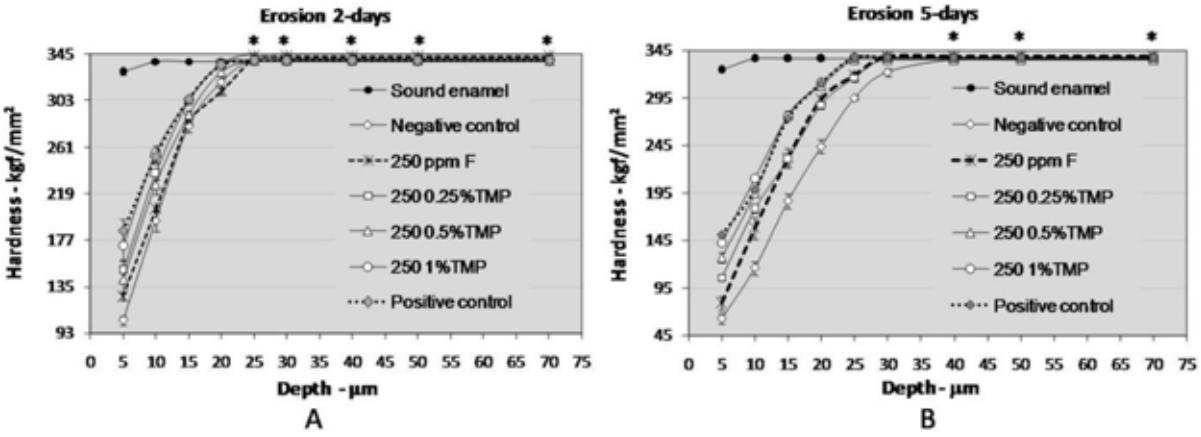


Figura 2. Depth profiles of mean hardness for each treatment according to time of erosion challenge

Anexos

ANEXO A

INSTRUÇÕES AOS AUTORES

INTERNATIONAL JOURNAL OF
PAEDIATRIC DENTISTRY

CrossCheck
The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL
International Journal of Paediatric Dentistry publishes papers on all aspects of paediatric dentistry including: growth and development, behaviour management, prevention, restorative treatment and issue relating to medically compromised children or those with disabilities. This peer-reviewed journal features scientific articles, reviews, clinical techniques, brief clinical reports, short communications and abstracts of current paediatric dental research. Analytical studies with a scientific novelty value are preferred to descriptive studies.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after acceptance of a manuscript for publication in International Journal of Paediatric Dentistry. Authors are encouraged to visit Wiley-Blackwell Author Services for further information on the preparation and submission of articles and figures.
In June 2007 the Editors gave a presentation on How to write a successful paper for the*International Journal of Paediatric Dentistry.*

2. ETHICAL GUIDELINES

Submission is considered on the conditions that papers are previously unpublished, and are not offered simultaneously elsewhere; that authors have read and approved the content, and all authors have also declared all competing interests; and that the work complies with the Ethical Policies of the Journal and has been conducted under internationally accepted ethical standards after relevant ethical review.

3. MANUSCRIPT SUBMISSION PROCEDURE

Articles for the *International Journal of Paediatric Dentistry* should be submitted electronically via an online submission site. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. Support is available by phone (+1 434 817 2040 ext. 167) or here. If you cannot submit online, please contact Reidin Vaughan in the Editorial Office by telephone (+44 (0)1865 476327) or by e-mail revaughan@wiley.com.

3.1. Getting Started

Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online submission site:http://mc.manuscriptcentral.com/ijpd
*Log-in or, if you are a new user, click on 'register here'.
*If you are registering as a new user.
- After clicking on 'Create Account', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.
- Enter your institution and address information as appropriate, and then click 'Next.'
- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click 'Finish'.
*If you are already registered, but have forgotten your log in details, enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.
*Log-in and select 'Author Center'.

3.2. Submitting Your Manuscript

After you have logged into your 'Author Center', submit your manuscript by clicking on the submission link under 'Author Resources'.
* Enter data and answer questions as appropriate.
* You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter. **Please note** that a separate *Title Page* must be submitted as part of the submission process as 'Title Page' and should contain the following:
• Word count (excluding tables)
• Authors' names, professional and academic qualifications, positions and places of work. They must all have actively contributed to the overall design and execution of the study/paper and should be listed in order of importance of their contribution
• Corresponding author address, and telephone and fax numbers and email address

*Click the 'Next' button on each screen to save your work and advance to

the next screen.
*You are required to upload your files.
- Click on the 'Browse' button and locate the file on your computer.
- Select the designation of each file in the drop down next to the Browse button.
- When you have selected all files you wish to upload, click the 'Upload Files' button.
* Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and a PDF document on upload and will be used for the review process. The text file must contain the entire manuscript including title page, abstract, text, references, tables, and figure legends, but no embedded figures. In the text, please reference figures as for instance 'Figure 1', 'Figure 2' to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below. Please note that any manuscripts uploaded as Word 2007 (.docx) is now accepted by IPD. As such manuscripts can be submitted in both .doc and .docx file types.

3.4. Review Process

The review process is entirely electronic-based and therefore facilitates faster reviewing of manuscripts. Manuscripts will be reviewed by experts in the field (generally two reviewers), and the Editor-in-Chief makes a final decision. *The International Journal of Paediatric Dentistry* aims to forward reviewers´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for 'fast-track publication' under special circumstances after consultation with the Editor-in-Chief.

3.5. Suggest a Reviewer

International Journal of Paediatric Dentistry attempts to keep the review process as short as possible to enable rapid publication of new scientific data. In order to facilitate this process, please suggest the names and current email addresses of a potential international reviewer whom you consider capable of reviewing your manuscript and their area of expertise. In addition to your choice the journal editor will choose one or two reviewers as well.

3.6. Suspension of Submission Mid-way in the Submission Process
You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission
After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status
You can access ScholarOne Manuscripts any time to check your 'Author Center' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts
Revised manuscripts must be uploaded within 2 months of authors being notified of conditional acceptance pending satisfactory revision. Locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision' to submit your revised manuscript. Please remember to delete any old files uploaded when you upload your revised manuscript. All revisions must be accompanied by a cover letter to the editor. The letter must a) detail on a point-by-point basis the author's response to each of the referee's comments, and b) a revised manuscript highlighting exactly what has been changed in the manuscript after revision.

3.10 Online Open
OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive.
For the full list of terms and conditions, seehttp://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms.
Any authors wishing to send their paper OnlineOpen will be required to complete the payment form available from our website athttps://authorservices.wiley.com/bauthor/onlineopen_order.asp

Prior to acceptance there is no requirement to inform an Editorial Office that you intend to publish your paper OnlineOpen if you do not wish to. All OnlineOpen articles are treated in the same way as any other article. They go through the journal's standard peer-review process and will be accepted or rejected based on their own merit.

4. MANUSCRIPT TYPES ACCEPTED

Original Articles: Divided into: Summary, Introduction, Material and methods, Results, Discussion, Bullet points, Acknowledgements, References, Figure legends, Tables and Figures arranged in this order. The summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions and should be less than 200 words. A brief description, in bullet form, should be included at the end of the paper and should describe Why this paper is important to paediatric dentists.

Review Articles: may be invited by the Editor.

Short Communications: should contain important, new, definitive information of sufficient significance to warrant publication. They should not be divided into different parts and summaries are not required.

Clinical Techniques: This type of publication is best suited to describe significant improvements in clinical practice such as introduction of new technology or practical approaches to recognised clinical challenges.

Brief Clinical Reports/Case Reports: Short papers not exceeding 800 words, including a maximum of three illustrations and five references may be accepted for publication if they serve to promote communication between clinicians and researchers. If the paper describes a genetic disorder, the OMIM unique six-digit number should be provided for online cross reference (Online Mendelian Inheritance in Man).

A paper submitted as a Brief Clinical/Case Report should include the following:

- a short **Introduction** (avoid lengthy reviews of literature);
- the **Case report** itself (a brief description of the patient/s, presenting condition, any special investigations and outcomes);
- a **Discussion** which should highlight specific aspects of the case(s), explain/interpret the main findings and provide a scientific appraisal of any previously reported work in the field.
- Please provide up to 3 bullet points for your manuscript under the heading: 1. Why this clinical report is important to paediatric dentists. Bullet points should be added to the end of your manuscript, before the references.

Letters to the Editor: Should be sent directly to the editor for consideration in the journal.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. Authors for whom English is a second language must have their manuscript professionally edited by an English speaking person before submission to make sure the English is of high quality. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found athttp://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

5.2. Structure

The whole manuscript should be double-spaced, paginated, and submitted in correct English. The beginning of each paragraph should be properly marked with an indent.

Original Articles (Research Articles): should normally be divided into: Summary, Introduction, Material and methods, Results, Discussion, Bullet points, Acknowledgements, References, Figure legends, Tables and Figures arranged in this order.

Summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions.

Introduction should be brief and end with a statement of the aim of the study or hypotheses tested. Describe and cite only the most relevant earlier studies. Avoid presentation of an extensive review of the field.

Material and methods should be clearly described and provide enough detail so that the observations can be critically evaluated and, if necessary repeated. Use section subheadings in a logical order to title each category or method. Use this order also in the results section. Authors should have considered the ethical aspects of their research and should ensure that the project was approved by an appropriate ethical committee, which should be stated. Type of statistical analysis must be described clearly and carefully.

(i) Experimental Subjects: Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried

out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

(ii) Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material. *International Journal of Paediatric Dentistry* encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials/, http://isrctn.org/. The clinical trial registration number and name of the trial register will then be published with the paper.

***(iii)DNA Sequences and Crystallographic Structure Determinations*:** Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

Results should clearly and concisely report the findings, and division using subheadings is encouraged. Double documentation of data in text, tables or figures is not acceptable. Tables and figures should not include data that can be given in the text in one or two sentences.

Discussion section presents the interpretation of the findings. This is the only proper section for subjective comments and reference to previous literature. Avoid repetition of results, do not use subheadings or reference to tables in the results section.

Bullet Points should include one heading:
*Why this paper is important to paediatric dentists.
Please provide maximum 3 bullets per heading.

Review Articles: may be invited by the Editor. Review articles for the *International Journal of Paediatric Dentistry* should include: a) description of search strategy of relevant literature (search terms and databases), b) inclusion criteria (language, type of studies i.e. randomized controlled trial or other, duration of studies and chosen endpoints, c)

evaluation of papers and level of evidence. For examples see: Twetman S, Axelsson S, Dahlgren H et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontologica Scandivaica 2003; 61: 347-355.
Paulsson L, Bondemark L, Söderfeldt B. A systematic review of the consequences of premature birth on palatal morphology, dental occlusion, tooth-crown dimensions, and tooth maturity and eruption. Angle Orthodontist 2004; 74: 269-279.

Clinical Techniques: This type of publication is best suited to describe significant improvements in clinical practice such as introduction of new technology or practical approaches to recognised clinical challenges. They should conform to highest scientific and clinical practice standards.

Short Communications: Brief scientific articles or short case reports may be submitted, which should be no longer than three pages of double spaced text, and include a maximum of three ilustrations. They should contain important, new, definitive information of sufficient significance to warrant publication. They should not be divided into different parts and summaries are not required.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.

5.3. References

A maximum of 30 references should be numbered consecutively in the order in which they appear in the text (Vancouver System). They should be identified in the text by bracketed Arabic numbers and listed at the end of the paper in numerical order. Identify references in text, tables and legends. Check and ensure that all listed references are cited in the text. Non-refereed material and, if possible, non-English publications should be avoided. Congress abstracts, unaccepted papers, unpublished observations, and personal communications may not be placed in the reference list. References to unpublished findings and to personal communication (provided that explicit consent has been given by the sources) may be inserted in parenthesis in the text. Journal and book references should be set out as in the following examples:

1. Kronfol NM. Perspectives on the health care system of the United Arab Emirates. East Mediter Health J. 1999; 5: 149-167.
2. Ministry of Health, Department of Planning. Annual Statistical Report.

Abu Dhabi: Ministry of Health, 2001.
3. Al-Mughery AS, Attwood D, Blinkhorn A. Dental health of 5-year-old children in Abu Dhabi, United Arab Emirates. Community Dent Oral Epidemiol 1991; 19: 308-309.
4. Al-Hosani E, Rugg-Gunn A. Combination of low parental educational attainment and high parental income related to high caries experience in preschool children in Abu Dhabi. Community Dent Oral Epidemiol 1998; 26: 31-36.

If more than 6 authors please, cite the three first and then et al. When citing a web site, list the authors and title if known, then the URL and the date it was accessed (in parenthesis). Include among the references papers accepted but not yet published; designate the journal and add (in press). Please ensure that all journal titles are given in abbreviated form.

We recommend the use of a tool such as Reference Manager for reference management and formatting. Reference Manager reference styles can be searched for here:www.refman.com/support/rmstyles.asp.

5.4. Illustrations and Tables

Tables: should be numbered consecutively with Arabic numerals and should have an explanatory title. Each table should be typed on a separate page with regard to the proportion of the printed column/page and contain only horizontal lines

Figures and illustrations: All figures should be submitted electronically with the manuscript via ScholarOne Manuscripts (formerly known as Manuscript Central). Each figure should have a legend and all legends should be typed together on a separate sheet and numbered accordingly with Arabic numerals. Avoid 3-D bar charts.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible).

For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone

and line images: >600 dpi.

Further information can be obtained at Wiley-Blackwell's guidelines for figures:http://authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it:http://authorservices.wiley.com/bauthor/eachecklist.asp.

NIH Public Access Mandate

For those interested in the Wiley-Blackwell policy on the NIH Public Access Mandate, please visit our policy statement

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the publisher.

Anexo B

Preparo dos blocos de esmalte bovino (4x4mm)

1. Coroa do dente bovino incisivo central inferior, separada da raiz por meio de disco diamantado de duas faces (KG Sorensen D 91), montado em motor de bancada (Nevoni), mantido sob refrigeração (água destilada/deionizada).

2. Secção da coroa utilizando disco diamantado (série 15 HC Diamond - n. 11-4244 Buehler) separando a superfície vestibular da lingual.

3. Face vestibular fixada na placa de acrílico.

4. Secção da face vestibular no sentido longitudinal, na porção mais plana, utilizando-se 2 discos diamantados (série 15 HC Diamond –n. 11-4244 Buehler), montados em cortadeira sob refrigeração com água destilada/deionizada e separados por um disco espaçador de alumínio com 4 mm de espessura. Em seguida, foi realizado o corte no sentido transversal.

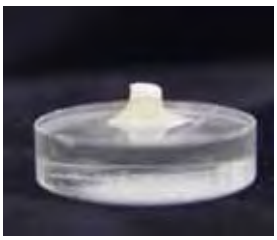
5. Fragmento vestibular do dente bovino, fixado sobre placa de resina. Ao lado, bloco de esmalte dentário.

Anexo c

Planificação da dentina e polimento do esmalte

1. Bloco de esmalte fixado em disco de resina acrílica pré-fabricada (± 3 cm de diâmetro por ± 11 mm de espessura), com auxílio de cera pegajosa (Kota Ind. e Com. LTDA), com a superfície dentinária voltada para cima.

2. Ajuste da dentina para obtenção de superfícies paralelas entre esmalte e dentina, utilizando Politriz Beta – Grinder – Polisher e Vector Power Head (Buehler, Lake Bluff, IL, USA) e lixas de granulação 320 (Carbimet Paper Discs, 30-5108-320, Buehler), durante 30 segundos sob baixa rotação e refrigeração.



3. Blocos fixados com a superfície do esmalte voltada para cima para serem polidos.

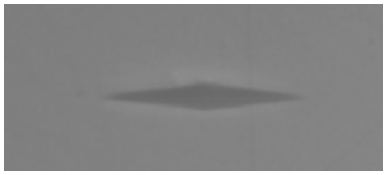
Seqüência do polimento de esmalte:

- ✓ Pedra-pomes, água deionizada e taça de borracha montada em contra-ângulo em baixa-rotação.
- ✓ Lixas de granulação 400 (30 segundos), 600 (20 segundos) e 1200 (30 segundos) e refrigeração a água. Limpeza em lavadora ultrassônica e água destilada/ deionizada por 2 minutos, entre cada lixa;
- ✓ Acabamento final com disco de papel feltro TEXMET 1000 (Buehler Polishing Cloth) durante 1 minuto com suspensão de diamante 1 micron base-água (Buehler);
- ✓ Lavagem durante 30 segundos com jato de água deionizada;
- ✓ Limpeza em lavadora ultrassônica Modelo 2110 (Branson, Danbury CT,USA) com água destilada/ deionizada (2minutos).

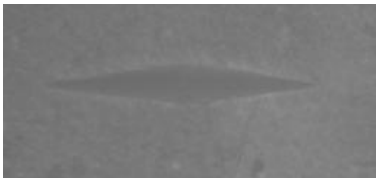
Anexo D

Análise da dureza de superfície do esmalte

1. Microdurômetro Micromet 5114 Hardness Tester (Buehler, Lake Bluff, USA e Mitutoyo Corporation, Kanagawa, Japan), com penetrador tipo Knoop, acoplado ao Software para análise de imagem Buehler OminMet (Buehler, Lake Bluff, USA).



2. Fotomicrografia da impressão inicial (SH*i*). (Aumento: 50x)

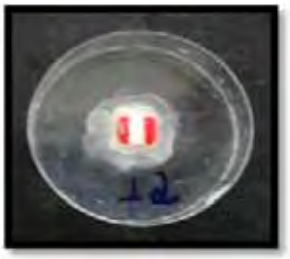


3. Fotomicrografia da impressão final *(*SH*f*). (Aumento: 50x)

Anexo E

Proteção dos blocos de esmalte

1. Seleção prévia dos blocos realizada através de microdureza e observação a olho nu da superfície de cada bloco.



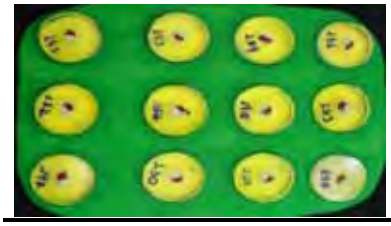
2. Esmalte cosmético foi utilizado para proteger uma área dos fragmentos necessária para realização dos testes de perfilometria.

Anexo F

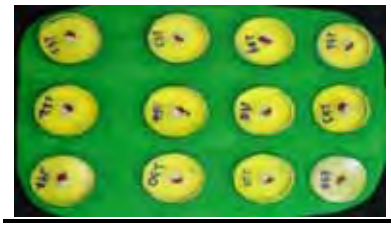
Esquema de desafio erosivo e tratamento

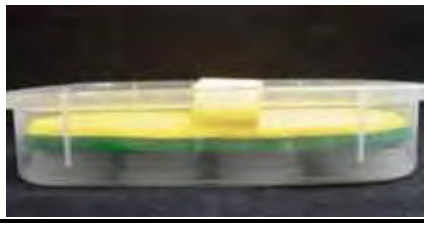
Anexo G

Sequência de erosão

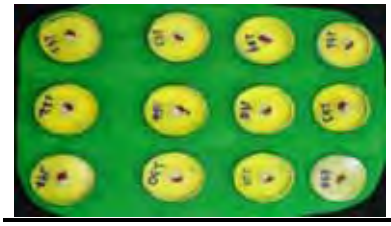


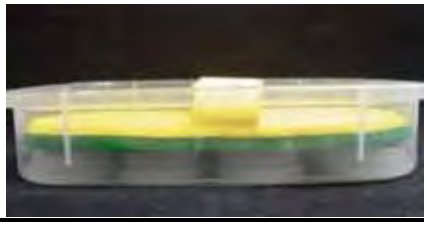
Dispositivo em E.V.A., com capacidade para 12 blocos, colocado em recipiente próprio com solução remineralizadora em intervalos de 2 horas.





Dispositivo em E.V.A., com capacidade para 12 blocos, colocado em recipiente com sprite zero® durante 5 minutos.

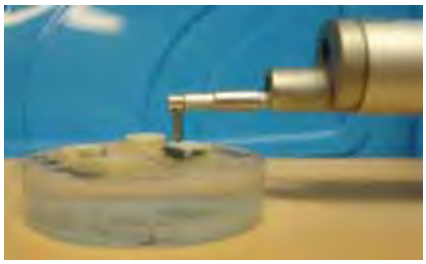




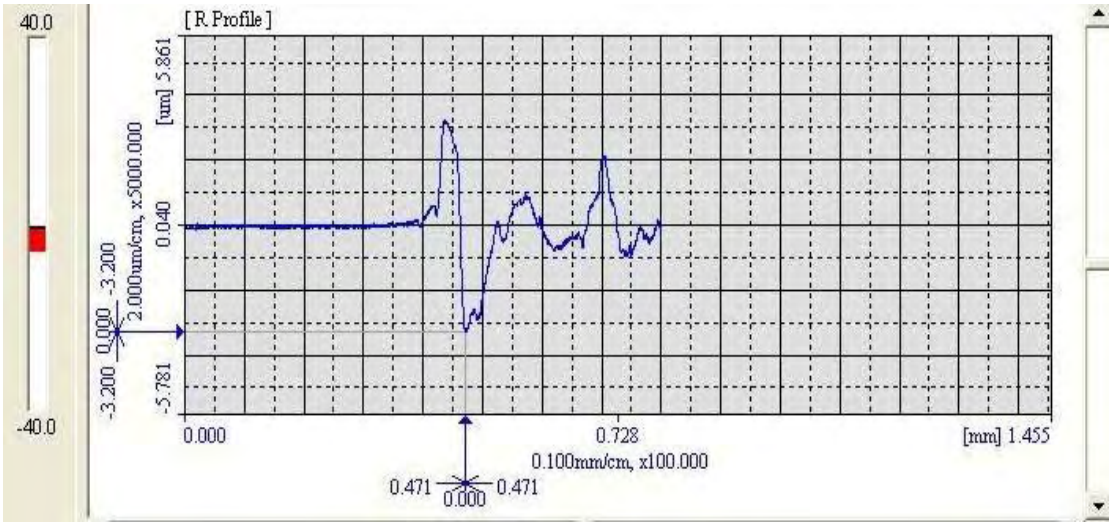
Dispositivo em E.V.A., com capacidade para 12 blocos, colocado em recipiente com slurry de dentifrício (1:3) durante 15 segundos.

Anexo H

Análise do desgaste



Rugosímetro – Mitutoyo (Mitutoyo American Corporation)



Surfpack-SJ versão 1.6

Anexo I

Análise da dureza em secção longitudinal

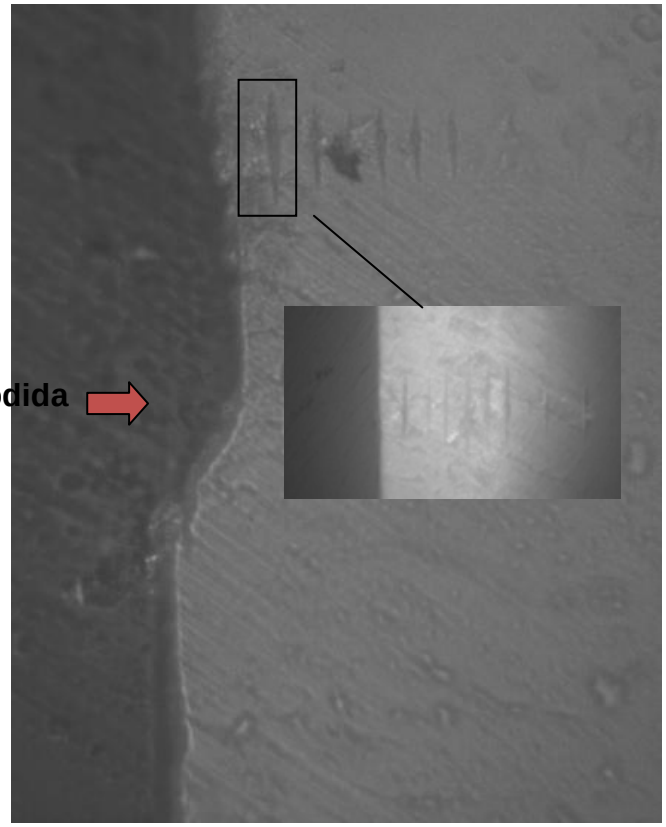
1. Embutir – utilizada para inclusão dos blocos de esmalte em 5 gramas de resina acrílica auto polimerizante (JET, Campo Limpo Paulista, Brasil). Os blocos foram fixados em posição com cola adesiva (Super Bonder – Loctite).

2. Corpo de prova – plano longitudinal voltado para a superfície da resina acrílica.

3. Microdurômetro Micromet 5114 Hardness Tester (Buehler, Lake Bluff, USA e Mitutoyo Corporation, Kanagawa, Japan), com penetrador tipo Knoop, acoplado ao Software para análise de imagem Buehler OminMet (Buehler, Lake Bluff, USA).

Seqüência do polimento de esmalte:

- ✓ Lixas de granulação 320 (1 minuto), 600 e 1200 (2minutos) e refrigeração a água. Limpeza em lavadora ultrassônica e água destilada/ deionizada por 2 minutos, entre cada lixa;
- ✓ Acabamento final com disco de papel feltro Microcloth Supreme PSA (Buehler) durante 2 minutos com suspensão de diamante 1/4 micron base-água (Buehler);
- ✓ Lavagem durante 30 segundos com jato de água deionizada;
- ✓ Limpeza em lavadora ultrassônica utilizando água destilada/deionizada (2 minutos).



Fotomicrografia das impressões. (Aumento: 1000x)

Anexo J

Esquema representativo da dosagem de F nos dentifrícios