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FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM
DEPARTAMENTO DE CLÍNICA ODONTOLÓGICA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

VANARA FLORÊNCIO PASSOS

INFLUÊNCIA DE AGENTES PROTETORES NA PREVENÇÃO DA EROSÃO EXTRÍNSECA OU INTRÍNSECA: ESTUDOS *IN VITRO* E *IN SITU*

FORTALEZA
2012

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como um dos requisitos para a obtenção do título de Doutor em Odontologia.

Área de Concentração: Clínica Odontológica.

Orientador: Prof. Dr. Sérgio Lima Santiago

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A **Deus** e **Nossa Senhora**,

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RESUMO

Considerando o declínio da prevalência da doença cárie e periodontal na sociedade, observa-se uma maior longevidade dentária. Assim, lesões cervicais não-cariosas têm sido observadas com maior freqüência. Portanto, produtos que possibilitem a redução do desgaste dentário, sendo de fácil acesso e de uso diário, são alternativas ideais para a redução da perda de tecido mineralizado. Dessa forma, esta tese é constituída por três artigos que objetivaram, respectivamente: (1) verificar o efeito preventivo de três pastas comerciais contendo AmF, AmF/SnF_2 or SnF_2 através de um modelo erosivo/abrasivo em dentina humana; (2) investigar o efeito do $Mg(OH)_2$ presente em dentifrícios comercializados na prevenção do processo erosivo no esmalte por ácidos extrínseco ou intrínseco, bem como, a influência do número de dias experimentais na progressão da erosão; (3) avaliar *in situ* a ação do $Mg(OH)_2$ e NaF na prevenção da erosão por ácidos de origem intrínseca em esmalte humano. Nos artigos 1 e 2, foram realizados modelos *in vitro*, cíclico, randomizado, cego, no qual os espécimes foram submetidos a processo de des- e remineralização. No artigo 1, foi simulado processo erosivo por ácido de origem intrínseca, enquanto no artigo 2, foi avaliada erosão por ácido de origem intrínseca e extrínseca. No artigo 1, foi acrescentado o processo abrasivo através da escovação. Para ambos os estudos, o ciclo foi repetido três vezes ao dia durante cinco dias. No artigo 3, foi realizado um estudo *in situ*, randomizado, duplo-cego, cruzado, em três fases de 5 dias cada, com a participação de 18 voluntários, que utilizaram dispositivos palatinos, contendo 2 blocos de esmalte dentário humano tratados com diferentes dentifrícios: controle (0 ppm F), $Mg(OH)_2$ (2%) e NaF (1450 ppm F). Os espécimes foram submetidos à erosão por imersão do dispositivo em um copo contendo HCl 0,01 M (pH=2) por 60 segundos, 4 vezes ao dia, em horários pré-determinados. Em seguida, os voluntários escovaram seus dentes por 25 segundos e, com o dispositivo na boca, bochecharam a suspensão dentifrício/saliva formada por 60 segundos. As alterações ocasionadas nos espécimes de todos os artigos foram avaliadas por testes de dureza e/ou perfilometria mecânica. Os dados obtidos foram testados usando ANOVA ($p < 0,05$). O teste de Tukey foi aplicado, quando necessário, em casos no qual ANOVA revelou diferença estatística. Os resultados do artigo 1 mostraram que dentifrícios contendo AmF/SnF_2 ou SnF_2 reduziram significativamente ($p<0,05$) a perda de superfície dentinária após o processo erosivo/abrasivo. No artigo 2, dentifrícios contendo $Mg(OH)_2$ ou NaF foram efetivos em reduzir a desmineralização ocasionada por ácido cítrico 0,05 M ($p<0,001$) comparados ao grupo controle. Entretanto, para simulação de erosão por origem intrínseca não houve prevenção para ambos os produtos. Os resultados do artigo 3 demonstraram que houve efeito dos dentifrícios testados na redução da perda de superfície de esmalte ($p = 0,021$). Entretanto, os mesmos não evidenciaram efeitos na redução da desmineralização ($p = 0,349$). Dessa forma, conclui-se que dentifrícios contendo fluoreto estanhoso ou hidróxido de magnésio são efetivos em realizar algum efeito protetor no substrato dentário após ação de ácidos de origem exógena ou endógena.

Palavras-chave: Erosão Dentária. Flúor. Hidróxido de Magnésio. Abrasão. Esmalte. Dentina

ABSTRACT

Considering the decline in the prevalence of caries and periodontal diseases in society, there is greater tooth longevity. Thus, non-carious cervical lesions have been observed with greater frequency. Therefore, products that enable the reduction of tooth wear, with easy access and daily use, are ideal alternatives for reducing the loss of mineralized tissue. This thesis consists of three papers that aim, respectively: (1) verify the preventive effect of three commercial AmF, AmF/SnF_2 or SnF_2-containing dentifrices through an erosive/abrasive model on human dentin; (2) investigate the effect of $Mg(OH)_2$ present in commercial dentifrices in preventing the erosion of enamel by extrinsic and intrinsic acids, as well as, the influence of the number of experimental days in erosion progression; (3) evaluate *in situ* the action of $Mg(OH)_2$ and NaF in prevention of intrinsic erosion on human enamel. In Articles 1 and 2, were conducted *in vitro* models, randomized, blind, cyclic, in which the specimens were subjected to the process of de- and remineralization. In Article 1, the erosion was simulated by intrinsic acid, while in Article 2, was intrinsic and extrinsic acids. In Article 1, was added the process of abrasion for toothbrushing. For both studies, the cycle was repeated three times daily for 5 days. In Article 3, the study was *in situ*, randomized, double-blind, crossover in three phases of five days each, with the participation of 18 volunteers who wore palatal appliances containing 2 blocks of human enamel treated with different toothpastes: control (0 ppm F), $Mg(OH)_2$ (2%) and NaF (1450 ppm F). The specimens were subjected to erosion by immersion them in a cup containing 0.01 M hydrochloric acid (pH = 2) for 60 seconds, 4 times a day, in predetermined times. Then, the volunteers brushed their teeth for 25 seconds with the device in the mouth, and the dilution dentifrice/saliva was rinsed mouthwash for 60 seconds. The alterations caused in specimens of all articles were evaluated by hardness and/or stylus profilometry. Data were tested using ANOVA ($p < 0.05$). The Tukey test was used when required, in cases in which ANOVA revealed difference statistically significant. The results showed that in the article 1, AmF/SnF_2 or SnF_2 significantly reduced ($p < 0.05$) the loss of dentin surface after erosion/abrasion. In Article 2, $Mg(OH)_2$ or NaF-containing dentifrices were effective in reducing demineralisation caused by citric acid 0.05 M ($p < 0.001$) compared to control. However, none prevention was observed for simulation of intrinsic acid, for both products. The results of the article 3 showed that tested dentifrices reduced the loss of surface enamel ($p = 0.021$). However, these products not present remineralizing effects ($p = 0.349$). Thus, it is concluded that dentifrices containing stannous fluoride or magnesium hydroxide are effective in performing some protective effect on the dental substrate after action of acids exogenous or endogenous.

Key-words: Tooth Erosion. Fluoride. Magnesium hydroxide. Abrasion. Dentin. Enamel.

SUMÁRIO

Introdução

1 INTRODUÇÃO

O declínio da prevalência da doença cárie e periodontal, devido a estratégias de promoção de saúde, tem permitido que outras patologias orais como o desgaste dentário sejam observadas com maior frequência, como evidenciado por estudos epidemiológicos presentes na literatura (LARSEN; POULSEN; HANSEN, 2005; JAEGGI; LUSSI, 2006). Na última década, muitas pesquisas têm sido realizadas a fim de obter melhor compreensão sobre o processo de desenvolvimento do desgaste dentário (HUYSMANS; CHEW; ELLWOOD, 2011). Esse processo caracteriza-se pela perda não-cariosa de tecido dentário, apresentando uma etiologia multifatorial com o envolvimento de processos interrelacionados incluindo abrasão durante a mastigação e a escovação, a atrição entre dentes antagonistas, dissolução química ocasionada pela erosão e a abfração (IMFELD, 1996).

O processo de erosão dentária ocorre como resultado da dissolução química do dente por ácidos não-bacterianos, no qual íons hidrogênio dos ácidos reagem com a hidroxiapatita $[Ca_{10}(PO_4)_6(OH)_2]$, resultando na liberação de íons minerais (Ca^{2+},OH^- e PO_4^{3-}) (BARTLETT 2005; LUSSI *et al.*, 2011). Os ácidos responsáveis por esse processo podem ser de origem intrínseca ou extrínseca. A dieta é a causa extrínseca mais comum de erosão dentária. Entretanto, medicamentos, como a vitamina C, e, mais raramente, a exposição aos ácidos no trabalho ou em ambientes de lazer também podem causar a desmineralização da superfície dentária (GANDARA; TRUELOVE, 1999; LUSSI; JAEGGI, ZERO, 2004; AMAECHI; HIGHAM, 2005; LUSSI; JAEGGI, 2008; LUSSI *et al.*, 2011; JOHANSSON *et al.*, 2012). O principal ácido envolvido neste processo é o ácido cítrico, um constituinte de muitos sucos de frutas e bebidas ácidas, com um potencial erosivo que pode induzir a desmineralização do tecido dentário. Adicionalmente, o ácido cítrico age como um quelante tendo seu efeito exacerbado pela sua união com o cálcio (SAURO *et al.*, 2008; SERRA; MESSIAS; TURSSI, 2009).

Os fatores responsáveis por erosão de origem intrínseca que ocasionam elevada acidez na cavidade bucal incluem vômitos, desordens alimentares, doença do refluxo gastroesofágico (DRGE), regurgitação, e ruminação (SERRA; MESSIAS; TURSSI, 2009; LAZARCHIL; FRAZIER, 2009; LUSSI *et al.*, 2011). Atualmente, a DRGE tem sido descrita como um importante fator etiológico da erosão dentária, sendo definido como um fluxo retrógrado de conteúdo gástrico para o esôfago que ocorre principalmente após as refeições (RANJITKAR; KAIDONIS; SMALES, 2012). Essa condição apresenta importante interesse sob o ponto de vista odontológico, pois a acidez do conteúdo gástrico pode apresentar um pH de aproximadamente 1,2 (RANJITKAR; KAIDONIS; SMALES, 2012), capaz de dissolver os cristais de hidroxiapatita do esmalte. Pace *et al*. (2008) mostraram, através de uma revisão sistemática, uma forte associação entre DRGE e erosão dentária, na qual observaram uma prevalência média de 24% de erosão em pacientes com o referido distúrbio. Devido aos maiores cuidados com a higiene bucal, alguns pesquisadores têm avaliado a associação dos processos erosivos e a escovação dentária (MAGALHÃES *et al*., 2007; SALES-PERES; PESSAN; BUZALAF, 2007; HUGLES *et al*., 2008, MAGALHÃES *et al*., 2008; MORETTO *et al*., 2010; AUSTIN *et al*., 2011; GANSS *et al*., 2011; ROCHEL *et al*., 2011). Diversos estudos comprovam a ocorrência de maior perda de estrutura dentária por meio da ação sinérgica dos processos erosivos e abrasivos, uma vez que, a superfície dentária submetida à erosão encontra-se amolecida sendo mais suscetível ao desgaste pelo processo abrasivo (HARA *et al*., 2008; MAGALHÃES *et al*., 2008; WIEGAND; EGERT; ATTIN, 2008; MORETTO *et al*., 2010; ROCHEL *et al*., 2011).

A avaliação da perda de estrutura dentária por erosão e/ou abrasão pode ser realizada por diversos métodos de análise. Dessa forma, a perfilometria é o método quantitativo mais comumente aplicado para determinar efeitos erosivos em esmalte ou dentina, seguida pela análise de dureza de superfície quando o substrato for o esmalte dentário e a micro-radiografia quando empregada a dentina (SCHLUETER *et al*., 2011). A dureza de superfície é uma técnica mais sensível para estágios iniciais da erosão, apresentando limitações em lesões mais avançadas, a passo que a perfilometria permite uma melhor observação em estágios avançados de erosão, verificando a perda de tecido dentário por erosão, abrasão ou sua combinação, mas não é sensível para detectar alterações iniciais de superfície (HARA; ZERO 2008; SCHLUETER *et al*., 2011).

Assim, estratégias preventivas à perda de tecido dentário têm sido avaliadas. Um método rotineiramente usado para higienização e de fácil acesso é o dentifrício, que apresenta-se como um veículo de agentes fluoretados ou produtos neutralizantes (HARA *et al*., 2009; HUYSMANS *et al.,* 2011; GANSS *et al.,* 2011; MESSIAS *et al.,* 2011; GHASSEMI *et al.,* 2012). Desse modo, o uso de dentifrícios fluoretados pode ser uma estratégia preventiva para a erosão, reduzindo a desmineralização ou perda de estrutura dentária (SAURO *et al*., 2008; BARLOW; SUFI; MASON 2009; FOWLER *et al*., 2009; HARA *et al*., 2009; MAGGIO *et al*., 2010; PASSOS *et al.,* 2010; GANSS *et al*., 2011; HUYSMANS *et al.,* 2011b; POGGIO *et al*., 2011; RIRATTANAPONG; VONGSAVAN; TEPVICHAISILLAPAKUL, 2011; TURSSI *et al*., 2011).

O efeito protetor do flúor frente à perda de substância dentária por erosão é devido à formação de fluoreto de cálcio (CaF_2) na superfície do esmalte (CHRISTOFFERSEN *et al*., 1988; LAGERWEIJ *et al*., 2006; GANSS *et al*., 2007). O uso de fluoreto estanhoso tem apresentado bons resultados no controle de lesões de erosão, provavelmente pela formação de uma camada protetora que contém produtos da reação do SnF_2 com a hidroxiapatita, como sais de Sn_2OHPO_4, $Sn_3F_3PO_4$, $Ca(SnF_3)_2$ ou CaF_2 (ADDY; MOSTAFA, 1988).

Do mesmo modo, substâncias neutralizantes também têm sido sugeridas para ajudar no controle da erosão por ácido hidroclorídrico e para minimizar os efeitos destrutivos em tecidos dentários. A literatura relata que o emprego de bochechos de bicarbonato de sódio ou suspensões neutralizantes como de hidróxido de magnésio ou hidróxido de alumínio pode reduzir a perda de esmalte dentário (MESSIAS *et al.,* 2010; LINDQUIST *et al.,* 2011; TURSSI *et al*., 2012). Esses compostos agem por neutralização dos ácidos da cavidade bucal e inibindo a redução do pH (MEURMAN *et al*., 1988). Contudo, poucos trabalhos avaliam o efeito de agentes neutralizantes contidos em cremes dentais na prevenção da erosão (MESSIAS; SERRA; TURSSI, 2008).

Outro fator que pode influenciar o processo de desgaste dentário é a saliva, que tem sido considerada o fator biológico mais importante na prevenção da erosão devido à habilidade de agir diretamente no agente erosivo por diluição, limpeza, neutralização e tamponamento de ácidos. A saliva permite a formação de uma membrana protetora, a película adquirida salivar, que age como uma barreira de difusão ou membrana de permeabilidade seletiva prevenindo o contato direto entre os ácidos e a superfície dentária. A saliva também reduz a taxa de desmineralização e age na remineralização por possuir cálcio, fosfato e flúor disponível para o esmalte e a dentina erosionadas (HARA; LUSSI; ZERO, 2006). De acordo com Hara *et al.* (2006), a película adquirida formada por duas horas sobre o esmalte apresenta potencial protetor frente à desmineralização.

A busca constante por produtos que possam prevenir ou até mesmo evitar a perda de estrutura dentária, que sejam de fácil disponibilidade, baixo custo e elevada eficácia, enfatiza a significância clínica dos estudos que compõe esta tese que objetivaram avaliar através de metodologias *in vitro* e *in situ* o efeito de diferentes dentifrícios fluoretados e de agentes neutralizantes disponíveis no comércio na prevenção da erosão associada ou não a abrasão.

Proposição

2 PROPOSIÇÃO

Os objetivos do presente estudo foram:

CAPÍTULO 1 - Avaliar *in vitro* o efeito do tipo de flúor presente nos dentifrícios – fluoreto de amina ou fluoreto estanhoso - na prevenção da perda de superfície após erosão de origem intrínseca e abrasão em dentina;

CAPÍTULO 2 - Avaliar *in vitro* o efeito do hidróxido de magnésio presente em dentifrícios na prevenção da desmineralização e perda de superfície de esmalte humano submetidos à erosão de origem extrínseca ou intrínseca, bem como avaliar o efeito do número de dias experimentais na progressão da erosão;

CAPÍTULO 3 - Avaliar *in situ* o efeito do fluoreto de sódio e do hidróxido de magnésio presentes em dentifrícios na redução da desmineralização e perda de superfície do esmalte humano submetidos à erosão por ácido hidroclorídrico.

Capítulos

3 CAPÍTULOS

Esta tese esta baseada no Artigo 46 do Regimento Interno do Programa de pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato. Por se tratarem de pesquisas envolvendo seres humanos, ou partes deles, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovado (Anexo A e B). Assim sendo, esta tese é composta três capítulos, sendo dois estudos *in vitro* e um *in situ,* conforme descrito abaixo:

✓ Capítulo 1

“EFFECT OF COMMERCIAL FLUORIDE DENTIFRICES AGAINST SIMULATED GASTRIC ACID IN AN EROSION-ABRASION MODEL**”**

Passos VF, Vasconcelos AA, Pequeno JHP, Rodrigues LKA, Santiago SL. Este artigo será submetido à publicação no periódico “*Clinical Oral Investigations*”.

✓ Capítulo 2

“MAGNESIUM HYDROXIDE-CONTAINING DENTIFRICE ON EXTRINSIC AND INTRINSIC ENAMEL EROSION MODEL**”**

Passos VF, Rodrigues LKA, Santiago SL. Este artigo será submetido à publicação no periódico “*Journal of Dentistry*”.

✓ Capítulo 3

“MAGNESIUM HYDROXIDE-BASED DENTIFRICE AS AN ANTI-EROSIVE AGENT IN AN IN SITU INTRINSIC EROSION MODEL”

Passos VF, Rodrigues LKA, Santiago SL. Este artigo será submetido à publicação no periódico “*Journal of Dentistry*”.

CAPÍTULO 1

3.1 Capítulo 1

EFFECT OF COMMERCIAL FLUORIDE DENTIFRICES AGAINST SIMULATED GASTRIC ACID IN AN EROSION-ABRASION MODEL

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Running title: **Dentinal erosive-abrasive inhibition by dentifrices**

Keywords: Abrasion; Dentifrice; Dentin Erosion; Hydrochloric Acid; Fluoride.

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Effect of commercial fluoride dentifrices against simulated gastric acid in an erosion-abrasion model

Abstract

Objectives: This study assessed the effect of three commercial dentifrices with different fluoride-containing compounds using an erosion-abrasion *in vitro* model. **Materials and Methods**: Dentin specimens were randomized into four groups (n=10): Control (no F); Elmex (1,400 ppm AmF); Meridol (1,400 ppm AmF/SnF_2) and Crest Pro-Health (1,100 ppm SnF_2). Dentin specimens were submitted to cycles of demineralization (HCl 0.01 M for 60s), remineralization (artificial saliva for 60 min), and immersion in 1:3 w/w of dentifrice/artificial saliva, followed by toothbrushing (150 brushing strokes). The cycle was repeated three times daily for five days. Surface loss was quantified by stylus profilometry. Data were submitted to one-way ANOVA and Tukey tests ($p<0.05$). **Results**: Wear (µm±SD) was: Control: 4.1±1.2; Elmex: 3.7±1.5; Meridol: 1.3±0.4; Crest Pro-Health: 2.1±0.7. So, all products (except Elmex) reduced mineral loss significantly ($p < 0.05$) when compared to the control. **Conclusion**: None of dentifrices avoid the erosive-abrasive process; however, dentifrices SnF_2-containing were effective in to reduce significantly dentin loss.

Clinical Relevance: Scientific literature shows evidence that fluoride can strengthen dental tissue against erosive acid damage. However, the beneficial effect of different fluorides presents in commercial dentifrices is questionable. So, the determination of an effective fluoride dentifrice may be beneficial in the reduction of erosive process in patients with gastric disorders.

Keywords: Abrasion; Dentifrice; Dentin Erosion; Hydrochloric Acid; Fluoride.

Introduction

Tooth erosion is a loss of dental hard tissues due to the chemical dissolution of teeth by non-bacterial acids, and is caused by both intrinsic and extrinsic factors. The intrinsic source of erosive acids is hydrochloric acid, which is produced by the stomach. The oral presence of this acid is frequently observed in patients who suffer from eating disorders, such as recurrent vomiting, regurgitation or gastro-esophageal reflux [1,2]. Moreover, the extrinsic sources responsible for erosion are acidic drinks and food [2-5]. According Packer [6], the changes in lifestyle have increased consumption of acid-containing products.

The excessive contact with dietary acids can be avoided through of oral health education. In the meantime, individuals presenting chronic reflux or gastrointestinal disorders that cause the secretion of the hydrochloric acid present in the gastric juice into the oral cavity require a prolonged medical treatment until stop to expose your teeth to acid contact [7]. According Ranjitkar, Kaidonis and Smales [7], the success of medical intervention is variable and the adequate treatment if difficult to manage. So, additionally to eliminate the cause, the use of products that reduce the effect of endogenous acid in dental surface should be beneficial.

Application of fluoride at high concentrations or metal ion fluoride may lead to the formation of mainly CaF_2- like products or may form a physical barrier, protecting it against acid attack [8-10]. In recent years, however, researchers have evaluated various regimens regarding fluoride levels existing in toothpastes, solutions, gels and varnishes and their effects on dental erosion and inconsistent results have been found [11-14].

An ideal preventive measure would be the use of fluoridated dentifrices, which are excellent vehicles for fluoride delivery [15] given their global use. Nevertheless, some degree of abrasivity is needed in all dentifrices to allow an adequate cleaning [16]. Searches have shown a minor mineral loss when brushing with fluoride as opposed to non fluoride dentifrices [17-19]. Therefore, new toothpastes with different composition have been developed for protection against caries and erosion. However, only little is known about preventive measures against tissue loss caused by combined erosive-abrasive impacts, in particular if the complex histological structure of eroded dentin is considered [20].

Consequently, this *in vitro* study aimed to investigate the effects of three commercial dentifrices (Elmex, Crest Pro-health and Meridol) containing respectively AmF, SnF_2 or AmF/SnF_2 compared with a non-fluoride dentifrice in the reduction of dentin loss after erosion/abrasion caused by intrinsic acid and toothbrushing. The null hypothesis tested was that there is no difference among the preventive effects of tested dentifrices on dentinal erosion/abrasion.

2. Materials and Methods

2.1 Ethical Aspects:

The protocol (# 131/2009) was approved by the local Ethics Committee. The extracted human teeth were collected after the patient's informed consent. The collected third molars were stored in 0.01% (w/v) thymol solution at 4°C and used within one month after extraction.

2.2. Dentin Sample Preparation

Root dentin slabs (4 x 4 x 2 mm) were obtained from freshly extracted and caries-free third molars. The dentin slabs were obtained using a water-cooled low-speed diamond disc mounted in a sectioning machine (IsoMet™ Low Speed Saw Buehler, Lake Bluff, IL, USA). Subsequently, the slabs were embedded in self-polymerized acrylic resin cylinders (Arotec SA Ind E Com, Cotia, SP, Brazil) with the external surface of the substrates exposed to facilitate handling. The slabs were ground flat with wet 320-, 600- and 1,200-grit silicon carbide paper (Extec, Enfiel, CT, USA) and polished with a diamond spray of 1 μm (Extec, Enfiel, CT, USA). After each flattening and polishing procedure, the specimens were sonicated for 2 min (Ultra Cleaner 1400, UNIQUE, Indaiatuba, SP, Brazil) in deionized distilled water, and polished surfaces free of visual defects or cracks were selected for this study.

An initial surface hardness (SH) test was performed with five indentations in the center of the slab with a Knoop diamond under a 25 g load for 5 s (FM7 AMRS; Future Tech, Tokyo, Japan) to select 50 slabs. The slabs, which presented microhardness values ranging from 52-64 Kg/mm^2, were randomly assigned into five groups (Table 1) according to a computer generated randomization list (n=10). The pH-values were obtained of slurry containing 1:3 w/w of dentifrice/artificial saliva and all the toothpastes present silica as abrasive.

In order to maintain reference surfaces for lesion depth determination, a layer of an acid-resistant varnish with a dark color (Colorama, CEIL Coml Exp Ind Ltd., São Paulo, Brazil) was applied on one-half of the surface of each slab.

2.3. Erosion-abrasion Cycling Model

Demineralization was performed by the immersion of each slab in 3 mL of hydrochloric acid (HCl 0.01 M, pH 1.96) for 60 s. Subsequently, each sample was remineralized in 5 mL of artificial saliva (1.45 mM Ca, 5.4 $mMPO_4$, 0.1 M Tris buffer, 2.2 g/L porcine gastric mucin, pH 7.0) under constant agitation for 60 min. The slabs were then immersed in 3 mL of 1:3 w/w of dentifrice/artificial saliva for 60 s. Next, the specimens were positioned in a brushing machine, (MSEt – 1500W – Marcelo Nucci ME – São Carlos, SP – Brazil**)** and brushed with 150 strokes (200-g load, 37°C). During this procedure, specimens were plated for suspensions of dentifrice and artificial saliva (1:3; w/w), according to each group. After each cycle, which was composed of demineralization, remineralization and toothbrushing, the specimens were again rinsed in deionized distilled water. All solutions were used at room temperature. The described cycle was repeated three times daily for five days at room temperature, as shown in Figure 1. After the daily cycles, the samples were stored overnight in artificial saliva at 37ºC.

2.4. Tissue Loss Measurements

After the experimental period, the nail varnish layer over the surfaces was carefully removed using acetone and a scalpel blade without touching the dentinal surfaces. The specimens were allowed to dry for 10 min before analysis in order to reduce the possible interference caused by the shrinkage of dentin organic content. Dentinal wear was determined in relation to the reference surfaces, using a profilometer (Hommel Tester T1000, Hommelwerke GmbH, Germany). Five readings were performed on each slab, at intervals of 100 µm. These profilometric traces were taken by moving the stylus from the reference to the exposed surfaces. For each sample, mean was calculated from the values obtained from the five traces.

2.6. Statistical Analysis

Mean and standard deviation (SD) values of wear per group were calculated. Statistical procedures were performed with the Statistical Package for Social Sciences (SPSS 17.0) for Windows. A Kolmogorov-Smirnov test was applied to all groups to test for the normal distribution of errors. Because the values were normally distributed across all of the groups, ANOVA and Tukey's post hoc tests were used for comparative purposes. The level of significance was set at 5%.

3. Results

Mean dentinal loss of all experimental groups is presented in Figure 2. After five days of erosion and abrasion cycles, tissue loss was highest in the control group, and all products (except Elmex) reduced mineral loss significantly in the order of 37 – 67% ($p < 0.05$ when compared to the control). However, Meridol, Crest Pro-health and Sensodyne Pronamel were statistically similar to each other. There were no no significant difference between control group and Elmex ($p = 0.948$).

4. Discussion

This study evaluated the effect of three commercial dentifrices containing AmF, SnF_2 or AmF/SnF_2 on reduction of dentin erosion using an erosive-abrasive model. The aim of erosion-abrasion model is to reduce the mineral loss by improving the characteristics of resistance of tooth surfaces. So, a preventive method that can be used daily and that acts against caries and erosion would be an excellent option.

Fluoride delivery by dentifrice has been evaluated as an alternative to reduce erosion [21] considering that dentifrices are used daily and can be easily accessed by the general population. Some authors have showed that fluoride dentifrices can enhance the resistance of the eroded dental substrate [17, 19, 22, 23]. However, the level of wear can be modulated not only by fluoride, but also by abrasiveness of dentifrice [17]. In this study, dentifrices tested presented values of Relative Dentin Abrasion raging from 70 to 144, below the limit of 250 recommend by ISO standard 11609 and ADA. This way, all dentifrices tested display a medium abrasivity [24].

From the present results, the hypothesis that there are no differences in the effect between dentifrices tested was rejected, because dentifrices containing fluoride in the forms of SnF_2 (Crest Pro-Health Enamel Shield) and AmF/SnF_2 (Meridol) reduced the tooth wear caused by hydrochloric acid on dentin *in vitro.* Thus, the results reinforce the relevance of dentifrices containing fluoride in the reduction of dental erosion, similar to other *in vitro* [21] and *in situ* studies [19, 23, 25].

To our knowledge, few studies have evaluated the erosion/abrasive effects of AmF-containing dentifrices on dentin. Application of AmF fluoride may lead the formation of CaF_2 products that can protect against acid attack. However, the formation of CaF_2- like products does not sufficiently resistant to avoid loss of dental structure after a strong erosive process occasioned by a simulated gastric acid and a brushing with dentifrice of medium abrasivity. Hara *et al.* [17] verified the interaction of fluoride and abrasivity of dentifrices obtained that in root dentin the abrasivity had a higher impact than fluoride in the reduction of tissue loss.

In respect of AmF, the results of the present study are corroborated with previous studies [13, 26], which have used different fluoridated solutions or dentifrices to evaluate the mineral loss in enamel and found no differences between AmF and the absence of any fluoride in inhibiting tooth wear. Otherwise, different results were demonstrated by Wiegand *et al.* [27] and Wegehaupt *et al.* [28], which showed a statistically significant capacity of AmF in preventing dental erosion. Probably, the result observed by Wiegand *et al.* [27] may be attributed to use of a weak acid (Sprite Zero) to perform the erosive challenge, which might have promoted a lower wear rate. Besides, the authors used a more acidic AmF solution (pH = 4.5) that may have increase the fluoride action, since the lower the pH of fluoride greater the formation of calcium fluoride [29]. Wegehaupt *et al.* [28], also, observed a beneficial effect, however the AmF solution present a concentration approximately ten times greater than ours.

The best effects were found when Sn-containing dentifrices were used, corroborating with other studies that assessed solutions or dentifrices containing same fluoride types [11, 14, 30-32]. Probably, this result can be attributed to the fact that when Sn-products are used, the dentinal surface is covered by a layer containing the reaction products of SnF_2 and hydroxyapatite, such as Sn_2OHPO_4, $Sn_3F_3PO_4$, $Ca(SnF_3)_2$ or CaF_2 salts, which obliterated patent dentinal tubules [10]. According to other studies, this distinct coating is capable of survival even after a 2-min immersion in citric acid solution [13] or a 50% decrease in the etching depths after acid exposure for 6 min on enamel [12]. In addition, the simple presence of the cited salts may be relevant for protection against erosion. Recently, Ganss *et al.* [33], showed that tin is retained in the demineralized organic matrix, beyond what is diffused through the dentin structure and is accumulated in the underlying mineralized tissues. These authors suggest that the main mechanism of action of SnF_2 is the uptake of tin in the underlying mineralized tissues, rather than surface precipitation.

Nevertheless, when Sn fluoride was used, the wear was modulated by the fluoride effect, and both Meridol and Crest Pro-health present similar wear rate. So, the kind of fluoride may affect the ability of dentifrices to remineralize eroded substrate, that was no observed when Am-F dentifrices were used. The results of study show that the protective effect of SnF_2 is effective even using strong acid challenge. These SnF_2 products can improve the fluoride action and minimize the effect of abrasivity, without harming the dental cleaning.

The *in vitro* experimental model used suggests that the kind of fluoride influences in the reduction of erosive process by simulated gastric acid, presenting a effective impact on the wear. So, considering the limitations of the study, it can be concluded that Meridol and Crest Pro-Health, both containing SnF_2, are effective in reducing mineral loss after erosion by intrinsic acid and abrasion in root dentin.

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Table

Table 1. Dentifrice product information and pH-values obtained of slurry (dentifrice/artificial saliva)

TRADENAME	FLUORIDE	pH	RDA	MANUFACTURER
Control	No F	7.33	70	Manufactured
Elmex	1,400 AmF	5.86	100	Gaba international, Münchenstein, Switzerland
Meridol	1,400 AmF/SnF_2	6.00	100	Gaba international, Münchenstein, Switzerland
Crest Pro-Health Enamel Shield	1,100 SnF_2	5.64	144	Crest, Procter and Gamble, Cincinnati, OH, USA

Figures

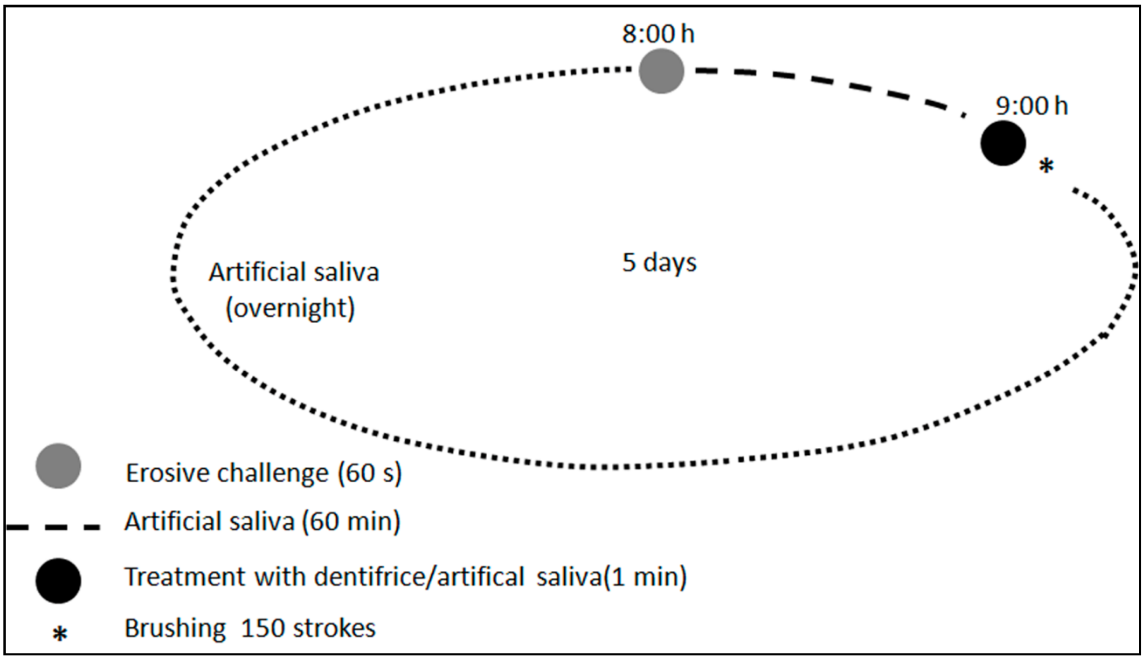

Figure 1. 24-hour cycle followed during experiment. This sequence was repeated during 5 days.

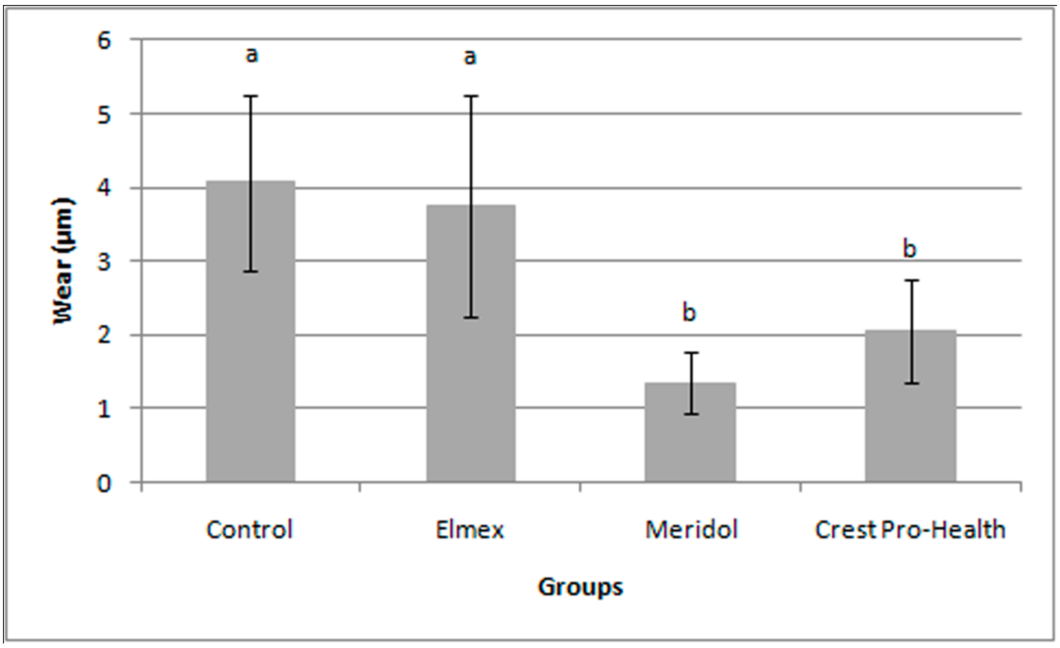

Figure 2 - Erosive/abrasive dentine loss after five days of various treatment procedures. Bars sharing the same letter are not significantly different.

Capítulo 2

3.2 Capítulo 2

Magnesium hydroxide-containing dentifrice on extrinsic and intrinsic enamel erosion model

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Running title: **Erosive inhibition by fluoride or magnesium hydroxide-dentifrices**

Keywords: Magnesium hydroxide; Dentifrice; Hydrochloric Acid; Sodium Fluoride; Citric acid.

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Abstract

Objective: To evaluate, *in vitro,* the effect of $MgOH_2$ dentifrice as well as the influence of the number of experimental days on extrinsic (citric acid - $C_6H_8O_7$) and intrinsic (hydrochloric acid - HCl) enamel erosion models. **Methods:** Human enamel slabs were selected by surface hardness and randomly assigned to 3 groups (n=9) as follows: non-fluoridated (control), NaF (1,450 ppm F) and $Mg(OH)_2$ (2%) dentifrices. In this study, the slabs were submitted daily to a previous 2 hour-acquired pellicle formation and, during 5 days, submitted to cycles (3x/day) of demineralization ($C_6H_8O_7$ 0.05 M, pH 3.75 or HCl 0.01 M, pH 2 for 30 s), treatment (1 min - 1:3 w/w of dentifrice/distilled water) and remineralization (artificial saliva for 120 min). Enamel changes were determined by surface hardness loss percentage (SHL) at each day and mechanical profilometry analysis. Data were analyzed by two-way ANOVA followed by Tukey's test to %SHL and one-way ANOVA to profilometry ($p<0.05$). **Results:** The number of experimental days influenced the erosion process in the two types of erosion ($p<0.001$). NaF and $Mg(OH)_2$-containing dentifrices were effective in reducing enamel extrinsic acid erosion for %SHL ($p<0.001$) compared to the control group, however, they were not effective for the intrinsic one ($p = 0.295$). With regard to surface wear, no statistically significant difference was found among treated and control groups for citric acid ($p=0.225$) and hydrochloric acid ($p=0.526$). **Conclusion:** The findings suggest that NaF and $MgOH_2$ dentifrices might protect enamel against slight erosion, but protection was not effective in the strong acid erosion.

Keywords: Magnesium hydroxide; Dentifrice; Hydrochloric Acid; Sodium Fluoride; Citric acid.

Introduction

The frequent ingestion of citrus fruits, pure acidic juices or carbonated sports drinks, as well as gastrointestinal disorders that cause the secretion of gastric acid into the oral cavity may lead to demineralization and loss of dental hard tissues.[1,2] According to Duffley *et al.*[3], adolescents consume an average of 360 ml of acid drinks per day, which shows a high ingestion of acid-containing products. A systematic review showed a median prevalence of 24% for tooth erosion in adult patients with gastro esophageal reflux disease,[4] proving that gastric acid is also an important etiological factor to dental erosion.

The avoidance of lifelong contact of erosive acidic contents with dental surfaces is an impossible task. Therefore, the development of early diagnostic methods and adequate preventive approaches should be searched.[1] Most preventive measures are based on the release of compounds by oral solutions or dentifrices due to daily use and easy access to over the counter merchandise. These products can introduce chemical protective substances into the oral cavity as buffer agents.[5] Sodium bicarbonate and magnesium hydroxide are the most commonly found buffer agents[6,7] available as solution or incorporated into dentifrices.

Different neutralizing products have been assessed to reduce intrinsic erosion.[5,8-10] In this regard, the protective effect of antacid products on dental erosion has been shown in some studies.[8-10] According to Lindquist *et al.*[9], these products increase the intra-oral pH after the erosive process, presenting a buffering effect.[6] Furthermore, this effect may relate to the fact that these products could react with the acid, forming a salt.[10] However, there is no published data on the effect of dentifrices containing magnesium hydroxide on enamel surfaces exposed to a simulated exogenous or endogenous erosive model.

The aim of the present study was to assess the effect of magnesium hydroxide, fluoridated and non-fluoridated based dentifrices in terms of reducing the progression of enamel surface erosion originated by extrinsic acid (experiment 1) or intrinsic acid (experiment 2). In addition, the influence of the number of experimental days in enamel surface softening. The null hypothesis tested was that there is no difference among the tested dentifrices against both kinds of acid contents.

Material and methods

Preparation of enamel samples

The study protocol was reviewed and approved in the local Research and Ethics Committee (protocol #75/12). Enamel slabs were obtained from caries free human third molar that had been stored in 0.01% (w/v) thymol solution at 4°C.[11] Enamel slabs (4 x 4 x 2 mm) were cut from the middle third of the coronal surface. Each slab was ground flat with the dentin and enamel surfaces planar parallel, and polished. The enamel surface was sequentially ground in a water-cooled mechanical grinder (Ecomet/Automet 250 Grinder-Polisher; Buehler, Lake Bluff, IL, USA) with 400-, 600-, and 1,200-grit Al_2O_3 papers and polished on cloths with a 1 µm diamond suspension (Alpha Micropolish; Buehler).

A total of fifty-four enamel slabs were randomly divided into experimental groups based upon their baseline surface hardness values (SHbas), using a computer generated list (Microsoft Excel 2007). The SHbas values were determined by placing five indentations, 100 µm apart from each other at the center of the specimens using Knoop indenter with a load of 50 g and a dwell time of 5 seconds (FM100, Future Tech, Tokyo, Japan). Enamel specimens presenting a mean hardness of 328.1 ± 13.1 Kg/mm² were selected and allocated to three groups to experiment 1 and to experiment 2 (n=9) generating balanced groups.

Subsequently, two parts of each specimen were covered with a dark-colored acid-resistant varnish (Jordana Cosmetics Corp., Los Angeles, CA, USA) to serve as the reference area for profilometry analysis. The exposed area of 2 x 4 mm in their central area was subjected to treatments. In experiment 1, the acid challenge was performed using 0.05 M citric acid (citric acid dehydrated, pH 3.75; Dinâmica®, Diadema, SP, Brazil), while in experiment 2, 0.01 M hydrochloric acid (pH 2.0; Merck, Darmstadt, Germany) was used. The experimental groups were: non-fluoridated (control, 0 ppm F; pH = 6.86), NaF (1450 ppm F; pH = 7.36) and $Mg(OH)_2$ (0 ppm F; 2%; pH = 9.96) dentifrices.

Pellicle formation

In each experimental day, fresh saliva samples were collected from groups of 15-20 volunteers without active carious lesions, erosions, or salivary dysfunction. The subjects did not eat or smoke during the 8-hour period before sampling. Saliva was stimulated with paraffin wax for 5 min. Saliva from the first minute of chewing was swallowed, and the rest was collected and deposited into a 50-ml centrifuge tubule. The saliva samples were centrifuged for 10 min at 2000 rpm in a pre-cooled centrifuge **(**4°C**)** *(5415R*, Eppendorf, Brazil). The clear fluid above the sediments was pooled and used for pellicle formation.[12] Each group of enamel slabs was independently immersed in clarified saliva and incubated during 2 hours before each experimental day, prior to erosive challenges, under agitation at 100 rpm (5 ml per slab) at 37°C to simulated oral cavity temperature.

Experimental procedure

The study consisted of 2 separate experiments. Both experiments consisted of cyclic procedures repeated over a five–day period, including pellicle formation, erosion, treatments with test dentifrices and remineralization with artificial saliva (1.5 mM Ca; 0.9 mM PO_4; 150 mM KCl and 0.1 M Tris buffer, pH 7.0)[13] (Figure 1).

In each experimental day, all procedures were performed under agitation at 100 rpm, at 37°C. All specimens were immersed in clarified saliva during 2 hours to allow formation of the acquired pellicle. Subsequently, each slab was submitted to citric acid or hydrochloric acid solution during 30 seconds. After, the specimens were also treated with fresh dentifrice slurry (5 ml for specimen) during 1 minute prepared from non-fluoridated, magnesium hydroxide or sodium fluoride dentifrices (1 part of toothpaste to 3 parts of distilled water solution, by weight). The slurries were freshly prepared at the beginning of each experimental day. Next, each slab was rinsed with distilled water and immersed in artificial saliva during 2 hours. This cycle was repeated three times a day during 5 days, in the end of each experimental day, the slabs were evaluated by surface hardness, as shown in Figure 1.

Measurement of enamel surface loss

Measurements were performed with a stylus profilometer Hommel Tester T1000 (Hommelwerke GmbH, Germany) after the last experimental day. The measurement of interest was the difference between the heights of the surfaces of the reference and the treated areas. Before the analysis, the nail varnish was carefully removed exposing the untreated references areas. On each sample, at intervals of 100 μm, five profile traces (1.5 mm in length) were recorded, being the levels of enamel wear determined in relation to the reference surfaces. For each sample, the mean values obtained from the five traces were calculated.

Percentage of surface hardness loss assessment

Immediately after completion of each experimental day, slabs were placed in the hardness machine and five new indentations (SHafter), similarly described for the baseline determinations, spaced 100 μm apart from the previous measurements (SHbas) were made with a Knoop diamond under a 50 g load for 5 s. The percentage of SH loss (% SHL) was then calculated for each day according to the equation:

$$\%SHL = [(SHbas. – SHafter) \times 100/ SHbas.]$$

Statistical analysis

Mean values of wear and %SHL were calculated. A Kolmogorov-Smirnov test was applied to all groups to test for the normal distribution of errors. Because the values were normally distributed across all of the groups, two-way ANOVA was carried out to analyze percentage of SH loss to evaluate the influence of treatment and of number of experimental days. One-way ANOVA was used to evaluate profilometry among the groups. Tukey’s post hoc test was applied, when necessary, in cases where ANOVA revealed significant differences. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 17.0) for Windows. The level of significance was set at 5%.

Results

In experiment 1, two-way ANOVA revealed a significant difference among the dentifrices tested ($p<0.001$; $F=26.0$), as well as the duration of demineralization represented by number of experimental days ($p<0.001$; $F=68.0$). However, the interaction between the factors was not significant ($p=0.982$; $F=0.24$). It was observed that with an increase in the number of experimental days, all specimens displayed a statistically significant surface softening from day 1 to day 3, which stabilizes at days 4 and 5. The results showed that magnesium hydroxide reduced significantly the %SHL compared to non-fluoridated ($p=0.0001$) and fluoridated dentifrices ($p=0.001$). Sodium fluoride reduced the percentage of surface loss compared to non-fluoridated dentifrice ($p=0.001$) [Figure 2]. The dentifrices tested showed no significant effect on wear compared to non-fluoride dentifrice (Table 1).

In experiment 2, two-way ANOVA did not show a significant difference among the tested dentifrices ($p=0.295$; $F=1.23$). However, the %SHL to the number of experimental days differed significantly ($p<0.001$; $F=48.43$). The interaction between factors (tested dentifrices and number of experimental days) was not significant ($p=0.326$; $F=1.16$). Demineralization influenced by number of experimental days was similar between experiment 1 (citric acid) and 2 (hydrochloric acid) (Figures 2 and 3). Dentifrices tested showed no significant preventive effect against wear compared to non-fluoride dentifrice (Table 1).

Discussion

Our *in vitro* de-remineralization cycling model investigated two products with respect to their capacity to protect enamel from intrinsic or dietary acids. This study confirmed the expected surface softening and enamel tissue loss due to action of citric acid or hydrochloric acid, even after the pellicle formation for 2 h before each experimental day. The use of an *in vitro* multiple-exposure acid model allows a better understanding of the erosive challenges faced by the dentition, and performed a controlled investigation, reducing experimental time and cost.[14]

The null hypothesis that there is no difference in the preventive effect among the tested dentifrices on extrinsic enamel erosion caused by citric acid was partially rejected, due to an observed difference in the percentage of surface hardness analysis. Magnesium hydroxide and sodium fluoride reduced the surface softening compared with a non-fluoridated dentifrice.

The results of this extrinsic erosion model reinforce the relevance of magnesium hydroxide and sodium fluoride-containing dentifrices in the reduction of enamel erosion caused by dietary acids. However, magnesium hydroxide was more effective than sodium fluoride in protecting the human enamel against citric acid erosion. This result may be explained through the acid buffering that occurs immediately after the contact of $Mg(OH)_2$ and $C_6H_8O_7$, that produce a salt and H_2O. The use of magnesium hydroxide-dentifrice may also help saliva to neutralize and clean erosive products from the oral cavity.[8] Lindquist *et al.*[9] showed that antacid products can increase the intra-oral pH after an erosive challenge, which is desirable to obtain acid neutralization. Similarly, Messias *et al.*[8] using sodium bicarbonate solution observed a preventive effect even in intrinsic erosion *in situ*.

Furthermore, the relevance of sodium fluoride-containing dentifrices in the reduction of enamel erosion caused by dietary acids was described by other *in vitro*[15,16] and *in situ* studies.[17,18] White *et al.*[19] verified that low concentrations (10 ug/g F^-) of sodium fluoride solutions are already capable of reducing hydroxyapatite dissolution in citric acid *in vitro*. Therefore, 1450 ppm F present in the NaF dentifrice even after dilution in saliva can be effective. The preventive effect of sodium fluoride dentifrice in erosive lesions is mainly based on the deposition of high amounts of CaF_2-like products on enamel surface, which can protect it against acid attack.[20,21] This layer is formed on enamel surfaces even within a short exposure time of 20 s - 2 min,[22] reducing initial erosion. However, the protective action will be reduced after repeated and excessive acid contact. Another point to consider is the pH of fluoride, which presents an important role in the efficacy of the preventive agent, since acidic fluoride increases the formation of CaF_2-like deposit. In the present study, sodium fluoride dentifrice may be less effective than magnesium hydroxide in the protection of enamel erosion, because the NaF dentifrice presented a neutral pH.

Meanwhile, sodium fluoride and magnesium hydroxide dentifrices were not effective to reduce the enamel loss. In fact, surface profilometry analysis is more indicated to evaluate advanced erosion,[23] which did not occur in experiment 1. Based on the present results, enamel surface loss was only of approximately 0.2 µm after five experimental days for extrinsic acid exposure, whereas intrinsic acid exposure produced a surface loss sevenfold more severe, proving a moderate action of acidic beverages. However, profilometric analysis is widely accepted as a technique to evaluate erosive challenge.[24] Despite the outcome of the profilometric analysis not properly assess the prejudicial effect of erosion in the case of experiment 1, this analysis serves as a complementary assessment to prove the loss of tooth structure at the end of experiment.

In both analyses of experiment 2, the tested dentifrices failed to reject the null hypothesis. A possible explanation for the lack of benefit of sodium fluoride and magnesium hydroxide dentifrice used in this *in vitro* report may be the severe action of hydrochloric acid, which may have masked the preventive effect of these products. Despite the methodological differences, studies using hydrochloric acid to simulate intrinsic erosion and using fluoride or antacid dentifrices agree with our findings.[5,21] Moreover, in the case of NaF dentifrice, the time of application (1 min) using a neutral sodium fluoride may be not sufficient to form a resistant CaF_2-layer able to prevent simulated intrinsic erosion. Besides, the use of diluted dentifrices (1:3) may have also reduced the formation of CaF_2, damaging the protective effect of sodium fluoride to strong acids.

Dental biofilm may serve as a reservoir of magnesium hydroxide. However, in the present *in vitro* report this complementary action is not observed, probably reducing the protecting effect exerted by $Mg(OH)_2$. Thus, further studies mimicking *in vivo* conditions and reproducing intra-oral influences as a constant action of saliva may be needed to verify the real effect of this agent. Turssi *et al.*[10] shows that magnesium hydroxide suspension can provide a significant reduction on surface enamel loss. Nevertheless, this effective result may be based on high concentration of magnesium hydroxide in the suspension used by the authors (80 mg ml^{-1}), while the concentration used in the present study was approximately 6 mg/ml. Besides, the erosive cycling model used was less aggressive (5 cycles) than the one used in our study (15 cycles).

In both experiments, the surface hardness and tissue loss results (Figure 2 and 3; Table 1) showed that all dentifrices were unable to avoid the simulated extrinsic or intrinsic erosive challenges on human enamel. Moreover, an increase in the number of experimental days generated a longer exposure time to acids, leading to greater softening of surface enamel, confirmed the progressive destruction of the enamel structures. According to Amaechi *et al.*[25] and West *et al.*[26], the frequent contact of the teeth with acidic products leads to the loss of a protective layer on the enamel surface, exposing a more vulnerable surface to future acid exposure. In addition, Creanor *et al.*[27], evaluating continuous or intermittent orange juice tooth exposure, observed that intermittent erosive protocol performed considerably greater lesion depths, being more realistic. Intermittent erosive challenge does not allow mineral ions dissolved from the tooth to influence the undersaturated condition generated by acid beverages. Hence, new acid solution constantly remove more calcium and phosphate from the tooth surface.

Conclusion

In conclusion, within the limitations of this *in vitro* study, the use of fluoride or hydroxide magnesium-containing dentifrices can present a protective role in extrinsic enamel erosion. However, the intrinsic erosive lesions caused by hydrochloric acid were not prevented by these therapeutic products. Furthermore, frequent and chronic contact of acids with the dentition progressively increases erosion, even in the presence of protective products.

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Figures

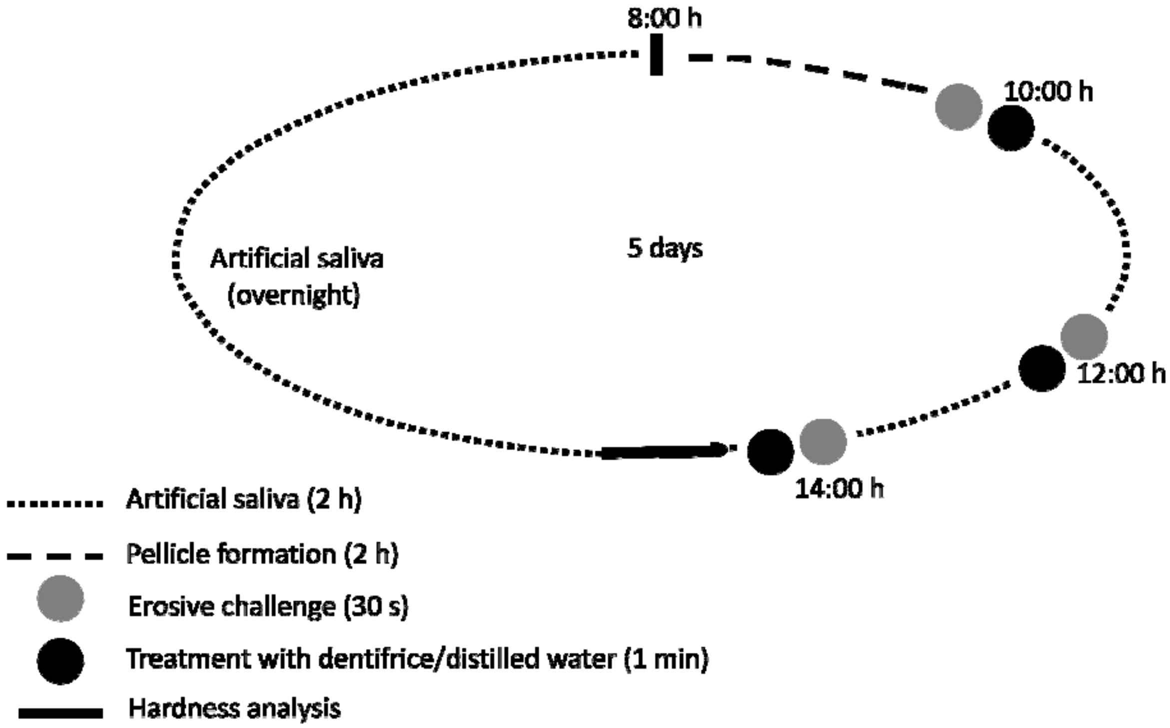

Figure 1. 24-hour cycle followed during experiments. This sequence was repeated during 5 days.

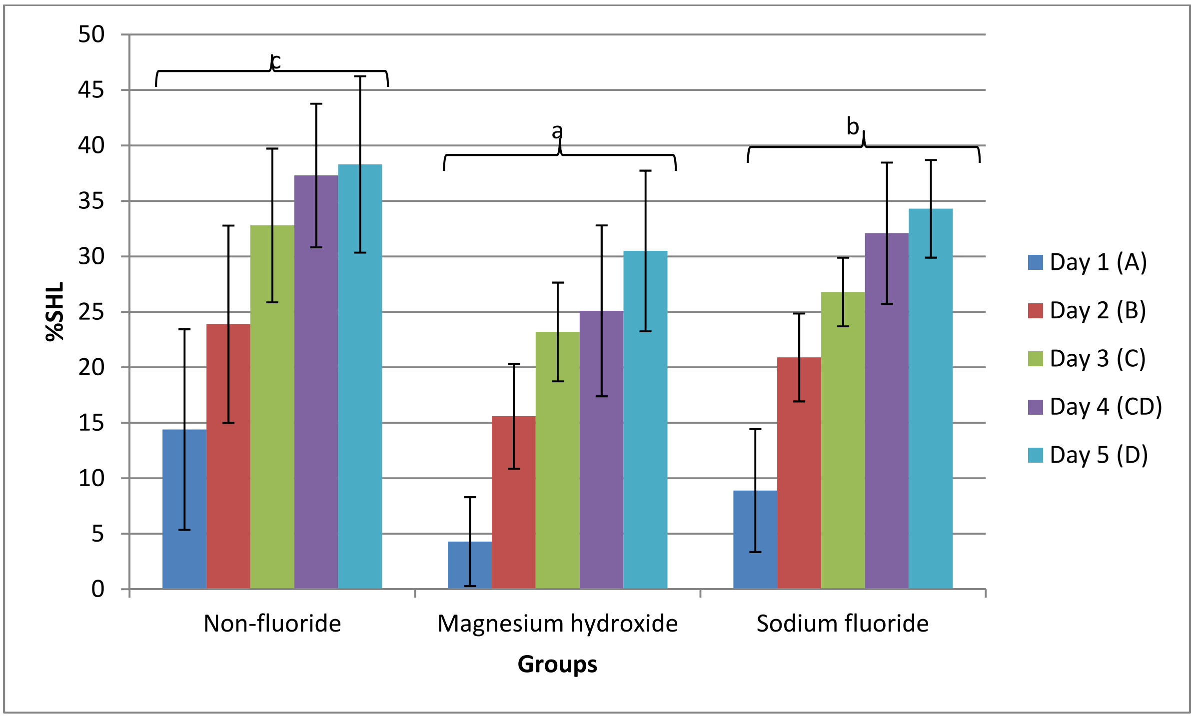

Figure 2. Percentage of surface hardness loss after each erosive day using citric acid (experiment 1) using two-way Anova and Tukey tests. Different capital letters imply statistical differences among the dentifrices and lower letters between each day.

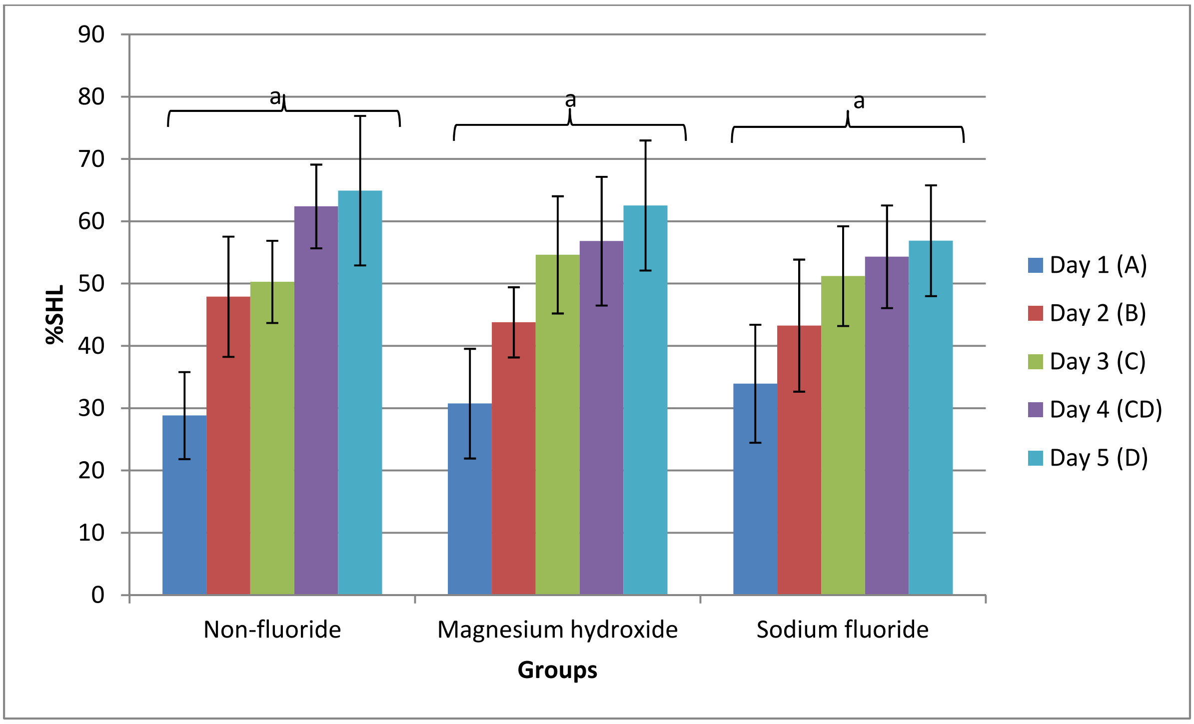

Figure 3. Percentage of surface hardness loss after each erosive day using hydrochloric acid (experiment 2) using two-way Anova and Tukey tests. Different capital letters imply statistical differences between each day.

Table 1. Mean enamel surface loss (SD) in µm of specimens subjected to extrinsic or intrinsic erosion in the presence of different dentifrices slurries after 5 days of experiment.

	Enamel surface loss			
Experiment	**Dentifrice**	**N**	**Wear (µm)**	**p-value**
1 (Citric acid 0.05 M , pH 3.75)	Non-fluoride	N=9	0.28 (0.10)	0.225
	Magnesium hydroxide	N=9	0.21 (0.07)	
	Sodium Fluoride	N=9	0.27 (0.09)	
2 (Hydrochloric acid 0.01 M, pH 2.0)	Non-fluoride	N=9	1.53 (0.63)	0.526
	Magnesium hydroxide	N=9	1.53 (0.54)	
	Sodium Fluoride	N=9	1.27 (0.50)	

The statistical test applied was ANOVA.

Capítulo 3

3.3 Capítulo 3

Magnesium hydroxide-based dentifrice as an anti-erosive agent in an *in situ* intrinsic erosion model

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Running title: ***In situ* erosive inhibition by fluoride or magnesium hydroxide-dentifrices**

Keywords: Gastric acid; Magnesium hydroxide; Erosion; enamel; Sodium fluoride.

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Abstract

Objective: To evaluate *in situ* a magnesium hydroxide [$Mg(OH)_2$] and sodium fluoride [NaF] based dentifrice on enamel erosion. **Methods:** Human dental enamel slabs were selected by surface microhardness and randomly assigned to one out of the following 3 groups (n=18): non-fluoride (control), NaF (1,450 ppm F) and $Mg(OH)_2$ dentifrices. Eighteen volunteers were enrolled in a randomized, crossover and double-blind study, with 3 phases of 5 days. They wore acrylic palatal appliances containing 2 human enamel slabs, which were treated with one of the three dentifrices. During each experimental phase, the specimens were subjected to erosion by immersion in 0.01 M HCl for 60 s, 4x/day, followed by treatment with the correspondent slurry (saliva/dentifrice) during one minute. Enamel changes were determined by percentage of surface hardness loss (%SHL) and mechanical profilometry analysis. Data were analyzed by ANOVA followed by Tukey's test ($p<0.05$). **Results:** The means (SD) for %SHL and surface wear (µm) were respectively: control [50.67 (17.48); 2.70 (1.24)], NaF [45.45 (15.44); 1.95 (0.70)] and $Mg(OH)_2$ [(53.94 (19.48); 1.95 (0.67)]. No statistically significant difference was found among treated and control groups for %SHL ($p=0.349$), however, for wear rates, a statistically significant difference was found between groups treated with NaF and $Mg(OH)_2$ and control group ($p=0.04$). **Conclusion:** In the context of dentifrice use and under the conditions of this study, NaF and $Mg(OH)_2$ dentifrices were effective in reducing enamel wear.

Key words: Gastric acid; Magnesium hydroxide; Erosion; enamel; Sodium fluoride.

Introduction

Dental erosion due to intrinsic factors is caused by gastric acid reaching the surfaces of the tooth as a result of recurrent vomiting, regurgitation, gastro-esophageal reflux or chronic alcoholism.[1] The main component of gastric juice is hydrochloric acid, which is produced by the parietal cells in the stomach.[2] Involuntary movements of stomach acids into the mouth might erode dental hard tissue.[3]

The association of some abnormality in the gastro-intestinal tract and tooth erosion has been underestimated by the general medical literature.[4] However, Pace *et al.*[5], in a systematic review, show a strong correlation between gastro-oesophageal reflux disease and dental erosion, in children and adults. Clinically, the consequences of intrinsic erosion are severe and need extensive treatments to re-establish loss of tooth structure and function. Although, the main method for preventing intrinsic erosion is eliminating its cause,[4] while treatment is being rendered, additional care should be taken to prevent dental erosion.

Although saliva and remineralizing foods have been shown to decrease enamel erosion,[6-9] preventive measures with improved characteristics to reduce tooth wear have been investigated. Considering that dentifrices are used daily and are acessible, preventive and therapeutic agents delivered by dentifrices have been evaluated as an alternate measure to reduce erosion.[10,11] The protection of ionic fluoride from NaF, amine fluoride (AmF) or stannous fluoride (SnF_2) dentifrices on enamel erosion has been shown in some studies,[10,11-15] but not in others.[16-18]

Products such as alkaline solutions have helped to control the harshness of hydrochloric acid and reduced the irreversible effects on enamel tissues.[19-21] However, there is a lack of investigation on the effect of antacid magnesium hydroxide-based dentifrice on enamel erosion *in situ*.

In situ and *in vitro* models have been used to analyze the challenges erosive in dental tissue, due to significant problems in conducting *in vivo* erosion's study. On the other hand, *in situ* studies allow a more realistic assessment of the effect of erosive and protective agents in a natural environment of saliva flow, pellicle development and routine care.[22] Consequently, this *in situ* study aimed to investigate the effect of dentifrices containing sodium fluoride and magnesium hydroxide compared with a non-fluoride dentifrice in the reduction of enamel erosion caused by hydrochloric acid. The null hypotheses tested were: (i) there is no difference in surface hardness among the tested dentifrices on enamel erosion; and (ii) there is no difference in surface loss among the groups.

Material and methods

Panelist and ethical aspects

The study protocol was reviewed and approved by the local Research and Ethics Committee (protocol #75/12). Eighteen healthy adult volunteers (13 female and 5 male, aged 20-32 years) living in an optimally fluoridated area (0.7 μg/ml), free from erosion and caries activity or periodontal disease, and able to comply with the experimental protocol were invited to participate, and signed a consent form. Subjects with gastro-oesophageal reflux disease or eating disorders, those wearing fixed or removable orthodontic devices or using drugs that could affect salivary flow rates were not included in the study.

Experimental design

This *in situ* study had a crossover, randomized and double-blind design. The study assessed the effects of three dentifrices: non-fluoride (control), sodium fluoride (NaF-1,450 ppm F) and magnesium hydroxide [$Mg(OH)_2$ - 0 ppm F] dentifrices. The study was double blinded to the person responsible for performing the sample and data analysis. The dentifrices were repackaged in plain white tubes and coded, to ensure blindness of the study to the volunteers, although they could be differentiated by the color and taste.

Statistical calculations estimated 11 volunteers would provide sufficient power (80%) to show statistically significant differences among the groups. However, prediction of potential dropouts and variations between volunteers lead to a total of eighteen volunteers and 108 enamel specimens that were randomly allocated to the treatment phases using a computer generated list (Microsoft Excel 2007). The experimental test was performed in three phases of 5 days each. During a 2-day lead-in, the volunteers used one of the dentifrices tested, following its use for the period of 5 days. After each phase, this was followed by 2-day washout, which the volunteers used the dentifrice of the next phase. In these three phases, the volunteers wore palatal appliances containing two enamel slabs, one on the right and one on the left side (Figure 1).

Four times a day the palatal appliances were immersed in 0.01 M hydrochloric acid aqueous solution (Merck, Darmstadt, Germany), pH 2.0, at room temperature for 60 s. The response variables were surface hardness (SH) and enamel surface loss measured by stylus profilometry (µm).

Enamel slabs and palatal appliances preparation

Caries free human third molars previously stored in 0.01% (w/v) thymol solution at 4°C were used in this study.[23] Enamel slabs (4 x 4 x 2 mm) were cut, flattened and polished. The slabs were sequentially ground in a water-cooled mechanical grinder (Ecomet/Automet 250 Grinder-Polisher; Buehler, Lake Bluff, IL, USA) with 400-, 600-, and 1,200-grit Al_2O_3 papers and polished on cloths with a 1 µm diamond suspension (Alpha Micropolish; Buehler). All specimens were sterilized by auto-claving. In order to standardize the tested specimens, the baseline surface hardness was determined (SHbas). Five indentations, 100 µm apart from each other were made at the center of the specimens using a Future-tech FM microhardness tester (FM100, Future Tech, Tokyo, Japan) coupled to software FM-ARS 900. Knoop diamond indenter was used with load of 50 g applied for 5 seconds. Enamel specimens presenting a mean hardness of 334.7 ± 15.0 Kg/mm² (ranged from 302.1 to 360.8) were selected and allocated to the treatments generating balanced groups (Table 1).

Acrylic custom-made palatal devices were constructed with two recession’s slots (5 x 5 x 3 mm), one in each side. The specimens were randomly assigned to each one of the two sites as described above and fixed with sticky wax. After, the specimens were coated with an acid-resistant varnish in a dark color (Jordana Cosmetics Corp., Los Angeles, CA, USA), leaving an exposed area of 2 x 4 mm in their central area to be subjected to the treatments. These covered areas provided reference surfaces for profilometry analysis.

Intraoral phase

During the lead-in and wash-out periods, and throughout the experimental phases, the volunteers brushed their teeth with the assigned dentifrice. Before the experimental phases, the appliances were worn for twelve hours to allow mineral equilibrium with saliva and also the formation and maturation of the salivary pellicle. According to Wiegand *et al.*,[24] a pellicle formed during 2 hours already exhibits a protective effect against erosion using a hydrochloric acid.

In each phase of 5 days, the erosive challenges were performed extraorally four times a day at 7:00, 12:00, 17:00 and 21:00 hours. In order to subject the enamel specimens to erosion, the volunteers were instructed to remove the appliance and immerse it in a cup containing 50 ml of a hydrochloric acid (0.01M, pH 2.0) at room temperature for 60 seconds. Afterwards, only the excess of acid was gently wiped out with absorbent paper. Immediately after the erosive attack, the volunteers were instructed to insert the appliances in their mouths and to brush their teeth with one of dentifrices according the protocol conducted by Zero *et al.*,[25] creating a slurry of dentifrice and saliva. During this time, the volunteers were advised not to brush the specimens. The slurry was swished in the mouth for 1 minute, allowing the contact with the specimens' surfaces and the subjects gently rinsed their mouths with 15 ml of tap water for 10 seconds. The same procedure was repeated for the subsequent phases, with a different dentifrice, according to the crossover experimental design.

The volunteers were instructed to avoid eating, drinking, or carrying out oral hygiene procedures using of intraoral devices. The devices should be worn continuously, including at night. When removed, during meals and for oral hygiene, the devices were kept moist in the plastic boxes. After each phase, new enamel specimens were inserted on the appliances, subjected to a new treatment and analyzed by surface hardness and stylus profilometry.

Measurement of enamel surface loss

The nail varnish was removed exposing the untreated references areas, and the levels of enamel wear were determined in relation to the reference surfaces by stylus profilometer

Hommel Tester T1000 (Hommelwerke GmbH, Germany). At intervals of 100 µm, five profile traces (1.5 mm in length) were recorded for each specimen. These profilometric traces were taken by moving the stylus from the reference surface to the exposed surface. For each sample, the mean values obtained from the five traces were calculated.

Percentage of surface hardness loss assessment

After the experimental phase, the enamel slabs were removed from the palatal device and the surface hardness was measured again, similarly to baseline determinations. Five indentations were made in the center of each specimen, in the experimental area (SHafter). The percentage of SH loss (% SHL) was calculated by the formula:

%SHL = [(SHbas. – SHafter) x 100/ SHbas.]

Statistical analysis

Mean values of wear and %SHL were calculated. A Kolmogorov-Smirnov test was applied to all groups to test for the normal distribution of errors. ANOVA were carried out to analyze the SH baseline, SH after the treatment, percentage of SH loss and also enamel surface loss data. Tukey’s post hoc test was applied, when necessary, in cases where ANOVA revealed significant differences. SHbas and SHafter were done by paired *t*-test. Statistical procedures were performed with the Statistical Package for Social Sciences (SPSS 17.0) for Windows. The level of significance was set at 5%.

Results

The values of SH baseline showed that the slabs were adequately randomized among the groups ($p=0.997$). However, the eroded enamel showed significantly lower results of SH than SHbas values, showing that none of dentifrices tested were able to refrain the hydrochloric acid action on enamel ($p<0.001$ to all tested dentifrices). The influence of tested dentifrice to reduction of intrinsic erosion was not significant for the values of SHafter ($p=0.151$) and %SHL ($p=0.349$) (Table 1).

A summary of the results related to surface loss is present in Figure 2. ANOVA indicate significant differences among treatments ($p = 0.021$) and Tukey test, exposure the enamel slabs to magnesium hydroxide and sodium fluoride dentifrices resulted in significantly lower wear, in which non-fluoridated differ from magnesium ($p = 0.041$) and from sodium fluoride ($p=0.040$), but $Mg(OH)_2$ and NaF did not differ ($p>0.05$).

Discussion

Upper gastrointestinal disorders are usually treated with antacid medications that reduce the acidity of the gastric contents.[4, 26] So, as a tactics to perform protection of dental erosion from endogenous acids, a daily method of therapeutic agents would be effective.[10,19, 26] The ideal choice would be the dentifrice, which present a worldwide contact and population use commonly. Fluoride dentifrices have been demonstrated to reduce dental hardness or loss during erosive challenge,[10-14] but there are no report about the antacid dentifrices against intrinsic erosion.

Fluoride and magnesium hydroxide based dentifrices were investigated using an *in situ* protocol, designed to simulate an intrinsic erosion, and consequently, surface-softening and surface loss. It is a method that allows approximating the results to *in vivo* conditions, to reproduce intra-oral influences as action of saliva, temperature, microorganisms, in a controlled study.[19,22]

The results failed to reject the primary null hypothesis. However, fluoride and magnesium hydroxide dentifrices performed preventive effect in intrinsic erosion observed through profilometry analysis. This leads us to reject the secondary null hypothesis. The use of surface hardness and profilometry analyses are broadly accepted to evaluate dental erosion.[27] The lack of preventive action from fluoride products or antacid agents were confirmed by Messias *et al.* [16] and Austin *et al.*[28] , showing that these products are not able to avoid enamel softening, which is in agreement with the results of the present study. Probably, this finding may be explained by two reasons: (i) the measure of indentation in highly eroded substrates may not be clearly defined, and the results become imprecise;[27,29] (ii) the remineralizing effect was not evident to reduce the gastric acid action.

Profilometry technique quantifies the loss of dental surface based in a reference area, being a reliable technique for quantifying high eroded surfaces.[27,29] So, even though therapeutic dentifrices did not show any evidence of erosion reduction for %SHL, a decrease in enamel surface loss was observed.

The effect of magnesium hydroxide dentifrice may be due to its alkalinity and buffering capacities that may act to neutralize acids in the oral cavity, inhibiting the decrease in pH,[30] leading to a reduction in surface loss, but not in %SHL, for not influencing the remineralization of the eroded enamel.[19] This finding was described by Turssi *et al.*[21] The authors using an *in vitro* study observed a preventive effect of magnesium hydroxide to gastric acid on enamel. Magnesium hydroxide neutralizes hydrochloric acid, by neutralizing each H^+ ion of the acid with OH^- ion of the base, forming $MgCl_2$ and H_2O.

The currently tested fluoride dentifrice was selected based in the fact that NaF is widely used in preventive dental care, particularly in caries prevention. Hooper *et al.*[26] and Messias *et al.*[16] observed similar results between non-fluoridated and NaF products in the erosion protection of enamel. Similarly to our study, other authors[10,12] using NaF-containing dentifrices observed a significant reduction in dentin or enamel loss after erosion in comparison to controls (no F). Moreover, the preventive effect of sodium fluoride dentifrice in erosive lesions may be generated by the deposition of high amounts of mainly CaF_2-like products on enamel surface, which can protect it against acid attack.[16,31] However, according Rochel *et al.*[32], the remaining demineralized layer of eroded enamel is considerably smaller compared to the bulk enamel loss, fluoride application predominantly aims to prevent further erosive tissue loss rather than to remineralize softened enamel. So, this explains the results from sodium fluoride of surface hardness analysis.

Regarding dental erosion, sodium fluoride or magnesium hydroxide dentifrices are not able to totally avoid endogenous erosion, since there was significant loss of hardness ($p<0.05$; *t*-test) and all dentifrices also presented some surface wear. Thus, the main prevention method against intrinsic erosion is the elimination of its cause. However, these agents are alternatives to reduce dental wear. For this reason, the use of either dentifrice contents neutralizing products or fluoride are interesting alternative in cases of gastroesophageal reflux or chronic vomiting.

A limitation of this study was not evaluating the role of dentifrice abrasion, since erosive tooth wear includes the combined effect of erosion and mechanical wear on the tooth surface.[14] Nonetheless, is better to understand each element individually (erosion or abrasion) and its specific effects,[33] than an association of factors which may compromise the real effect of the active ingredients of dentifrices. Thus, it is adequate to isolate one or more of these elements through an experimental appropriate design, as was done in this study.

Conclusion

In conclusion, our findings suggest that sodium fluoride and magnesium hydroxide dentifrices are effective in reducing hydrochloric acid enamel wear, under *in situ* conditions.

Acknowledgments

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Figures

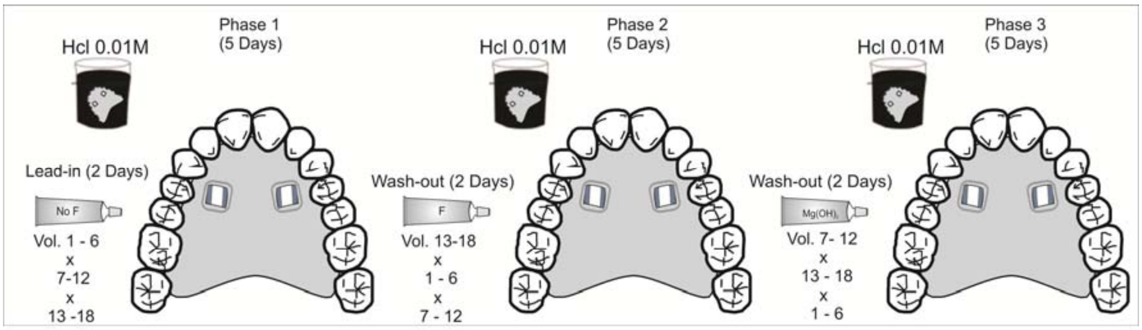

Figure 1. Experimental design

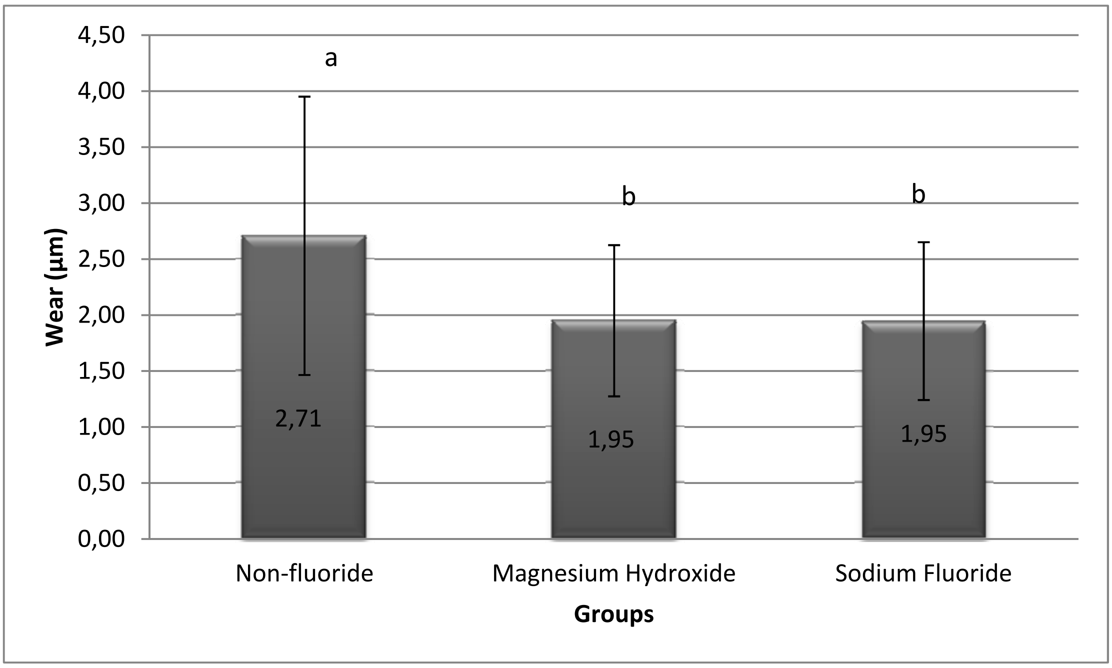

Figure 2. Data showing mean surface loss (µm) for human enamel specimens treated with diferent dentifrice, after which specimens were subjected to hydrocloric acid 0.01M using Anova and Tukey tests. Erros bars are standard deviations. Different letters imply statistical differences between groups (p=0.04).

Table 1. Mean (SD) of enamel surface hardness (SH) before (baseline), after the erosive challenge/dentifrices treatments, and percentage of loss (%SHL) using ANOVA test.

	Enamel surface hardness		
Dentifrices	**Baseline**	**After**	**%SHL**
Non-fluoride (n=18)	334.88 (15.06)a	164.3 (63.56)a	50.67 (17.48)a
Magnesium Hydroxide (n=18)	334.67 (15.11)a	154.18 (70.80)a	53.94 (19.48)a
Sodium fluoride (n=18)	334.62 (15.16)a	181.56 (54.12)a	45.45 (15.44)a
p-values for the factor dentifrice	**0.997**	**0.151**	**0.349**

For all dentifrices, SH values after the erosive challenge were significantly lower than at baseline ($P < 0.05$; paired *t*-test).

Conclusão geral

4 CONCLUSÃO GERAL

Diante dos resultados destes estudos e considerando as condições experimentais empregadas, pode-se concluir que:

- Dentifrícios contendo fluoreto estanhoso ou fluoreto de amina adicionado ao fluoreto de estanho são efetivos em prevenir a perda de superfície dentinária após desafio erosivo e abrasivo por ácido hidroclorídrico simulando erosão intrínseca;
- A ação intermitente e, bem como, o tempo de exposição a ácidos exógenos ou endógenos permitem a progressão do processo erosivo no esmalte dentário, mostrando que o contato da dentição de forma frequente e crônica a ácidos ocasiona uma tendência a maior desmineralização e possivelmente perda de estrutura dentária;
- Hidróxido de magnésio presente em dentifrícios de uso diário é efetivo no controle da erosão do esmalte humano submetido a procedimentos erosivos que simulam a ação de ácidos endógenos ou exógenos;
- Diferentes produtos disponíveis no mercado podem ser utilizados para a prevenção de erosão dentária, contudo, nenhum destes evita completamente o processo erosivo, mas evita a progressão da desmineralização e perda de estrutura dentária.

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APÊNDICES

APÊNDICE A – Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado(a) a participar, como voluntário, de uma pesquisa a ser realizada na Universidade Federal do Ceará. Após ser esclarecido(a) sobre as informações a seguir e caso aceite fazer parte do estudo, assine ao final deste documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. A qualquer momento, você poderá desistir de participar da pesquisa e retirar seu consentimento. Sua recusa não trará nenhum prejuízo em sua relação com o pesquisador ou com a instituição. Em caso de dúvida, você pode entrar em contato com o pesquisador responsável através do telefone e endereço que constam neste termo ou procurar o Comitê de Ética em Pesquisa da Universidade Federal do Ceará pelo telefone (85) 33668344.

1. DADOS DE IDENTIFICAÇÃO DO PACIENTE E/OU RESPONSÁVEL LEGAL

<table>
<tr><td colspan="5">Nome do paciente:</td></tr>
<tr><td>Documento de identidade nº:</td><td colspan="2">Gênero:</td><td colspan="2">Data de nascimento: ___/___/_____</td></tr>
<tr><td colspan="2">Endereço:</td><td colspan="2">Cidade:</td><td>UF:</td></tr>
<tr><td colspan="3">Telefones para contato:</td><td colspan="2">CEP:</td></tr>
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<tr><td colspan="5">Nome do responsável legal:</td></tr>
<tr><td>Documento de identidade nº:</td><td colspan="2">Gênero:</td><td colspan="2">Data de nascimento: ___/___/_____</td></tr>
<tr><td colspan="2">Endereço:</td><td colspan="2">Cidade:</td><td>UF:</td></tr>
<tr><td colspan="5">Natureza (grau de parentesco, tutor, curador etc):</td></tr>
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2. INFORMAÇÕES SOBRE A PESQUISA (PROCEDIMENTOS, RISCOS E BENEFÍCIOS)

Pesquisador responsável: Vanara Florêncio Passos

Endereço: Rua Capitão Francisco Pedro s/n - Rodolfo Teófilo - Curso de Odontologia FFOE (UFC).

Telefone para contato: (85) 87702227

Título do Projeto: Efeito de dentifrício contendo hidróxido de magnésio na erosão do esmalte dentário.

A erosão é ocasionada por ácidos intrínsecos e extrínsecos, como bebidas ácidas e ácido gástrico. Como a escovação com pasta dental é o hábito mais comum de higiene bucal e os produtos ativos existentes em sua composição tem impacto no efeito preventivo destes produtos. Neste estudo serão utilizados dispositivos palatinos que deverão ser imersos em bebida ácida quatro vezes ao dia para verificar o efeitos destes produtos na prevenção da erosão dentária. Os materiais utilizados encontram-se disponíveis no mercado e não demonstram nenhum risco à integridade do ser humano.

Confidencialidade: Os registros individuais dos seus dados serão mantidos em sigilo (confidencial). As informações a respeito dessa pesquisa poderão ser publicadas em revista científica. Apenas os resultados envolvendo médias serão divulgados ou, em pequeno número e de forma ilustrativa, fotografias sem a sua devida identificação.

3. ASSINATURAS

Eu, ____, concordo em participar da pesquisa e declaro que fui devidamente informado(a) e esclarecido(a) sobre o tipo de pesquisa, os procedimentos nela envolvidos, assim como os possíveis riscos e benefícios decorrentes da minha participação. Foi esclarecido que posso retirar meu consentimento a qualquer momento, sem que isto leve a qualquer penalidade.

Fortaleza, ___ de _____de 2012 ____

Sujeito da Pesquisa

Confirmamos a solicitação de consentimento, esclarecimentos sobre a pesquisa e aceite do sujeito em participar:

______________________________ ___________________________________

Pesquisador Responsável Testemunha

Nome:

Identidade nº:

APÊNDICE B – Termo de Doação de Dentes

<u>TERMO DE DOAÇÃO DE DENTES</u>

Pelo presente instrumento que atende às exigências legais, o Sr. (a) ____, após ter tomado conhecimento do protocolo de pesquisa **"Efeito de dentifrício contendo hidróxido de magnésio na erosão do esmalte dentário"** que tem como objetivo analisar através de um estudo *in situ* a associação da abrasão e erosão ocasionado pelo uso de dentifrícios e bebidas ácidas no esmalte dentário humano**,** vem na melhor forma de direito **DOAR** à CD **Vanara Florêncio Passos __** dentes, declarando, sob as penas da lei, que os dentes objeto da presente doação foram extraídos por indicação terapêutica, cujos históricos circunstanciados fazem parte dos prontuários dos pacientes de quem se originam.

Data:___/___/___

Assinatura:____

RG: ____

ANEXOS

ANEXO A – Aprovação do Comitê de Ética em Pesquisa – Capítulos 1 e 3

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 221/12 Fortaleza, 05 de julho de 2012.
Protocolo COMEPE nº 75/12
Pesquisador responsável: Vanara Florêncio Passos.
Título do Projeto: "Efeito de dentifrício contendo hidróxido de magnésio na erosão do esmalte dentário"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a *pesquisa em seres humanos*, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do projeto supracitado na reunião do dia 04 de julho de 2012.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório parcial e final do referido projeto.

Atenciosamente,

Dr. Fernando A. Frota Bezerra
Coordenador do Comitê
de Ética em Pesquisa
COMEPE/UFC

ANEXO B – Aprovação do Comitê de Ética em Pesquisa – Capítulos 2

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 112/09 Fortaleza, 15 de maio de 2009
Protocolo COMEPE nº 131/ 09
Pesquisador responsável: Sérgio Lima Santiago
Deptº./Serviço: Departamento de Odontologia/ UFC
Título do Projeto: "Avaliação da alteração estrutural do esmalte dentário promovida pelo uso de dentifrício e bebida ácida- Estudo in vitro"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o projeto supracitado na reunião do dia 14 de maio de 2009.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório final do referido projeto.

Atenciosamente,

Mirian Parente Monteiro

Dra. Mirian Parente Monteiro
Coordenadora Adjunta do Comitê
de Ética em Pesquisa
COMEPE/UFC