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AVALIAÇÃO DA ALTERAÇÃO ESTRUTURAL DO ESMALTE DENTÁRIO PROMOVIDA POR EROSÃO/ABRASÃO

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Dissertação 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 Mestre em Odontologia.

Área de Concentração: Clínica Odontológica.

Orientador: Prof. Dr. Sérgio Lima Santiago

Co-orientadora: Profa. Dra. Lidiany Karla Azevedo Rodrigues

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Dissertação apresenta à Coordenação do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará como requisito parcial para a obtenção do Título de Mestre em Odontologia. Área de concentração: Clínica Odontológica.

Aprovada em: ___/___/___

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À Deus.

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RESUMO

O desgaste dentário é a perda não cariosa de tecido dentário caracterizado por uma etiologia multifatorial tendo como principal fator a interação entre erosão ocasionada por ácidos e forças abrasivas intra-orais. Essa dissertação é constituída por dois artigos que objetivam, respectivamente: (1) revisar criticamente a literatura disponível sobre as técnicas de análises de alterações micromorfológicas da estrutura dentária submetida a processos de erosão e/ou abrasão; (2) avaliar o efeito do NaF e MFP presentes em dentifrícios comercializados na prevenção da desmineralização da estrutura dentária por processos erosivos ou erosivos e abrasivos. No artigo 1, a literatura científica pertinente ao assunto foi obtida usando a base de dados nacionais e internacionais e busca manual de referências citadas em artigos científicos. No artigo 2, foi realizado um estudo *in situ*, randomizado, duplo-cego, cruzado, boca-dividida, em três fases de 5 dias cada, com a participação de 15 voluntários, que utilizaram dispositivos palatinos, contendo 4 blocos de esmalte dental humano tratados com diferentes dentifrícios: controle (11,2 ppm F, silica), MFP (1450 ppm F, silica) e NaF (1450 ppm F, silica). Os blocos foram submetidos à erosão por imersão em bebida do tipo cola (Coca-Cola®) por 60 s, 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 o dentifrício/saliva por 60 segundos, sendo posteriormente um lado do dispositivo (2 blocos) escovado com uma pequena porção de dentifrício por 40 movimentos de vai-e-vem. As alterações no esmalte foram avaliadas por testes de microdureza e por microscopia eletrônica de varredura. Os dados obtidos foram testados usando ANOVA ($p < 0,05$). A análise crítica do artigo 1 mostrou que a literatura apresenta diferentes métodos para análise de desgaste dentário, variando de técnicas bem estabelecidas a técnicas de uso recente, sendo seus conhecimentos necessários para o desenvolvimento de estudos futuros. Os resultados do artigo 2 demonstraram que não houve diferença no efeito da remineralização dos dentifrícios fluoretados nas condições de erosão e erosão associada à abrasão em relação ao grupo controle ($p > 0,05$). Contudo, os dados de dureza referentes à condição (erosão ou erosão + abrasão) apresentaram-se diferentes estatisticamente ($p < 0.0001$). Conclui-se que o conhecimento sobre técnicas de análise acerca do desgaste dentário é indispensável para a sua determinação e que é premente a realização de mais estudos para avaliação do efeito do flúor, na forma de NaF ou MFP, presente em dentifrícios comercializados utilizando técnicas complementares que permitam a medição do desgaste.

Palavras-chave: Erosão Dentária. Esmalte Dentário. Escovação Dentária. Flúor.

ABSTRACT

Dental wear is the non-carious loss of dental hard tissue, characterized by a multifactorial etiology with the main factor the interaction between erosion caused by acids from diet or endogenous and abrasives forces intra-oral. This dissertation consisting of two articles, which aim, respectively: (1) to critically review the available literature about the techniques of analysis of micromorphological changes in structure dental subject to erosion and/or abrasion, (2) to evaluate the effect of NaF and MFP in dentifrices available in market in the prevention of demineralization of tooth structure by erosive or erosive and abrasive process. In study 1, the scientific literature to the issue was searched using base of data nationals and internationals and manual tracing of references cited in scientific papers. In study 2, a *in situ* study, randomized, double-blind, crossover, slipt-mouth was conducted in three phases of 5 days each, with the participation of 15 volunteers who used palatal devices, containing 4 blocks of human tooth enamel treated with different dentifrices: control (11,2 ppm F, silica), MFP (1450 ppm F, silica) and NaF (1450 ppm F, silica). The slabs were subjected to erosion by immersion in a cola drink (Coca-Cola®) for 60 s, 4 times a day, at predetermined times. Then, the volunteers brushed their teeth, for 25 seconds and, with the device in the mouth, swished the dentifrice/saliva slurry for 60 seconds, after on side of appliance (2 blocks) was brushed with a small portion of the dentifrice by 40 brushing strokes. The enamel changes were evaluated for microhardness test and scanning electron microscopy. Data scores were submitted to ANOVA ($p < 0,05$). The critical review presented in study 1 showed which the literature presents different methods for analysis of dental wear, ranging from techniques well-established to techniques of recent use, therefore their expertise is needed for the development of future studies. The results of study 2 demonstrated that there was no differences in remineralization effect of fluoride dentifrices in the condition of erosion and erosion plus abrasion in relation of control group ($p > 0,05$). However, the harness data concerning to condition (erosion or erosion + abrasion) showed different statistically ($p < 0.0001$). The results of these studies indicate the knowledge about techniques of analysis of dental wear is essential for its determination. Moreover, it is imperative the realization of more studies to evaluate the fluoride effect in form of NaF or MFP, present in dent
available in the market using complementary techniques that allow the measurement of

Key-words: Tooth Erosion. Dental Enamel. Toothbrushing. Fluorine.

SUMÁRIO

1 INTRODUÇÃO GERAL

A incidência de cárie dentária tem declinado em países desenvolvidos (BROWN; WALL; LAZAR, 2000) e em desenvolvimento. Entretanto, tem-se observado o aumento de outras lesões como o desgaste dentário (IMFELD, 1996), que se caracteriza pela perda não-cariosa de tecido dentário, apresentando uma etiologia multifatorial com o envolvimento de processos inter-relacionados. Esses processos incluem a abrasão por fricção de materiais exógenos durante a mastigação e a escovação, a atrição entre dentes antagonistas e a dissolução química ocasionada pela erosão. Entretanto, segundo o estudo de Hooper *et al.* (2003), o efeito da erosão apresenta-se dominante na ocorrência do desgaste.

A erosão é a perda de estrutura dentária resultante da dissolução química do dente por ácidos não-bacterianos, de origem intrínseca ou extrínseca. Os fatores intrínsecos são representados pela ação do ácido clorídrico estomacal causando erosão em pacientes que apresentam vômito crônico decorrente de bulimia, alcoolismo, gravidez ou pacientes com refluxo gastroesofágico involuntário. Em relação aos fatores extrínsecos, a dieta é a fonte mais comum de ácidos, sendo estes provenientes de sucos de frutas, refrigerantes, bebidas esportivas, chás ou alimentos ácidos. (BARBOUR; REES, 2006; DAVID, 2006; GANDARA; TRUELOVE, 1999; LARSEN, 2008; LUSSI; JAEGGI; ZERO, 2004; SCHEUTZEL, 1996; ZERO, 1996; ZERO; LUSSI, 2000).

Devido ao aumento do consumo de refrigerantes e o maior cuidado com a higiene dentária, alguns pesquisadores têm buscado avaliar o processo de erosão e abrasão ocasionados pelo uso de refrigerantes e dentifrícios. (ATTIN; BUCHALLA; PUTZ, 2001; HARA *et al.*, 2003; MAGALHÃES *et al*., 2007; MAGALHÃES *et al*., 2008a; 2008b; RIOS *et al*., 2006a, 2006b; TURSSI *et al*., 2004, 2005). Estudos *in vitro* e *in situ* permitem observar a maior perda de estrutura dentária pela ação conjunta da erosão e abrasão, (HOOPER *et al.*, 2003; ATTIN; BUCHALLA; PUTZ, 2001; HARA *et al*., 2003; RIOS *et al*., 2006a, 2006b; GANSS *et al*., 2007; KIELBASSA *et al*., 2005; LUSSI *et al.*, 2004; VIEIRA *et al*., 2006a, 2006b; WIEGAND; KÖWING; ATTIN, 2007) pois o tecido dentário submetido a processos erosivos encontra-se amolecido e, quando seguido por um processo abrasivo, observa-se um efeito sinérgico, resultando em extrema perda de tecido dentário.

Analisando-se a ocorrência do desgaste dentário, tem-se observado maior prevalência em pessoas de faixa etária jovem e de países industrializados. (NUNN, 2000; JAEGGI; LUSSI, 2006). Dugmore e Rock (2003) examinaram 1753 adolescentes ingleses e determinaram a presença de erosão em 56,3% dos adolescentes aos 12 anos e 64,1% aos 14 anos. Neste estudo, foi observado que 12,3% das crianças livres de erosão aos 12 anos desenvolveram erosão em dois anos subsequentes. Com relação aos adolescentes brasileiros, Auad *et al.* (2007) realizaram uma avaliação clínica em 459 jovens, entre 13 a 14 anos, de 14 escolas brasileiras, observando presença de erosão em 34,1% das mesmas, envolvendo apenas esmalte e principalmente situada em incisivos centrais superiores. A partir destes estudos de avaliação clínica sobre a prevalência de erosão, observa-se a importância da determinação de medidas preventivas.

O flúor é uma forma de medida preventiva capaz de reduzir a cárie, sendo também, recentemente, aplicado com o objetivo de prevenir perda de tecido dentário por processos que promovem o desgaste, embora o efeito preventivo do flúor ainda encontre-se em estudo. Diferentes tipos de soluções fluoretadas, como verniz de flúor e fluoretação intensa, são também exemplos importantes para a prevenção da erosão. (GANSS *et al.,* 2001, 2004, 2007; VIEIRA, 2006b; HOVE *et al.,* 2008; LAGERWEIJ *et al.,* 2006; SCHLUETER *et al.,* 2007; VIEIRA; RUBEN; HUYSMANS, 2005). É importante mencionar que são escassas avaliações que comparam o efeito de diferentes tipos de flúor presentes em dentifrícios comercializados em relação ao desgaste dentário. (LUSSI *et al*., 2008).

Bartlett *et al*. (1994), Ganss *et al.* (2004), Zero *et al*. (2006) e Magalhães *et al*. (2007) mostraram um efeito benéfico de dentifrícios fluoretados em comparação ao placebo, observando que a presença do flúor reduziu a perda de estrutura dentária. Magalhães *et al.* (2008) verificaram que dentifrícios com flúor reduzem o desgaste em aproximadamente 27,5% tanto em processos erosivos como quando associado a abrasão.

Os tipos mais comuns de flúor encontrados em dentifrícios comercializados são o monofluorfosfato de sódio (MFP) e fluoreto de sódio (NaF) que liberam flúor para os fluidos orais por mecanismos diferentes. Especificamente, o MFP requer hidrólise para liberar flúor na boca, diferindo do dentifrício que possui NaF, que já apresenta flúor na forma livre. Este passo de hidrólise tem sido postulado como a maior razão para dentifrícios contendo NaF geralmente mostrarem maiores concentrações de flúor na saliva e placa que dentifrícios contendo MFP. (EKSTRAND, 1997; VOGEL *et al*.,2000).

Além do flúor, outro fator que pode influenciar no processo de desgaste dentário é a saliva, que tem sido considerado o fator biológico mais importante influenciando 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 pode proteger contra erosão agindo como uma barreira de difusão ou membrana de permeabilidade seletiva prevenindo o contato direto entre 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 formada por duas horas em esmalte apresenta potencial protetor contra a desmineralização.

A avaliação da perda ou da alteração micromorfológica da superfície dentária necessita de uma adequada escolha de métodos de análise. Desta forma, existem diferentes metodologias capazes de quantificar a erosão ocasionada por bebidas ácidas, a abrasão por produtos de higiene bucal e a ação de produtos que podem ser utilizados na prevenção ou redução de perda de estrutura dentária. Estas técnicas podem obter informações qualitativas ou quantitativas, que permitem determinar o que pode ser prejudicial para a longevidade e função do dente, levando à determinação de métodos preventivos adequados.

Alguns destes métodos utilizados para determinar a alteração ocasionada pela erosão isolada ou associada com a abrasão envolvem a análise da estrutura dentária por dureza, (MAGALHÃES *et al*., 2007, 2008a; RIOS *et al.*, 2006a, 2006b; WIEGAND; KÖWING; ATTIN, 2007; LUSSI *et al*., 2008; ZERO *et al*., 2006; VAN EYGEN; VANNET; WEHRBEIN, 2005; HARA; ZERO, 2008; SALES-PERES; PESSAN; BUZALAF, 2007; WONGKHANTEE *et al*., 2006) microscopia de força atômica, (BARBOUR *et al*., 2003, 2006; LIPPERT; PARKER; JANDT, 2004) rugosidade, (TURSSI *et al*., 2005; MENEZES *et al*., 2004; WORSCHECH *et al.*, 2006) microscopia eletrônica de varredura, (RIOS *et al.*, 2006b; VIEIRA *et al*., 2006b; SHELLIS *et al*., 2005) perfilometria, (HOOPER *et al*., 2003, 2007; HARA *et al*., 2003; MAGALHÃES *et al.*, 2007, 2008a, 2008b; RIOS *et al*., 2006a, 2006b; TURSSI *et al.*, 2004, 2005; GANSS *et al.*, 2007; KIELBASSA *et al.*, 2005; VIEIRA *et al.*, 2006a, 2006b; LAGERWEIJ *et al*., 2006; HARA; ZERO, 2008; BARBOUR *et al*., 2006; MENEZES *et al*., 2004; EISENBURGER; ADDY, 2003; EISENBURGER; SHELLIS; ADDY, 2003; JOINER *et al*., 2005; PICKLES *et al*., 2005; WETTON *et al*., 2006; WIEGAND; BEGIC; ATTIN, 2006) microradiografia, (KIELBASSA *et al*., 2000, 2005; GANSS *et al*., 2004; SCHLUETER *et al*., 2007) entre outras tecnologias que ainda são pouco utilizadas como microscopia confocal (AMAECHI *et al*., 1999).

Para a realização de estudos sobre desgastes dentários, podem ser executadas metodologias *in vitro* (TURSSI *et al*., 2005; GANSS *et al*., 2001, 2007; VIEIRA *et al.*, 2006a, 2006b; WIEGAND; KÖWING; ATTIN, 2007; LAGERWEIJ *et al*., 2006; SCHLUETER *et al*., 2007; VIEIRA; RUBEN; HUYSMANS, 2005; VAN EYGEN; VANNET; WEHRBEIN, 2005; BARBOUR *et al*., 2003, 2006; LIPPERT; PARKER; JANDT, 2004; MENEZES *et al*., 2004) ou *in situ*. (HOOPER *et al*., 2003; HARA *et al*., 2003; MAGALHÃES *et al*., 2007, 2008a, 2008b; RIOS *et al*., 2006a, 2006b; TURSSI *et al*., 2004; GANSS *et al.*, 2004, 2007; LUSSI *et al*., 2004; HOVE *et al*., 2008; ZERO *et al*., 2006; HARA *et al*., 2006; HARA; ZERO, 2008; SALES-PERES; PESSAN; BUZALAF, 2007; JOINER *et al*., 2005; PICKLES *et al.*, 2005). Modelos *in vitro* são extremamente utilizados para demonstrar a propensão erosiva de uma substância, mas não reproduzem perfeitamente a cavidade bucal com todas as suas variações biológicas. Já os estudos *in situ* permitem interações entre bactérias, saliva e tecidos duros na cavidade bucal, enquanto retém a sensibilidade de análises laboratoriais.

O desenvolvimento de um trabalho *in situ* serve como um elo entre a situação clínica não controlada e a situação laboratorial, sendo bastante utilizado para estudos de avaliação de desgaste dentário, pois permite reproduzir o que ocorre no processo clínico de perda de tecido duro em um limitado número de sujeitos. Estudos *in situ* permitem avaliar também o efeito do flúor contido em dentifrícios comercializados na prevenção ou redução do desgaste. Como o MFP predomina em 90% dos dentifrícios do mercado brasileiro e o efeito relativo dele na erosão/abrasão do esmalte é desconhecido, há a necessidade de estudos que avaliem se existe diferença do efeito de dentifrícios contendo MFP ou NaF na redução do desgaste, uma vez que estes são os mais consumidos pela população durante as práticas de higiene oral.

A busca incessante da comunidade científica por produtos que possam evitar o desgaste dentário, que sejam de fácil disponibilidade, baixo custo e eficaz, enfatiza a significância clínica deste estudo que objetiva revisar a literatura sobre as metodologias disponíveis para uma adequada análise de processos erosivo/abrasivos e avaliar o efeito de dois tipos de flúor presentes em dentifrícios disponíveis no comércio na prevenção da erosão associada ou não a abrasão.

2 PROPOSIÇÃO

Os objetivos do presente estudo foram:

a) revisar a literatura disponível sobre as técnicas de análises de alterações micromorfológicas de estrutura dentária submetida a processos de erosão e/ou abrasão;

b) avaliar *in situ* o efeito do tipo de flúor presentes nos dentifrícios – fluoreto de sódio ou monofluorfosfato de sódio – na redução da desmineralização por erosão associada ou não à abrasão em dentes submetidos à erosão por ácido de origem extrínseca presente em um refrigerante do tipo cola.

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). Assim sendo, esta dissertação é composta de um capítulo de revisão de literatura que será submetido ao periódico Brazilian Dental Journal e outro capítulo será enviado para publicação no Journal of Dentistry, conforme descrito abaixo:

✓ Capítulo 1

"METHODOLOGIES TO ANALYZE THE MICROMORPHOLOGICAL ALTERATIONS OF ENAMEL SUBJECTED TO ABRASION/EROSION"

Passos VF, Santiago SL. Este artigo será submetido à publicação no periódico "General Dentistry".

✓ Capítulo 2

"EFFECT OF NAF AND MFP-DENTIFRICE ON ENAMEL EROSION, ASSOCIATED OR NOT TO ABRASION"

Passos VF, Vasconcellos AA, Cury JÁ, Hara AT, Tenuta LMA, Rodrigues LKA, Santiago SL. Este artigo será submetido à publicação no periódico "Journal of Denistry".

3.1 Capítulo 1

METHODOLOGIES TO ANALYZE THE MICROMORPHOLOGICAL ALTERATIONS OF ENAMEL SUBJECTED TO ABRASION/EROSION

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Abstract

The incidence of caries has declined; however, other dental lesions such as dental wear are becoming increasingly important. Dental wear is a multifactorial process that may encompass erosion and abrasion, and combinations thereof. Therefore, various methodologies have been applied to evaluate the loss of dental hard tissue and the surface-softened zone in enamel induced by abrasive and erosive challenges. In this review, different techniques to evaluate alterations in enamel are analyzed, such as microhardness, surface profilometry, surface roughness, microradiography, atomic force microscopy (AFM), AFM nanoindentation, scanning electron microscopy, white light interferometer and confocal laser scanning microscopy. Thereby, the knowledge about these techniques is indispensable to the choice of methods to measure dental wear.

Introduction

Improved oral care, including the use of fluorited toothpastes and an increased oral hygiene of the population have led to a reduction of the number of caries in industrialized countries. However, this has been followed by the increase in other dental lesions, such as tooth wear.[1]

Tooth wear is produced by non-carious destructive processes and is likely to be a multifactorial phenomenon. It is a cumulative lifetime process, which may lead to a substantial tooth surface loss. The term tooth wear describes the processes of erosion, attrition and abrasion.[2] Dental erosion is defined as the chemical dissolution of teeth by acids of non-bacterial origin or chelating. It is caused by acids of either intrinsic or extrinsic origin. The most common intrinsic source of erosive acid is hydrochloric acid produced by the stomach, it is frequently observed in patients with conditions that promote chronic vomiting, such as bulimia, alcoholism, and, to a lesser extent, pregnancy. In the modern society, the extrinsic factors have been more important due to the higher consumption of acids drinks such as soft drinks, juices, sport drinks and others.[3-5]

A mechanical factor in the mouth like toothbrush abrasion has a synergistic effect with erosion. Therefore, exposure of enamel to acid is more vulnerable to abrasion and this may result in extreme tooth material loss. Abrasion is a physical wear as a result of mechanical processes involving foreign substances or objects, such as excessive brushing or effect of abrasives in toothpastes. Many studies have assessed the largest loss of tooth structure by action of erosion and abrasion.[6-11] Thereby, efforts have been made to elucidate how erosive/abrasive lesions can be prevented.[12-17]

Evaluating of dental wear depends on the correct choice of a method to measure the micromorphological alteration of dental structure. In the meantime, there are different methodologies able to quantify the erosion occasioned by intrinsic and extrinsic acids, the abrasion by oral hygiene substances and the action of products which can be utilized to prevention or reduction of dental structure loss. The techniques can obtain quantitative and qualitative data, determining products that can be harmful to teeth longevity and function, and allowing the knowledge of preventive methods.

This study critically analyzes different methodologies to evaluate the dental wear and allows the choice of an ideal method for measure the micromorphological alteration of enamel.

Literature review

Different methodologies are developed to analyze dental wear (see Table 1). *In vitro* models are extremely useful for demonstrate the erosive capacity of a substance,[9,11,18-28] but cannot replicate the oral environment with all its biological variations.[29] *In situ* studies allow interactions to take place among bacteria, saliva and hard tissues in the oral environment, while retaining the sensitivity of laboratory analysis.[6-8,10,13,15,29-33]

The determination of micromorphological alteration in enamel dental occasioned by erosion or erosion plus abrasion, can be realized for different methods, involving analyses of dental structure by hardness, atomic force microscopy (AFM), microradiography, roughness, scanning electron microscopy (SEM), surface profilometry and other techniques.

Some studies use chemical analyses that allow determining the amount of mineral dissolved from teeth during erosion.[20,34] Nevertheless, no information about the effect of acids on the microstructure of enamel can be obtained with this method.[35] In the following, the techniques most widely utilized to evaluate the microstructural alteration as well as emerging methods will be described.

1. Microhardness

The effect of extrinsic and intrinsic acids in dental erosion can be investigated by microhardness test. This technique can evaluate early stages of enamel and dentin dissolution, which are associated with weakening of the surface.[36]

In this test, a pyramid shaped fine diamond tip of known geometry is pushed into the enamel or dentin surface with a defined load. The tip result in an indentation in the surface of tooth, and the measure of diameters of the indentation allows to know the microhardness, which is measured with a micrometer scale incorporated in the ocular of the microscope.

The microhardness is the value of resistance of the enamel or dentine against local plastic deformation. Frequently, microhardness methods are the Knoop hardness and the Vickers hardness test. The difference between these is the shape of the diamond probes. The Knoop diamod results in a rhomboid indentation, while Vickers in a tetra-pyramidal one (Figure 1 and 2).

The Knoop microhardness test is more sensitive to surface hardness than many other conventional hardness tests and it is suitable for the measurement materials such as hydroxyapatite, the main component of dental enamel.[35]

The formula utilized to convert the indentation length to KHN (Knoop Hardness Numbers) is $KHN = 14.230 \times P / IL^2$, where KHN is in Kg/mm^2, P is the load in g, IL is the indentation length in micrometers, and 14.230 is a constant utilized to converting grams into kilograms and micrometer into millimeters.[37] Additionally, the formula to VHN (Vickers Hardness Numbers) is $VHN = 1.854\ Q/L^2$, where Q is the load in kgf and L is the arithmetic mean of the two diagonals, *d1* and *d2* in mm.[38]

Hara and Zero[20] assessed the effect of some beverages - extrinsic acids - with and without calcium in the hardness of enamel after demineralization in different times (0, 5, 10, 30, 60 and 120 min). The results showed that beverages containing calcium had reduced capacity to demineralize enamel. In relation to time, after 60 minutes of demineralization, the values for percentage of surface microhardness changes (%SMHC) were not detected because of the highly demineralized surface caused by some beverages. This way, the microhardness test is more sensitive for the initial stages of erosion but have limitations in the analysis of advanced lesions. Therefore, the analysis was complemented using optical profilometry.

Evaluating the influence of erosion and erosion plus abrasion through Knoop microhardness, Rios *et al.*[8] verified that only erosion occasioned %SMHC of 91,61 and the erosion plus abrasion was 58,77. The results showed a possible removal of a softened layer resulting in exposure of a harder enamel surface.

Zero *et al.*[33] evaluated through surface microhardness the ability of a experimental dentifrice containing potassium nitrate (1150 ppm F) to enhance the remineralization of enamel after erosion and whether the substrate remineralized in presence of the experimental toothpaste is more resistant to subsequent erosive challenges. In this study, test dentifrice (1150 ppm F + 5% KNO3; TD) was compared to placebo dentifrice (0 ppm F; PD), dose response control dentifrice (250 ppm F; DD) and clinically tested fluoride dentifrice (1100 ppm F; FD). The results showed that both TD and FD were more effective in enhancing remineralization than PD. TD showed higher resistance against the second erosive challenge than PD and DD dentifrices. These results may be essentially attributed to the presence of fluoride in the dentifrice.

Knoop microhardness test also determines the loss of substance by abrasion. By means of the indentations is possible to calculate the depth of the indentation. The difference between the depth before and after abrasion provided a direct measurement for the loss of substance.[10,22,24,26,30,31] The substance loss (Δd) is calculated from the change in indention length (Δl) using the geometrical formula: Δd=0,032772Δl. Utilizing the analysis in context, Joiner *et al.*[30] verified the effect in enamel of three dentifrices with different relative dentine abrasivity (RDA) and relative enamel abrasivity (REA) using an *in situ* model. The results showed that the dentifrice with the higher REA has a higher level of enamel wear. Unfortunately, measurements of the amount of substance directly removed by an erosive attack could not be performed with this method, since the acid also removes some substance from the body of the indentation and not only from its surrounding, because the main principle of this method is that the body of the indentation in not changed and not removed by the abrasion.[36]

The microhardness test have a relatively low costs and this can be combined with others measure because is a non-destructive technique.

2. Surface Profilometry

Profilometry is a method to measure enamel or dentin loss caused by erosion and/or abrasion, which can be for contact or non-contact form. In this technique, the samples are covered on one side with a protective tape, creating a so-called reference area and the other part of the sample remains uncovered to determine the dental wear. This reference area can be a thin layer of composite resin, with no acid etching done and no adhesive system (7) or can be used nail varnish.[15] Thus, the profilometry measures the difference in height (Δh) between the exposed area and the reference. The main advantage of profilometry is the speediness of measurements which take in order of 1 min per sample.[35]

Mechanical profilometry (MP) is widely utilized to evaluate changes in surface of teeth after abrasion and/or erosion.[6-8,14,15,23,32,39] However, the force applied by probe in surface softened by erosion, can collapse the area. This effect would be especially strong on samples with a relatively severe demineralization, so the erosive effect of a soft drink measured would be exaggerated by the result measured by contact profilometry.[35]

Magalhães *et al.*[15] assessed by MP the effect of dentifrices with different concentrations of fluoride on dentin subjected to erosion or erosion plus abrasion. This analysis determined that both erosion and erosion plus abrasion wear was higher for placebo dentifrice (0 ppm F) than for the 1100 ppm F and 5000 ppm F dentifrices, but the fluoride dentifrices did not differ from each other. In this study, it is possible that the lack of difference between fluoridated dentifrices might be explained for the erosive effect that was realized 4 times a day for 60 s during 7 days, promoting a strong demineralization.

This disadvantage of quantify erosion by MP is avoided by using a non-contact profilometry - optical or laser - guided for a computer, which may determine the loss surface with high precision.[9,11,19,20,31] However, these equipments are very expensive and it is necessary a long experience with the system.

Hara and Zero[20] evaluated the wear occasioned by different acid beverages with or without calcium, as cited previously. The authors used two methods to analyze the micro-structural alteration (microhardness and optical profilometry). In this study, the optical profilometry was the most appropriate method to quantify erosion at advanced stages. The results showed that calcium beverages, expect one, did not present any trend for enamel surface loss with increase in demineralization time.

Kielbassa *et al.*[9] assessed, using laser profilometry, the abrasive effects of toothpastes (low, medium and high-abrasive paste), acidic gels (fluoride and fluoride-free) and water (control) on sound and demineralized enamel. These authors observed that abrasion was about 50% less on sound than the demineralized enamel and that the greatest wear was obtained with high-abrasive paste. With the sound and demineralized enamel specimens, the lowest abrasion was observed after brushing with water and with the nonfluoridated acidic gel.

3. Surface Roughness (SR)

The surface roughness is a method, which may be adopted for evaluate the superficial alteration of texture in enamel/dentine after different erosive or abrasive treatment.[23,27,39] The data of roughness measure is expressed in Ra values (Roughness average – µm). Baseline and final roughness is the average of measures in the samples before and after the treatment, respectively. The roughness is calculated by subtracting the baseline measurements from the post-treatment values.[39] In the meantime, this measure no allows to know the structure loss.

Turssi *et al.*[39] used a profilometer equipped with a diamond stylus of 2 mm radius, at a constant speed of 0,05 mm/second with a force of 0,7 mN to evaluate wear depth and roughness of dentifrices (regular, baking soda, tartar control, whitening and distilled water) on enamel exposed to an acid soft drink (Sprite Diet®) or distilled water. The authors concluded that there was no significant difference in depth of enamel loss between the sound samples and the specimens subjected to acidic challenge, and no difference was observed by dentifrices. However, the surface roughness revealed significant effect. The exposure to Sprite Diet yielded higher roughness than did distilled water and the tartar control dentifrice had higher surface roughness than those brushed either with distilled water or with the whitening dentifrice.

Similarly, Menezes *et al.*[23] utilized SR and MP to evaluate the effect of different dentifrices (control, regular, baking soda, whitening and tartar control) on root dentine previously exposed to erosive challenges. The results obtained with SR and MP were also different, because the roughness assess the alteration in microstructural surface and not the surface loss.

This technique is little utilized, with scarce recent studies, because it has superficial information, therefore accurate results, about erosion or erosion plus abrasion, are not obtained, and this is not the best method to evaluate preventive products.

4. Microradiography

Microradiography is used for quantification of mineral loss based on the attenuation of X-ray irradiation transmitting dental hard tissue. Microradiographs are obtained with a digital image analyzing system interfaced to a universal microscope and a personal computer. This can be longitudinal when the X-rays are parallel to direction of process. The transversal is when the X-rays are perpendicular to the direction of lesion progression.[40]

For transverse microradiography (TMR) thin sections are obtained perpendicular to the sample surface and radiographer with a Nickel-filtered Cu Kα-line perpendicular to the cut surface. TMR is a valid tool for quantitative assessment of the mineral content as a function of depth from surface. From in-depth profiles, the lesion depth and mineral loss integrated over the entire depth (ΔZ) of the lesion can be calculated. Lesion depth usually is defined up to that point, where the mineral content reaches 95% of the mineral content of sound enamel or dentin. TMR for erosive mineral loss determination depends on the use of reference area not subjected to an erosive challenge. However, longitudinal microradiography enables the use of thicker specimens up to 4 mm thickness and the changes in mineral content can be calculated using pixel by pixel comparison of gray values of a radiography after treatment with the gray values of the reference radiograph. The main advantage of microradiograpgy is that the method enables to simultaneously determine surface loss and demineralization of the eroded samples.[36]

Kielbassa *et al.*[21] evaluated for transversal microradiografy the effect of saliva substitutes (Artisial, Glandosane, Oralube, Saliva medae, Mineral water, Biòtene and

Meridol) on the mineral content of pre-demineralized and sound enamel. The data determine that Biotene and Glandosane demineralized the sound enamel, however all other solutions revealed a significant mineral gain.

Schlueter *et al.*[16] assessed the effect of TiF_4 and NaF on mineral loss on enamel and dentin using longitudinal microradiography (LMR), the results of this study showed the reduction of enamel and dentine mineral loss by both fluoride solutions. Ganss *et al.*[12] used the same technique to evaluate the effect of toothpaste fluoridation and intensive fluoridation (toothpaste, mouth-rinse and gel) in prevention of erosion on enamel and dentine, observing that intensive fluoridation is effective in preventing enamel and dentine mineral loss on erosive conditions.

5. Atomic Force Microscopy (AFM) And AFM Nanoindentation

Recently, the application of atomic force microscopy (AFM) and especially AFM nanoindentation in biological research have been conducted. AFM provides a powerful tool to investigate the surface morphology of a variety of biological samples with nanometer resolution. There are two types of AFM scanning, such as tapping mode and contact mode.[41] Tapping mode AFM has been successfully applied to study alterations in enamel. AFM is capable of delivering high-resolution images of tooth enamel and, thus, unlike mechanical profilometry, allows quantifying the enamel loss caused by erosion.[35] Figure 3 and 4 show the micromorphological alteration of enamel after seven days of erosion (immersion in Coca for 5 minutes three times in a day) and immediate abrasion for 30 strokes.

AFM nanoindentation allows the measurement of nanomechanical properties such as surface hardness and reduced elastic modulus for indentation depths of less than 100 nm and has been shown useful for studying the mechanical properties of surface softened enamel.[19,22] The nanoindentaion sensibility allows to study very early stages of enamel erosion.[19]

Lippert *et al.*[22] investigated the effect of demineralization (1, 2 or 3 minutes), exposition to artificial saliva and toothbrush abrasion using tapping mode AFM and AFM nanoindentation. The AFM investigations observed that demineralization revealed the prismatic structure of enamel and resulted in a grainy surface structure, while the exposure to artificial saliva led to the deposition of a mineral phase with random orientation. In the images after brushing treatment, the prismatic structure was still identifiable and appeared smoother than prior the brushing treatment. The AFM nanoindenation investigations showed that toothbrushing of surface softened enamel leads to minor changes in the surface morphology and nanomechanical properties and the amount of enamel lost due to toothbrushing was independent of the demineralization time.

Barbour *et al.*[18] investigated the dissolution of human enamel in citric acid solutions over a wide range of pH ($2.30 \leq pH \leq 6.30$) through of a nanoindentation study. This analysis was used for this study because it is extremely sensitive to the early stages of enamel dissolution. Using this technique is possible distinguish enamel dissolution after short exposure times. The results showed that below pH 2.90, the enamel have the lowest possible hardness value.

Barbour *et al.*[19] assessed by AFM nanoindentaion the nanomechanical properties of enamel exposure to two different non-carbonated soft drinks at 4, 25, 50 and 75 °C. The analysis concluded that the nanohardness decreased, approximately linearly with the increase of temperature. This technique is utilized because it is more sensitive to changes in the thickness of the enamel softened layer.

In the last five years, AFM and nanoindentation have revolutionized the investigation of food induced enamel erosion. In addition to a fundamental understanding of dental erosion, the results of these studies were applied to develop new soft drinks with reduced erosive potential and it will be useful to investigation of erosive challenges.[35]

6. Scanning Electron Microscopy (SEM)

This method only allows subjective and qualitative assessment. Some studies utilizing SEM analysis to illustrate the surface subjected to erosive and abrasive challenges on dental structure.[8,11,25] Enamel/dentine samples which are examined with SEM need to be coated with a conductive layer, normally consisting of gold. Furthermore SEM does not allow quantitative measurements of enamel loss.[35]

Vieira *et al.*[11] evaluated the samples of bovine enamel pretreated with 4% TiF_4, AmF, fluoride varnish (FV), fluoride-free varnish, FV and subsequently submitted to varnish removal and a control submitted to erosive and/or abrasive challenges. The authors utilized SEM to determine the presence of fluoride or no fluoride varnish after the treatments, showing that sample treated with fluoride varnish had approximately 2 μm varnish layer well attached to the enamel surface and the sample treated with fluoride-free varnish had a surface layer with a thickness of approximately 1 μm partially detached from the enamel. This result was important to show that fluoride varnish is a mechanical barrier permitting the protective effect.

7. White Light Interferometer (WLI)

A white light interferometer is a computerized optical microscope that uses interference to produce a topographic image of the surface. Digital WLI images are typically shown as a topographic map where various colors denote different heights for the pixel, as recorded by the WLI software. By subtracting the original image from the image obtained after the experiment, a difference image created which show how much enamel had been removed during the erosive challenges. Using WLI is also possible to calculate the mean roughness in surface roughness due the etching of enamel. Thereby, many authors have utilized new techniques for develop the best manner to quantify the dental erosion.

Hove *et al.*[28] compared the protective effect of three fluoride substances (TiF_4, SnF_2 and NaF) on the development of erosion lesions in human enamel measured by a white light interferometer (WLI) *in vitro.* In this study, all the fluoride solutions protected the surface against exposure acid; however the TiF_4 solution provided the best protection. Hove *et al.*[13] compared the same products through an *in situ* study, however in this analysis, NaF had no protective effect and the TiF_4 showed also the best protection against acid attack. Previous studies show that TiF_4 form a protective surface layer or glaze, and this glaze is responsible for the protective effect against acid. This study has been demonstrated that WLI can be used to measure erosion lesions.

8. Confocal Laser Scanning Microscopy

Confocal microscopy is a non-destructive, 3-dimensional microscopic topography technique for obtaining high-resolution images. The confocal principle is based on the elimination of stray light from out-of-focus planes by confocal apertures. Images are obtained by scanning over the sample with a spot-size laser beam and recording the light reflected from the in-focus plane. In-depth imaging (tomography) is possible by recording series of consecutive images either in the optical x-y plane (optical section parallel to the surface) or x-z plane (optical section perpendicular to the surface).[42]

Duschner *et al.*[42] used this technique for investigating the early processes of erosion in dental enamel and according to the confocal images the enamel surface without a pellicle seemed to be relatively vulnerable to an acidic beverage and the 7-day in-vitro pellicle seemed to provide a very good protection against the action of the acidic components of the carbonated cola.

In the study conducted by Amaechi *et al.*,[43] the thickness of acquired salivary pellicle within the arches was investigated by the confocal laser microscopy technique. The results showed the pellicle was thinner in the upper anterior palatal surface when compared with the lower anterior lingual surface, and in the upper posterior palatal surface when compared with the lower posterior lingual surface. It has also shown that this variation can determine the sites and severity of erosion within the arches.

Confocal laser scanning microscopy is used in erosion studies, provides histotomographic images allowing for qualitative assessment and interpretation of hard tissue destruction or mineral dissolution, since light reflection and light scattering of hard tissue samples are influenced by microhistological changes within a tooth sample.[36]

Conclusion

The literature review points out different methods to analyze the dental wear, some already most established as well as emerging methods, raging from simple to complex techniques. Therefore, the knowledge about these techniques is indispensable to the choice of methods to measure dental wear.

References

1. Imfeld T. Dental erosion: Definition, classification and links. Eur J Oral Sci 1996;104:151-155.

2. Bartlett D, Smith BGN. Definition, classification and clinical assessment of attrition, erosion and abrasion of enamel and dentine. In: Tooth wear and sensitivity. Addy M, Edgar WM, Orchardson R, Embery G. 1st ed. London: Martin Dunitz; 2000. p 83-92.

3. Barbour ME, Rees GD. The role of erosion, abrasion and attrition in tooth. J Clin Dent 2006;17:88-93.

4. Lazarchik DA, Frazier KB. Dental erosion and acid reflux disease: An overview. Gen Dent 2008; 57:151-156.

5. Lussi A, Jaeggi T, Zero DT. The role of diet in the aetiology of dental erosion. Caries Res 2004;38:34-44.

6. Hooper S, West NX, Pickles MJ, Joiner A, Newcombe RG, Addy M. Investigation of erosion and abrasion on enamel and dentine: a model in situ using toothpastes of different abrasivity. J Clin Periodontol 2003;30:802-808.

7. Hara AT, Turssi CP, Teixeira ECN, Serra MC, Cury JA. Abrasive wear on eroded root dentine after different periods of exposure to saliva *in situ*. Eur J Oral Sci 2003;111:423-427.

8. Rios D, Honório HM, Magalhães AC, Buzalaf MAR, Palma-Dibb RG, Machado MAAM, Silva SMB. Influence of toothbrushing on enamel softening and abrasive wear of eroded bovine enamel: an *in situ* study. Braz Oral Res 2006;20:148-154.

9. Kielbassa AM, Gillmann L, Zantner C, Meyner-Lueckel H, Hellwig E, Schulte-Mönting J. Profilometric and microradiographic studies on the effects of toothpastes and acidic gel abrasivity on sound and demineralized bovine dental enamel. Caries Res 2005;39:380-386.

10. Lussi A, Jaeggi T, Gerber C, Megert B. Effect of amine/sodium fluoride rinsing on toothbrush abrasion of softened enamel in situ. Caries Res 2004;38:567-571.

11. Vieira A, Lugtenborg M, Ruben JL, Huysmans MCDNJM. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. Caries Res 2006;40:224-230.

12. Ganss C, Klimek J, Brune V, Schürmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561-566.

13. Hove LH, Holme B, Young A, Tveit AB. The protective effect of TiF_4, SnF_2 and NaF against erosion-like lesions in situ. Caries Res 2008;42:68-72.

14. Hooper SM, Newcombe RG, Faller R, Eversole S, Addy M, West NX. The protective effects of toothpaste against erosion by orange juice: studies *in situ* and *in vitro.* J Dent 2007;35:476-481.

15. Magalhães AC, Rios D, Moino AL, Wiegand A, Attin T, Buzalaf MAR. Effect of different concentrations of fluoride in dentifrices on dentin erosion subjected or not to abrasion in situ/ ex vivo. Caries Res 2008;42:112-116.

16. Schlueter N, Ganss C, Mueller U, Klimek J. Effect of titanium tetrafluoride and sodium fluoride on erosion progression in enamel and dentine in vitro. Caries Res 2007;41:141-145.

17. Vieira A, Ruben JL, Huysmans MCDNJM. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371-379.

18. Barbour ME, Parker DM, Allen GC, Jandt KD. Human enamel dissolution in citric acid as a function of pH in the range 2.30 ≤ pH ≤ 6.30 – a nanoindentation study. Eur J Oral Sci 2003;111:258-262.

19. Barbour ME, Finke M, Parker DM, Hugles JA, Allen GC, Addy M. The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. J Dent 2006;34:207-213.

20. Hara AT, Zero DT. Analysis of the erosive potential of calcium-containing acidic beverages. Eur J Oral Sci 2008;116:60-65.

21. Kielbassa AM, Shohadai SP, Schulte-mönting J. Effect of saliva substitutes on mineral content of demineralized and sound dental enamel. Support Care Cancer 2000;9:40-47.

22. Lippert F, Parker DM, Jandt KD. Toothbrush abrasion of surface softened enamel studied with tapping mode AFM and AFM nanoindentation. Caries Res 2004;38:464-472.

23. Menezes M, Turssi CP, Hara AT, Messias DCF, Serra MC. Abrasion of eroded root dentine brushed with different toothpastes. Clin Oral Invest 2004;8:151-155.

24. Philpotts CJ, Weader E, Joiner A. The measurement *in vitro* of enamel and dentine wear by toothpastes of different abrasivity. Int Dent J 2005;55:183-187.

25. Shellis RP, Finke M, Eisenburger M, Parker DM, Addy M. Relationship between enamel erosion and liquid flow rate. Eur J Oral Sci 2005;113:232-238.

26. Wiegand A, Wegehaupt F, Werner C, Attin T. Susceptibility of acid-softened enamel to mechanical wear – ultrasonication versus toothbrushing abrasion. Caries Res 2007;41:56-60.

27. Worschech CC, Rodrigues JA, Martins LRM, Ambrosano GMB. Brushing effect of abrasive dentifrices during at-home bleaching with 10% carbamide peroxide on enamel surface roughness. JCDP 2006;7:1-9.

28. Hove L, Holme B, Øgaard B, Willumsen T, Tveit AB. The protective effect of TiF_4, SnF_2 and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res 2006;40:440-443.

29. West, NX, Jandt KD. Methodologies and instrumentation to measure tooth wear: future perspectives. In: Tooth wear and sensitivity. Addy M, Edgar WM, Orchardson R, Embery G. 1st ed. London: Martin Dunitz; 2000. p 105-119.

30. Joiner A, Pickles MJ, Tanner C, Weader E, Doyle P. An in situ model study the toothpaste abrasion of enamel. J Clin Periodontol 2004;31:434-438.

31. Pickles MJ, Joiner A, Weader E, Cooper YL, Cox TF. Abrasion of human enamel and dentine caused by toothpastes of differing abrasivity determined using an *in situ* wear model. Int Dent J 2005;55:188-193.

32. Turssi CP, Faraoni JJ, Rodrigues Jr AL, Serra MC. An in situ investigation into the abrasion of eroded dental hard tissues by a whitening dentifrice. Caries Res 2004;38:473-477.

33. Zero DT, Hara AT, Kelly SA, González-Cabezas C. Evaluation of a desensitizing test dentifrice using an *in situ* erosion remineralization model. J Clin Dent 2006;17:112-116.

34. Low IM, Alhuthali A. In-situ monitoring of dental erosion in tooth enamel when exposed to soft drinks. Mater Sci Eng 2008;xx:1-4.

35. Jandt KD. Probing the future in functional soft drinks on the nanometer scale – towards tooth friendly soft drinks. Trends in Food Science & Technology 2006;17:263-271.

36. Attin T. Methods for assessment of dental erosion. In: Dental Erosion: from diagnosis to therapy. Lussi, A. 1st ed. Switzerland: Karger 2006. p 152-172

37. Eygen IV, Vannet BV, Wehrbein H. Influence of a soft drink with low pH on enamel surfaces: an in vitro study. Am J Orthod Dentofacial Orthop 2005;128:372-377.

38. Elias CN, Lopes HP. Mechanical tests. In: Scientific Methodology. Estrela C. 1 st Ed. São Paulo: Artes Médicas 2001. p 249-273.

39.Turssi CP, Messias DCF, Menezes M, Hara AT, Serra MC. Role of dentifrices on abrasion of enamel exposed to an acidic drink. Am J Dent 2005;18:251-255.

40. Thomas RZ, Ruben JL, Vries J, ten Bosch JJ, Huysmans MCDNJM. Transversal wavelength-independent microradiography, a method for monitoring caries lesions over time, validated with transversal microradiography. Caries Res 2006;40:281-291.

41. Santos NC, Castanho MARB. An overwiew of the biophysical applications of atomic force microscopy. Biophysical Chemistry 2004;107:133-149.

42. Duschner H, Götz H, Walker R, Lussi A. Erosion of dental enamel visualized by confocal laser scanning microscopy. In: Tooth wear and sensitivity. Addy M, Edgar WM, Orchardson R, Embery G. 1st ed. London: Martin Dunitz; 2000. p 67-73.

43. Amaechi BT, Higham SM, Edgar WM, Milosevic A. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. J Dent Res 1999;78:1821-1828.

FIGURES

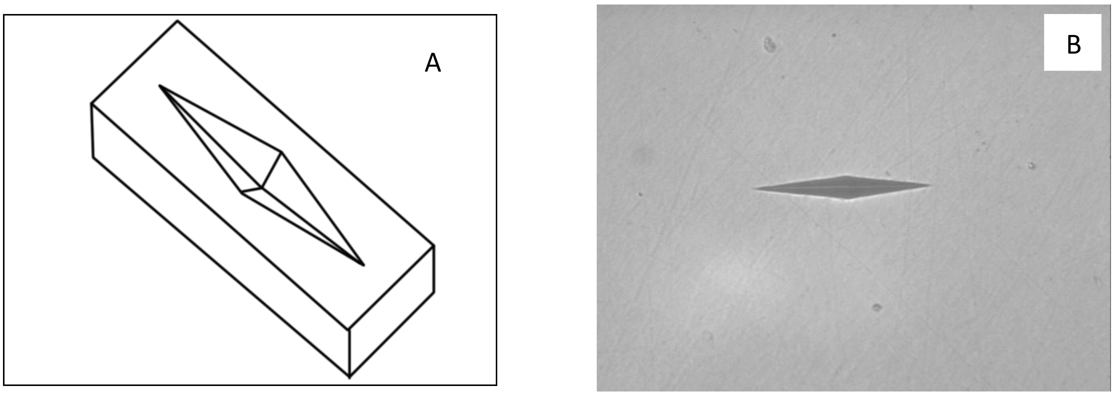

Figure 1: Knoop indentation. A: Squematic image; B: Indentation in enamel.

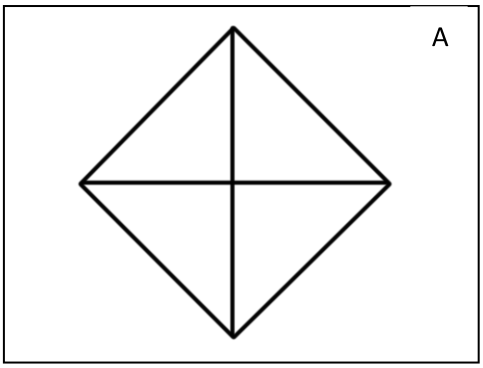

Figure 2: Vickers indentation. A: Squematic image;

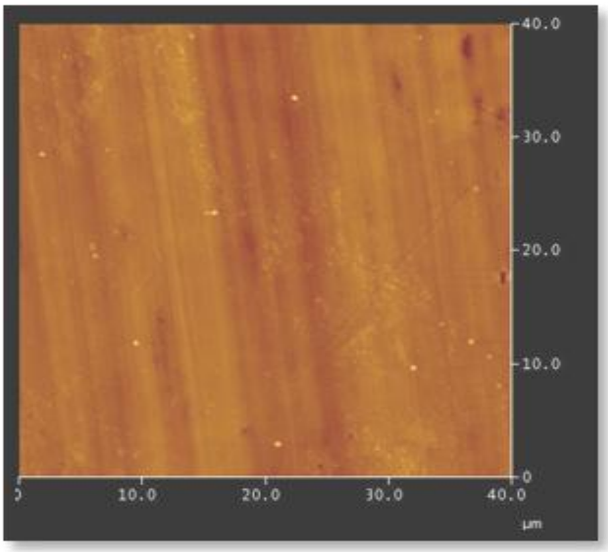

Figure 3- control.

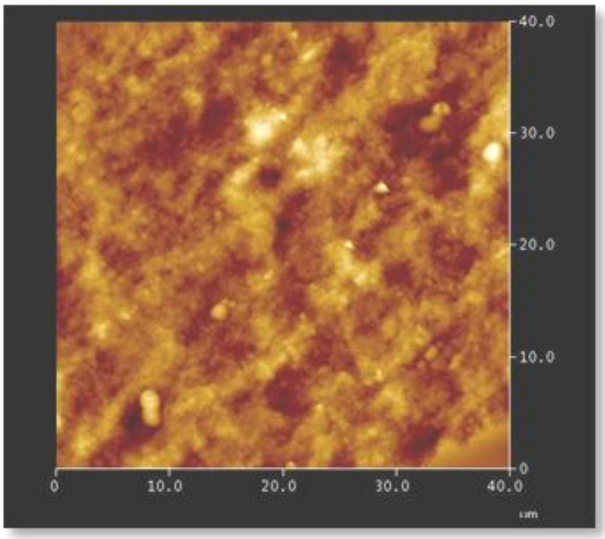

Figure 4 - erosive and abrasive challenges.

Table 1: Survey of the methods described in the text with respect to advantages and disadvantages, suitability for use with erosion (after few minutes of acidic challenge), as well as to type of analysis (quantitative or qualitative).

Methods	Advantages	Disadvantages	Suitability for early erosion	Type of analysis
Microhardness	-Relatively low costs -Nondestructive technique	- Not evaluate wear	Suitable	Quantitative
Surface Profilometry	-Nondestructive technique -Evaluate wear - Not time-consuming	- Perfectly flat and polished specimens - Mechanical profilometry could damage surface	Limitedly suitable	Quantitative
Surface roughness	- Evaluate texture - Not time-consuming	-	Suitable	Quantitative
Microradiography	-Evaluate mineral loss and demineralization	- Destructive technique	Not suitable	Quantitative
Atomic Force Microscopy and	-Nondestructive technique - Very sensitive	- Time-consuming - Long experience -High costs	Suitable	Qualitative
AFM Nanoindentation	-Allow the measurement of nanomechanical properties such as surface hardness and reduced elastic modulus - Nondestructive technique	- Time-consuming - Demanding sample preparation	Suitable	Quantitative
SEM	-High resolution - Evaluate micromorphological alteration	-Destructive technique -Specimens need be coat with a conductive layer - High costs	Suitable	Qualitative
White Light interferometer	- Nondestructive technique	-Emerging technique	Limitedly suitable	Quantitative

	-Could evaluate native surfaces -Determine roughness and wear	-Long experience		
Confocal Laser Scanning Microscopy	-High resolution -Nondestructive technique	- Long experience	Suitable	Qualitative

3.2 Capítulo 2

EFFECT OF NAF AND MFP-DENTIFRICE ON ENAMEL EROSION, ASSOCIATED OR NOT TO ABRASION.

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Short title: Effect of fluorides in prevention of erosion

Key Words: Erosion, Toothbrush abrasion, Fluoride, Enamel, Soft drinks.

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EFFECT OF NAF AND MFP-DENTIFRICE ON ENAMEL EROSION, ASSOCIATED OR NOT TO ABRASION.

Abstract

Objectives: To determine the protective effect of dentifrices containing sodium monofluorophosphate (MFP) or sodium fluoride (NaF) on eroded enamel subjected or not to brushing abrasion. **Methods**: A randomized, crossover, split-mouth and double-blind study was performed in three phases of 5 days each, in which 15 volunteers wore acrylic palatal appliances containing 4 human enamel slabs, of known surface microhardness (SMH), two of them placed of each side of the appliance. The slabs were daily subjected to erosion with cola soft drink 4 times a day for 60 s followed by treatment with one of following three dentifrice: non-fluoride, MFP and NaF, both containing 1450 ppm F. The slabs on one side of the appliance were subjected only to dentifrice slurry, evaluating erosion, but those of the other side were also brushed aiming to evaluate erosion plus abrasion. After each phase, SMH was again determined and the percentage of surface microhardness loss %SMHL) was calculated. The effect of the treatments was also observed by scanning electronic microscopy (SEM).. **Results**: The effect of erosive condition was significant ($p<0.01$) but of dentifrice was not ($p=0.06$). The %SMHL was lower when the enamel was subjected to erosion than to erosion+abrasion, which was confirmed by SEM showing that enamel surface softened by the acid erosion was removed by brushing. **Conclusion**: The data suggest that NaF dentifrice may chemically reduce the effect of erosion on enamel surface but this effect is eliminated by the mechanical wear provoked by brushing.

Key Words: Erosion, Toothbrush abrasion, Fluoride, Enamel, Soft drinks.

Introduction

The acid attack leads to the irreversible loss of dental hard tissue accompanied by a progressive softening of the surface, because during the erosion by acids and /or chelators, these agents interact with the surface of the mineral crystals by the hydrogen ion leading to surface etching[1].

In the modern society, the consumption of potentially erosive foodstuffs and drinks, e. g. fruits, salads, sport and soft drinks, is increasing for a variety of reasons including dietary recommendations by various especially health-minded groups.[2-6] Usually, these groups of persons are well instructed in oral hygiene consequently, their risk of loosing tooth substance due to dental cleansing is high, since abrasion or attrition immediately after an acid attack is greater than without previous exposure of the teeth to erosive agents.[7,8-10]

Eliminating the causal effect of erosion may be difficult, once a time people are exposed to extrinsic acids for physiologic motives or professional inclinations. Therefore, the better is promoting prevention programs, changing habits and protecting hard tissues, which can warn of the occurrence and limit the damage. The prevention manner is using remineralizing agents as the fluoride present in dentifrices[3,5,11] that increase the hardness and the acid resistance of enamel decreasing the structure loss by erosion and abrasion processes.[12-16] The main form of fluoride in dentifrices is NaF or MFP. However, there are not studies evaluating the effect of them in the control of dental loss after erosion and erosion associated to abrasion.

However, the MFP fluoride has a different reaction mechanism, it needs to be hydrolyzed by saliva or plaque, releasing fluoride ions to react with enamel whereas NaF does not. This hydrolysis step has been postulated as the major reason for the observation that NaF dentifrices generally show higher salivary and whole plaque fluoride concentrations than MFP dentifrices.[17,18] MFP form is present in 90% of dentifrices available in the Brazilian market and the relative effect of them in erosion/abrasion in enamel is not known.

Taking into account the association between erosion and abrasion and the importance of preventive methods, the purpose of this research is to test the null hypothesis that there is no difference in the effect of NaF and MFP on enamel erosion with or without the influence of toothbrushing.

Material and Methods

Panelists and Ethical Aspects

This study protocol was approved by the Research and Ethics Committee of Federal University of Ceará Medical School (protocol #092/2007). Fifteen healthy adult volunteers (9 females and 6 males, aged 18-29 years) residing in the same fluoridated area (0.70 mg F/l), who fulfilled the inclusion criteria described below, took part in this study. The subjects were free from erosive lesions or untreated carious cavities, able to comply with the experimental protocol were invited to participate in this study. Moreover, the subjects who use fixed or removable orthodontic devices, or have utilized in the last two months medicines that can affect the salivary flow rates were excluded from the study. Consent forms were signed prior to enrollment in the study.

Experimental Design

This study consisted of a factorial 2 x 3, conducted according to a randomized complete block design. The factors under study were condition at two levels (erosion or erosion plus abrasion) and dentifrice at three levels (non-fluoritaded, MFP and NaF).

Combination of these two factors originated six experimental groups, in which 180 specimens were randomly assigned. This crossover, split-mouth to erosion and erosion plus abrasion and double-blind study was performed in three phases of 5 days each, between each phase the volunteers used for two days the dentifrice that would be used in next phase, avoiding carry-over effect (Figure 1). Fifteen volunteers wore acrylic palatal appliances with 4 human enamel slabs divided in two rows: erosion and erosion plus abrasion and they used dentifrices encoded by A, B or C to allow a blind study. The split-mouth design in the same intra-oral palatal appliance was supported by the absence of any possible carry-across effect between abraded and non-abraded enamel slabs. It should be emphasized that the split-mouth design was not used for dentifrice treatments because of the possibility of carry-across effects. Therefore, each dentifrice treatment was performed in different phases.

The tested dentifrices were: non-fluorited (NF), MFP and NaF, the mean total fluoride concentrations and the Relative Enamel Abrasivity (REA) are described in Table 1. The mean total fluoride concentrations of the agents were checked prior to the *in situ* phase using a specific electrode (Orion 96-09, Orion Research., Beverly, Mass., USA).

Erosion was performed with cola soft drink (60 s) and the abrasion by toothbrushing with the respective dentifrice, 4 times a day. After each phase, the surface loss of enamel microstructure were determined by microhardness

Preparation of the Enamel Specimens.

Enamel slabs (4 x 4 x 2 mm) were obtained from caries-free third molars, which were stored in 0.01% (v/v) thymol solution at 4°C. The slabs were free of enamel defects or macroscopic cracks as assessed by visual examination. One hundred and eighty enamel slabs were obtained using a water-cooled low-speed diamond disc (No. 11-4244,series 15 HC - Diamond Buehler, Lake Bluff, IL, USA.) mounted in a cut machine (IsoMet^{TM} Low Speed

Saw, Buehler). The enamel surfaces of the slabs were ground flat with wet 320, 600 and 1,200-grit silicon carbide paper (3M do Brasil, Sumaré, SP, Brazil) and polished with diamond spray (1 µm; Buehler). After each flattening and polishing procedure, the specimens were sonicated for 2 min (Ultra Cleaner 1400, UNIQUE, Indaiatuba, São Paulo) in a detergent solution (Buehler, Lake Bluff, IL, USA). After preparation, all slabs were sterilized by autoclaving. A surface microhardness initial test (SMH) were performed with five indentations in the centre of the slab with a Knoop diamond under a 50 g load for 5 s (FM7 AMRS; Future Tech, Tokyo, Japan) to select the slabs. Specimens were allocated to treatments by stratified randomization according to the mean surface microhardness. All groups showed a mean of hardness of 325 ± 21 Kg/mm^2.

Palatal Device Preparation

Custom-made acrylic palatal devices were produced with four sites (5 x 5 x 3 mm) recessed into the polished surface of each appliance. One dental slab was randomly assigned to each one of the four sites and attached with wax. The position of each condition (erosion or erosion plus abrasion) in the device was randomly determined for each volunteer, using the coin flipping method. The side of the device in which was performed the abrasive procedure was colored with a blue wax.

In order to maintain reference surfaces to scanning electronic microscopy, a thin layer of composite resin (TPH; Dentsply, Petropolis, RJ, Brazil) was applied over the specimens' surfaces, leaving a window of 2 x 4 mm in their central area[19] after the slabs were fixed with wax in the palatal devices. No acid etching was carried out in the slab and no adhesive system was used before the composite resin application. The composite resin was light-cured for 40 s (Elipar™ Freelight™ 2, 3M ESPE, St. Paul, MN, USA)

Intraoral phase

During the lead-in period (2-days), and throughout the experimental phases, the volunteers brushed their teeth with one of the respective dentifrices. In this crossover protocol, the volunteers were randomly allocated, according to a computer generated list, to the treatments and participated in 3 phases. Twelve hours before the experimental phases, the device was worn and specimens were not subjected to erosive/abrasive processes, to allow the formation of a salivary pellicle. On the following five days, erosive and abrasive challenges were made extraorally 4 times[14,15,20,21] a day at predetermined times (7:00, 12:00, 17:00 and 21:00 h).

For erosion of the enamel samples, the volunteers were instructed to remove the appliance and immerse it in a cup containing 150 ml of a freshly opened bottle of regular Coke (Coca-cola®, Nossa Refrigerantes Ltda, Teresina, Piauí, Brazil) at room temperature for 60 s[15,21]. After each erosive challenge, the subjects were instructed to insert the appliances in their mouths and to brush their teeth with one of the dentifrice using a soft end-rounded toothbrush (Professional Clear, Colgate-Palmolive Ind. e Com. Ltda., São Paulo, SP, Brazil) with a small portion of the dentifrice (approximately 0.3 g), for 25 seconds, creating a dentifrice/saliva slurry.[16] During this moment, the toothbrush did not touch the specimens. The slurry was swished around the appliance for one minute, allowing contact to the experimental surfaces of the specimens. Subsequently, the appliance was removed, and more toothpaste was included in the toothbrush to the brushing procedure in two specimens of the blue row, followed by 40 brushing strokes.[19] Volunteers were trained and instructed to carefully perform this procedure. After this toothbrush, subjects gently rinsed their mouths with 15 ml of tap water for 10 seconds. The same procedure was repeated for the subsequent phases, only changing the dentifrice provided to the subject, according to the crossover experimental design.

The volunteers received instructions to wear the appliances continuously, including at night, but to remove them during meals. When removed devices were kept moist in the plastic boxes.

After each phase, new slabs of dental enamel were installed on the device to be subjected to a new treatment, and the slabs treated were analyzed by microhardness.

Percentage of superficial microhardness loss assessment

In the morning of 6^{th} day, the volunteers stopped wearing the palatal devices. The composite resin over the reference surfaces was carefully detached and the slabs were removed from the device. Afterwards, surface microhardness of the enamel slabs was measured again using a microhardness tester (Future Tech) with a Knoop diamond under a 50 g load for 5 s. Five indentations were made on the center of each specimen, on the experimental area (SMH_1). Using, SMH and SMH_1 measurements, the percentage of surface microhardness loss (%SMHL) was calculated using the equation:

$$\%SMHL= [[SMH-SMH_1] \times 100/ SMH].$$

Scanning electron microscopy

Eighteen specimens were selected for scanning electron microscopic observations. The specimens were coated with a thin layer of gold (approximately 10-12 nm in thickness) with a Denton Vacuum Desk II (Moorestown, NJ, USA). Observations were then made with a JEOL JSM-5600 LV Scanning Electron Microscope (Jeol Inc., Peabody, MA, USA) at 15 kV and magnifications up to 3000 ×.

Statistical analysis

Data of percentage of surface microhardness change were transformed to the square root in order to fit the assumptions of homocedasticity and normal distribution of errors. A split-plot analysis of variance (ANOVA) was used to compare the effect of toothpastes (as the plot) and condition of abrasion (as the subplot). The SAS program (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used for the analysis and the significance level set at $P < 0{,}05$.

Results

A total of 14 volunteers completed the study. Only one volunteer did not complete the three experimental phases due to lack of compliance with the study.

The percentage of loss in surface microhardness is shown in Table 2. The percentage surface microhardness loss between the factors toothpaste and condition did not differ statistically ($P = 0{,}7288$). Considering the effect of toothpaste, ANOVA did not show a statistical difference among groups, since $p = 0.0616$. The conditions of erosion and erosion+abrasion were significantly different from each other ($p < 0.0001$).

SEM images showed distinct patterns of the control areas of enamel, which had been covered by composite resin, and those submitted to erosion or erosion+abrasion (Figure 2-1 and 2-3). In the Figure 2-3, the presence of lines resultants of toothbrush is much apparent. At higher magnification of the test area (x 3000) of erosion group, the dissolution of the interprismatic spaces is suggestive, with possible loss of crystallinity of the structure of prisms, dissolution of minerals and amorphous reprecipitation (Figure 2-2). The erosion plus abrasion experimental area showed surface roughening, with enamel prisms clearly seen (Figure 2-4).

Discussion

In order to simulate the everyday situation as close as possible, an *in situ* model was chosen in the present study to evaluate erosion and erosion plus abrasion on enamel. Since, these models allow interactions among saliva and hard tissues in the oral environment, while retaining the sensitivity of laboratory analysis. The pattern of wear of an *in situ* model consists of specimens of human/bovine enamel or dentine mounted in the human mouth with intra-oral dispositive and subjected to erosive and/or abrasive process.

In this study, the findings (Table 2) showed that the fluoride dentifrices had no significant effect in the demineralization control, therefore the null hypothesis that there is no difference between the dentifrices used was accepted. These results are in agreement with Magalhães *et al.*[20] (2008), Rios *et al.*[21] (2008) and Lagerweij *et al.*[22] (2006). Similarly, Hove *et al.*[23] (2008) showed that fluoride solution of 2.1% NaF used 3 times for 2 min, during the experimental period (9 days) after erosion, compared with the control, TiF_4 and SnF_2, had no significant protective effect, showing that the NaF even at high concentrations was unable to reduce the effect caused by erosion. However, our results demonstrated that the effect of dentifrices was almost significant ($p = 0.0616$), probably because MFP requires a hydrolysis step to release fluoride to the mouth, whereas NaF does not. This step might have been the reason for NaF has promoted more control of erosive effect than MFP dentifrice in the enamel subjected to erosive challenges.

Others studies, such as Bartlett *et al.*[12] (1994), Ganss *et al.*[13] (2004), Zero *et al.*[16] (2006), Magalhães *et al.*[14] (2007) and Magalhães *et al.*[15] (2008) showed that fluoride might reduce the wear after erosion differently from the current research. This difference might be due to methodological differences used or by performing different analyses. In the present study, the slurry dentifrice/saliva get in contact with eroded enamel surfaces during only 60 seconds, that might have been insufficient to exposed the specimens to the highest fluoride concentrations as may occur with the subject’s teeth. On the other hand, in the study of Ganss *et al.*[12] (2004), the time of contact with slurry (fluoridation) was around 5 minutes, so much intense that the time utilized in our study and the analysis was microrradiography. In the studies conducted by Zero *et al.*[16] (2006) and Magalhães *et al.*[14] (2007), the time of demineralization was higher than the used in our research (25 and 5 minutes, respectively), which caused higher erosion, possibly promoting an effective action of fluoride in the remineralization. Magalhães *et al.*[15] (2008) assessed the fluoride effect on dentin and performed mechanical profilometry to evaluate their data, therefore, the force applied by probe of equipment in surface softened by erosion, can collapse the area and can be obtained overestimated results of wear, thereby this methodology may not be the best way to evaluated wear.

The enamel slabs subjected to erosion alone presented higher %SMHC than the specimens subjected to erosion plus abrasion and the last in some cases won hardness, in agreement with the study of Rios *et al.*[8] (2006). Thereby, the erosion treatment might promote a superficial softened zone that was lost, and in addition, may let an underlying softened layer, thus resulting in lower microhardness. So, in erosion plus abrasion, the hardness might obtain incorrect interpretation, once this softened layer may have been removed by the brush procedure, resulting in exposure of a harder enamel surface, that may be interpreted as increase of resistance to erosion, however, the specimen had most wear, so reaching a surface of most hardness. This data suggest that the remineralization in enamel have little clinic significance when simultaneously occur abrasion, because the remineralized layer will be removed by toothbrushing procedures.

The scanning electronic microscope is a qualitative method and it was utilized in this study only to illustrate the enamel surface after an erosive challenge, which present areas of dissolution of interprismaitc regions (Figure 2-1 and 2-2). However, the images of specimens submitted to erosion and abrasion presented a minor alteration of enamel when compared with the groups that had only erosion (Figure 2-3 and 2-4). The microhardness evaluated the alteration of microstructural surface, and showed that fluoride dentifrice no reduced the demineralization. However, this test is not capable of detected the wear. Therefore, these results can be underestimating in relation of the influence of fluorides dentifrices.

Conclusion

In the context of dentifrice use and under the conditions of this study, no type of fluoride was capable of control erosion or erosion + abrasion in human enamel. In addition, brushing teeth immediately after ingestion of cola soft drink may cause more alterations on enamel surface. Thereby, other studies should be realized to evaluate the effect of dentifrice containing NaF or MFP, using a complementary method to measure the wear.

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References

1. Hara AT, Ando M, Gonzaléz-Cabezas C, Cury JÁ, Serra MC, Zero DT. Protective effect of the dental pellicle against erosive challenges *in situ*. *Journal of Dental Research* 2006;**85**:612-16.

2. Santiago SL, Vieira, AHM, Passos VF. Restorations of non-carious cervical lesions: problems and solutions. *Pro-odonto Estética* 2008;**2**;83-138.(Portuguese)

3. Gandara BK, Ttruelove EL. Diagnosis and management of dental erosion. *The Journal of Comtemporary Dental Practice* 1999;**1**:1-15.

4. Larsen MJ. Erosion of the teeth. In: Fejerskov O, Kidol E, editors. Dental Caries: The disease and its clinical management, 2. Ed. Oxford: Blackwell Munksgaard, 2008. p. 234-247.

5. Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. *Caries Research* 2004;**38**:34-44.

6. Zero DT, Lussi A.Etiology of enamel erosion: intrinsic and extrinsic factors. In: Addy M, Embery G, Edgar WM, Orchardson R, editors. Tooth wear and sensitivity. London: Martin Dunitz; 2000. p. 121-40.

7. Hooper S, West NX, Pickles MJ, Joiner A, Newcombe RG, Addy M. Investigation of erosion and abrasion on enamel and dentine: a model in situ using toothpastes of different abrasivity. *Journal of Clinical Periodontology* 2003;**30**:802-808.

8. Rios D, Honório HM, Magalhães AC, Buzalaf MAR, Palma-Dibb RG, Machado MAAM, Silva SMB. Influence of toothbrushing on enamel softening and abrasive wear of eroded bovine enamel: an *in situ* study. *Brazilian Oral Research* 2006;**20**:148-54.

9. Vieira A, Overweg E, Ruben JL, Huysmans MCDNJM. Toothbrush abrasion, simulated tongue friction and attrition of eroded bovine enamel in vitro. *Journal of Dentistry* 2006;**34:**336-42.

10. Wiegand A, Köwing L, Attin T. Impact of brushing force on abrasion of acid-softened and sound enamel. *Archives of Oral Biology* 2007;52:1043-47.

11. Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. *Journal of Dentistry* 2005;**33**:243-52.

12. Bartlett DW, Smith BGN, Wilson RF. Comparison of the effect of fluoride and non-fluoride toothpaste on tooth wear *in vitro* and the influence of enamel fluoride concentration and hardness of enamel. *British Dental Journal* 1994;**176**:346-48.

13. Ganss C, Klimek J, Brune V, Schürmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. *Caries Research* 2004;**38**:561-66.

14. 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 Research* 2007;**41**:77-79.

15. Magalhães AC, Rios D, Moino AL, Wiegand A, Attin T, Buzalaf MAR. Effect of different concentrations of fluoride in dentifrices on dentin erosion subjected or not to abrasion in situ/ ex vivo. *Caries Research* 2008;**42**:112-16.

16. Zero DT, Hara AT, Kelly SA, González-Cabezas C. Evaluation of a desensitizing test dentifrice using an *in situ* erosion remineralization model. *The Journal of Clinical Dentistry* 2006;**17**:112-16.

17. Vogel GL, Mao Y, Chow LC, Proskin HM. Fluoride in plaque fluid, plaque, and saliva measured for 2 hours after a sodium fluoride monofluorophosphate rinse. *Caries Research* 2000**;34**:404-11.

18. Ekstrand J. Fluoride in plaque fluid and saliva after NaF or MFP rinses. *European Journal of Oral Sciences* 1997;**105**:478-84.

19. Hara AT, Turssi CP, Teixeira ECN, Serra MC, Cury JA. Abrasive wear on eroded root dentine after different periods of exposure to saliva *in situ*. *European Journal of Oral Sciences* 2003;**111**:423-27.

20. Magalhães AC, Rios D, Martinho CCR, Delbem ACB, Buzalaf MAR, Machado MAAM. The influence of residual salivary fluoride from dentifrice on enamel erosion: an *in situ* study. *Brazilian Oral Research* 2008;**22**:67-71.

21. 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. *The Journal of the American Dental Assosiation* 2008;**139**:1652-56.

22. Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. *Caries Research* 2006;**40**:148-53.

23. Hove LH, Holme B, Young A, Tveit AB. The protective effect of TiF_4, SnF_2 and NaF against erosion-like lesions in situ. *Caries Research* 2008;**42**:68-72.

TABLES

Table 1: Mean (SD; n=3) of fluoride concentrations and relative enamel abrasivity (REA) of the dentifrices used

Dentifrices	**Fluoride concentration (μg F/g) as**			**REA**
	Ion F	**MFP**	**TOTAL**	
Non-Fluoride	0.0	0.0	0.0	5.3 ± 0.4
NaF	*1465.0 ± 8.9	0.0	1475.0 ± 0.0	7.0 ± 0.1
MFP	62.5 ± 0.1	*1372.8 ± 17.8	1434.9 ± 0.0	12.8 ± 0.8

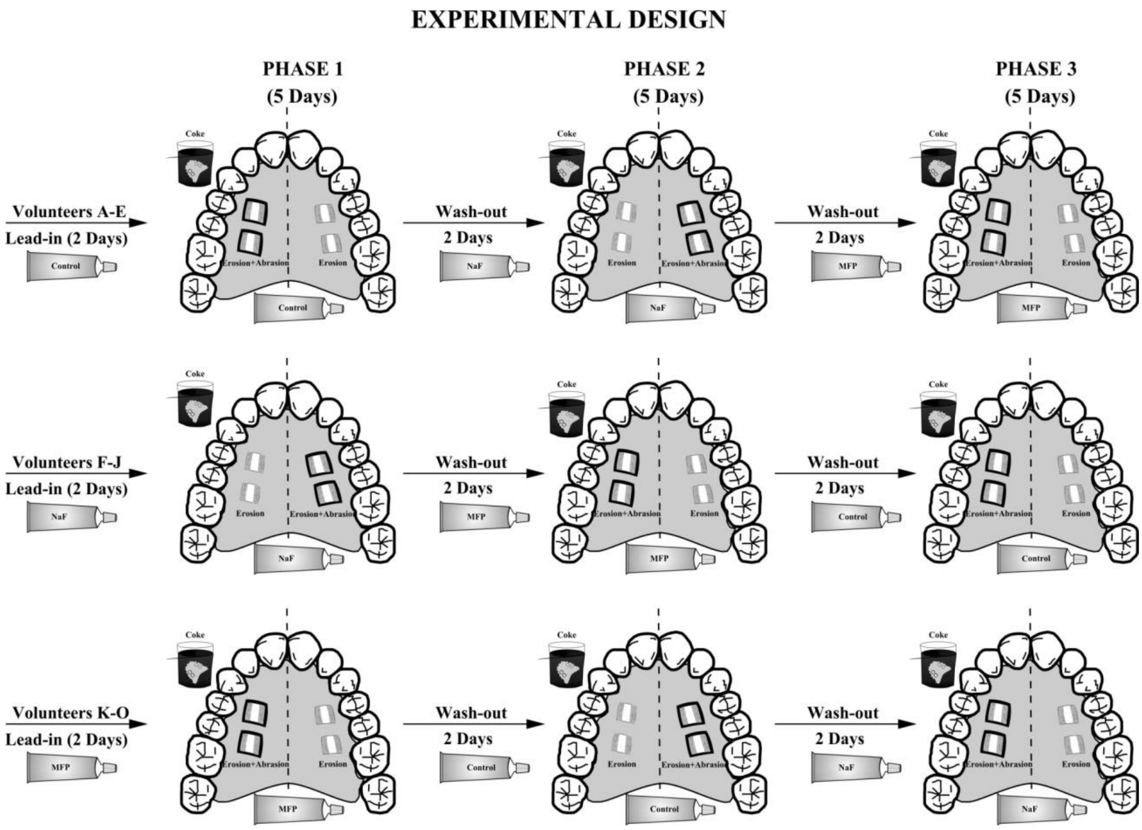
*Expected = 1450 ppm F

Table 2: Percentage of surface microhardness loss according to the erosive conditions and the dentifrices used (means ± SD, n=14)

***Surface Treatment**	****Dentifrices**		
	Non-Fluoride	**NaF**	**MFP**
Erosion	52.4 ± 18.7	42.9 ± 21.2	52.3 ± 21.9
Erosion+Abrasion	-0.1 ± 11.8	-7.5 ± 8.8	-4.3 ± 16.5

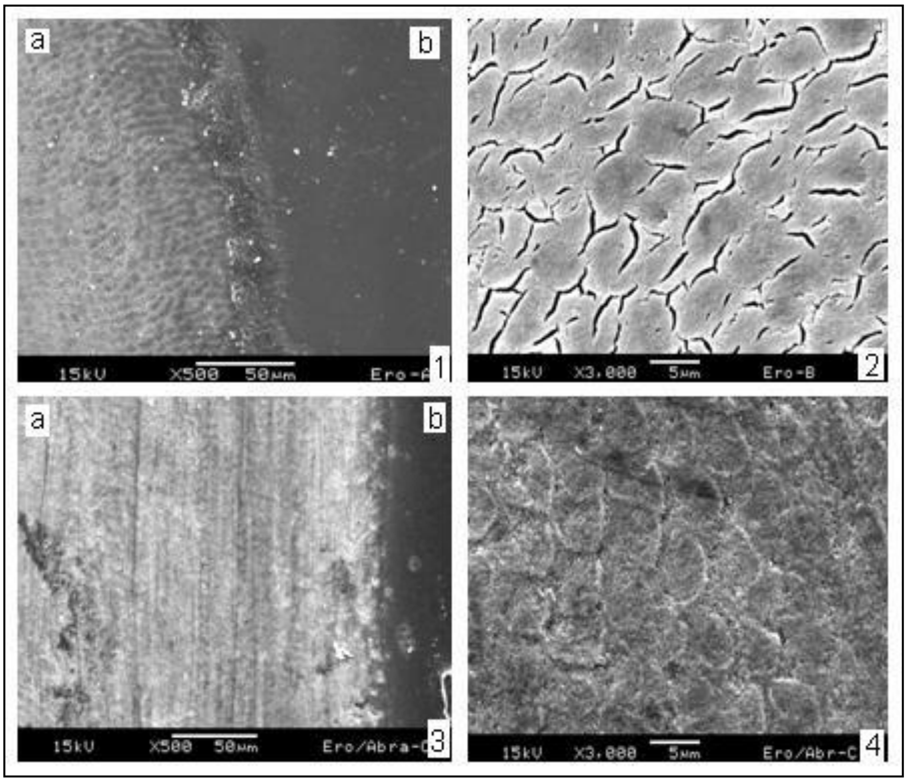
*p < 0.0001; ** p = 0.0616 and p = 0.7288 for the interaction dentifrice*condition

FIGURE 1



EXPERIMENTAL DESIGN
PHASE 1
(5 Days)
PHASE 2
(5 Days)
PHASE 3
(5 Days)
Volunteers A-E
Lead-in (2 Days)
Control
Coke
Erosion+Abrasion
Erosion
Control
Wash-out
2 Days
NaF
Erosion
Erosion+Abrasion
NaF
Wash-out
2 Days
MFP
Erosion+Abrasion
Erosion
MFP
Volunteers F-J
Lead-in (2 Days)
NaF
Erosion
Erosion+Abrasion
NaF
MFP
Erosion+Abrasion
Erosion
MFP
Control
Erosion+Abrasion
Erosion
Control
Volunteers K-O
Lead-in (2 Days)
MFP
Erosion+Abrasion
Erosion
MFP
Control
Erosion
Erosion+Abrasion
Control
NaF
Erosion+Abrasion
Erosion
NaF

FIGURE 2



SEM images. 1: SEM of the areas protected (B) and subjected to erosion (A). Bar represents 50 μm (500 ×). 2: SEM (3000 x) of the area subjected to erosion. Bar represents 5 μm. 3: SEM of the areas protected (B) and subjected to erosion+abrasion (A). Bar represents 50 μm (500 ×) 4: SEM (3000 x) of area subjected to erosion plus abrasion. Bar represents 5 μm.

4 CONCLUSÃO GERAL

Da avaliação dos resultados obtidos nesta dissertação, pode-se concluir que:

a) a literatura apresenta diferentes metodologias para o estudo de erosão e/ou abrasão tanto em esmalte como em dentina, que variam desde técnicas simples e de fácil realização, até processos mais complexos que exijam treinamento e habilidade. Dessa forma, é importante o conhecimento das diversas técnicas que possam ser empregadas para a avaliação da alteração micro-estrutural do tecido dentário, permitindo a seleção de uma metodologia adequada para o objetivo do estudo.

b) dentifrícios fluoretados, contendo NaF ou MFP não foram efetivos no controle da erosão do esmalte humano submetido a procedimentos erosivos ou erosivos e abrasivos.

c) é necessária a realização de mais estudos para avaliação do efeito do flúor, na forma de NaF ou MFP, na remineralização de esmalte dentário submetido a procedimentos erosivos ou erosivos e abrasivos, utilizando-se técnicas complementares que permitam a medição do desgaste, com o intuito de confirmar o efeito destes produtos possibilitando uma extrapolação clínica dos dados.

REFERÊNCIAS

AMAECHI, B. T.; HIGHAM, S. M.; EDGAR, W. M.; MILOSEVIC, A. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. **J. Dent. Res.**, v. 78, n. 12, p. 1821-1828, Dec. 1999.

ATTIN, T.; BUCHALLA, W.; PUTZ, B. In vitro evaluation of different remineralization periods in improving the resistance of previously eroded bovine dentine against tooth-brushing abrasion. **Arch. Oral Biol.**, v. 46, p. 871-874, 2001.

AUAD, S. M.; WATERHOUSE, P. J.; NUNN, J. H.; STEEN, N.; MOYNIHAN, P. J. Dental erosion amongst 13- and 14-year-old Brazilian schoolchildren. **Int. Dent. J.**, v. 57, n. 3, p. 161-167, June 2007.

BARBOUR, M. E.; FINKE, M.; PARKER, D. M.; HUGHES, J. A.; ALLEN, G. C.; ADDY, M. The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. **J. Dent.**, v. 34, n. 3, p. 207-213, Mar. 2006.

BARBOUR, M. E.; PARKER, D. M.; ALLEN, G. C.; JANDT, K. D. Human enamel dissolution in citric acid as a function of pH in the range $2.30 \leq pH \leq 6.30$ – a nanoindentation study. **Eur. J. Oral Sci.**, v. 111, n. 3, p. 258-262, June 2003.

BARBOUR, M. E.; REES, G. D. The role of erosion, abrasion and attrition in tooth. **J. Clin. Dent.**, v. 17, p. 88-93, 2006.

BARTLETT, D. W.; SMITH, B. G.; WILSON, R. F. Comparison of the effect of fluoride and non-fluoride toothpaste on tooth wear *in vitro* and the influence of enamel fluoride concentration and hardness of enamel. **Br. Dent. J.**, v. 176, n. 9, p. 346-348, May 1994.

BROWN, L. J.; WALL, T. P; LAZAR, V. Trends in total caries experience: permanent and primary teeth. **J. Am. Dent. Assoc.**, v.131, p. 223-231, 2000.

DAVID, B. Intrinsic causes of erosion. In: LUSSI, A. **Dental erosion:** from diagnosis to therapy. Basel: Karger, 2006. cap. 8, p. 119-139.

DUGMORE, C. R.; ROCK, W. P. The progression of tooth erosion in a cohort of adolescents of mixed ethnicity. **Int. J. Paediatr. Dent.**, v. 13, n. 5, p. 295-303, Sept. 2003.

EISENBURGER, M.; ADDY, M. Influence of liquid temperature and flow rate on enamel erosion and surface softening. **J. Oral. Rehabil.**, v. 30, n. 11, p. 1076-1080, Nov. 2003.

EISENBURGER, M.; SHELLIS, R. P.; ADDY, M. Comparative study of wear of enamel induced by alternating and simultaneous combinations of abrasion and erosion in vitro. **Caries Res.**, v. 37, n. 6, p. 450-455, Nov./Dec. 2003.

EKSTRAND, J. Fluoride in plaque fluid and saliva after NaF or MFP rinses. **Eur. J. Oral Sci.**, v. 105, n. 5, pt. 2, p. 478-484, Oct. 1997.

GANDARA, B. K.; TRUELOVE, E. L. Diagnosis and management of dental erosion. **J Contemp. Dent. Pract.**, v. 1, n. 1, p. 16-23, Nov. 1999.

GANSS, C.; KLIMEK, J.; BRUNE, V.; SCHÜRMANN, A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. **Caries Res.**, v. 38, n. 6, p. 561-566, Nov./Dec. 2004.

GANSS, C.; KLIMEK, J.; SCHÄFFER, U.; SPALL, T. Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. **Caries Res.** v. 35, n. 5, p. 325-330, Sept./Oct. 2001.

GANSS, C.; SCHLUETER, N.; FRIEDRICH, D.; KLIMEK, J. Efficacy of waiting periods and topical fluoride treatment on toothbrush abrasion of eroded enamel in situ. **Caries Res.**, v. 41, n. 2, p. 146-151, 2007.

HARA, A. T.; ANDO, M.; GONZÁLEZ-CABEZAS, C.; CURY, J. A.; SERRA, M. C.; ZERO, D. T. Protective effect of the dental pellicle against erosive challenges *in situ*. **J. Dent. Res.**, v. 85, n. 7, p. 612-616, 2006.

HARA, A. T.; LUSSI, A.; ZERO, D. T. Biological factors. In: LUSSI, A. **Dental erosion:** from diagnosis to therapy. Basel: Karger, 2006. cap. 7.1.2, p. 88-99.

HARA, A. T.; TURSSI, C. P.; TEIXEIRA, E. C.; SERRA, M. C.; CURY, J. A. Abrasive wear on eroded root dentine after different periods of exposure to saliva *in situ*. **Eur. J. Oral Sci.**, v. 111, n. 5, p. 423-427, Oct. 2003.

HARA, A. T.; ZERO, D. T. Analysis of the erosive potential of calcium-containing acidic beverages. **Eur. J. Oral Sci.**, v. 116, n. 1, p. 60-65, Feb. 2008.

HOOPER, S. M.; NEWCOMBE, R. G.; FALLER, R.; EVERSOLE, S.; ADDY, M.; WEST, N. X. The protective effects of toothpaste against erosion by orange juice: studies *in situ* and *in vitro.* **J. Dent.**, v. 35, n. 6, p. 476-481, June 2007.

HOOPER, S.; WEST, N. X.; PICKLES, M. J.; JOINER, A.; NEWCOMBE, R. G.; ADDY, M. Investigation of erosion and abrasion on enamel and dentine: a model in situ using toothpastes of different abrasivity. **J. Clin. Periodontol.**, v. 30, n. 9, p. 802-808, Sept. 2003.

HOVE, L. H.; HOLME, B.; YOUNG, A.; TVEIT, A. B. The protective effect of TiF_4, SnF_2 and NaF against erosion-like lesions in situ. **Caries Res.**, v. 42, n. 1, p. 68-72, 2008.

IMFELD, T. Dental erosion: Definition, classification and links. **Eur. J. Oral Sci.**, v. 104, p. 151-155, 1996.

JAEGGI, T.; LUSSI, A. Prevalence, incidence and distribution of erosion. In: LUSSI, A. **Dental Erosion:** from diagnosis to therapy. [S.l.]: Karger, 2006. cap. 5, p. 44-65.

JOINER, A.; COLLINS, L. Z.; COX, T. F.; PICKLES, M. J.; WEADER, E.; LISCOMBE, C.; HOLT, J. S. The measurement of enamel and dentine abrasion by tooth whitening products using an *in situ* model. **Int. Dent. J.**, v. 55, n. 3, suppl. 1, p. 194-196, 2005.

KIELBASSA, A. M.; GILLMANN, L.; ZANTNER, C.; MEYER-LUECKEL, H.; HELLWIG, E.; SCHULTE-MÖNTING, J. Profilometric and microradiographic studies on the effects of toothpastes and acidic gel abrasivity on sound and demineralized bovine dental enamel. **Caries Res.**, v. 39, n. 5, p. 380-386, Sept./Oct. 2005.

KIELBASSA, A. M.; SHOHADAI, S. P.; SCHULTE-MÖNTING, J. Effect of saliva substitutes on mineral content of demineralized and sound dental enamel. **Support Care Cancer**, v. 9, n. 1, p. 40-47, Jan. 2000.

LAGERWEIJ, M. D.; BUCHALLA, W.; KOHNKE, S.; BECKER, K.; LENNON, A. M.; ATTIN, T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. **Caries Res.**, v. 40, n. 2, p. 148-153, 2006.

LARSEN, M. J. Erosion of the teeth. In: FEJERSKOV, O.; KIDOL, E. **Dental caries:** The disease and its clinical management. 2nd ed. Oxford: Blackwell Munksgaard, 2008. cap. 13, p. 234-247.

LIPPERT, F.; PARKER, D. M.; JANDT, K. D. Toothbrush abrasion of surface softened enamel studied with tapping mode AFM and AFM nanoindentation. **Caries Res.**, v.38, n. 5, p. 464-472, Sept./Oct. 2004.

LUSSI, A.; JAEGGI, T.; GERBER, C.; MEGERT, B. Effect of amine/sodium fluoride rinsing on toothbrush abrasion of softened enamel in situ. **Caries Res.**, v. 38, n. 6, p. 567-571, Nov./Dec. 2004.

LUSSI, A.; JAEGGI, T.; ZERO, D. The role of diet in the aetiology of dental erosion. **Caries Res.**, v. 38, suppl. 1, p. 34-44, 2004.

LUSSI, A.; MEGERT, B.; EGGENBERGER, D.; JAEGGI, T. Impact of different toothpastes on the prevention of erosion. **Caries Res.**, v. 42, n. 1, p. 62-67, 2008.

MAGALHÃES, A. C.; RIOS, D.; DELBEM, A. C.; BUZALAF, M. A..; MACHADO, M. A. Influence of fluoride dentifrice on brushing abrasion of eroded human enamel: an in situ/ex vivo study. **Caries Res.**, v. 41, n. 1, p. 77-79, 2007.

MAGALHÃES, A. C.; RIOS, D.; MARTINHON, C. C.; DELBEM, A. C.; BUZALAF, M. A.; MACHADO, M. A. The influence of residual salivary fluoride from dentifrice on enamel erosion: an *in situ* study. **Braz. Oral Res.**, v. 22, n. 1, p. 67-71, Jan./Mar. 2008a.

MAGALHÃES, A. C.; RIOS, D.; MOINO, A. L.; WIEGAND, A.; ATTIN, T.; BUZALAF, M. A. Effect of different concentrations of fluoride in dentifrices on dentin erosion subjected or not to abrasion in situ/ ex vivo. **Caries Res.**, v. 42, n. 2, p. 112-116, 2008b.

MENEZES, M.; TURSSI, C. P.; HARA, A. T.; MESSIAS, D. C.; SERRA, M. C. Abrasion of eroded root dentine brushed with different toothpastes. **Clin. Oral Invest.**, v. 8, n. 3, p. 151-155, Sept. 2004.

NUNN, J. H. Prevalence and distribution of tooth wear. . In: ADDY, M.; EMBERY, G.; EDGAR, W. M.; ORCHARDSON, R. **Tooth wear and sensitivity**. London: Martin Dunitz, 2000. cap. 9, p. 93-103.

PICKLES, M. J.; JOINER, A.; WEADER, E.; COOPER, Y. L.; COX, T. F. Abrasion of human enamel and dentine caused by toothpastes of differing abrasivity determined using an *in situ* wear model. **Int. Dent. J.**, v. 55, n. 3, suppl. 1, p. 188-193, 2005.

RIOS, D.; HONÓRIO, H. M.; MAGALHÃES, A. C.; BUZALAF, M. A.; PALMA-DIBB, R. G.; MACHADO, M. A.; DA SILVA, S. M. Influence of toothbrushing on enamel softening and abrasive wear of eroded bovine enamel: an *in situ* study. **Braz. Oral Res.**, v. 20, n. 2, p. 148-154, Apr./June 2006b.

RIOS, D.; HONÓRIO, H. M.; MAGALHÃES, A. C.; DELBEM, A. C.; MACHADO, M. A.; SILVA, S. M.; BUZALAF, M. A. Effect of salivary stimulation on erosion of human and bovine enamel subjected or not to subsequent abrasion: an in situ/ex vivo study. **Caries Res.**, v. 40, n. 3, p. 218-223, 2006a.

SALES-PERES, S. H.; PESSAN, J. P.; BUZALAF, M. A. Effect of an iron mouthrinse on enamel and dentine erosion subjected or not to abrasion: na *in situ/ex vivo* study. **Arch. Oral Biol.**, v. 52, n. 2, p. 128-132, Feb. 2007.

SCHEUTZEL, P. Etiology of dental erosion – intrinsic factors. **Eur. J. Oral Sci.**, v. 104, p. 178-190, 1996.

SCHLUETER, N.; GANSS, C.; MUELLER, U.; KLIMEK, J. Effect of titanium tetrafluoride and sodium fluoride on erosion progression in enamel and dentine in vitro. **Caries Res.**, v. 41, n. 2, p. 141-145, 2007.

SHELLIS, R. P.; FINKE, M.; EISENBURGER, M.; PARKER, D. M.; ADDY, M. Relationship between enamel erosion and liquid flow rate. **Eur. J. Oral Sci.**, v. 113, n. 3, p. 232-238, June 2005.

TURSSI, C. P.; FARAONI, J. J.; RODRIGUES JR, A. L.; SERRA, M. C. An in situ investigation into the abrasion of eroded dental hard tissues by a whitening dentifrice. **Caries Res.**, v. 38, n. 5, p. 473-477, Sept./Oct. 2004.

TURSSI, C. P.; MESSIAS, D. C.; DE MENEZES, M.; HARA, A. T.; SERRA, M. C. Role of dentifrices on abrasion of enamel exposed to an acidic drink. **Am. J. Dent.**, v. 18, n. 4, p. 251-255, Aug. 2005.

VAN EYGEN, I.; VANNET, B. V.; WEHRBEIN, H. Influence of a soft drink with low pH on enamel surfaces: an in vitro study. **Am. J. Orthod. Dentofacial Orthop.**, v. 128, n. 3, p. 372-377, Sept. 2005.

VIEIRA, A.; LUGTENBORG, M.; RUBEN, J. L.; HUYSMANS, M. C. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. **Caries Res.**, v. 40, n. 3, p. 224-230, 2006b.

VIEIRA, A.; OVERWEG, E.; RUBEN, J. L.; HUYSMANS, M. C. Toothbrush abrasion, simulated tongue friction and attrition of eroded bovine enamel in vitro. **J. Dent.**, v. 34, n. 5, p. 336-342, May 2006a.

VIEIRA, A.; RUBEN, J. L.; HUYSMANS, M. C. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. **Caries Res.**, v. 39, n. 5, p. 371-379, Sept./Oct. 2005.

VOGEL, G. L.; MAO, Y.; CHOW, L. C.; PROSKIN, H. M. Fluoride in plaque fluid, plaque, and saliva measured for 2 hours after a sodium fluoride monofluorophosphate rinse. **Caries Res.**, v. 34, n. 5, p. 404-411, Sept./Oct. 2000.

WETTON, S.; HUGHES, J.; WEST, N.; ADDY, M Exposure time of enamel and dentine to saliva for protection against erosion: a study in vitro. **Caries Res.**, v. 40, n. 3, p. 213-217, 2006.

WIEGAND, A.; BEGIC, M.; ATTIN, T. In vitro evaluation of abrasion of eroded enamel by different manual, power and sonic toothbrushes. **Caries Res.**, v. 40, n. 1, p. 60-65, 2006.

WIEGAND, A.; KÖWING, L.; ATTIN, T. Impact of brushing force on abrasion of acid-softened and sound enamel. **Arch. Oral Biol.**, v. 52, n. 11, p. 1043-1047, Nov. 2007.

WONGKHANTEE, S.; PATANAPIRADEJ, V.; MANEENUT, C.; TANTBIROJN, D. Effect of acidic food and drinks on surface hardness of enamel, dentine, and tooth-coloured filling materials. **J. Dent.**, v. 34, n. 3, p. 214-220, Mar. 2006.

WORSCHECH, C. C.; RODRIGUES, J. A.; MARTINS, L. R.; AMBROSANO, G. M. Brushing effect of abrasive dentifrices during at-home bleaching with 10% carbamide peroxide on enamel surface roughness. **J. Contemp. Dent. Pract.**, v. 7, n. 1, p. 25-34, Feb. 2006.

ZERO, D. T. Etiology of dental erosion – extrinsic factors. **Eur. J. Oral Sci.**, v. 104, p. 162-177, 1996.

ZERO, D. T.; HARA, A. T.; KELLY, S. A.; GONZÁLEZ-CABEZAS, C.; ECKERT, G. J.; BARLOW, A. P.; MASON, S. C. Evaluation of a desensitizing test dentifrice using an *in situ* erosion remineralization model. **J. Clin. Dent.**, v. 17, n. 4, p. 112-116, 2006.

ZERO, D. T.; LUSSI, A. Etiology of enamel erosion: intrinsic and extrinsic factors. In: ADDY, M.; EMBERY, G.; EDGAR, W. M.; ORCHARDSON, R. **Tooth wear and sensitivity**. London: Martin Dunitz, 2000. cap. 11, p. 121-140.

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) 3366 8338.

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

<table>
<tr><td colspan="4">Nome do paciente:</td></tr>
<tr><td colspan="2">Documento de identidade nº:</td><td>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>
</table>

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) 9988-2039

Título do Projeto: **AVALIAÇÃO DA ALTERAÇÃO ESTRUTURAL DO ESMALTE DENTÁRIO PROMOVIDA POR EROSÃO/ABRASÃO.**

A abrasão é definida como um desgaste dentário por processos mecânicos envolvendo objetos ou substâncias repetidamente introduzidos na cavidade bucal e em contato com o dente. E a erosão é ocasionada por ácidos intrínsecos e extrínsecos, como bebidas ácidas. Como a escovação com pasta dental é o hábito mais comum de higiene bucal e sua ação deriva da ação mecânica da escova e das propriedades da pasta, esse fator tem impacto potencial na abrasão do tecido dentário. Neste estudo serão utilizados dispositivos palatinos que deverão ser escovados com três tipos diferentes de cremes dentais e imersos em bebida ácida quatro vezes ao dia. 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 2008

____
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

TERMO DE DOAÇÃO DE DENTES

Pelo presente instrumento que atende às exigências legais, o Sr. (a) ____, após ter tomado conhecimento do protocolo de pesquisa **"**AVALIAÇÃO DA ALTERAÇÃO ESTRUTURAL DO ESMALTE DENTÁRIO PROMOVIDA POR EROSÃO/ABRASÃO**"** 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** a 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: ____

ANEXO A – Aprovação do Comitê de Ética em Pesquisa

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 705/08 Fortaleza, 28 de novembro de 2008
Protocolo COMEPE nº 92/ 07
Pesquisador responsável: Vanara Florêncio Passos
Deptº./Serviço: Departamento de odontologia/ UFC
Título do Projeto: "Avaliação da abrasão do esmalte por dentifrícios através de um estudo in situ"

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 a alteração do projeto supracitado na reunião do dia 27 de novembro de 2008.

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

Atenciosamente,

Mirian Parente Monteiro

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