ANA CAROLINA MAGALHÃES

INFLUÊNCIA DE DIFERENTES ÍONS SOBRE A EROSÃO DENTÁRIA

Tese apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus Araçatuba, para obtenção do título de Doutor em Odontopediatria.

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DEDICATÓRIA

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“...É melhor tentar e falhar que preocupar-se e ver a vida passar; É melhor tentar, ainda em vão, que não fazer nada até o final. Eu prefiro na chuva caminhar, que em dias tristes em casa me esconder. Prefiro ser feliz, embora louco, que em conformidade viver...”

Martin Luther King

RESUMO GERAL

Magalhães AC. Influência de diferentes íons sobre a erosão dentária**.** [Tese] Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2008.

Esta tese teve como objetivo mostrar a ação de diferentes íons sobre a erosão dentária. O 1º Capítulo abordou o efeito da suplementação de um refrigerante (Sprite Light®), pela adição de cálcio 1 mM, flúor 0,047 mM, ferro 1 mM e fósforo 1 mM associados ou não (9 grupos, n=10 blocos/grupo), na redução da erosão do esmalte dentário bovino *in vitro*. A ciclagem de pH foi realizada por 24 h. Os blocos foram imersos no refrigerante puro ou modificado, seis vezes ao dia (1 min), sendo que nos intervalos (59 min) e nas 18 h restantes foram mantidos em saliva artificial. A alteração do esmalte foi avaliada por perfilometria e microdureza superficial. Somente os grupos como Ca e Ca+F foram efetivos em reduzir o desgaste do esmalte. O 2º Capítulo relatou o efeito da aplicação tópica de uma solução de tetrafluoreto de titânio (TiF_4 4%) sobre a erosão do esmalte humano *in situ*. Para tal, 10 voluntários utilizaram aparelho palatino com 2 blocos de esmalte divididos em 2 fileiras correspondentes à: erosão sem aplicação prévia de TiF_4 (ERO+ no-F); e erosão com aplicação prévia de TiF_4 (ERO+F). No 1º dia da fase *in situ*, os voluntários utilizaram o aparelho para permitir a formação da película adquirida. No 2º dia, aplicou-se a solução em uma fileira (F) durante 1 min enquanto na outra nada foi realizado (controle). Na seqüência, o aparelho foi recolocado na boca dos voluntários. Do 3º ao 7º dia, o desafio erosivo foi realizado por meio da imersão do aparelho em refrigerante tipo cola por 5 min, 4x/dia. A alteração do esmalte foi avaliada por microdureza superficial, perfilometria e microscópio eletrônico de varredura com espectroscopia por energia dispersiva (MEV-EED). A aplicação da solução de TiF_4 4% potencializou a perda de esmalte pela erosão, sendo possível detectar titânio e observar microtrincas em parte das superfícies tratadas. Os 3º e 4º Capítulos testaram o efeito de um verniz experimental de TiF_4 a 4% sobre a cárie e erosão dentária *in vitro*, respectivamente. Blocos de esmalte bovino foram divididos em 4 grupos de acordo com o tipo de verniz (Duraphat®, Duofluorid®, TiF_4 e placebo). No Capítulo 3, blocos hígidos e com lesão de cárie artificial foram submetidos à ciclagem de pH de acordo com Vieira et al. [2005]. No Capítulo 4, os

blocos foram submetidos a 6 ciclagens por dia (10 min Coca-cola® e 50 min saliva artificial), durante 4 dias. O verniz de TiF_4 foi efetivo para a cárie dentária (microdureza superficial e longitudinal), mas não para a erosão dentária (microdureza superficial e perfilometria). Por isso, no 5º Capítulo foi reduzido o tempo de desafio erosivo, assim como foram utilizados uma “boca de artificial” e um novo perfilômetro. Neste caso, foi possível observar a efetividade do verniz experimental sobre a erosão dentária.

Palavras chaves: cálcio; desmineralização; erosão de dente; esmalte dentário; flúor; íons; titânio.

GENERAL ABSTRACT

Magalhães AC. Influence of different ions on dental erosion. [Tese] Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2008.

The objective of this thesis was to show the effect of different ions on dental erosion. In the 1st Chapter, the effect of a modified soft drink (Sprite Light®) with 1 mM calcium, 0.047 mM fluoride, 1 mM iron and 1 mM phosphorus alone or together (9 groups, n= 10 samples/group) on the inhibition of dental bovine enamel erosion was evaluated *in vitro*. During 24 h, the samples were subjected to 6 pH-cycles. In each cycle, the samples were immersed in modified or pure drink (1min) and in artificial saliva (59 min). In the remaining 18 h, the samples were stored in artificial saliva. The enamel alteration was evaluated by profilometry and surface microhardness. Only groups with Ca and Ca+F were effective on enamel wear reduction. The 2nd Chapter showed the effect of a titanium tetrafluoride solution (4% TiF_4) on human enamel erosion *in situ*. For this, 10 volunteers wore palatal appliances with 2 enamel samples divided into 2 rows: Erosion without previous TiF_4 application (ERO+ no-F) and erosion with previous TiF_4 application (ERO+F). During the 1st day, the formation of a salivary pellicle was allowed. In the 2nd day, the TiF_4 solution was applied on one row (ERO+F), whereas on the other row no treatment was performed (ERO+ no-F). From the 3rd until 7th day, the samples were subjected to erosion by immersion of the appliance in cola drink (5 min), 4 times a day. The enamel alterations were evaluated by surface microhardness, profilometry, scanning electron microscopy and x-ray energy dispersive spectroscopy (SEM-EDS). The application of 4% TiF_4 solution increased the enamel loss by erosion. It was possible to detect titanium and to see a surface coating with micro cracks on the part of treated enamel. The 3th and 4th Chapters showed the effect of an experimental 4% TiF_4 varnish on dental caries and erosion *in vitro*, respectively. Bovine enamel samples were divided into 4 groups of different varnishes (Duraphat®, Duofluorid®, TiF_4 and placebo). In the 3th Chapter, sound and artificial carious enamel were subjected to pH cycles according to Vieira et al. [2005]. In the 4th Chapter, the samples were subjected to 6 pH-cycles per day (10 min Coca-cola® and 50 min artificial saliva), for 4 days. The TiF_4 varnish was effective in reducing dental caries (as shown by surface and cross-sectional

microhardness), but not for inhibition of dental erosion (surface microhardness and profilometry). Due to these results, in the study reported in the 5th Chapter the erosive challenges were performed with a reduction in acid exposure time, using an “artificial mouth” and a new profilometry device. In this case, it was possible to observe a beneficial effect of the experimental varnish on dental erosion.

Keywords: calcium; demineralization; dental enamel; dental erosion; fluoride; ions; titanium.

SUMÁRIO

1 Introdução Geral

Nas últimas décadas, foi constatada uma redução na incidência de cárie dentária em alguns países, incluindo o Brasil [Petterson e Brathall, 1996; Narvai et al., 2006]. Dessa forma, os dentes permanecem por mais tempo na boca, o que os torna susceptíveis a outros tipos de lesões, sendo uma delas a erosão dentária [Lussi, 2006].

A erosão dentária é definida como uma perda irreversível de estrutura dentária, devido a um processo químico, sem envolvimento de microorganismos [Lussi, 2006], desencadeado por ácidos de origem intrínseca ou extrínseca. Os ácidos intrínsecos são oriundos do estômago, em pacientes que apresentam anorexia, bulimia nervosa e problemas gastroesofágicos [Bartlett, 2006]. Os ácidos de origem extrínseca incluem alimentos, bebidas, medicamentos e produtos ácidos advindos do ambiente de trabalho [Lussi e Jaeggi, 2006].

Atualmente, os ácidos extrínsecos têm sido considerados os principais fatores relacionados com a ocorrência de erosão dentária em função de uma mudança dos hábitos dietéticos da população, que tem consumido com maior freqüência alimentos ácidos, tais como sucos de frutas e refrigerantes [Lussi et al., 2004b; Lussi, 2006]. De acordo com dados do IBGE [2004], o consumo de refrigerantes aumentou em 490% no Brasil de 1995 a 2003.

Para minimizar a ocorrência da erosão, recomenda-se a diminuição na freqüência de consumo de alimentos ácidos [Lussi, 2006; Lussi e Jaeggi, 2006]; a ingestão de bebidas ácidas em canudos [Lussi, 2006; Lussi e Jaeggi, 2006]; o aumento do fluxo salivar pela mastigação de chicletes [Rios et al., 2006] e a ingestão de alimentos alcalinos e ricos em cálcio, como o leite e o queijo, logo após o consumo de alimentos ácidos [Gedália et al., 1991; Lewinstein et al., 1993].

No entanto, estas estratégias preventivas dependem da colaboração do paciente que, em muitas ocasiões, é de difícil obtenção, principalmente tratando-se de crianças e adolescentes. O interessante seria conseguir minimizar a erosão pela modificação dos produtos. Entretanto, esta alternativa é pouco explorada pela possibilidade de modificação do sabor e pH dos alimentos.

O efeito da adição de flúor em bebidas ácidas tem sido analisado a partir de vários estudos *in vitro* e em animais [Wiegand e Attin, 2003]. No entanto, o flúor nas bebidas, exceto em concentrações tóxicas, não é capaz de impedir a erosão dentária [Larsen e Nyvad, 1999; Larsen e Richards, 2002]. Já em relação à adição de cálcio, outros estudos *in vitro* e *in situ* têm mostrado bons resultados na diminuição da perda de minerais [West et al., 1999; Hughes et al., 1999a; Hughes et al., 1999b; Hughes et al., 2002; Hunter et al., 2003].

Trabalhos mais recentes têm sugerido que íons metálicos, especialmente o ferro, possuem propriedades anticariogênicas, através da interferência no processo de desmineralização e remineralização do esmalte [Martinhon et al., 2006]. De acordo com Brookes et al. [2004], a adição de $FeSO_4$ a 10 mmol/L e 1,25 mmol/L à solução ácida inibiu em 51% e 10%, respectivamente, a dissolução da hidroxiapatita sintética. Efeito similar foi encontrado para o pó de esmalte bovino, com uma inibição da desmineralização de 40 e 13% para as concentrações de $FeSO_4$ de 10 mmol/L e 1,25 mmol/L, respectivamente, utilizando um modelo abiótico com ácido acético. A inibição máxima da dissolução, ao redor de 50% de diminuição na perda de fósforo, foi encontrada para a dose de 15 mmol/L, não havendo uma inibição adicional pelo incremento da dose até 120 mmol/L [Buzalaf et al., 2006].

Portanto, parece possível que a modificação na formulação de bebidas ácidas com ferro possa minimizar a erosão dentária e ainda colaborar para o controle de anemia [Lynch, 2005]. Kato et al. [2007b] mostraram que a adição de $FeSO_4$ a 10 mM a um refrigerante à base de cola reduziu a dissolução do pó de esmalte bovino, mas não reduziu quando adicionado a um refrigerante à base de limão. Isto mostra que os sais adicionados podem ter interação com o tipo de ácido presente no refrigerante. A inibição da erosão também foi obtida com a adição de Fe a um refrigerante à base de cola, usando blocos de esmalte bovino submetidos à ciclagem de pH [Kato et al., 2007a].

Na tentativa de potencializar este efeito do ferro, seguindo os trabalhos de Attin et al. [2003] e Attin et al. [2005] os quais mostraram que a adição de diversos íons (cálcio, flúor e fosfato) minimizou a erosão produzida pelo ácido cítrico e refrigerante à base de limão, respectivamente, o **1º Capítulo** deste trabalho abordará o efeito da adição de cálcio, flúor, fosfato e ferro, em concentrações consideradas não tóxicas, isoladamente ou combinados a um refrigerante à base de limão sobre a erosão do esmalte dentário bovino *in vitro* (Este artigo será submetido

ao *Archives of Oral Biology* com o título *"Impact of mineral supplements to an erosive soft drink on inhibition of enamel erosion in vitro".* Normas do periódico em ANEXO A).

A erosão dentária não causa apenas uma perda irreversível de estrutura, mas também uma desmineralização do tecido dentário que fica mais susceptível à ação de distúrbios mecânicos, como a atrição e a abrasão, que podem potencializar o dano ao tecido dentário [Attin et al., 2001 a,b; Attin et al, 2004; Wiegand et al., 2007]. De acordo com trabalhos *in situ*, há um aumento na perda de estrutura dentária quando o esmalte erodido é imediatamente escovado [Rios et al., 2006; Magalhães et al., 2007]. Para minimizar a abrasão do tecido dentário é recomendado esperar em torno de 1 h, após o consumo de bebidas ácidas, para a realização da escovação [Jaeggi e Lussi, 1999; Attin et al, 2001 a,b; Rios et al., 2006]. Este tipo de recomendação é difícil de ser seguida na vida cotidiana, já que geralmente se recomenda a escovação imediata para a prevenção da cárie dentária e doença periodontal.

Por outro lado, esta condição faz com que o esmalte também esteja mais susceptível à ação de meios remineralizadores, como a saliva e flúor [Rios et al., 2006; Wiegand e Attin, 2003]. Na tentativa de minimizar esta perda, o flúor tem sido utilizado em suas diferentes formas de aplicação (gel, dentifrício e solução) [Lussi et al., 2004a; Ganss et al., 2004; Lagerweij et al., 2006]. No entanto, tem sido relatado que o flúor do dentifrício pode minimizar a abrasão do esmalte erodido, mas não consegue prevení-la completamente [Magalhães et al., 2007].

Em contrapartida, vários estudos têm mostrado que agentes com alta concentração de flúor são mais efetivos na prevenção da erosão [Lussi et al., 2004a; Ganss et al., 2004; Lagerweij et al., 2006]. Dentre os sais de fluoreto, a solução de tetrafluoreto de titânio (TiF_4) tem mostrado ser mais eficaz que outros sais fluoretados na prevenção da erosão dentária. A ação preventiva do TiF_4 não se deve somente ao flúor, pela formação da barreira de CaF_2, mas também devido a uma possível ação adicional do titânio, pela formação de um glaze de dióxido de titânio sobre a superfície do esmalte [Buyukyilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005; Hove et al., 2006; Schlueter et al., 2007]. No entanto, não existem trabalhos testando o uso da solução de TiF_4 em protocolos *in situ*, que simulam de forma mais adequada as condições *in vivo*. Portanto, o **2º Capítulo** abordará um trabalho *in situ*, previamente aprovado pelo Comitê de Ética da FOB-USP (ANEXOS

B e C), o qual testou o efeito da solução de TiF_4 a 4% sobre a erosão do esmalte humano, pela perda de dureza e desgaste do esmalte. Além disso, foram realizadas avaliações da superfície do esmalte, para o entendimento mais detalhado da interação deste produto com o substrato dentário, através da microscopia eletrônica de varredura - MEV e espectroscopia por energia dispersiva (Este artigo será submetido ao *Archives of Oral Biology* com o título "*Effect of 4% titanium tetrafluoride solution on dental erosion by a soft drink: an in situ/ ex vivo study*". Normas do periódico em ANEXO A).

O resultado do uso da solução de TiF_4 a 4 % *in situ* não foi satisfatório. Na tentativa de melhorar o resultado do uso deste produto, o sal de TiF_4 foi incorporado a um verniz com as mesma formulações do Duofluorid (FGM), exceto pelo sal de flúor. O verniz tem sido considerado um excelente veículo para aplicação de flúor, uma vez que se adere ao esmalte dentário, permitindo que o sal de fluoreto (no caso TiF_4) possa agir por mais tempo, formando mais produtos de reação sobre a superfície dentária [Vieira et al., 2005]. Com a possibilidade de gerar patente, foram realizados dois estudos com o verniz de TiF_4 a 4%, abordando o seu efeito sobre a prevenção e tratamento da cárie dentária (**3º Capítulo**) e sobre a prevenção da erosão dentária (**4º Capítulo**) *in vitro*. O **3º Capítulo** será submetido ao *Journal of Dentistry* com o título "*Effect of 4% titanium tetrafluoride (TiF_4) varnish on demineralization and remineralization of bovine enamel in vitro*" (Normas do periódico em ANEXO D). O **4º Capítulo** também será submetido para publicação no *Journal of Dentistry* com o seguinte título "*Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink"* (Normas do periódico em ANEXO D).

O verniz de TiF_4 foi eficaz na prevenção e tratamento da cárie dentária *in vitro*, através da avaliação da microdureza superficial e longitudinal. Entretanto, este veículo não apresentou a mesma efetividade para a erosão dentária *in vitro*, através da avaliação de microdureza superficial e perfilometria, devido aos altos desafios erosivos aplicados. Na tentativa de simular melhor as condições *in vivo*, o verniz de TiF_4 foi novamente testado para a prevenção da erosão dentária, utilizando um equipamento chamado "boca artificial", que permite intercalar a solução de desmineralização e remineralização com o controle da velocidade, volume e temperatura. Além disso, as alterações da superfície das amostras foram analisadas com um novo perfilômetro, equipamento que mede o perfil da superfície e calcula o

desgaste e por MEV. Neste caso, foi possível observar a efetividade do verniz experimental sobre a erosão dentária. Este trabalho será apresentado no **5º Capítulo**. O artigo foi intitulado "*The effect of an experimental 4% TiF_4 varnish compared to NaF varnishes and 4% TiF_4 solution on dental erosion in vitro*" e será submetido ao periódico *Caries Research* (Normas do periódico em ANEXO E).

Referências Bibliográficas*

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 2001a;46**:**871-874.

Attin T, Knofel S, Buchalla W, Tütüncü R: In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res 2001b;35:216-222.

Attin T, Meyer K, Hellwig E, Buchalla W, Lennon AM: Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol 2003;48:753-759.

Attin T, Siegel S, Buchalla W, Lennon AM, Hannig C, Becker K: Brushing abrasion of softened and remineralised dentin: an in situ study. Caries Res 2004;38:62-66.

Attin T, Weiss K, Becker K, Buchalla W, Wiegand A: Impact of modified acidic soft drinks on enamel erosion. Oral Dis 2005;11:7-12

Bartlett D: Intrinsic causes of erosion. Monogr Oral Sci 2006;20:119-139.

Brookes SJ, Shore RC, Robinson C, Wood SR, Kirkham J: Inhibitory effect of metal ions on acid demineralisation. Caries Res 2004;38:401.

Buyukyilmaz T, Øgaard B, Rolla G: The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci 1997;105:473-477.

Buzalaf MA, de Moraes Italiani F, Kato MT, Martinhon CC, Magalhães AC: Effect of iron on inhibition of acid demineralisation of bovine dental enamel in vitro. Arch Oral Biol 2006;51:844-848.

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

Gedalia I, Dakuar A, Shapira L, Lewinstein I, Goultschin J, Rahamim E: Enamel softening with coca-cola and rehardening with milk or saliva. Am J Dent 1991;4:120-122.

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.

Hughes JA, Jandt KD, Baker N, Parker D, Newcombe RG, Eisenburger M et al: Further modification to soft drinks to minimise erosion. Caries Res 2002;36:70-74.

Hughes JA, West NX, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink in vitro and in situ 1: comparison with orange juice. J Dent 1999a;27:285-290.

Hughes JA, West NX, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink. 3. Final drink and concentrate, formulae comparisons in situ and overview of the concept. J Dent 1999b;27:345-350.

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

Instituto Brasileiro de Geografia e Estatística [IBGE]. Ministério do planejamento, orçamento e gestão. Pesquisa de orçamentos familiares 2003-2003: Brasil e grandes regiões. Rio de Janeiro 2004.

Jaeggi T, Lussi A: Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. Caries Res 1999;33:455-461.

Kato MT, de Carvalho Sales-Peres SH, Buzalaf MA: Effect of iron on acid demineralisation of bovine enamel blocks by a soft drink. Arch Oral Biol 2007a;52:1109-1111.

Kato MT, Maria AG, Sales-Peres SH, Buzalaf MA: Effect of iron on the dissolution of bovine enamel powder in vitro by carbonated beverages. Arch Oral Biol 2007b;52:614-617.

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 Res 2006;40:148-153.

Larsen MJ, Nyvad B: Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Res 1999;33:81-87.

Larsen MJ, Richards A: Fluoride is unable to reduce dental erosion from soft drinks. Caries Res 2002;36:75-80.

Lewinstein I, Ofek L, Gedalia I: Enamel rehardening by soft cheeses. Am J Dent 1993;6:46-48.

Lussi A, Jaeggi T, Megert B: Effect of various fluoride regimes on toothbrush abrasion of softened enamel in situ. Caries Res 2004a;38:393.

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

Lussi A, Jaeggi T: Chemical factors. Monogr Oral Sci 2006;20:77-87.

Lussi A: Erosive tooth wear - a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006;20:1-8.

Lynch SR: The impact of iron fortification on nutritional anaemia. Best Pract Res Clin Haematol 2005;18:336-346.

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

Martinhon CC, Italiani FM, Padilha PM, Bijella MF, Delbem AC, Buzalaf MA: Effect of iron on bovine enamel and on the composition of the dental biofilm formed "in situ". Arch Oral Biol 2006;51:471-475.

Narvai PC, Frazao P, Roncalli AG, Antunes JL: Dental caries in Brazil: decline, polarization, inequality and social exclusion. Rev Panam Salud Publ 2006;19:385-393.

Peterson G, Bratthall D: The caries decline: a review of reviews. Eur J Oral Sci 1996;104:436-443.

Rios D, Honório HM, Magalhães AC, Delbem ACB, Machado MAAM, Silva SMB et al: 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 2006;40:218-223.

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.

van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J: Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment *in vitro*. Eur J Oral Sci 2003;11:253-257.

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

West NX, Hughes JA, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink. 2. Comparison with a conventional blackcurrant juice drink and orange juice. J Dent 1999;27:341-344.

Wiegand A, Attin T: Influence of fluoride on the prevention of erosive lesions – a review: Oral Health Prev Dent 2003;1:245-253.

Wiegand A, Kowing L, Attin T: Impact of brushing force on abrasion of acid-softened and sound enamel. Arch Oral Biol 2007;52:1043-1047.

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2 Capítulo 1

Impact of mineral supplements to an erosive soft drink on inhibition of enamel erosion in vitro

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Running title: Effect of minerals on dental erosion

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2.1 Abstract

(1)Objective: The objective of the present in vitro study was to evaluate the effect of low levels of calcium, fluoride, iron and phosphate supplemented alone or in combination to Sprite light® on dental erosion. *(2)Design:* Ninety enamel samples (4X4X3 mm) were randomly allocated to nine groups (n=10): G1 – pure Sprite light® (control); G2 – with 1 mM Ca; G3 – with 0.047 mM F; G4 – with 1 mM Fe; G5 – with 1 mM P; G6 - with 1 mM Ca and 0.047 mM F; G7 – with 1 mM Ca and 1 mM P; G8 – with 1 mM Fe and 0.047 mM F; and G9 – with 1 mM Ca, 1 mM P, 0.047 mM F and 1.0 mM Fe. During 24h, the samples were subjected to 6 pH-cycles. In each cycle, the samples were immersed in pure or modified Sprite light® (1 min) and in artificial saliva (59 min). During the remaining time (18 h), the samples were maintained in artificial saliva. Enamel alterations were assessed by profilometry (µm) and microhardness (%SMHC) tests. Data were tested using ANOVA and Tukey's tests ($p<0.05$). *(3)Results:* The highest enamel losses were observed in the control group (G1) and in the groups containing Fe (G4 and G8). The groups containing Ca (G2 and G6) showed significantly less wear compared to the control. With regard to enamel softening, all groups revealed similar values of %SMHC, except for G5 (P). *(4)Conclusion:* The modification of an erosive soft drink with low concentrations of Ca with or without F may exert a protective effect against enamel erosion.

Keywords: calcium; dental erosion; enamel; fluoride; iron; phosphate

2.2 Introduction

Dental erosion is the loss of tooth substance by chemical processes not involving bacteria[1,2]. Although a multitude of factors seem to be involved in this process, the most important factors are dietary acids[3,4] and intrinsic acids from the stomach[5,6]. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor for the development of erosive wear[2,3].

As it is difficult to control possible etiological factors, such as the intake of acidic beverages or special drinking habits[7], many strategies have been developed for the prevention of dental erosion. Studies have shown that the consumption of cheese or milk after an erosive challenge[8,9], the application of fluoride gels and varnishes[10-12] and salivary stimulation by chewing sugar-free gums[13] improve the remineralisation of erosive lesions. However, in the daily life situation, these strategies may be of limited impact as they are highly dependent on the cooperation of the patient. Thus, it seems to be of great interest to develop preventive strategies, which are less dependent on the patient's behavior.

Taking into account these considerations, one preventive strategy might be the reduction of the erosive potential of acidic beverages by mineral supplementation. The addition of calcium, fluoride and phosphate has been shown to reduce the erosive potential of pure acids and acidic drinks[14-21]. Moreover, it has also been shown that the supplementation with iron seems to decrease the erosive potential of acidic solutions[22,23]. It is important to highlight that in the studies involving iron supplementation of soft drinks or acid solutions, high concentrations of iron were used[22,23], which might exhibit toxic effects[24].

Due to the possibility of a synergistic effect among different ions it could be speculated that it might be possible to increase their benefic effects with much lower doses by using the adequate combination of these ions. Therefore, the aim of the present in vitro study was to evaluate the effect of low and no toxic levels of calcium, fluoride, iron and phosphate supplemented alone or in combination to Sprite light® on dental erosion.

2.3 Material and Methods

2.3.1 Enamel samples preparation

Ninety enamel samples (4X4X3 mm) were prepared from extracted bovine incisors, which were previously stored in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. One sample was cut from each crown using ISOMET Low Speed Saw cutting machine (Buehler Ltda., Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer. The enamel surface was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet by diamond spray (1 µm; Buehler). This procedure resulted in a removal of about 100 µm depth of enamel. The surface microhardness was determined by performing five indentations (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan) for selection and analysis purposes. Enamel samples with a microhardness ranging from 307 to 374 KHN (337±17) were randomly distributed into 9 groups (n=10): G1 – pure Sprite Light® (control); G2 – with 1 mM Ca; G3 – with 0.047 mM F; G4 – with 1 mM Fe; G5 – with 1 mM P; G6 - with 1 mM Ca and 0.047 mM F; G7 – with 1 mM Ca and 1 mM P; G8 – with 1 mM Fe and 0.047 mM F; and G9 – with 1 mM Ca, 1 mM P, 0.047 mM F and 1 mM Fe. Two layers of nail varnish were applied on half of the surface of the enamel in order to maintain reference surfaces for lesion depth determination.

2.3.2 Preparation of beverages and erosive pH-cycling

Prior to the experiment, the basic concentration of the different ions in pure Sprite light® was measured using an ion-selective electrode (0.4 mg F/L), atomic absorption spectrometry (9.414 mg Ca/L and 0.132 mg Fe/L) and a colorimetric method (1.68 mg P/L). Considering the initial concentrations of the different ions, the respective ions were added until the final concentrations were obtained. In group 1, the pure Sprite light® remained unchanged and served as control. In groups 2 to 5, calcium (1 mM) was added as Ca lactate (Sigma-Aldrich, St Louis, USA), fluoride (0.047 mM) as NaF (Merck, Rio de Janeiro, Brazil), iron (1 mM) as $FeSO_4$ (Synth, Diadema, Brazil) and phosphate (1 mM) as $NaH_2PO_4.2H_2O$ (Merck, Darmstadt, Germany), respectively. In groups 6-9, the ions were added in combination to the beverage in the same concentrations as described above.

During 24 h, the samples were subjected to 6 pH cycles. In each cycle, the samples were immersed in pure or modified Sprite light® drink (citric acid, Coca-Cola Company, Spal, Porto Real, RJ, Brazil) for 1 min (30 mL per block). The Sprite light® (pH 2.87) presented a buffering capacity of 0.375±0.01, which is equivalent to 0.375 mL of 0.2 M NaOH/ 3mL beverage to increase in one pH unit[25].

Between the erosive challenges, the samples were immersed in artificial saliva [1.5 mM $Ca(NO_3)_2.4H_2O$, 0.9 mM $NaH_2PO_4.2\ H_2O$, 150 mM KCl, 0.1 M Tris buffer, 0.03 ppm F, pH 7.0][26] for 59 min (30 mL per block) at room temperature. During the remaining time (18 h), the samples were maintained in artificial saliva.

2.3.3 Microhardness and wear assessments

Initially, enamel surface microhardness was measured as described above. Five indentations with a distance of 100 µm from each other were performed on enamel and the mean surface microhardness (SMH) per sample was calculated. After pH cycling, the nail varnish over the surfaces was carefully cleaned with acetone-soaked cotton wool[27] and the final microhardness test (SMH1) was performed as describe above. The percentage of surface microhardness change (%SMHC) was calculated as follows: $100(SMH_1 - SMH)/SMH$[13,28]. The %SMHC values of each group were averaged. The enamel wear was determined in relation to the reference surface by profilometry (Hommel Tester T1000, VS, Schwenningen, Germany)[13,28]. Five readings were performed on each specimen by scanning from the reference to the exposed surface. The wear values of each group were averaged.

2.3.4 Statistical analysis

The software GraphPad Instat for Windows (San Diego, CA, USA) was used. The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Since the assumptions were satisfied, ANOVA and Tukey's tests were carried out for statistical comparisons and the significance limit was set at 5%.

2.4 Results

Table 1 shows the mean %SMHC (±SD) and the mean wear (µm ± SD). Significantly higher enamel losses (0.76-0.97 µm) were recorded for groups G8 (Fe

and F), G4 (Fe), G1 (control) and G7 (Ca and P) when compared to all other groups. These did not differ significantly from each other. Mean enamel losses in groups G3 (F), G5 (P) and G9 (all ions) amounted to 0.5 to 0.67 µm, but these groups were not significantly different from the control (G1). Only groups 2 (Ca) and 6 (Ca and F) revealed significantly less enamel wear (±0.34 µm) compared to the control ($p<0.05$). With respect to %SMHC (softening), only group 5 (P) showed a significantly higher softening when compared to the control, while the remaining groups presented a similar softening, which was not different from the control group ($p>0.05$).

2.5 Discussion

The present study was conducted in order to minimize the erosivity of a commercial soft drink by admixture of low levels of calcium (1 mM), phosphate (1 mM), fluoride (0.047 mM) and/or iron (1 mM). Sprite light is a common soft drink, which contains citric acid and was shown to exhibit severe erosive potential[25]. In contrast to the study by Attin et al.[14], which evaluated the effect of different mineral ions supplemented to pure acid, the present study modified a commercial soft drink. This procedure more closely resembles the clinical situation, not only for the addition of the ions directly to the soft drink, but also for the use of these ions in non-toxicological concentrations[14,24].

During the pH cycling the samples were briefly eroded (60 s) to simulate the sipping or drinking of an erosive beverage. The saliva used allows for rehardening of the samples between the erosive challenges[8]. In this study, both the enamel softening (%SMHC) and the enamel wear were used as response variables. The %SMHC reflects the enamel softening of the outermost enamel layer, but it is not able to reveal the cumulative effect of erosive challenges. Although the addition of P leads to significantly higher softening values, the %SMHC was very high in all the groups, indicating that this method is not able to reveal important differences among the treatments. In contrast, the profilometry technique is able to measure the complete dental loss induced by pH-cycles, thus reflecting the cumulative effect of the erosive challenges.

The supplementation with Ca (G2 and G6) resulted in significantly less enamel loss compared to the other groups. On the other hand, the single admixture of F and P did not significantly reduce the enamel loss. This finding is in accordance to Attin et

al.[14], who showed that Ca reduced the enamel loss significantly more than F and P, when these ions were mixed to citric acid. Moreover, our results confirm the findings of the studies by Larsen and Nyvad[29] and Larsen and Richards[30], which showed that fluoride admixtures in a concentration excluding toxicological side effects seem unable to reduce erosive lesions. The supplementation of low levels of these minerals was not effective in decreasing the erosive potential of solutions with a pH below 4.0. Also, the addition of Ca and P together did not prevent dental erosion. One hypothesis might be that these ions reacted with each other within the beverage, thus reducing the amount of "free" ions to react with apatite. Interestingly, in the present study, the combination of all ions also did not reduce the enamel loss. This finding is in disagreement to Attin et al.[15], who showed that the combination of low levels of Ca (0.5 mM), P (0.5 mM) and F (0.037 mM) is able to reduce enamel loss. However, these authors[15] did not test the effect of iron. It might be speculated that, in the present study, the salts also reacted with each other within the beverage and that the presence of iron may has increased this effect, since the groups with Fe (G4 and G8) presented higher enamel loss compared to control (n.s.).

Previous studies showed that iron participates in the remineralisation of human enamel and in the nucleation of apatite, in the substitution of calcium in apatite and in inhibition of demineralization[31-33]. In addition, rinsing with an iron solution after an erosive attack followed or not by an abrasive episode, significantly reduced the dentin wear in situ[34]. However, the effect of iron added in combination with other ions on inhibition of dental erosion was not investigated previously. The present study showed that 1 mM Fe alone or in combination with other ions was not able to minimize enamel loss.

Buzalaf et al.[22] investigated the protective effect of crescent concentrations (0-120 mmol/L) of iron on dissolution of enamel by acetic acid and showed that the 15 mM Fe was able to reduce the enamel dissolution. Kato et al.[23] showed that iron (10 mM Fe) can interfere with the dissolution of dental enamel powder in the presence of acidic beverages. This effect seems to be modulated by the type of acid. Interestingly, these authors[23] found that higher concentrations of Fe were effective to inhibit dental erosion by a cola drink (Coke, phosphoric acid), but not by a drink containing citric acid (Sprite). The opposite was showed by Attin et al.[15], who revealed that the mixture of Ca alone or in combination with P and F was effective to reduce the dental loss by Sprite, but not by Coke. The results of the study by Kato et

al.[23] and the present study suggest that some chemical reactions may occur between the Fe salt and the acid present in the beverage (Sprite drink).

However, the supplementation of iron may lead to a metallic taste of the soft drink. The admixture of 1 mM Fe or 1 mM Ca lactate leads to a distinct change of the soft drinks taste, as tested prior to the experimental phase (data not shown). Moreover, Fe might affect the tooth colour and the taste of other foods[24]. It is important to consider that the concentration of Fe used in this study is below the toxic threshold[24], but did not have a beneficial effect on dental erosion. In contrast, the supplementation of 1 mM Ca was able to reduce the erosivity of the soft drink. Attin et al.[15] showed that the modification of Sprite Light® with a low concentration of Ca (1 mM) or the combination of Ca (0.5 mM), P (0.5 mM) and F (0.037 mM) may exert a significant preventive effect on erosion, without changing the taste of the soft drink. However, in the above-mentioned study[15], Ca was applied as $CaCl_2$-dihydrated, while in the present study Ca was mixed in the form of Ca-lactate. This salt was used in the present study, because the pilot study showed a better effect of Ca-lactate added into beverage than $CaCl_2$-dihydrated on enamel erosion in vitro (data not shown). Further studies should analyse whether the taste of Ca-lactate can be reduced.

In conclusion, the modification of an erosive soft drink with low concentrations of Ca or Ca and F may exert a protective effect on enamel erosion, but was not able to prevent it completely.

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2.7 References

1. Imfeld T. Dental erosion. Definition, classification and links. *Eur J Oral Sci* 1996; **104**: 151-155.
2. Lussi A. Erosive tooth wear - a multifactorial condition of growing concern and increasing knowledge. *Monogr Oral Sci* 2006; **20**: 1-8.
3. Lussi A, Jaeggi T, Zero D. The role of diet in the etiology of dental erosion. *Caries Res* 2004; **38**: 34-44.

4. Lussi A, Jaeggi T. Chemical factors. *Monogr Oral Sci* 2006; **20**: 77-87.
5. Bartlett D. Intrinsic causes of erosion. *Monogr Oral Sci* 2006; **20**: 119-139.
6. Scheutzel P. Etiology of dental erosion-intrinsic factors. *Eur J Oral Sci* 1996; **104**: 178-190.
7. Johansson AK, Lingström P, Imfeld T, Birkhed D. Influence of drinking method on tooth-surface pH in relation to dental erosion. *Eur J Oral Sci* 2004; **112**: 484-489.
8. Gedalia I, Dakuar A, Shapira L, Lewinstein I, Goultschin J, Rahamim E. Enamel softening with coca-cola and rehardening with milk or saliva. *Am J Dent* 1991; **4**: 120-122.
9. Lewinstein I, Ofek L, Gedalia I. Enamel rehardening by soft cheeses. *Am J Dent* 1993;**6**: 46-48.
10. Ganss C, Klimek J, Brune V, Schumann A. Effects of two fluoridation measures in erosion progression on enamel and dentine in situ. *Caries Res* 2004; **38**: 561-566.
11. 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 Res* 2006; **40**: 148-153.
12. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. *Caries Res* 2005; **39**: 371-379.
13. Rios D, Honório HM, Magalhães AC, Delbem ACB, Machado MAAM, Silva SMBS et al. 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* 2006; **40**: 218-223.
14. Attin T, Meyer K, Hellwig E, Buchalla W, Lennon AM. Effect of mineral supplements to citric acid on enamel erosion. *Arch Oral Biol* 2003; **48**: 753-759.
15. Attin T, Weiss K, Becker K, Buchalla W, Wiegand A. Impact of modified acidic soft drinks on enamel erosion. *Oral Dis* 2005; **11**: 7-12
16. Hughes JA, Jandt KD, Baker N, Parker D, Newcombe RG, Eisenburger M et al. Further modification to soft drinks to minimise erosion. *Caries Res* 2002; **36**: 70-74.

17. Hughes JA, West NX, Addy M. The protective effect of fluoride treatments against enamel erosion in vitro. *J Oral Rehabil* 2004; **31**: 357-363.

18. Hughes JA, West NX, Parker DM, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant juice drink in vitro and in situ 1: comparison with orange juice. *J Dent* 1999; **27**: 285-290.

19. Hughes JA, West NX, Parker DM, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant juice drink. 3. Final drink and concentrate, formulae comparisons in situ and overview of the concept. *J Dent* 1999; **27**: 345-350.

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

21. West NX, Hughes JA, Parker DM, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant juice drink. 2. Comparison with a conventional blackcurrant juice drink and orange juice. *J Dent* 1999; **27**: 341-344.

22. Buzalaf MA, de Moraes Italiani F, Kato MT, Martinhon CC, Magalhães AC. Effect of iron on inhibition of acid demineralisation of bovine dental enamel in vitro. *Arch Oral Biol* 2006; **51**: 844-848.

23. Kato MT, Maria AG, Sales-Peres SH, Buzalaf MA. Effect of iron on the dissolution of bovine enamel powder in vitro by carbonated beverages. *Arch Oral Biol* 2007; **52**: 614-617.

24. Goldhaber SB. Trace element risk assessment: essentiality vs. toxicity. *Regul Toxic Pharma* 2003; **38**: 232-242.

25. Sales-Peres SHC, Magalhães AC, Machado MAAM, Buzalaf MAR. In vitro evaluation of variables related to the erosive potential of five soft drinks. *Eur J Dent* 2007; **1**: 10-13.

26. Vieira AEM, Delbem ACB, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. *Caries Res* 2005; **39**: 514-520.

27. Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. *Caries Res* 2000; **34**: 48-52.

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

29. Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. *Caries Res* 1999; **33**: 81-87.

30. Larsen MJ, Richards A. Fluoride is unable to reduce dental erosion from soft drinks. *Caries Res* 2002; **36**: 75-80.

31. Bachra BN, Van Harskamp GA. The effect of polyvalent metal ions on the stability of a buffer system for calcification *in vitro*. *Calc Tissue Res* 1970; **4**: 358-365.

32. Rao SVC. Preparation of solid solutions of calcium and iron hydroxylapatites. *J Inst Chem Calcutta* 1974; **46**: 30-31.

33. Okasaki M, Takahashi J, Kimura H. Iron uptake of hydroxyapatite and fluoridated apatites. *J Dent Res* 1985; **64**: 301 (Abs. 1141).

34. Sales-Peres SH, Pessan JP, Buzalaf MAR. Effect of an iron mouthrinse on enamel and dentin erosion subjected or not to abrasion: an in situ/ ex vivo study. *Arch Oral Biol* 2007; **52**: 128-132.

2.8 Table 1. Enamel alterations (wear and softening) for each experimental group

Groups	Response Variables (±SD)	
	Wear (µm)	%SMHC (softening)
G1 - pure Sprite	0.79±0.21 a,b	47.92±7.45 a,c
G2 – Sprite with Ca	0.36±0.10 c,d	47.06±5.11 a
G3 – Sprite with F	0.50±0.18 b,c	53.94±4.58 a,d
G4 – Sprite with Fe	0.79±0.26 a,b	56.04±7.46 b,c,d,e
G5 – Sprite with P	0.50±0.15 b,c	60.90±4.62 b,d,f
G6 – Sprite with Ca and F	0.32±0.11 c	47.23±4.99 a
G7 – Sprite with Ca and P	0.76±0.22 a,b	49.10±6.96 a,e
G8 – Sprite with Fe and F	0.97±0.23 a	52.91±4.67 a,e,f
G9 – Sprite with all ions	0.67±0.38 a,b,d	46.14±6.80 a,e

Values in the same column followed by different letters indicate statistical significance ($p<0.05$)

3 Capítulo 2

Effect of 4% titanium tetrafluoride solution on dental erosion by a soft drink: an in situ/ex vivo study

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Running title: Effect of titanium tetrafluoride on dental erosion

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3.1 Abstract

(1)Objective: This in situ/ex vivo study assessed the effect of titanium tetrafluoride (TiF_4) on permanent human enamel subjected to erosion. *(2)Design:* Ten volunteers took part in this study performed in two phases. In the first phase (ERO), they wore acrylic palatal appliances containing 2 enamel blocks, divided into two rows: TiF_4 (F) and no-TiF_4 (no-F). During the 1st day, the formation of a salivary pellicle was allowed. In the 2nd day, the TiF_4 solution was applied on one row (ERO+F), whereas on the other row no treatment was performed (ERO+no-F). From 3rd until 7th day, the blocks were subjected to erosion, 4X/day. In the second phase (no-ERO), the volunteers wore acrylic palatal appliances containing one enamel block, during 2 days, to assess the effect of TiF_4 only (no-ERO+F). Enamel alterations were determined using profilometry (wear), microhardness (%SMHC) tests, scanning electron microscope and microprobe analysis. The %SMHC and wear were tested using ANOVA and Tukey´s *post hoc* tests ($p<0.05$). *(3)Results:* The mean %SMHC and wear (µm) values (±SD) were, respectively: ERO+F –73.32±5.16^{A} / 2.40±0.60^{a}; ERO+no-F –83.49±4.59^{B} / 1.17± 0.48^{b} and no-ERO+F – 67.92±6.16^{A} / 0.21±0.09^{c}. In microscope analysis, the no-F group showed enamel with honeycomb appearance. For F groups, a surface coating with micro cracks was observed. The microprobe analysis revealed the presence of the following elements (%) in groups ERO+F, ERO+no-F and no-ERO+F, respectively: Ca (69.9, 72.5, 66.25); P (25.9, 26.5, 26.06) and Ti (3.0, 0, 5.93). *(4)Conclusions:* The TiF_4 was unable to reduce dental erosion.

Keywords: erosion; fluoride; soft drinks; titanium.

3.2 Introduction

With the decline in the prevalence of dental caries in most developed countries[1], as well as in Brazil[2], an increasing interest in other dental disorders, including tooth wear, has arisen[3]. The term tooth wear is used to describe the processes of erosion, attrition and abrasion[4-6].

Dental attrition and abrasion are mechanical wear by tooth to tooth contact[4,6] and by abrasive substances[4,6,7], respectively. Dental erosion is the loss of tooth substance by chemical processes not involving bacteria[3,8,9]. The etiology of erosion is multifactorial and not fully understood. The most important sources of acids are those found in the diet (acidic foods and drinks)[10,11] and from the stomach (gastric acids from regurgitation and reflux disorders)[12,13]. In modern societies, the increased consumption of acid drinks as soft drinks, sport drinks, fruit juices and fruit teas is an important factor of tooth wear[8,10].

For erosion prevention, many strategies have been used, such as the topical application of fluoride. Although the preventive action of fluoride on dental caries is well known[14,15], its role in erosion is still controversially discussed[16,17], since the deposited calcium fluoride-like material from topical fluoride application is supposed to dissolve readily in most acidic drinks[17]. However, high-concentrated fluoride applications, as oral rinses, gels or varnishes, have been demonstrated, in some cases, to increase abrasion resistance and decrease the development of erosion in enamel[18-20].

The fluoride agents that have been assessed in most in vitro studies for erosion prevention are those that have been used over years for caries prevention, such as: sodium fluoride (NaF), acidulated phosphate fluoride (APF), stannous fluoride (SnF_2) or amine fluoride (AmF). More recently, other agents, as titanium tetrafluoride (TiF_4), have been investigated for erosion prevention[21-27].

With respect to TiF_4, in vitro studies have shown its inhibitory effect on erosion[21-23,25], which has been attributed not only to the effect of fluoride, but also to the action of titanium[28,29]. The low pH of TiF_4 (around 1.2) favors the linking between titanium and oxygen of the group phosphate, thus leading to the formation of a titanium dioxide glaze-like layer on the surface[21,24]. Vieira et al.[25] showed that the application of 4% TiF_4 resulted in the formation of a dense layer. However, other studies have not found a protective effect of TiF_4 against erosion[26,27].

Thus, additional studies involving protocols closer to the clinical situation are necessary to clarify this issue. Taking into account these considerations, the purpose of this study was to assess the effect of TiF_4 solution on permanent human enamel subjected to erosion in situ/ex vivo.

3.3 Material and Methods

3.3.1 Experimental Design

This study was approved by the Research and Ethics Committee of the Bauru School of Dentistry, University of São Paulo (Proc nº 78/2005). It involved a crossover design performed in two phases. Ten healthy adult volunteers (aged 19-30 yr) with normal salivary flow, living in a fluoridated area (0.70 mgF/L) and using the same fluoridated dentifrice during the experiment took part in this study. In the first phase (ERO), they wore acrylic palatal appliances containing two permanent human enamel blocks, divided into two rows: TiF_4 (ERO+F) and no-TiF_4 (ERO+no-F). In the first 24 hours of the intraoral phase, the formation of a salivary pellicle was allowed. In the 2nd day, the fluoride solution was applied on one row, whereas on the other row no treatment was performed. From 3rd until 7th days, the blocks were subjected to erosion, 4 times a day. In the second phase (no-ERO), the volunteers wore acrylic palatal appliances containing one permanent human enamel block, during 2 days, to assess only the effect of TiF_4 (no-ERO+F). The same procedures made in the first two days of 1st phase were repeated. Between the two phases, a washout period of 7 days was allowed. Enamel alterations were determined by profilometric and surface microhardness tests. Moreover, the surface of enamel blocks from 2 volunteers (double of samples/phase) was examined using scanning electron microscopy (SEM) and microprobe analysis.

3.3.2 Enamel blocks and palatal appliance preparation

Permanent human enamel blocks (4X4X3mm) were prepared from freshly extracted third molars sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature[30,31]. The teeth were cut using ISOMET Low Speed Saw cutting machine (Buehler Ltda., Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer. The

enamel surface of the blocks was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 µm; Buehler), resulting in removal of about 100 µm depth of the enamel which was controlled with a micrometer. The surface microhardness determination was performed by five indentations in different regions of the blocks (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan).

In the first phase (ERO), twenty and four permanent enamel blocks with a mean surface microhardness around 368.24±11.57 KHN were randomly divided into two groups (ERO+F and ERO+no-F). In the 2nd phase (no-ERO), twelve permanent enamel blocks with a mean surface microhardness around 362.11±12.12 KHN were used for the group treated with TiF_4 without erosion (no-ERO+F). In order to maintain reference surfaces for lesion depth determination, two layers of nail varnish were applied on half of the surface of each block. On the left and right sides of the acrylic palatal appliances, one cavity of 5X5X3 mm was made and in each of them one enamel block was fixed with wax in the 1st phase. In the 2nd phase, one block was fixed in one cavity and the other cavity was filled with wax.

3.3.3 Treatment

In the first 24 hours of the 1st intraoral phase, the blocks were not subjected to the erosive process, to allow the formation of a salivary pellicle[30,31]. In the second day, the application of fluoride solution (4% TiF_4) was made extraorally by one of the researchers. For preparing the 4% (1.29 M F) TiF_4 solution, solid TiF_4 (Sigma Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in deionized water. The pH of the solution (1.2) was checked by glass electrode (Orion). The application was made in drops with a cotton roll, during 1 min on one row (ERO+F), whereas on the other row no treatment was performed (ERO+no-F). The drop was left undisturbed until the surface appeared dry. Additional drops were applied in the same manner until 1 min had elapsed[22]. After that, the excess of product was removed carefully on the enamel surface with cotton swab and, the appliance was replaced into the mouth.

From 3rd until 7th days, erosive challenges were made extraorally 4 times a day and at predetermined times (8:00, 12:00, 16:00 and 20:00 h). In order to subject the enamel blocks to erosion, 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® (Spal, Porto Real, RJ, Brazil) at room temperature, for 5 minutes[30-32]. In sequence, the appliance was replaced into the mouth.

In the 2nd phase, the volunteers wore acrylic palatal appliances containing one permanent human enamel block, during 2 days, to assess the effect of TiF_4 on sound enamel (no-ERO+F). The same procedures that were made in the first two days of the 1st phase were repeated.

The volunteers received instructions to wear the appliances continuously, including at night, but to remove them during meals (3 times a day, 1 h per meal) and oral hygiene. Oral hygiene was performed right after meals with a fluoride dentifrice (1,500 ppm F, Sorriso®, Brazil). In this period the appliance was stored in wet gauze. The volunteers received oral and written information to refrain from using any fluoridated product, except the dentifrice.

3.3.4 Percentage of superficial microhardness change (%SMHC) assessment

The permanent enamel blocks were removed from the appliances and the nail varnish over the surfaces was carefully cleaned with acetone-soaked cotton wool[33]. Enamel surface microhardness was measured as described earlier and an average of each group per volunteer was obtained. Ten indentations on each specimen were made, five on the previously protected enamel surface (SMH) and five on the experimental area (SMH1). Using these measurements, the percentage of superficial microhardness change (%SMHC) was calculated, as follows: %SMH= {[(SMH1-SMH)/SMH]*100}[30,31].

3.3.5 Wear assessment

Initially, the nail varnish was removed and the enamel blocks were dried. In sequence, the wear was determined in relation to the reference surface by profilometry (Hommel Tester T1000, VS, Schwenningen). Five readings were performed on each specimen through scanning from the reference to the exposed surface. The difference between the interface of reference (not eroded) and exposed (eroded) area was measured, and an average of each group per volunteer was obtained (µm).

3.3.6 SEM and microprobe analysis

The enamel blocks from two volunteers, not protected by nail varnish, were then coated with carbon and examined with a scanning electron microscope (SEM) (Carl Zeiss, DSM 940A, German at 20KV) using secondary electron (SE) and backscattered electron detectors (BSE) to make the images. The presence of Ca, P and Ti was detected by means of x-ray energy dispersive spectroscopy (EDS) coupled to the SEM (Link ISIS 300, OXFORD, England). The elements weight percent determination was made using quantitative analysis with ZAF correction.

3.3.7 Statistical Analysis

The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Since the assumptions were satisfied, ANOVA and Tukey's post hoc tests were carried out for statistical comparisons and the significance limit was set at 5%.

3.4 Results

The mean %SMHC for groups ERO+F and ERO+no-F were -73.40 ± 5.12 and -83.40 ± 4.64, respectively. The group ERO+F resulted in significant less softening when compared to ERO+no-F group. With respect to wear, the eroded permanent enamel pre-treated with TiF_4 (ERO+F) had a significantly higher loss of dental structure in comparison to no-F group (ERO+F – 2.40 ± 0.60 and ERO+no-F –1.17 ± 0.48 µm) (Table 1).

In order to confirm the loss of dental structure caused by the use of TiF_4, the 2nd phase was conducted. In this phase, only the effect of TiF_4 was assessed (no-ERO+F). The mean %SMHC and wear (µm) values (±SD) were -67.8 ± 6.39 and 0.21 ± 0.09, respectively. The fluoride group (no-ERO+F) had a significantly smaller %SMHC when compared to the erosion only group (ERO+no-F), but did not significantly differ from the ERO+F group. The wear of no-ERO+F group was significantly smaller when compared to ERO+no-F and ERO+F groups ($p<0.05$). In respect to microprobe analysis, the Table 1 shows the percentage of the elements found (Ca, P, K, Mg, Cl and Ti).

Regarding microscopy analysis, for ERO+no-F group (Figure 1), the enamel surface exhibited a honeycomb appearance, which is characteristic of erosion. For F-treated groups (ERO+F and no-ERO+F), besides the honeycomb appearance for the group ERO+F (Figure 3), a surface layer coating with micro cracks was observed in both groups, corresponding to the titanium deposits (Figures 2 and 4).

3.5 Discussion

In order to simulate the everyday situation as closely as possible, an in situ/ ex vivo model was chosen in the present study to test the effect of titanium tetrafluoride (TiF_4) solution on permanent human enamel subjected to erosion. To the best of our knowledge, this is the first study about the effect of TiF_4 on dental erosion which was conducted in situ/ ex vivo.

The TiF_4 solution has been tested in several in vitro studies of dental erosion. Some publications attest its inhibitory effect on erosion[21,22,24,34,35], while others have shown the opposite[26,27]. The probable explanations for these contrasting results are the methodological differences among these studies as well as the distinct response variables analyzed. For example, in most of the studies, the solution was applied many times (1x/day) or the period of erosive challenge was shorter (1 day), which does not simulate the clinical condition. In the present study, the solution was applied only once and its effect was tested after 5 days in order to simulate the clinical situation with a single professional application. Since the use of fluoride has been advocated for high risk patients of dental erosion, a high erosive challenge was conducted (4 x 5 min per day, during 5 days).

van Rijkom et al.[22] compared the solutions of 1% sodium fluoride (NaF) and 1% TiF_4 applied 4 minutes prior to the erosion. The TiF_4 was more effective, what is in agreement to Schlueter et al.[35], who compared NaF to TiF_4 applied after erosion. In both studies, the variables analyzed were calcium loss and longitudinal microhardness, respectively. However, none of these studies evaluated surface enamel loss. Because of this, in the present study the enamel wear was assessed.

In the present study, the application of TiF_4 only reduced the %SMHC (enamel softening), probably due to the interaction of titanium with the surface of the enamel, which was confirmed by microprobe analysis (Table 1). A surface layer coating with micro cracks, which closely resembles the images reported by Buyukyilmaz et al.[21],

was observed. However, this study was not able to show which compound was formed on the enamel surface.

The formation of the described surface layer coating was not sufficient to prevent the subsequent loss of dental structure (wear). It is possible to depict from Figure 3 that a part of this glaze was removed by erosion. This is in agreement with results by Vieira et al.[27], who showed in vitro that the TiF_4 did not have a significant protective effect against erosive wear. The values of wear found by Vieira et al.[27] (TiF_4 - 4.56 µm and Control - 4.40 µm) were higher than that in this present study (TiF_4 - 2.4 µm and Control - 1.7 µm). This difference may be due to the distinct equipments used for profilometric analysis and also to the fact that the enamel loss is more pronounced in vitro.

It is known that the low pH (1.2) of TiF_4 solution causes a reasonable amount of enamel loss at application[27]. Therefore, in our study, a period of 24 h was allowed after the application of 4% TiF_4, in order to allow for remineralization by saliva before the first erosive attack could take place. Interestingly, this enamel loss at application did not occur. When the TiF_4 solution was applied alone, without erosion by the cola drink, the wear was insignificant (0.21 µm), but a reduction in surface microhardness could be detected. However, one intriguing question arises: how can TiF_4 have worsened the wear when the erosive challenge was performed? At first glance, this question seems difficult to answer. One probable hypothesis is that the low pH of TiF_4 caused a softening of enamel at application, thus turning this enamel more susceptible to wear after the “titanium dioxide glaze-like layer” was removed. This can be confirmed by the analysis of the table, which shows a reduction in enamel microhardness when the TiF_4 solution was applied alone and also by Figures 2 and 3. In these figures, on samples treated with Ti and subjected to subsequent erosive challenge, some areas with a “titanium dioxide glaze-like layer” could be seen. However, most of the areas had a honeycomb appearance.

Our study was not able to show the presence of fluoride on enamel, because there was no specific electron probe for fluoride. However, some studies have reported less fluoride retention in enamel after application of TiF_4 when compared to other fluoride compounds[23,24]. This fact is probably caused by the dissolution of the deposited material from topical TiF_4 application by acidic drinks.

In conclusion, according to the protocol of this study, the TiF_4 solution was not able to prevent dental erosion in situ. The addition of TiF_4 to other vehicles, such as a

varnish should be tested, in order to form a more resistant compound on dental enamel and to reduce the negative effects of the enamel softening at application.

3.6 Acknowledgements

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3.7 References

1. Peterson G, Bratthall D. The caries decline: a review of reviews. *Eur J Oral Sci* 1996; **104**: 436-443.
2. Souza SMD. CPO-D brasileiro aos 12 anos tem redução de 53.22%. *Jornal da ABO Nacional* 1996; **8**.
3. Imfeld T. Dental erosion. Definition, classification and links. *Eur J Oral Sci* 1996; **104**: 151-155.
4. Addy M, Shellis RP. Interaction between Attrition, Abrasion and Erosion in Tooth Wear. *Monogr Oral Sci* 2006; **20**: 17-31.
5. Eccles JD. Tooth surface loss from abrasion, attrition and erosion. *Dent Update* 1982; **9**: 373-381.
6. Litonjua LA, Andreana S, Bush PJ, Cohen RE. Tooth wear: attrition, erosion, and abrasion. *Quintessence Int* 2003; **34**: 435-446.
7. Bartlett D, Smith BGN. Definition, classification and clinical assessment of attrition, erosion and abrasion of enamel and dentine. Addy A, Embery G, Edgar WM, Orchardson R. In: *Tooth wear and sensivity-Clinical advances in restorative dentistry.* Martin Dunitz, 2000.
8. Lussi A. Erosive tooth wear - a multifactorial condition of growing concern and increasing knowledge. *Monogr Oral Sci* 2006; **20**: 1-8.
9. Moss SJ. Dental erosion. *Int Dent J* 1998; **48**: 529-539.
10. Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. *Caries Res* 2004; **38**: 34-44.
11. Lussi A, Jaeggi T. Chemical factors. *Monogr Oral Sci* 2006; **20**: 77-87.

12. Bartlett D. Intrinsic causes of erosion. *Monogr Oral Sci* 2006; **20**: 119-139.
13. Scheutzel P. Etiology of dental erosion-intrinsic factors. *Eur J Oral Sci* 1996; **104**: 178-190.
14. Øgaard B. Effects of fluoride on caries development and progression in vivo. *J Dent Res* 1990; **69**: 813-819.
15. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *Eur J Oral Sci* 1997; **105**: 461-465.
16. Larsen MJ, Richards A. Fluoride is unable to reduce dental erosion from soft drinks. *Caries Res* 2002; **36**: 75-80.
17. Wiegand A, Attin T. Influence of fluoride on the prevention of erosive lesions – a review. *Oral Health Prev Dent* 2003; **1**: 245-253.
18. Buchalla W, Lagerweij M, Kohnke S, Becker K, Lennon AM, Attin T. Fluoride is able to reduce erosive and erosive/abrasive enamel loss under severe erosive conditions. *Caries Res* 2004; **38**: 291.
19. Ganss C, Klimek J, Brune V, Schumann A. Effects of two fluoridation measures in erosion progression on enamel and dentine in situ. *Caries Res* 2004; **38**: 561-566.
20. Lussi A, Jaeggi T, Megert B. Effect of various fluoride regimes on toothbrush abrasion of softened enamel in situ. *Caries Res* 2004; **38**: 393.
21. Buyukyilmaz T, Øgaard B, Rolla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. *Eur J Oral Sci* 1997; **105**: 473-477.
22. van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment *in vitro*. *Eur J Oral Sci* 2003; **11**: 253-257.
23. Schlueter N, Ganss C, Ort R, Schmidt R, Klimek J. Retention of fluoride and titanium on enamel under erosive conditions in vitro. *Caries Res* 2004; **38**: 392.
24. Tveit AB, Hals E, Isrenn R, Totdal B. Highly acid SnF_2 and TiF_4 solution. *Caries Res* 1983; **17**: 412-418.
25. Vieira A, Ruben JL, Stokroos I, Huysmans MCDNJM. Surface and surface analysis of eroded bovine enamel pretreated with fluoride. *Caries Res* 2004; **38**: 391.

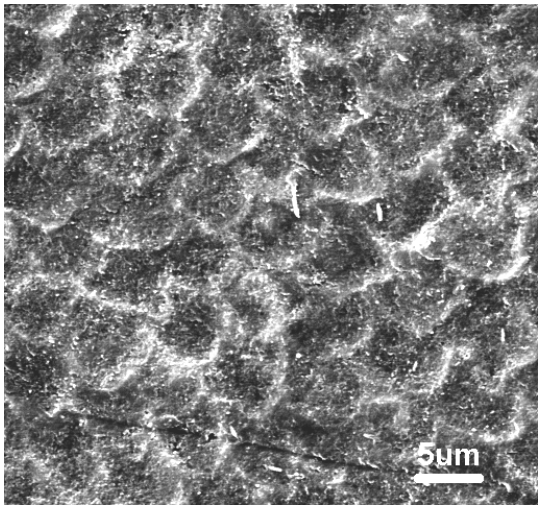
26. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. *Caries Res* 2005; **39**: 371-379.
27. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. *Caries Res* 2006; **40**: 224-230.
28. Buyukyilmaz T, Øgaard B, Duschner H, Ruben J, Arends J. The caries-preventive effect of titanium tetrafluoride on root surfaces in situ as evaluated by microradiography and confocal laser scanning microscopy. *Adv Dent Res* 1997; **11**: 448-452.
29. Tezel H, Ergucu Z, Onal B. Effects of topical fluoride agents on artificial enamel lesion formation in vitro. *Quintessence Int* 2002; **33**: 347-352.
30. Magalhães AC, Rios D, Delbem ACB, Buzalaf MAR, Machado MAAM. Influence of fluoride dentifrice on brushing abrasion of eroded human enamel: an in situ/ex vivo study. *Caries Res* 2007; **41**: 77-79.
31. Rios D, Honório HM, Magalhães AC, Machado MAAM, Delbem ACB, Silva SMB et al. Effect of salivary stimulation on erosion subjected or not to abrasion in human and bovine enamel: an in situ/ex vivo study. *Caries Res* 2006; **40**: 218-223.
32. Sales-Peres SHC, Pessan JP, Buzalaf MA. Effect of an iron mouthrinse on enamel and dentine erosion subjected or not to abrasion: an in situ/ex vivo study. *Arch Oral Biol* 2007; **52**: 128-132.
33. Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. *Caries Res* 2000; **34**: 48-52.
34. 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.
35. 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.

3.8 Table and Figures

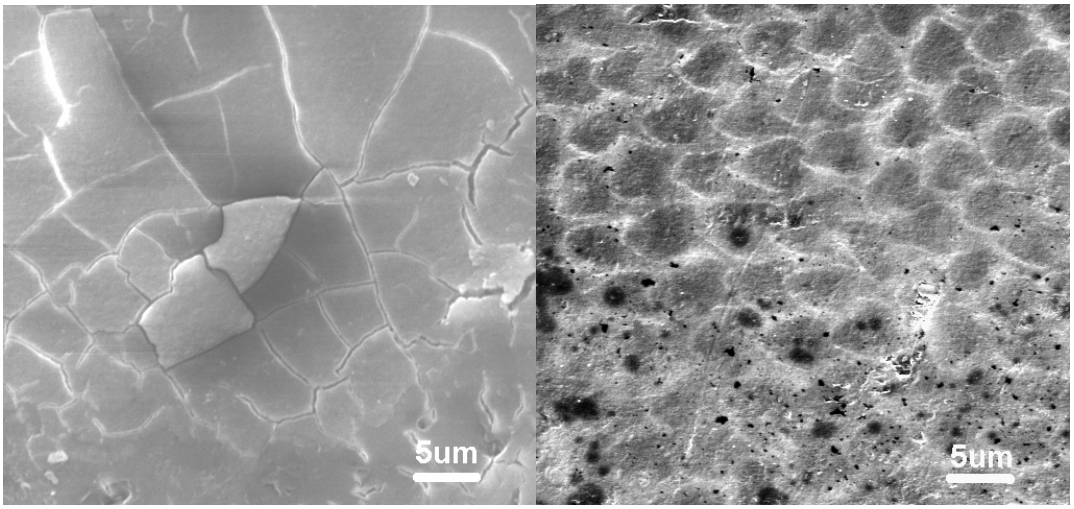
3.8.1 Table 1. Enamel alterations for each experimental group.

GROUPS / ANALYSIS	ERO+F	ERO+no F	no ERO+F
%SMHC	-73.40 ±5.12[a]	-83.40±4.64[b]	-67.8±6.39[a]
Wear (µm)	2.40±0.60[a]	1.17±0.48[b]	0.21±0.09[c]
Ca (%)	69.9	72.5	66.25
P (%)	25.9	26.5	26.06
Ti (%)	3.0	0	5.93
K (%)	0.4	0	0.94
Mg (%)	0	0.2	0.21
Cl (%)	0.8	1.10	0.56

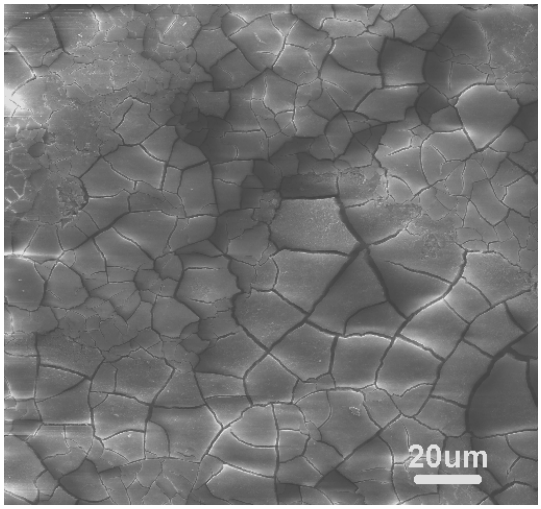
Values in the same line followed by different superscripts indicate statistical significance (p<0.05)



3.8.2 Figure 1. Enamel from group ERO+ no-F showing a honeycomb appearance.



3.8.3 Figures 2 and 3. Enamel treated with TiF_4 and subsequently eroded (group ERO+F) showing a surface coating with micro-cracks, corresponding to the titanium deposits and another surface far to titanium deposits (honeycomb appearance), respectively .



3.8.4 Figure 4. Enamel treated with TiF_4 only showing a surface coating with micro-cracks, corresponding to the titanium deposits.

4 Capítulo 3

Effect of 4% titanium tetrafluoride (TiF_4) varnish on demineralisation and remineralisation of bovine enamel in vitro

Short title: Effect of TiF_4 on enamel caries

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Keywords: dental caries; enamel; fluoride; titanium

Effect of 4% titanium tetrafluoride (TiF_4) varnish on demineralisation and remineralisation of bovine enamel in vitro

4.1 Summary

Objectives: This in vitro study assessed the effect of a 4% TiF_4 varnish on demineralisation and remineralisation of sound enamel and artificial carious lesions, respectively. *Methods:* Bovine sound and carious enamel were randomly allocated to each type of varnish: Duraphat®-D (NaF, 2.26%F, pH 4.5, Colgate-Brazil, n=30), Duofluorid®-F (NaF, 2.71% F, pH 8.0, FGM-Brazil, n=30), TiF_4-T (2.45%F, pH 1.0, FGM-Brazil, n=30) and no-fluoride–P (FGM-Brazil, pH 5.0, n=20). For the formation of artificial enamel caries, half of the blocks were immersed in 32 mL buffer acetate solution (16 h), whereas the other half was maintained sound. The varnishes were applied onto the enamel surfaces. Thus, the samples were subjected to pH cycles (37° C) for 7 days. The response variables tested were surface and cross-sectional hardness. Data were tested using Kruskal-Wallis test ($p<0.05$). *Results:* All F varnishes significantly reduced demineralisation and increased remineralisation in comparison to placebo. The TiF_4 did not significantly reduce the surface enamel softening when compared with the other F varnishes, but it decreased the loss of subsurface hardness to the same extent. In enamel blocks with previous artificial carious lesions, the TiF_4 significantly improved the rehardening compared to the other varnishes up to 30 µm depth. *Conclusions:* The TiF_4 varnish was able to decrease the demineralisation and increase the remineralisation of previously sound and carious enamel, respectively. It was equally effective compared to NaF varnishes on reducing the demineralisation at subsurface, but it was more effective on improving the remineralisation at surface and subsurface.

4.2 Introduction

Fluoride (F) is regarded as the major factor responsible for the dramatic caries reduction in children and young adults in most industrialized countries during the last decades[1]. It is deposited on the enamel by the formation of a CaF_2-like reservoir. During a cariogenic challenge, F released from this reservoir may diffuse into the enamel promoting reformation of apatite[2,3].

Agents with a high concentration of fluoride, such as varnish and gels, promote much larger amounts of CaF_2 formation on the enamel surface than do low F concentration agents[3,4]. Thus, professionally applied topical F agents, such as gels or varnishes, are recommended for individuals who present moderate to severe caries activity, with the objective to prevent or arrest new or recurrent dental cavities[1].

The use of F varnish has been supported by a great number of investigations that have evaluated the enamel resistance to acid etching by increasing fluoride incorporation in the enamel or by decreasing the enamel solubility in acids *in vitro*[5,6]. However, there are only clinical studies testing the use of F varnish to the treatment of non-cavity caries lesion (white spot lesions), and most of them use visual analysis as the outcome variable[7-11]. However, although clinical experience endorses the use of varnishes for the treatment of white spot lesions, *in vitro* studies testing its effect on enamel rehardening have not been conducted so far.

The F compound present in the varnish, in most of the commercially available products, is sodium fluoride (NaF). More recently, the titanium tetrafluoride (TiF_4) solution, has been investigated for the prevention of dental demineralisation[12-16]. The TiF_4 solution seems to have an inhibiting effect on caries lesion formation[17,18]. However, there are no studies testing the use of TiF_4 incorporated into varnish for the prevention and treatment of dental caries.

Taking into account these considerations, the purpose of this study was to assess the effect of an experimental titanium tetrafluoride (4% TiF_4) varnish on the demineralisation of initially sound enamel and remineralisation of previously carious enamel in vitro.

4.3 Material and Methods

4.3.1 Enamel blocks preparation

Enamel blocks (4X4X2.5 mm) were prepared from incisor bovine teeth, freshly extracted, sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature[19]. The teeth were cut using ISOMET Low Speed Saw cutting machine (Bulher Ltda., Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer. The enamel surface of the blocks was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 µm; Buehler), resulting in removal of about 100 µm depth of the enamel. This was controlled with a micrometer. The surface hardness determination was performed by five indentations (Knoop diamond, 25 g, 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan).

One hundred and ten blocks were selected (330 to 390 KHN) and half of them were subjected to formation of artificial caries lesion by immersion in 32 mL of 50 mM buffer acetate solution [1.28 $mmolL^{-1}$ $Ca(NO_3)_2.4H_2O$, 0.74 $mmolL^{-1}$ $NaH_2PO_4.2\ H_2O$, 0.03 ppm F, pH 5.0, 37 °C], during 16 h[20]. After that, the hardness was again evaluated and the percentage of surface hardness change was calculated for these blocks [%SHC lesion = 100*(SH lesion – SH)/SH]. Blocks with mean %SHC around 65-90 were selected and randomly allocated for the groups. In order to evaluate if this solution was able to produce caries lesion, 10 blocks were analyzed for longitudinal hardness (Figure 2).

4.3.2 Treatment and pH-cycling

Sound enamel blocks with hardness ranging from 330 to 390 KHN and carious enamel blocks with %SHC between 65 and 90 were randomly distributed into 4 groups (Mean KHN 367±17/ Mean %SHC 85±7): Duraphat® - D (NaF, 2.26%F, pH 4.5, Colgate, São Bernardo, SP, Brazil, n=30), Duofluorid® - F (NaF, 2.71% F, pH 8.0, FGM, Joinvile, Santa Catarina, Brazil, n=30), TiF_4 - T (2.45%F, pH 1.0, FGM, Joinvile, Santa Catarina, Brazil, n=30) and no-fluoride – P (pH 5.0, FGM, Joinvile, Santa Catarina, Brazil, n=20). The basic formulation of F, T and P was the same, except for the fluoride compound. While Duofluorid® is transparent, the other varnishes present a yellow color. A thin layer of varnish was applied with a

microbrush on the enamel surface once, immediately before the first 6h-demineralisation treatment. After 6h, the varnish layer was removed before to start the 18h-remineralization treatment. The varnishes were carefully removed using a surgical blade and cotton swabs soaked in acetone[6]. The complete removal of the varnishes was checked microscopically.

The blocks were subjected to a pH-cycling model for 7 days, according to Vieira et al.[21]. During 5 days, the blocks were immersed in demineralisation solution [2.0 $mmolL^{-1}$ $Ca(NO_3)_2.4H_2O$, 2.0 $mmolL^{-1}$ $NaH_2PO_4.2\ H_2O$, 0.075 $mmolL^{-1}$ acetate buffer, 0.02 ppm F, pH 4.7] for 6 h (30 mL per block) and in remineralisation solution [1.5 $mmolL^{-1}$ $Ca(NO_3)_2.4H_2O$, 0.9 $mmolL^{-1}$ $NaH_2PO_4.2\ H_2O$, 150 $mmolL^{-1}$ KCl, 0.1 $molL^{-1}$ Tris buffer, 0.03 ppm F, pH 7.0] for 18 h (15 mL per block). In the last 2 days, the blocks were maintained in remineralisation solution only.

4.3.3 Hardness determination

Initially, enamel surface hardness was measured as described earlier (Knoop diamond, 25 g, 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Five indentations were made in the center of enamel blocks (SH) at distances of 100 µm from each other. For the blocks with previous artificial enamel caries, the SH lesion was evaluated again to calculate the percentage of surface hardness change [%SHC lesion = 100*(SH lesion – SH)/SH].

After the treatments, final hardness test (SH1) was made. In the TiF_4 group, final hardness measurement was performed on surface areas, which appeared free from any glaze-like structures (checked by 40x magnification). The percentage of surface hardness change for the sound and previous carious enamel was calculated as follows: %SHC sound enamel = 100*(SH_1 – SH)/SH and %SHC carious enamel = (SH1 – SH lesion)/(SH – SH lesion)*100].

To perform cross-sectional hardness (CSH) tests, the blocks were longitudinally sectioned through the center, embedded and polished. Three rows of 8 indentations each were made, one in the central region of the dental enamel exposed and the other two 100 µm below and above this, under a 25-g load for 10 s. The indentations were made at 10, 30, 50, 70, 90, 110, 220 and 330 µm from the outer enamel surface. The mean values at all 3 measuring points at each distance from the surface were then averaged.

4.3.4 Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Kruskal-Wallis and Dunn's tests were carried out for statistical comparisons and the significance limit was set at 5%.

4.4 Results

The mean %SHC for sound and carious enamel is described in Table 1. With respect to %SHC, all fluoride varnishes reduced the softening and improved the rehardening of enamel in comparison to placebo varnish ($p<0.05$). The behavior of D and F varnishes was similar. The TiF_4 promoted higher remineralisation of carious enamel than D and F. In contrast, the TiF_4 group was statistically less effective than both the D and F treatment groups at reducing surface softening ($p=0.01$).

The mean cross-sectional hardness of previous sound and carious enamel, respectively, is shown in Figures 1 and 2. For sound enamel, all fluoride varnishes significantly reduced mineral loss in comparison to placebo until 70 µm depth and no significant difference among the fluoride varnishes was detected (Figure 1, $p<0.001$). For carious enamel, all fluoride varnishes significantly increase cross-sectional hardness in comparison to placebo until 70 µm depth. In this case, there was a significant difference among the fluoride varnishes. The TiF_4 group was significantly more effective than both the D and F treatment groups until 30 µm depth (Figure 2, $p<0.001$).

4.5 Discussion

Many investigations have been conducted to define the best F therapy for the prevention of dental caries[22,23]. Frequent application of low F concentration agents has been considered as the most beneficial treatment regime. However, in situations of high risk of caries, the use of a method that employs high concentration of F, such as the professionally applied products, has been recommended[8]. Gels and varnishes are the most commonly used highly fluoridated agents. The F varnish has been indicated because it decreases the enamel solubility in acids in vitro[5,6,24], reduces the incidence of dental caries and remineralizes white spot lesions in clinical studies[7-11]. This beneficial effect is attributed to the pronounced adherence to enamel and the

calcium fluoride layer, which acts as a long-term reservoir of F. Especially for children, the varnish is also the agent of choice in function of its easy application and safety in comparison to other types of topical fluoride treatments (such as gels and rinses)[9]. Besides, Duraphat varnish seems to be equally or more effective than APF gel[25].

This is the first study that evaluated the effect of TiF_4 incorporated into varnish on enamel de- and remineralisation. The protective action of this compound incorporated into a solution has been attributed not only to the action of F, but also to the action of titanium[17.18]. According to previous studies, the low pH of TiF_4 (around 1.2), favors the linking between titanium and oxygen of the phosphate group, thus leading to the formation of a titanium dioxide glaze-like layer on the surface[12,14]. It is possible that this anticariogenic effect is improved when TiF_4 is incorporated into the varnish.

As the objective of the present study was to focus on the chemical effect of the NaF and TiF_4 varnishes rather than on the mechanical protection, the varnishes were removed carefully after 6 h, to simulate the clinical situation in which the varnishes might be removed by toothbrushing or mastication after some hours. In order to avoid damaging of the enamel surface, the scalpel only touched the varnish. The enamel surface was cleaned with a cotton slab and acetone, which is not able to remove fluoride deposits from the enamel[6].

So far, the studies in the literature tested the effect of commercial varnishes containing NaF. These in vitro and in vivo studies have shown a reduction of dental caries around 30%[4-6,24]. The results obtained in this study for D and F are similar and in agreement with the literature. It is known that the formation of the CaF_2 reservoir is increased under acidic compared to neutral conditions[3]. However, the difference between the pH of NaF varnishes did not influence their behavior in this protocol. This may be due to the fact that the enamel blocks were immersed in an acid solution (demineralisation, pH 4.7) for 6 h immediately after application of the varnishes.

Regarding the TiF_4 varnish, it seems to have a slightly lower and similar effect on the reduction of superficial and longitudinal hardness loss of previously sound enamel in comparison to the other NaF varnishes, respectively. For previously carious enamel, the TiF_4 group showed higher hardness values up to 30 µm depth when compared to the other F varnishes indicating a better remineralizing action. It is

possible that the pre-demineralisation as well as the protocol of pH cycling used in this study have produced more softened surfaces (Figures 1 and 2) than it would be expected in the clinical situation. This may have allowed the titanium glaze layer to penetrate into the subsurface, which could explain the beneficial effect of the TiF_4 varnish in the cross sectional hardness, for both types of enamel substrates. These observations may be taken into account when the results of this study are intended to be extrapolated to the clinical situation. It is possible that the results might be different in the clinical situation, especially with regard to the effect of TiF_4. Thus, further in situ and in vivo studies are necessary to confirm the results of the present study.

Some studies have shown a higher fluoride uptake into demineralised enamel when compared to sound enamel[2,18]. As for fluoride, the titanium may have also been more incorporated in the previously carious than in the sound enamel. The porosities of enamel can have allowed for a deeper penetration of fluoride (for all fluoridated groups) and also for titanium (TiF_4 varnish). In the TiF_4 group, the penetration of F and Ti might have permitted the formation of a new harder compound on the surface and until 30 µm depths. This can explain the different behavior of the TiF_4 varnish for sound and carious enamel at the subsurface. It might also account for the rehardening of the carious enamel for all fluoridated varnishes under pH cycling.

However, Chevitarese et al.[26] showed that there was no significant difference between sound and carious enamel regarding the titanium penetration, but titanium penetrated more deeply into sound enamel compared to artificially decayed enamel. In this study, the authors used TiF_4 solution. It is possible that the behavior of the varnish is different from the solution regarding the fluoride and titanium penetration in enamel. According to our protocol, the varnishes did not have a mechanical protective effect on enamel de- and remineralisation. Probably the F varnishes reacted with enamel chemically during 6h, producing a F (for all F varnishes) and Ti-rich (TiF_4 varnish) layer on the surface. Thus, further studies testing the titanium and fluoride penetration in enamel after varnish application could be instructive to clarify this issue. Besides, for application of this new varnish in the clinical situation, more studies must be conducted using in situ protocols.

4.6 Conclusion

The TiF_4 varnish was able to decrease the demineralization and increase the rehardening of previously sound and carious enamel, respectively. It was equally effective compared to NaF varnishes on reducing the demineralisation at subsurface, but it was more effective on improving the remineralisation at surface and subsurface.

4.7 Acknowledgements

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4.8 References

1. Axelsson P. Preventive materials, methods and programs. Chicago: Quintessence Publishing, 2004: 656p.

2. Øgaard B, Rolla G, Helgeland K. Fluoride retention in sound and demineralized enamel in vivo after treatment with fluoride varnish (Duraphat). *Scandinavian Journal of Dental Research* 1984; **92**: 190-7.

3. ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. *Acta Odontologica Scandinavia* 1999; **57**: 325-9.

4. Seppa L. Fluoride varnishes in caries prevention. *Medical Principles Practice* 2004; **13**: 307-11.

5. Demito CF, Vivaldi-Rodrigues G, Ramos AL, Bowman SJ. The efficacy of fluoride varnish in reducing enamel demineralisation adjacent to orthodontic brackets: an in vitro study. *Orthodontic Craniofacial Research* 2004; **7**: 205-10.

6. Delbem ACB, Bergamaschi M, Sassaki KT, Cunha RF. Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralisation: pH-cycling study. *Journal of Applied Oral Science* 2006; **14**: 88-92.

7. Richards D. Fluoride varnish should be part of caries prevention programs. *Evidence Based Dentistry* 2006; **7**: 65-6.

8. Tranaeus S, Al-Khateeb S, Bjorkman S, Twetman S, Anqmar-Mansson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. *European Journal of Oral Sciences* 2001; **109**: 71-5.

9. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD et al. Fluoride Varnish efficacy in preventing early childhood caries. *Journal of Dental Research* 2006; **85**: 172-6.

10. Øggard B, Duschner H, Ruben J, Arends J. Microradiography and confocal laser scanning microscopy applied to enamel lesions formed in vivo with and without fluoride varnish treatment. *European Journal of Oral Sciences* 1996; **104**: 378-83.

11. Seppa L, Hausen H, Tuuti H, Luoma H. Effect of a sodium fluoride varnish on the progress of initial caries lesions. *Scandinavian Journal of Dental Research* 1983; **91**: 96-8.

12. Buyukyilmaz T, Øgaard B, Rolla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. *European Journal of Oral Sciences* 1997; **105**: 473-7.

13. van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment *in vitro*. *European Journal of Oral Sciences* 2003; **11**: 253-7.

14. Tveit AB, Hals E, Isrenn R, Totdal B. Highly acid SnF_2 and TiF_4 solution. Effect on and chemical reaction with root dentin in vitro. *Caries Research* 1983; **17**: 412-8.

15. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. *Caries Research* 2005; **39**: 371-9.

16. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. *Caries Research* 2006; **40**: 224-30.

17. Buyukyilmaz T, Øgaard B, Duschner H, Ruben J, Arends J. The caries-

preventive effect of titanium tetrafluoride on root surfaces in situ as evaluated by microradiography and confocal laser scanning microscopy. *Advances in Dental Research* 1997; **11**: 448-52.

18. Tezel H, Ergucu Z, Onal B. Effects of topical fluoride agents on artificial enamel lesion formation in vitro. *Quintessence International* 2002; **33**: 347-52.

19. Dibdin GH, Shellis RP. Physical and biochemical studies of streptococcus mutans sediments suggest new factor linking with cariogenicity of plaque with its extracellular polysaccharide content. *Journal of Dental Research* 1988; **67**: 890-5.

20. Queiroz CS. [In vitro study to evaluate the effect of fluoride on demineralisation and remineralisation of enamel and dentine] thesis. Piracicaba, Faculdade de Odontologia de Piracicaba, 2004.

21. Vieira AEM, Delbem ACB, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. *Caries Research* 2005; **39**: 514-20.

22. American Dental Association Council on Scientific Affairs. Professional applied topical fluoride: evidence-based clinical recommendations. *Journal of the American Dental Association* 2006; **137**: 1151-9.

23. Petersson LG, Twetman S, Dahlgren H, Norlund A, Holm AK, Nordenram G et al. Professional fluoride varnish treatment for caries control: a systematic review of clinical trials. *Acta Odontologica Scandinavia* 2004; **62**: 170-6

24. Hicks J, Wild T, Flaitz CM, Seybold S. Fluoride varnishes and caries development in primary tooth enamel: an in vitro study. *ASDC Journal of Dentistry for Children* 2001; **68**: 304-10.

25. Seppa L. Effects of a sodium fluoride solution and a varnish with different fluoride concentrations on enamel remineralisation in vitro. *Scandinavian Journal of Dental Research* 1988; **96**: 304-9

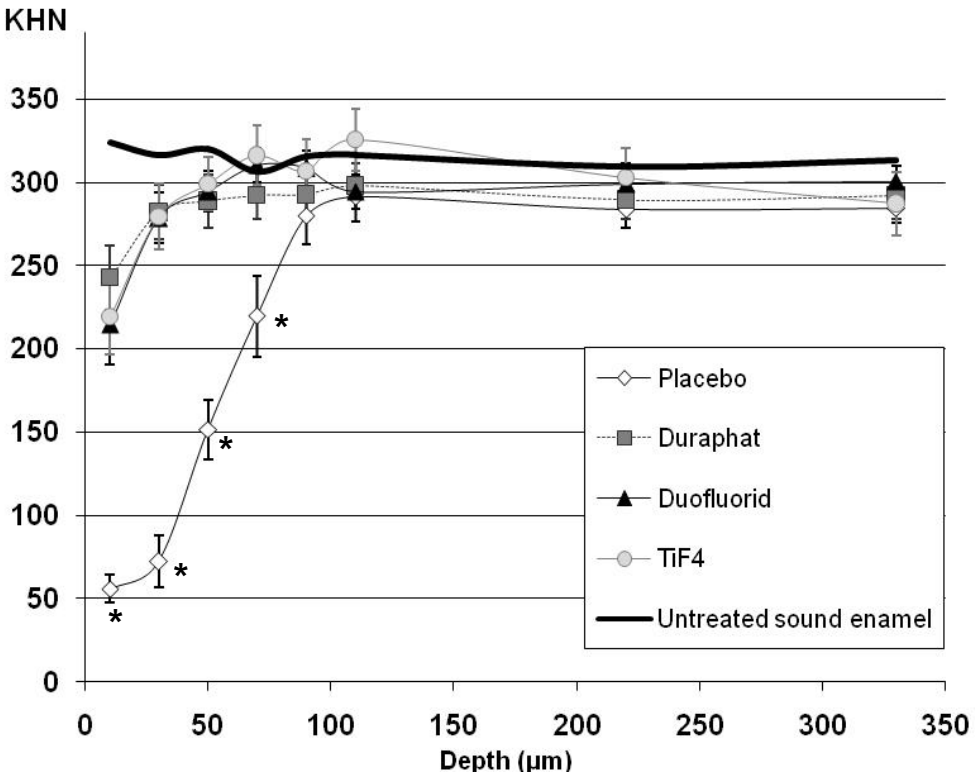
26. Chevitarese AB, Chevitarese O, Chevitarese LM, Dutra PB. Titanium penetration in human enamel after TiF_4 application. *Journal of Clinical Pediatric Dentistry* 2004; **28**: 253-6.

4.9 Table and Figures

4.9.1 Table 1. Mean % SHC of sound and carious enamel blocks according to the type of varnish

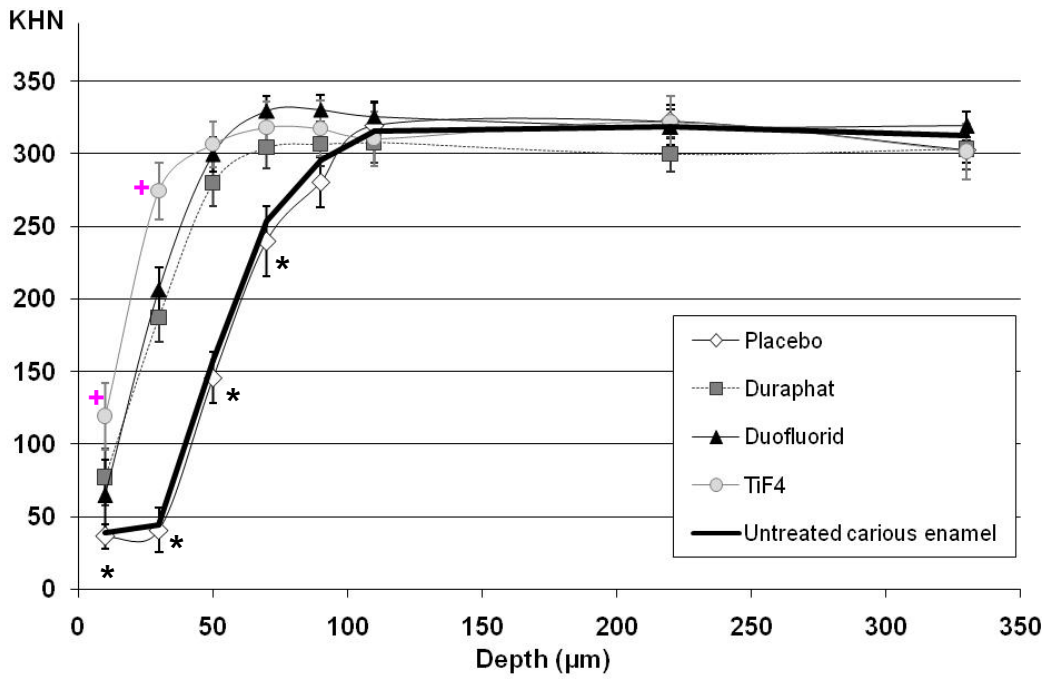
Treatment	Sound enamel	Carious enamel
Duraphat®-D	-27±4[a]	+13±4[a]
Duofluorid®-F	-27±6[a]	+14±4[a]
TiF_4-T	-38±8[b]	+27±4[b]
Placebo-P	-87±12[c]	-10±4[c]

Treatments whose means are followed by distinct letters in the same column differ significantly ($p<0.05$).



4.9.2 Figure 1. Mean of cross-sectional hardness vs. distance results for previous sound enamel.

* indicates significant less hardness values for the placebo treatment (up 70 µm) compared to all other groups, which in turn did not differ significantly from each other.



4.9.3 Figure 2. Mean of cross-sectional hardness vs. distance results for previous carious enamel.

* indicates significant less hardness values for the placebo treatment (up 70 µm) compared to all other groups. However, up to 30 µm depth, the TiF_4 treatment lead to significantly higher hardness values compared to the NaF vanishes (marked by +).

5 Capítulo 4

Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink

Short title: Effect of TiF_4 varnish on enamel erosion

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Keywords: dental erosion; enamel; fluoride; in vitro; titanium

Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink

5.1 Summary

Objectives: This *in vitro* study assessed the effect of an experimental 4% TiF_4 varnish on enamel erosion. *Methods:* Sixty bovine enamel blocks were randomly allocated to each type of varnish: Duraphat®-D (NaF, 2.26%F), Duofluorid®-F (NaF, 2.71% F), TiF_4-T (2.45%F) and no-fluoride–P. After the application of the varnishes, the blocks were subjected to 6 sequential pH cycles (cola drink for 10 min and artificial saliva for 50 min, each) per day, during 4 days. After the pH cycles, the blocks were maintained in artificial saliva for 18 h. Enamel alterations were determined in the 2nd and 4th days, using profilometry (wear) and microhardness (%SMHC) tests. Data were tested using ANOVA and Tukey`s tests ($p<0.05$). *Results:* The mean %SMHC (±SD) at the 2nd and 4th day was, respectively: D (-77.26±5.04^{a} and -88.59±5.11^{A}), F (-76.79±7.82^{a} and -88.78±6.10^{A}), T (-88.28±3.19^{b} and -92.04±2.54^{A,B}) and P (-87.96±2.23^{b} and -94.15±1.14^{B}). The mean wear (µm, ±SD) at the 2nd and 4th day was, respectively: D (3.16±0.32^{a} and 7.56±0.90^{A}), F (3.35±0.78^{a,b} and 7.92±0.98^{A}), T (3.81±0.43^{b} and 7.69±0.76^{A}) and P (3.43±1.13^{a,b} and 7.31±0.53^{A}). *Conclusions:* The NaF varnishes reduced the softening, but had no effect on the reduction of the wear. The TiF_4 varnish was not able to reduce the softening and wear.

5.2 Introduction

With the decline in the prevalence of dental caries in most developed countries[1], as well as in Brazil[2], an increasing interest in other dental disorders including tooth wear has arisen[3]. The term tooth wear is used to describe the processes of erosion, attrition and abrasion[4-6].

Dental attrition and abrasion are mechanical wear by tooth to tooth contact[4-6] and by abrasive substances[4-6], respectively. Dental erosion is the loss of tooth substance by chemical processes not involving bacteria[3,7,8]. The etiology of erosion is multifactorial and not fully understood. The most important sources of acids are those found in the diet, as acidic foods and drinks[9,10], and from the stomach, as gastric acids from regurgitation and reflux disorders[11,12]. In modern societies, the increased consumption of acid drinks as soft drinks, sport drinks, fruit juices and fruit teas is becoming a more important factor of tooth wear[7,9].

Many strategies have been used for prevention of dental erosion, such as the topical application of fluoride. Although the preventive action of fluoride on dental caries is well known[13,14], its role in erosion is still controversially discussed[15,16], since the deposited calcium fluoride-like material from topical fluoride application is supposed to dissolve readily in most acidic drinks[16]. However, high-concentrated fluoride applications, as oral rinses, gels or varnishes, have been demonstrated to increase abrasion resistance and decrease the development of erosion in enamel[17].

The fluoride agents that have been assessed in most *in vitro* studies for erosion prevention are those that have been used over years for caries prevention, such as sodium fluoride (NaF), acidulated phosphate fluoride (APF), stannous fluoride (SnF_2) or amine fluoride (AmF). More recently, other agents such as titanium tetrafluoride (TiF_4) have been investigated for erosion prevention[18-25]. With respect to TiF_4 solution, *in vitro* studies have shown its inhibitory effect on erosion[18,19,20,21,23]. However, other studies have shown the opposite[24,25].

As fluoride varnishes may be more effective than solution and gels in prevention of erosive defects[16] due to their better capability to adhere on the tooth surface and create a calcium fluoride reservoir[26], it seems to be interesting to analyze the effect of an experimental TiF_4 varnish on dental erosion. Taking into account these considerations, the purpose of this study was to assess the effect of an

experimental TiF_4 varnish compared to commercial NaF varnishes on bovine enamel subjected to erosion *in vitro.*

5.3 Material and Methods

5.3.1 Experimental design

Sixty enamel blocks were obtained from bovine teeth, polished and subjected to initial surface microhardness analysis. Enamel samples with microhardness ranging from 330 to 380 KHN were selected and randomly distributed into 4 groups: Duraphat® - D (NaF, 2.26%F, pH 4.5, Colgate, São Bernardo, São Paulo, Brazil, n=15), Duofluorid® - F (NaF, 2.71% F, pH 8.0, FGM, Joinvile, Santa Catarina, Brazil, n=15), TiF_4 -T (2.45%F, pH 1.0, FGM, Joinvile, Santa Catarina, Brazil, n=15) and no-fluoride – P (pH 5.0, FGM, Joinvile, Santa Catarina, Brazil, n=15). The basic formulation of F, T and P was the same, except for the fluoride compound. While Duofluorid® is transparent, the other varnishes present a yellow color. The varnishes were applied onto the enamel surfaces. In sequence, the blocks were subjected to 6 pH cycles per day for 4 days at 25°C. In each cycle, demineralization and remineralization were performed by immersion in cola drink (10 min) and artificial saliva (50 min), respectively. Each day, the 6 cycles were conducted sequentially (totalized 6 h) and the blocks were then immersed in artificial saliva for 18 h. Enamel alterations were determined at the 2nd and 4th day, using profilometry (wear) and microhardness (%SMHC) tests.

5.3.2 Enamel blocks preparation

Enamel blocks (4X4X2.5mm) were prepared from incisor bovine teeth, freshly extracted, sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. The teeth were cut using ISOMET Low Speed Saw cutting machine (Buehler Ltda., Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer. The enamel surfaces of the blocks were ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 µm; Buehler), resulting in removal of about 100 µm depth of the enamel which was controlled with a micrometer. The surface microhardness determination was performed by five indentations on center of the

surface of the blocks (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Enamel blocks with microhardness ranging from 330 to 380 KHN were randomly distributed into 4 groups. In order to maintain reference surfaces for lesion depth determination, two layers of nail varnish were applied on half of the surface of each block.

5.3.3 Treatment and pH-cycling

A thin layer of fluoride varnishes was applied with a microbrush on the enamel surface. In sequence, the blocks were subjected to 6 pH-cycles at the 1st day. In separate containers, the blocks were immersed in cola drink (Coca-Cola®, Spal, Porto Real, RJ, Brazil) at room temperature for 10 minutes (30 mL per block) and in artificial saliva [1.5 $mmolL^{-1}$ $Ca(NO_3)_2.4H_2O$, 0.9 $mmolL^{-1}$ $NaH_2PO_4.2\ H_2O$, 150 $mmolL^{-1}$ KCl, 0.1 $molL^{-1}$ Tris buffer, 0.03 ppm F, pH 7.0] for 50 min (15 mL per block)[27]. The Coca-Cola® (pH 2.9) presented a buffering capacity of 0.1 which is equivalent to 0.1 mL of 0.2 M NaOH/ 3 mL beverage to increase in one pH unit. After the first 6 hours, the varnishes were removed carefully using a surgical blade and cotton swabs soaked in acetone solution (1 pure acetone: 1 water)[28]. After that, the nail varnish was applied again on the reference surface.

This pH-cycling model was performed for additional 3 days. Each day, the 6 cycles were conducted sequentially and the blocks were then maintained in artificial saliva for 18 h (overnight).

5.3.4 Microhardness and wear determinations

Initially, enamel surface microhardness was measured as described above (Knoop diamond, 25 g, 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Five indentations, at distances of 100 µm from each other, were made in the center of enamel blocks (SMH). At 2nd and 4th day, the nail varnish over the surfaces was carefully cleaned with acetone-soaked cotton wool[29] and final microhardness test (SMH_1) was made. The percentage of surface microhardness change was calculated for both days as follows: $\%SMHC = 100(SMH_1 - SMH)/SMH$.

The enamel blocks were dried and the wear was determined in relation to the reference surface by profilometry using a rugosimeter (Hommel Tester T1000, VS, Schwenningen). At the 2nd and 4th day, five readings were performed on each specimen through scanning from the reference to the exposed surface and an

average of each group was obtained (µm). At the 2nd day, after the analysis, two layers of nail varnish were again applied on the reference surface of each block.

5.3.5 Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested. Since the assumptions were satisfied, ANOVA and Tukey's test were carried out for statistical comparisons and the significance limit was set at 5%.

5.4 Results

Tables 1 and 2 show the mean %SMHC (±SD) and the mean wear (µm, ±SD) at the 2nd and 4th day, respectively. The mean %SMHC (±SD) at the 2nd and 4th day was: D (-77.26±5.04^{a} and -88.59±5.11^{A}), F (-76.79±7.82^{a} and -88.78±6.10^{A}), T (-88.28±3.19^{b} and -92.04±2.54^{A,B}) and P (-87.96±2.23^{b} and -94.15±1.14^{B}). The mean wear (µm, ±SD) at the 2nd and 4th day was: D (3.16±0.32^{a} and 7.56±0.90^{A}), F (3.35±0.78^{a,b} and 7.92±0.98^{A}), T (3.81±0.43^{b} and 7.69±0.76^{A}) and P (3.43±1.13^{a,b} and 7.31±0.53^{A}).

The experimental varnish (T) did not significantly differ from the placebo varnish for both variables and periods evaluated. The commercial fluoride varnishes were able to significantly reduce the enamel softening at both 2nd and 4th days when compared to the placebo varnish ($p<0.0001$ and $p=0.0012$, respectively), but had no effect on the reduction of the enamel wear at both 2nd and 4th days ($p>0.05$).

5.5 Discussion

The TiF_4 solution has been tested in *in vitro* studies of dental erosion. Some publications attest its inhibitory effect on erosion[18,19,20,21,23], while others have shown the opposite[24,25]. The probable explanation for these contrasting results is the distinct protocol used in the diverse studies, as well as the distinct response variables analyzed. In the present study, in order to simulate the clinical situation when a professional application is conducted, the varnishes were applied only once. Since

the use of fluoride has been advocated for high erosion risk patients, a high erosive challenge was conducted (6 X 10 min per day, during 4 days).

As previously mentioned, professionally applied topical fluorides varnishes are recommended for individuals who present moderate to severe dental wear, to prevent or arrest new or recurrent dental wear[16]. This is the first study that evaluated the effect of a TiF_4 varnish on erosion. Most of the studies tested the effect of commercial varnishes that contain NaF, showing a reduction of erosion *in vitro* and *in situ* protocols[30,31]. In the present study, the commercial fluoride varnishes (NaF, positive controls) were able to reduce the enamel softening significantly, but they did not reduce the enamel wear. In the studies that show a protective effect of fluoride varnishes against dental erosion[30,31], the varnishes were not completely removed during the experimental period and this mechanic protection may have played a role on the protective effect found. In addition, the erosive challenges applied in these studies were less pronounced than the protocol we used. Since fluoride varnishes are professionally applied in high erosion risk patients, studies which simulate a high erosive challenge in a long experimental period should be conducted.

According to our protocol, the varnishes did not have a mechanical protective effect on enamel erosion, because they were removed after 6 h. This procedure simulates *in vivo* conditions, in which the varnish is gradually removed by toothbrushing and by mastication. Probably, the commercial NaF varnishes reacted chemically with enamel during the 6 h of contact, diminishing the %SMHC (softening), but this was not enough to reduce the enamel loss provoked by the high erosive attack. The both NaF varnishes presented the same F concentration; the difference between the pH of NaF varnishes did not influence their behavior in this protocol. Despite the fact that the NaF varnishes caused a significant reduction of softening, the %SMHC was very high in all the groups, indicating that the varnish application might not be effective in the clinical situation.

The experimental varnish had no effect on the reduction of enamel softening and enamel wear by erosion, when compared to the commercial varnishes. In fact, the TiF_4 varnish was similar to the placebo varnish. As previously mentioned, we are unaware of studies testing TiF_4 varnish, which makes the discussion of the results difficult. According to the literature, the beneficial action of TiF_4 solution on dental erosion has been attributed to its low pH (around 1.2), favoring the linking between titanium and oxygen of the group phosphate, thus leading to the formation of a

titanium dioxide glaze-like layer on the surface[18,21,22]. It is probable that in the present study this protective layer have been rapidly removed due to the high erosive challenge, thus not allowing the observation of such a protective effect.

5.6 Conclusion

In this *in vitro* protocol, the experimental TiF_4 varnish was not able to prevent dental erosion, while the commercial NaF varnishes had only a partial protective effect, probably due to the high erosive challenge used. Thus, more studies must be conducted before the fluoride varnishes can be widely used in the clinical situation as a preventive measure for dental erosion.

5.7 Acknowledgements

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5.8 References

1. Peterson G, Bratthall D. The caries decline: a review of reviews. *European Journal of Oral Sciences* 1996; **104**: 436-43.

2. Narvai PC, Frazao P, Roncalli AG, Antunes JL. Dental caries in Brazil: decline, polarization, inequality and social exclusion. *Revista Panamericana Salud Publica* 2006; **19**: 385-93.

3. Imfeld T. Dental erosion. Definition, classification and links. *European Journal of Oral Sciences* 1996; **104**: 151-5.

4. Addy M, Shellis RP. Interaction between Attrition, Abrasion and Erosion in Tooth Wear. *Monographs in Oral Science* 2006; **20**: 17-31.

5. Eccles JD. Tooth surface loss from abrasion, attrition and erosion. *Dental Update* 1982; **9**: 373-81.

6. Litonjua LA, Andreana S, Bush PJ, Cohen RE. Tooth wear: attrition, erosion, and abrasion. *Quintessence International* 2003; **34**: 435-46.

7. Lussi A. Erosive tooth wear - a multifactorial condition of growing concern

and increasing knowledge. *Monographs in Oral Science* 2006; **20**: 1-8.

8. Moss SJ. Dental erosion. *International Dental Journal* 1998, **48**: 529-39.

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

10. Lussi A, Jaeggi T. Chemical factors. *Monographs in Oral Science* 2006; **20**: 77-87.

11. Bartlett D. Intrinsic causes of erosion. *Monographs in Oral Science* 2006; **20**: 119-39.

12. Scheutzel P. Etiology of dental erosion-intrinsic factors. *European Journal* of Oral *Sciences* 1996; **104**:178-90.

13. Øgaard B. Effects of fluoride on caries development and progression in vivo. *Journal of Dental Research* 1990; **69**: 813-9.

14. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *European Journal of Oral Sciences* 1997; **105**: 461-5

15. Larsen MJ, Richards A. Fluoride is unable to reduce dental erosion from soft drinks. *Caries Research* 2002; **36**: 75-80.

16. Wiegand A, Attin T. Influence of fluoride on the prevention of erosive lesions – a review: *Oral Health & Preventive Dentistry* 2003;**1**: 245-53.

17. Ganss C, Klimek J, Brune V, Schumann A. Effects of two fluoridation measures in erosion progression on enamel and dentine in situ. *Caries Research* 2004; **38**: 561-6.

18. Buyukyilmaz T, Øgaard B, Rolla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. *European Journal of Oral Sciences* 1997; **105**: 473-7.

19. van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment *in vitro*. *European Journal of Oral Sciences* 2003; **11**: 253-7.

20. 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 Research* 2007; **41**: 141-5.

21. Tveit AB, Hals E, Isrenn R, Totdal B. Highly acid SnF_2 and TiF_4 solution. *Caries Research* 1983; **17**: 412-8.

22. Vieira A, Ruben JL, Stokroos I, Huysmans MCDNJM. Surface and surface analysis of eroded bovine enamel pretreated with fluoride. *Caries Research* 2004; **38** (Suppl): 391.

23. Ganss C, Schlueter N, Muller U, Klimek J. Effect of TiF_4 on erosion progression in enamel in vitro. *Caries Research* 2004, **38** (Suppl): 392.

24. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. *Caries Research* 2005; **39**: 371-9.

25. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. *Caries Research* 2006; **40**: 224-30.

26. Seppa L. Fluoride varnishes in caries prevention. *Medical Principles Practice* 2004; **13**: 307-11.

27. Vieira AEM, Delbem ACB, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. *Caries Research* 2005; **39**: 514-20.

28. Delbem ACB, Bergamaschi M, Sassaki KT, Cunha RF. Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study. *Journal of Applied Oral Science* 2006; **14**: 88-92.

29. Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. *Caries Research* 2000; **34**: 48-52.

30. Sorvari R, Meurman JH, Alakuijala P, Frank RM. Effect of fluoride varnish and solution on enamel erosion in vitro. *Caries Research* 1994; **28**: 227-32.

31. Vieira A, Jager DHJ, Ruben JL, Huysmans MCDNJM. Inhibition of erosive wear by fluoride varnish. *Caries Research* 2007; **41**: 61-7

5.9 Tables

5.9.1 Table 1. Mean and standard deviation of %SMHC for each experimental group.

%SMHC mean* ± SD				
Day	**Duraphat**	**Duofluorid**	**TiF_4**	**No-F**
2nd	-77.26 ± 5.05 [a]	-76.79± 7.82 [a]	-88.28 ± 3.20 [b]	- 87.96 ± 2.23 [b]
4th	-88.59 ± 5.11 [A]	-88.78± 6.11 [A]	-92.04 ± 2.55 [A,B]	-94.15± 1.14 [B]

Values in the same line followed by distinct superscripts indicate statistical significance ($p<0.05$)

5.9.2 Table 2. Mean and standard deviation of wear (µm) for each experimental group.

Wear (µm) mean* ± SD				
Day	**Duraphat**	**Duofluorid**	**TiF_4**	**No-F**
2nd	3.16 ± 0.32 [a]	3.35± 0.78 [a,b]	3.81 ± 0.43 [b]	3.43 ± 1.13 [a,b]
4th	7.56 ± 0.90 [A]	7.92± 0.98 [A]	7.69 ± 0.76 [A]	7.31± 0.53 [A]

Values in the same line followed by distinct superscripts indicate statistical significance ($p<0.05$)

6 Capítulo 5

The effect of 4% titanium tetrafluoride varnish and solution on dental erosion in vitro

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6.1 Abstract

This in vitro study assessed the effect of an experimental 4%TiF_4 varnish compared to commercial NaF varnishes and 4%TiF_4 solution on enamel erosion. For this, bovine enamel samples (n=72) were randomly allocated to the following treatments: Duraphat® (NaF, 2.26%F, Colgate-Brazil), Duofluorid® (NaF, 2.71%F, CaF_2, 2.92% F, FGM-Brazil), 4% TiF_4 varnish (2.45%F, FGM-Brazil), placebo varnish (FGM-Brazil), 4% TiF_4 solution (2.45%F) and control (not treated). The varnishes were applied in a thin layer and removed after 6 h. The solution was applied onto the enamel surface for 1 min. Then, the samples were transferred to a so-called artificial mouth for 5 days at 37°C, allowing alternating re- and demineralization (6 times/day). Demineralization was performed with the beverage Sprite® (1 min, 3 mL/min) and remineralization with artificial saliva (at day: 59 min, 0.5 mL/min, during night: 0.1 mL/min). Enamel wear was determined by profilometry each day. Regarding the cumulative enamel loss, the best preventive effect was observed for the TiF_4 varnish. The NaF varnishes were not different from each other, but showed a better protective effect when compared to the TiF_4 solution, placebo varnish and control. When the enamel loss was analyzed per day, only TiF_4 varnish was able to reduce the progression of erosion up to the 3rd day. The TiF_4 solution, however, was not able to reduce the erosive enamel wear. In conclusion, the TiF_4 varnish seems to be a promising treatment to reduce enamel loss under mild erosive conditions.

6.2 Introduction

Dental erosion is the loss of tooth substance by chemical processes not involving bacteria [Imfeld, 1996; Moss, 1998; Lussi, 2006]. Although a multitude of factors seem to be involved in this process, the most important factors are dietary acids [Lussi et al., 2004; Lussi and Jaeggi, 2006] and intrinsic acids from the stomach [Scheutzel, 1996; Bartlett, 2006]. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor for the development of erosive wear [Lussi et al., 2004; Lussi, 2006].

As it is difficult to control possible etiological factors, many strategies have been developed for the prevention of erosion, such as the topical application of fluoride. Although the preventive action of fluoride on dental caries is well known [ten Cate, 1997], its role in erosion is still controversially discussed [Larsen and Richards, 2002; Wiegand and Attin, 2003], since the deposited calcium fluoride-like material from topical fluoride application is supposed to readily dissolve in most acidic drinks [Ganss et al., 2007].

The formation of the CaF_2-like layer and its protective effect on demineralization depend on the pH, F concentration and type of F salt of the agent [Saxegaard and Rölla 1988]. High-concentrated fluoride applications, such as oral rinses, gels or varnishes, have been demonstrated to increase abrasion resistance and decrease the development of enamel erosion in vitro and in situ [Ganss et al., 2004; Lagerweij et al. 2006; Vieira et al. 2006]. Most in vitro studies focusing on the preventive effect of fluoride on erosion used fluoride compounds which have been used over years in caries prevention, such as NaF, AmF, SnF_2 or acidulated phosphate fluoride (APF). More recently, other agents, as titanium tetrafluoride (TiF_4), have been investigated for erosion prevention [Tveit et al., 1983; Buyukyilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005; Vieira et al., 2006; Hove et al., 2006; Hove et al., 2007; Schlueter et al., 2007].

With regard to TiF_4 solution, several in vitro studies have shown an inhibitory effect on dental erosion [Buyukyilmaz et al., 1997; van Rijkom et al., 2003; Hove et al., 2006; Hove et al., 2007; Schlueter et al., 2007], which is attributed not only to the effect of fluoride, but also to the action of titanium [Buyukyilmaz et al., 1997; Tezel et al., 2002]. It is speculated that the titanium ions might play an important role as they might substitute calcium in the apatite lattice and show a strong tendency to complex

with phosphate groups, forming stable titanium dioxide layer [Mundorf et al., 1972; Tveit et al., 1983; Buyukyilmaz et al., 1997; Tezel et al., 2002; Ribeiro et al., 2006; Magalhães et al., 2007a]. Moreover, it is suggested that titanium interacts with the enamel surface, due to the low pH of the agent, thus leading to an increased fluoride uptake by enamel [Mundorf et al., 1972; Gu et al., 1996]. However, other studies did not found a protective effect of TiF_4 against erosion or combined erosion and abrasion [Vieira et al., 2005; Vieira et al., 2006].

Due to their better capability to adhere on the tooth surface and create a calcium fluoride reservoir [Sorvari et al., 1994; Vieira et al., 2007], fluoride varnishes might be more effective than solutions and gels in the prevention of erosive defects [Sorvari et al., 1994; Vieira et al., 2007]. Thus, it was proposed the use of an experimental TiF_4 varnish for the prevention of erosive wear. In a previous study, the TiF_4 varnish was not effective to reduce enamel loss under severe erosive conditions (10 min erosion, 6 times daily) [Magalhães et al., 2007b]. Thus, the present study aimed to test the same TiF_4 varnish compared to a TiF_4–solution (actually the most investigated form of TiF_4 application) and commercial NaF varnishes (as positive controls) in an in vitro model characterized by a mild erosive challenge.

6.3 Material and Methods

6.3.1 Samples preparation

Seventy-two crowns of bovine incisors were embedded in acrylic resin cylinders (Paladur, Heraeus Kulzer, Wehrheim, Germany), and the labial surfaces were ground flat and polished with water-cooled carborundum paper (500, 800, 1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrat, Germany). Thereby, approximately 200 µm of the outer enamel layer was removed. Surface microhardness of enamel samples was determined as a criterion for stratified allocation of the samples among 6 groups (n = 12 specimens/group). Prior to the experiment, surface baseline scans were obtained from the samples by profilometry. Reference areas on the polished enamel surface were covered with parallel strips of adhesive tape (Tesa, Beiersdorf, Hamburg, Germany), at 1.5 mm apart. After preparation, the samples were stored in water until used for the experiment to avoid dehydration.

6.3.2 Fluoride pre-treatment

Prior to acid exposure, the samples were pre-treated with one of the respective fluoride varnishes (Duraphat®: NaF, 2.26% F, pH 4.5, Colgate-Brazil; Duofluorid®: NaF, 2.71% F, CaF_2, 2.92% F, pH 8.0, FGM-Brazil; 4% TiF_4 varnish: 2.45 % F, pH 1.0, FGM-Brazil; placebo varnish: no fluoride, pH 5.0, FGM-Brazil) or the 4% TiF_4 solution (2.45% F, pH 1.0). While Duofluorid® is transparent; the other varnishes present a yellow color. The basic composition of the Brazilian varnishes (FGM), according to the manufacturer, is: colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol. As for Duraphat®, the composition informed by the manufacturer is: 2.26% NaF, 33.14% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. For preparing the 4% (1.29 M F) TiF_4 solution, solid TiF_4 (Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in deionized water. The pH of all solution/varnishes was measured by indicator paper (±0.5 unit).

The application of a thin layer of varnishes and the solution was done with a microbrush on the enamel surface. The solution was left on the surface undisturbed until the surface appeared dry. Additional drops were applied in the same manner until 1 min had elapsed [van Rijkom et al., 2003]. After 1 min, the excess of solution was removed from the surface by a cotton roll and the samples were stored in artificial saliva [Klimek et al., 1982].

The varnishes were applied in a thin layer and the samples were stored in artificial saliva. After 6 h, the varnishes were carefully removed using acetone and a scalpel blade, taking care to avoid touching of the enamel surface [Delbem et al., 2006; Vieira et al., 2006]. The complete removal of the varnish layer was checked microscopically. Prior to cycling treatment, surface wear due to application of the varnishes or solution was determined by profilometry (Table 1). Twelve enamel samples were not pre-treated with any fluoride varnish or solution and served as control.

6.3.3 pH-cycling in the artificial mouth

For pH cycling, the specimens were mounted in a so-called artificial mouth [Attin et al., 2003; Lennon et al., 2006; Lagerweij et al., 2006] for 5 days allowing for alternating de- and remineralization treatment. The artificial mouth consisted of 12 chambers, which were heated to 37°C, and was equipped with two automatic

multichannel pumps (IPC/IPC-N Kassetten-Schlauchpumpen, Ismatec SA, Glattbrugg-Zürich, Switzerland). Temperature and pumps were controlled by a computer and customized software. The artificial mouth was programmed so that the specimens were rinsed with Sprite (3 mL/min, Coca-Cola Company, USA, pH 2.6) 6 times a day for 1 min each. Among the erosive challenges, the specimens were rinsed continuously with artificial saliva [Klimek et al., 1982] (0.5 mL/min, 59 min). The composition of the artificial saliva was: 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and ascorbic acid. After 6 de- and remineralization treatments, enamel surface loss was determined profilometrically, and the samples were submitted to the artificial mouth overnight (artificial saliva, 0.1 mL/min, 18 h).

6.3.4 Profilometric measurement of enamel loss

Surface profiles of the enamel samples were obtained with a contact profilometer (Mahr Perthometer, Göttingen, Germany) at baseline (prior to the experiment), after the respective fluoride pre-treatment and after each 5 daily de- and remineralization cycles. The reference areas, which remained protected by tape during fluoride pre-treatment and during the entire daily de- and remineralization cycling, were marked with a scalpel blade on the outer surface to allow for exact reposition of the tape. Prior to the experiment, five equidistant baseline surface scans of each specimen were performed. For determination of enamel loss the tape was removed and five profiles were recorded at exactly the same sites as for baseline measurement. For this, the enamel samples were provided with identification marks, which allows for the exact reposition of the stylus at each measurement. The profile scans were performed in the centre of each specimen at intervals of 250 µm. Pre- and post-treatment scans were superimposed and the average depth of the area under curve in the eroded area was calculated with specially designed software. The results of the five scans at each day were averaged for each specimen.

6.3.5 Scanning Electron Microscopy (SEM)

The surfaces of two specimens treated with TiF_4 varnish and TiF_4 solution were checked by SEM. The specimens were mounted and sputter-coated with palladium-gold in a Hammer VI cathodic evaporator (Anatech LTD, Alexandria, USA).

They were then examined and photographed in a JEOL JSM T220A scanning electron microscope operating at 10kV.

6.3.6 Statistical analysis

The softwares GraphPad Prism 4 version 4.0 for Windows and GraphPad InStat version 3.0 for Windows, Graph Pad Software (San Diego, CA, USA) were used. The assumptions of equality of variances and normal distribution of data were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. Since the assumptions were satisfied, two-way repeated measures ANOVA and Bonferroni *post hoc* test were used. The significance level was set at 5%.

6.4 Results

Figure 1 shows the mean cumulative wear (µm, ±SD) at each day. Two-way repeated measures ANOVA revealed a significant difference among the treatments ($F=18$, $p<0.0001$) and among the days ($F=1,47$, $p<0.0001$). The interaction between these criteria was also significant ($F=22$, $p<0.0001$). The best preventive effect was observed for the TiF_4 varnish, which significantly reduced the wear during the whole experimental period when compared to the placebo varnish, control and TiF_4 solution ($p<0.05$). When compared to the NaF varnishes (Duraphat and Duofluorid), the TiF_4 varnish significantly reduced the cumulative wear at days 2-5 ($p<0.05$). Both NaF varnishes had a similar behavior, for all the tested days (n.s.). The TiF_4 solution was not significantly different from the placebo varnish and control throughout the experimental period (n.s.). In addition, at day 5, the cumulative wear in the presence of the TiF_4 solution was significantly higher when compared to the NaF varnishes ($p<0.05$).

Table 1 shows the daily enamel wear (µm, ±SD). The two-way repeated measures ANOVA revealed a significant difference among the treatments ($F=32.57$, $p=0.000000$), but not among the days ($F=1.07$, n.s.). The interaction between these criteria was also significant ($F=4.70$, $p=0.00000$). The best preventive effect was observed for the TiF_4 varnish, which significantly reduced the progression of the enamel wear up to the 3rd day when compared to the placebo varnish, control and TiF_4 solution ($p<0.05$). However, when compared to the NaF varnishes (Duraphat

and Duofluorid), the TiF_4 varnish presented the same wear progression at days 2-5 ($p<0.05$). Both NaF varnishes had a similar behavior, for all the tested days (n.s.), but they did not differ from the control and placebo varnish (n.s.). The TiF_4 solution was not significantly different from the placebo varnish and the control up to the 4th day (n.s.).However, on day 5, the progression of enamel wear was significantly higher for the TiF_4 solution than for the other groups ($p<0.05$).

The SEM images show that for enamel treated with the TiF_4 varnish, the surface presented more "CaF_2 –like" globules compared to the control area of the same sample (Figures 2 and 3). In contrast to the TiF_4 varnish, the TiF_4 solution induced a demineralised surface with microcracks (Figure 4), which is not seen at the control area of the same sample (Figure 5).

6.5 Discussion

TiF_4 solutions have been tested in in vitro studies about dental erosion. Some publications attest their inhibitory effect on erosion [Buyukyilmaz et al., 1997; van Rijkom et al., 2003; Hove et al. 2006; Schlueter et al., 2007; Hove et al., 2007], while others have shown no preventive impact [Vieira et al., 2005; Vieira et al., 2006, Magalhães et al., 2007a]. The probable explanation for these conflicting results is the different protocol used in the diverse studies, as well as the different response variables analyzed.

In the present study, the varnishes and the solution were applied only once in order to simulate the clinical situation with a single professional application by a dentist. A mild erosive challenge of the samples was conducted during 5 days to simulate a frequent acid contact in the in vivo situation. As done in several studies before [Attin et al., 2003; Lagerweij et al., 2006; Lennon et al., 2006], enamel erosion was assessed by contact profilometry. It must be taken into consideration that the stylus might be able to scratch the acid softened surface [Barbour and Rees, 2004]. However, even considering that the stylus might damage the surface to a small extent, this phenomenon would occur in all the groups, thus not interfering in the final results.

In order to improve the erosion protective efficacy of TiF_4 by increasing the adherence to dental hard tissues, the TiF_4 was applied in the form of a varnish. Most of the studies tested the effect of commercial varnishes containing NaF and found a

reduction of erosion in vitro and in situ protocols [Sorvari et al., 1994; Vieira et al., 2005; Vieira et al., 2006; Vieira et al., 2007]. In the present study, the NaF-varnish Duraphat was able to reduce the cumulative enamel loss significantly when compared to the placebo varnish and control especially at the 1st day and after the 3rd day, while for Duofluorid this was only observed at the 5th day. The different behavior of the varnishes, which contain similar amounts of F in form of NaF, may be explained by their different pHs. Duraphat has an acidic pH, while Duofluorid has an alkaline pH. It is known that the formation of the CaF_2 reservoir is increased under acidic compared to neutral conditions [Saxegaard and Rölla, 1988; ten Cate, 1997].

However, when the progression of enamel wear was considered for each day, the NaF varnishes did not differ from each other, neither from the placebo varnish and the control. Even when the NaF varnishes were shown to reduce enamel erosion compared to the placebo and the control when considering cumulative wear, they failed to reach significance when considering progression of enamel loss. Thus, it might be assumed that their efficacy is due to the first day and that these commercial varnishes are less effective in reducing the progression of enamel erosion for long time.

In contrast to the most of the studies showing a protective effect of fluoride varnishes against dental erosion [Vieira et al., 2005; Vieira et al., 2006; Vieira et al., 2007], the varnishes were completely removed after 6 h in the present study [Delbem et al., 2006]. This was done because it was focused on the chemical effect of the varnishes (NaF x TiF_4) rather than on the mechanical protection. Thus, the varnishes were removed to simulate the clinical situation in which the varnishes might be removed by toothbrushing or mastication after some hours. This fact is important especially for eroded surfaces (vestibular, lingual and occlusal), which are usually more susceptible to mechanical forces.

The experimental TiF_4 varnish showed the best protective effect in the present model, especially in reducing the progression of enamel wear up to the 3rd day, which resulted in a reduction of cumulative enamel loss for the whole experimental period. Thus, probably, in the clinical situation this experimental varnish has to be applied frequently for being effective. The durability of the protective effect of TiF_4 varnish has to be evaluated in further studies, using experimental times higher than 5 days.

It might be assumed that a protective “layer” has been formed by application of TiF_4 varnish, which acted protectively against enamel erosion during the erosive

challenges. In a previous study performed by our research group [Magalhães et al, 2007b], no protective effect of a TiF_4 varnish under severe erosive conditions (240 min of erosion by cola drink) could be observed. In contrast, the erosive challenge (30 minutes of erosion by sprite) applied in the present study is supposed to be not able to completely remove the protective glaze layer of the varnish.

On the other hand, the TiF_4 solution did not present a preventive effect during the 5 days of de- and remineralisation. Enamel samples treated with the TiF_4 solution presented more enamel loss when compared to the other groups at day 5. It seems that the "layer" formed after application of the TiF_4 solution might have been removed, leading to the exposure of the subjacent enamel, which is softened due to the low pH of the TiF_4 solution and thus more prone to erosion. This hypothesis is supported by the study by Magalhães et al. [2007a], which showed high amounts of titanium after the application of a 4% TiF_4 solution on human enamel surface by SEM-EDS; but less than half could be detected after 5 days of erosion in situ.

A possible explanation for the different behavior of the TiF_4 when employed in the form of a varnish or solution is that a thicker and/or more stable layer has been formed upon application of the varnish. The SEM images show that for enamel treated with the TiF_4 varnish, the surface presented more "CaF_2 –like globules" compared to the control area (Figures 2 and 3). However, SEM analysis does not allow for determination of the type of compound formed on the enamel surface. In contrast to the TiF_4 varnish, the enamel treated with TiF_4 solution showed a demineralized surface with microcracks (Figure 4) compared to the control area (Figure 5). In agreement to the results of Magalhães et al [2007a], the TiF_4 solution induced some enamel loss during application, as it was showed in the present study (Table 1).

These different findings of the SEM analysis might be related to the better capability of the varnish to adhere onto the tooth surface, which allows for an increased contact time with the enamel (6 h for the varnish versus 60 s for the solution), reducing the effects of pH of the solution/varnish (pH 1.0) on the surface and increasing the reaction between the TiF_4 and enamel [Vieira et al., 2006; 2007].
Since Vieira et al. [2005; 2006] did not find a protective effect of a TiF_4 solution on combined enamel erosion and abrasion, it would be interesting to test if the efficacy of this experimental varnish is affected by abrasive forces too. Moreover, the effect of this varnish on human enamel should be evaluated. Hove et al. [2007] showed that

that the protective effect of TiF_4 solution was better for bovine compared to human enamel, especially when a salivary pellicle was present on the surface.

Under the conditions of the present in vitro protocol, it can be concluded that the TiF_4 varnish, but not the TiF_4 solution, was able to reduce erosive bovine enamel wear. Moreover, the TiF_4 varnish showed better results when compared to the commercial NaF varnishes. However, more studies must be conducted in situ and in vivo, before the TiF_4 varnish can be widely used in the clinical situation.

6.6 Acknowledgements

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6.7 References

Attin T, Meyer K, Hellwig E, Buchalla W, Lennon AM: Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol 2003;48:753-759.

Bartlett D: Intrinsic causes of erosion. Monogr Oral Sci 2006;20:119-139.

Barbour ME, Rees JS: The laboratory assessment of enamel erosion: a review. J Dent 2004;32:591-602.

Buyukyilmaz T, Øgaard B, Rolla G: The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci 1997;105:473-477.

Delbem ACB, Bergamaschi M, Sassaki KT, Cunha RF: Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study. J Appl Oral Sci 2006;14:88-92.

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

Ganss C, Schlueter N, Friedrich D, Klimek J: Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions- A comparison of in vitro and in situ results. Arch Oral Biol 2007;52:9-14.

Gu Z, Li J, Söremark R: Influence of tooth surface conditions on enamel fluoride uptake after topical application of TiF_4 in vitro. Acta Odontol Scand 1996;**54**:281.

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.

Hove LH, Young A, Tveit AB: An in vitro study on the effect of TiF_4 treatment against erosion by hydrochloric acid on pellicle-covered enamel. Caries Res 2007;41:80-84.

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

Klimek J, Hellwig E, Ahrens G: Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. Caries Res 1982;16:156-161.

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 Res 2006;40:148-153.

Larsen MJ, Richards A: Fluoride is unable to reduce dental erosion from soft drinks. Caries Res 2002;36:75-80.

Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T: Effect of a casein/calcium phosphate containing tooth cream and fluoride on enamel erosion in vitro. Caries Res 2006;40:154-157.

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

Lussi A, Jaeggi T: Chemical factors. Monogr Oral Sci 2006;20:77-87.

Lussi A: Erosive tooth wear - a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006;20:1-8.

Magalhães AC, Rios D, Honório HM, Jorge Junior AM, Delbem ACB, Buzalaf MAR: Effect of 4% titanium tetrafluoride solution on dental erosion by a soft drink: an in situ/ex vivo study. Arch Oral Biol. 2007a:*in press*.

Magalhães AC, Stancari FH, Rios D, Buzalaf MAR: Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink. J Dent 2007b;35: 858-861.

Moss SJ: Dental erosion. Int Dent J 1998,48:529-539.

Mundorff SA, Little MF, Bibby BG: Enamel dissolution. 2. Action of titanium tetrafluoride. J Dent Res 1972;51:1567-1571.

Ribeiro CC, Gibson I, Barbosa MA: The uptake of titanium ions by hydroxiapatite particles - structural changes and possible mechanisms. Biomaterials 2006;27:1749-1761.

Saxegaard E, Rolla G: Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res 1988;96:523-535.

Scheutzel P: Etiology of dental erosion-intrinsic factors. Eur J Oral Sci 1996;104:178-190.

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.

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

ten Cate JM: Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci 1997;105:461-465.

Tezel H, Ergucu Z, Onal B: Effects of topical fluoride agents on artificial enamel lesion formation in vitro. Quintessence Int 2002;33:347-352.

Tveit AB, Hals E, Isrenn R, Totdal B: Highly acid SnF_2 and TiF_4 solution. Caries Res 1983;17:412-418.

van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J: Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment *in vitro*. Eur J Oral Sci 2003;11:253-257.

Vieira A, Jager DHJ, Ruben JL, Huysmans MCDNJM: Inhibition of erosive wear by fluoride varnish. Caries Res 2007;41:61-67.

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

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

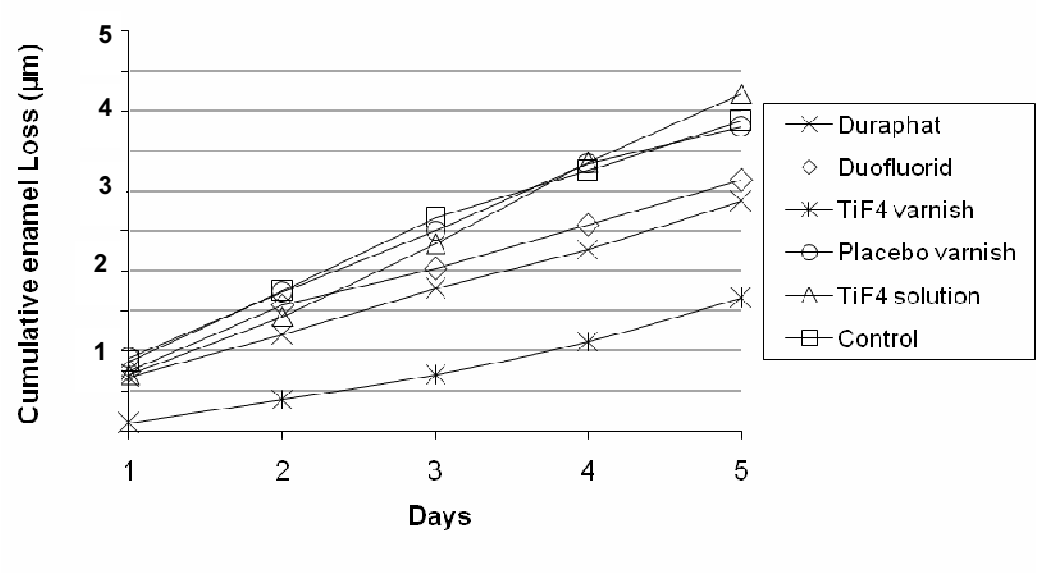
Wiegand A, Attin T: Influence of fluoride on the prevention of erosive lesions – a review. Oral Health Prev Dent 2003;1:245-253.

6.8 Table and Figures

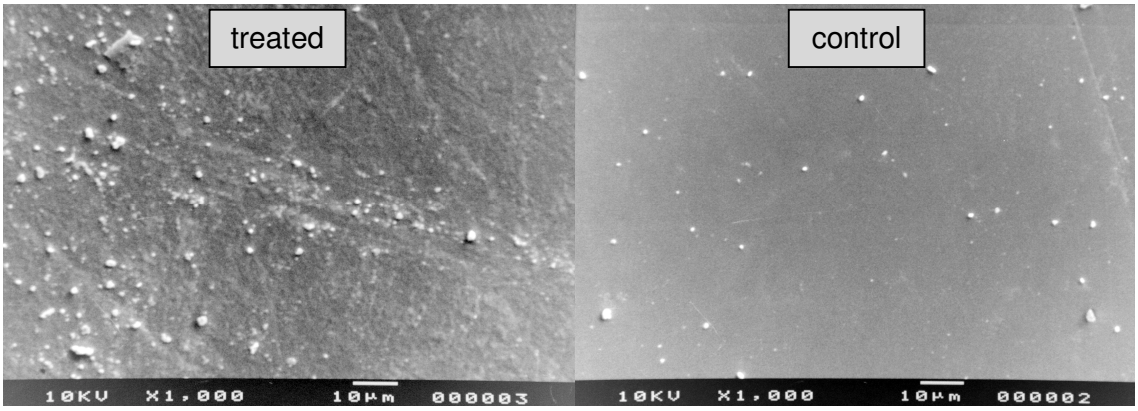
6.8.1 Table 1. Mean and standard deviation of enamel loss (µm) per day for each experimental group during the 5 days.

Wear (µm) mean* ± SD						
Days	**Duraphat**	**Duofluorid**	**TiF_4 varnish**	**Placebo varnish**	**TiF_4 solution**	**Control**
Treatment	0.01±0.10	-0.01±0.10	0.12±0.18	-0.02±0.10	-0.24±0.14	_____
1st	-0.68±0.27 a	-0.68±0.23 a	-0.10±0.25 b	-0.91±0.32 a	-0.73±0.17 a	-0.90±0.23 a
2nd	-0.59±0.18 a,b	-0.83±0.27 a	-0.27±0.16 b	-0.88±0.21 a	-0.78±0.20 a	-0.94±-0.34 a
3th	-0.57±0.18 b,d	-0.45±0.12 b,c	-0.27±0.13 b	-0.78±0.14 a,c,d	-0.92±0.22 a,d	-0.91±-0.21 a,d
4th	-0.49±0.17 b,c	-0.55±0.21 b,c	-0.41±0.17 b	-0.85±0.24 a,c	-1.02±0.21 a	-0.61±-0.15 b
5th	-0.58±0.10 a	-0.59±0.24 a	-0.49±0.28 a	-0.48±0.14 a	-0.86±0.21 b	-0.58±0.35 a

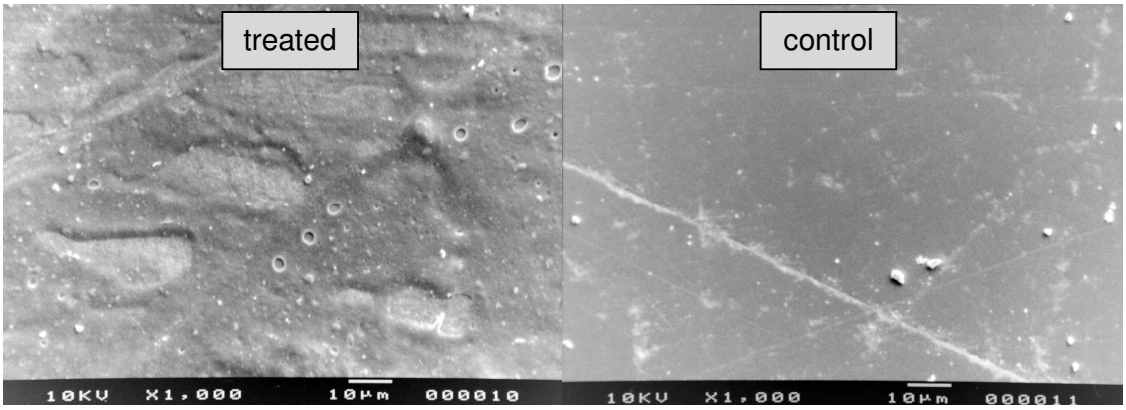
Values in the same line followed by different superscripts indicate statistical significance (n=12, $p<0.05$).



6.8.2 Figure 1. Mean of the cumulative enamel loss (µm) for each experimental group during the 5 days. The TiF_4 varnish significantly reduced the wear during the whole experimental period when compared to the placebo varnish, control and TiF_4 solution ($p<0.05$). When compared to the NaF varnishes, the TiF_4 varnish significantly reduced the cumulative wear at days 2-5 ($p<0.05$).



6.8.3 Figures 2 and 3. SEM-Images from samples treated with 4 % TiF_4 varnish, showing more "CaF_2-like" globules when compared to the control area of the same sample.



6.8.4 Figures 4 and 5. SEM-Images from samples treated with 4% TiF_4 solution, showing demineralized areas and microcracks when compared to the control area of the same sample.

Anexo A

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Anexo B

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
PABX (0XX14)3235-8000 – FAX (0XX14)3223-4679

Comitê de Ética em Pesquisa

Processo nº 47/2005

Bauru, 05 de julho de 2005.

Senhora Professora,

O projeto de pesquisa encaminhado a este Comitê de Ética em Pesquisa em Seres Humanos, denominado "**Influência de diferentes íons na erosão dentária**", de autoria de Ana Carolina Magalhães, que será desenvolvido sob sua orientação, foi enviado ao relator para avaliação.

Na reunião de 01 de julho de 2005 o parecer do relator, **aprovando o projeto**, foi aceito pelo Comitê, considerando que não existem infrações éticas pendentes.

Informamos que após o envio do trabalho concluído, este Comitê enviará o parecer final, que será utilizado para publicação do trabalho.

Atenciosamente,

Prof. Dr. José Henrique Rubo
Coordenador

Ilmª Srª **Profª Drª Marília Afonso Rabelo Buzalaf**
DD. Docente do Departamento de Ciências Biológicas

Anexo C

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
PABX (0XX14)3235-8000 – FAX (0XX14)3223-4679

Comitê de Ética em Pesquisa (3235-8356)

Processo nº 78/2005

Bauru, 30 de setembro de 2005.

Senhor Professor,

Informamos que após o envio da documentação solicitada referente ao projeto de pesquisa encaminhado a este Comitê de Ética em Pesquisa **"Avaliação do efeito do tetrafluoreto de titânio na diminuição da erosão do esmalte humano permanente e decíduo"** de autoria de Ana Carolina Magalhães, que será desenvolvido sob sua orientação, foi novamente analisado e considerado **APROVADO** em reunião deste Comitê realizada no dia 28 de setembro de 2005.

Informamos ainda, que após o envio do trabalho concluído, este Comitê enviará o parecer final, que será utilizado para publicação do trabalho.

Atenciosamente,

Prof. Dr. José Henrique Rubo
Coordenador

Ilmª Srª **Profª Drª Marília Afonso Rabelo Buzalaf**
DD. Docente do Departamento de Ciências Biológicas

Anexo D

JOURNAL OF DENTISTRY – GUIDE FOR AUTHORS

Submissions

The requirements for submission are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," Annals of Internal Medicine, 1977, **126**, 36-47.

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- Review articles: maximum length 10 printed pages, approximately 33 typescript pages, including illustrations and tables.
- Short communication for rapid publication: maximum length 2 printed pages, approximately 7 typescript pages, including illustrations.
- Letters providing informed comment and constructive criticism of material previously published in the Journal.
-Authors are urged to write as concisely as possible.

Articles should be arranged in the following order. *Title, Summary, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, References, Tables* and *Legends to Illustrations*.

Summary: should not exceed 250 words and should be presented under the following subheadings: Objectives, Methods; Results; Conclusions (For Reviews: Objectives; Data; Sources; Study selection;

Conclusions). These subheadings should appear in the text of the summary. Please repeat the title of the article at the top of the abstract page.

Introduction: must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question; the essence of existing knowledge and understanding pertinent to the issue; and the aims and objectives of the research being reported.

Keywords: up to 10 keywords should be supplied.

Abbreviations and acronyms: terms and names to be referred to in the form of abbreviations or acronyms must be given in full when first mentioned.

Units: SI units should be used throughout. If non-SI units must be quoted, the SI equivalent must immediately follow in parentheses.

The complete names of individual teeth must be given in the test. In tables and legends for illustrations individual teeth should be identified using the FDI two-digit system.

Illustrations: The following are acceptable ways to present illustrations: white card or plastic; high quality computer generated line drawings; unmounted glossy photographs.
Illustrations should be clearly labeled on the back with the title of the article, the figure number and an arrow to indicate the top edge.

When preparing illustrations authors should consider that the majority of illustrations will be reduced to the width of a single column (approximately 85 mm). Authors can indicate if they feel an illustration should be full page width.

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- to put the name of the Journal in Italics
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Anexo E

CARIES RESEARCH – GUIDE FOR AUTHORS

Aims and Scope

'Caries Research' is an international journal, the aim of which is to promote research in dental caries and related fields through publication of original research and critical evaluation of research findings. The journal will publish papers on the aetiology, pathogenesis, prevention and clinical control or management of dental caries. Papers on health outcomes related to dental caries are also of interest, as are papers on other disorders of dental hard tissues, such as dental erosion. Aspects of caries beyond the stage where the pulp ceases to be vital are outside the scope of the journal.

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Copies of any 'in press' papers cited in the manuscript must accompany the submission. Manuscripts reporting on clinical trials must be accompanied by the CONSORT checklist (see below).

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Reviews can have a freer format but should nevertheless commence with a Title page, an Abstract and an Introduction defining the scope.

Current topics are concise articles that present critical discussion of a topic of current interest, or a fresh look at a problem, and should aim to stimulate discussion.

Letters to the Editor, commenting on recent papers in the journal, are published occasionally, together with a response from the authors of the paper concerned.

Preparation of Manuscripts

Text should be one-and-a-half-spaced, with wide margins. All pages should be numbered, starting from the title page. A conventional font, such as Times New Roman or Arial, should be used, with a font size of 11 or 12. Avoid using italics except for Linnaean names of organisms and names of genes.

Manuscripts should be prepared as a text file plus separate files for illustrations. The text file should contain the following sequence of sections: Title page; Declaration of interests; Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgements; References; Legends; Tables. Each section should start on a new page, except for the body of the paper (Introduction to Acknowledgements), which should be continuous.

Title page: The first page of each manuscript should show, in order:

- the title, which should be informative but concise;
- the authors' names and initials, without degrees or professional status, followed by their institutes;

- a short title, maximum length 60 characters and spaces, for use as a running head;
- a list of 3-10 keywords, for indexing purposes;
- the name of the corresponding author and full contact details (postal address, telephone and fax numbers, and e-mail address).

Declaration of Interests: Potential conflicts of interest should be identified for each author or, if there are no such conflicts, this should be stated explicitly. Conflict of interest exists where an author has a personal or financial relationship that might introduce bias or affect their judgement. Examples of situations where conflicts of interest might arise are restrictive conditions in the funding of the research, or payment to an investigator from organisations with an interest in the study (including employment, consultancies, honoraria, ownership of shares). The fact that a study is conducted on behalf of a commercial body using funds supplied to the investigators' institution by the sponsor does not in itself involve a conflict of interest. Investigators should disclose potential conflicts to study participants and should state whether they have done so.

The possible existence of a conflict of interest does not preclude consideration of a manuscript for publication, but the Editor might consider it appropriate to publish the disclosed information along with the paper.

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Acknowledgements: Acknowledge the contribution of colleagues (for technical assistance, statistical advice, critical comment etc.) and also acknowledge the source of funding for the project. The position(s) of author(s) employed by commercial firms should be included.

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(c) Monographs: Matthews DE, Farewell VT: Using and Understanding Medical Statistics. Basel, Karger, 1985.

(d) Edited books: DuBois RN: Cyclooxygenase-2 and colorectal cancer; in Dannenberg AJ, DuBois RN (eds): COX-2. Prog Exp Tum Res. Basel, Karger, 2003, vol 37, pp 124-137.

(e) Patents: Diggens AA, Ross JW: Determining ionic species electrochemically. UK Patent Application GB 2 064 131 A, 1980.

(f) World Wide Web: Chaplin M: Water structure and behavior. www.lsbu.ac.uk/water, 2004.

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