

**UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU**

ANA CAROLINA MAGALHÃES

**Efeito preventivo de um verniz experimental de tetrafluoreto de
titânio sobre a cárie e erosão dentárias: estudos *in vitro*, *in situ* e
revisões bibliográficas**

**BAURU
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Efeito preventivo de um verniz experimental de tetrafluoreto de titânio sobre a cárie e erosão dentárias: estudos *in vitro*, *in situ* e revisões bibliográficas

Tese apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo como parte das exigências para o Concurso Público de Títulos e Provas visando a obtenção do Título de Livre-Docência, junto ao Departamento de Ciências Biológicas, Disciplina de Bioquímica.

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DEDICATÓRIA

À minha família

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“...Eu fico com a pureza da resposta das crianças
É a vida, é bonita e é bonita
Viver e não ter a vergonha de ser feliz
Cantar.. (E cantar e cantar...) A beleza de ser um eterno aprendiz
Ah meu Deus!
Eu sei... (Eu sei...) Que a vida devia ser bem melhor e será
Mas isso não impede que eu repita
É bonita, é bonita e é bonita...”

Luiz Gonzaga do Nascimento Júnior

Resumo

RESUMO

O objetivo geral desta tese foi reunir os resultados de estudos realizados *in vitro* e *in situ*, os quais avaliaram o efeito de uma aplicação única de um verniz experimental de tetrafluoreto de titânio (TiF_4) a 4%, comparando-o a vernizes à base de fluoreto de sódio (NaF), e às respectivas soluções fluoretadas, sobre os processos de des- e remineralização por desafios ácidos cariogênicos em esmalte (2 artigos, 1 *in vitro* e 1 *in situ*) e erosivos associados ou não aos abrasivos em esmalte e dentina radicular (6 artigos, 5 *in vitro* e 1 *in situ*). Ainda foi o objetivo deste trabalho incluir revisões de literatura publicadas pela pesquisadora (3 artigos), as quais abordaram estratégias preventivas, em especial para a erosão dentária, com enfoque no efeito dos fluoretos, para proporcionar o embasamento final para a conclusão desta tese e diretrizes para estudos futuros sobre este tópico. No estudo sobre cárie utilizando modelo abiótico, o verniz de TiF_4 apresentou efeito similar aos vernizes comerciais à base de NaF na redução da desmineralização, porém foi mais efetivo que os vernizes à base de NaF na remineralização de lesão artificial de cárie. Na fase *in situ*, com acúmulo de biofilme, simulou-se a desmineralização (com exposição à sacarose) e a remineralização do esmalte dentário. Os vernizes NaF , TiF_4 e a solução TiF_4 foram capazes de reduzir significativamente a desmineralização superficial e de subsuperfície do esmalte dentário em comparação à solução NaF , verniz placebo e controle. O verniz e a solução de TiF_4 foram capazes de aumentar significativamente a remineralização superficial do esmalte previamente desmineralizado em comparação ao NaF , verniz placebo e controle. Entretanto, o protocolo experimental não permitiu uma adequada remineralização de subsuperfície. Para erosão dentária associada ou não à abrasão, o verniz de TiF_4 foi tão ou mais efetivo que os vernizes comerciais à base de NaF e a solução de TiF_4 na prevenção da perda do esmalte e dentina por desafios erosivos brandos (30 minutos) *in vitro* e *in situ*, porém quando os desafios erosivos se estenderam por mais de 120 minutos, os vernizes fluoretados foram incapazes de reduzir a erosão do esmalte. De acordo com as revisões de literatura, o fluoreto é um dos agentes mais estudados para a prevenção da erosão dentária, em especial os fluoreto metálicos. O TiF_4 tem apresentado resultados positivos, em função do titânio que pode interagir com a apatita dentária, formando uma camada ácido-resistente e pela

maior incorporação de fluoreto ao esmalte. Em relação à cárie dentária, há algumas evidências clínicas do efeito positivo deste sal fluoretado, porém para a erosão dentária, os trabalhos ainda são restritos aos experimentos de laboratório. Em conclusão, o verniz de TiF_4 tem potencial similar ou superior aos vernizes à base de NaF na prevenção da desmineralização, porém, mais estudos são necessários para melhor entendimento do seu mecanismo de ação e de como otimizar o efeito.

Palavras-chaves: Cárie dentária. Desmineralização. Erosão de dente. Esmalte dentário. Flúor. Remineralização. Titânio.

Abstract

ABSTRACT

Preventive effect of an experimental titanium tetrafluoride varnish on dental caries and erosion: *in vitro* and *in situ* studies and bibliographic review

The aim of this thesis was to show the results of *in vitro* and *in situ* studies, which evaluated the effect of a single application of the experimental 4% titanium tetrafluoride (TiF₄) varnish, compared to sodium fluoride (NaF) varnishes and the respective fluoride solutions, on de and re-mineralization processes by cariogenic challenges on enamel (2 manuscripts, 1 *in vitro* and 1 *in situ*) and erosive challenges associated or not with abrasion on enamel and root dentine (6 manuscripts, 5 *in vitro* and 1 *in situ*). Besides, some bibliographic reviews about preventive measures, in special for dental erosion, were included (3 manuscripts), to allow the final considerations and ideas for further researches. In the caries research, using an abiotic model, TiF₄ presented similar effect as the commercial NaF varnishes on the demineralization decrease; however, it was more effective than the NaF varnishes on the remineralization of artificial enamel caries lesion. In the *in situ*, using biofilm accumulation, the enamel demineralization (sucrose exposition) and the remineralization were simulated. The TiF₄, NaF varnishes and TiF₄ solution were able to significantly reduce surface and subsurface enamel demineralization in comparison to NaF solution, placebo varnish and control. The TiF₄ varnish and solution significantly increased the surface remineralization of demineralized enamel compared to NaF, placebo varnish and control. However, the protocol could not provide a subsurface remineralization. For dental erosion associated or not with abrasion, TiF₄ varnish was equally or more effective than NaF varnishes and TiF₄ solution on the prevention of enamel and dentine loss when mild erosive challenges were performed (30 minutes) *in vitro* and *in situ*. However, when the erosive challenges were more aggressive (120 minutes), the effect of the fluoride varnishes was lost. In accordance with the bibliographic reviews, fluoride is one of the preventive measures more useful for prevention of dental erosion, in special the metal fluorides. TiF₄ has showed positive results, due to the effect of titanium that can interact with apatite, leading to a formation of acid-resistant layer and also by the

higher fluoride incorporation to the enamel. Regarding dental caries, there are some clinical evidences of the TiF_4 effect; however, for dental erosion, most researches have been done in the Laboratory. As conclusion, the TiF_4 varnish has similar or higher potential than NaF varnishes to prevent dental demineralization; however, more studies are required for better understanding of its mechanism of action and how to improve its effect.

Key-words: Demineralization. Dental caries. Dental enamel. Fluoride. Remineralization. Titanium. Tooth erosion.

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1 Introdução

Geral

1 INTRODUÇÃO GERAL

A cárie dentária é uma lesão causada por desmineralização dos tecidos dentários promovida pela exposição a ácidos de origem bacteriana, que se estabelece em pacientes que apresentam biofilme dentário cariogênico (*S. não mutans* em estágios iniciais e *S. mutans*, *Lactobacilos* e *Bifidobactérias* em estágios mais avançados) e dieta rica em açúcar, em especial a sacarose (TEN CATE et al., 2003; TAKAHASHI; NYVAD, 2011). O principal ácido envolvido na cárie dentária é o ácido láctico, cujo pH é em torno de 4,5-5,0, o qual associado à dinâmica do biofilme em termos de concentração de fluoreto, cálcio e fosfato, pode gerar uma desmineralização superficial (em estágios precoces) ou de subsuperfície (em estágios mais avançados), que é caracterizada clinicamente como mancha branca (lesão não cavitada). Durante este estágio, ainda é possível reverter o processo, evitando a cavitação e consequentemente a necessidade restauradora (FEJERSKOV; NYVAD; KIDD, 2003; TEN CATE et al., 2003).

Nas últimas décadas, têm-se observado ganhos nos níveis de saúde bucal na maioria dos países industrializados e em desenvolvimento, como o Brasil, com expressiva redução na prevalência e na severidade das lesões cariosas, isto é, redução de lesões cavitadas (NARVAI et al., 2006; BÖNECKER et al., 2010). A partir da constatação da queda nos níveis de cárie dentária, muito tem sido discutido quanto aos fatores associados a este fenômeno, dentre eles os fatores sociais e de comportamento (PERES; BASTOS; LATORRE, 2000).

Apesar da expressiva redução na prevalência das lesões cariosas, esse declínio ocorreu acompanhado de um fenômeno conhecido como polarização da doença que consiste na concentração da maior parte das lesões cariosas ou das necessidades de tratamento odontológico em uma pequena parcela da população. Como consequência, as necessidades de tratamento também passaram a se concentrar nesta pequena parcela da população, caracterizando significativas inequidades em saúde (SHEIHAM, 1984; PATTUSSI et al., 2001; CARDOSO et al., 2003; NARVAI et al., 2006), fato que requer a atenção de autoridades e adequadas intervenções na área da saúde (SHEIHAM, 1984; PATTUSSI et al., 2001).

Paralelamente à redução da prevalência e severidade da cárie dentária, outra lesão causada por desmineralização que tem ganhado destaque nas últimas duas

décadas é a erosão dentária. Apesar dos dados de prevalência da erosão dentária em crianças e adolescentes serem ainda inconsistentes, os estudos mostram uma tendência ao aumento da prevalência desta lesão com a idade nesta fase (KREULEN et al, 2010) e na fase adulta (van't SPIJKER et al., 2009). A erosão dentária é definida como perda irreversível de estrutura dentária, devido a um processo químico, sem envolvimento de microrganismos, desencadeado por ácidos de origem intrínseca e/ou extrínseca (LUSSI, 2006). Os ácidos intrínsecos (ácido clorídrico) são oriundos do estômago em pacientes que apresentam anorexia, bulimia nervosa e problemas gastro-esofágicos (BARTLETT, 2006). Os ácidos de origem extrínseca (ácido fosfórico, cítrico, acético) incluem aqueles presentes nos alimentos, bebidas, medicamentos e produtos ácidos advindos do ambiente de trabalho (LUSSI et al., 2004). 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 frequência bebidas ácidas, como refrigerantes e sucos de fruta (LUSSI, 2006). Como a cárie dentária, a etiologia da erosão dentária é multifatorial, sendo os três fatores mais importantes: químicos (tipos de ácidos e suas características, como pH, capacidade tampão, concentração, quantidade de minerais), comportamentais (hábitos alimentares, prática de bulimia) e biológicos (características do dente e da saliva) (LUSSI, 2006; LUSSI; JAEGGI, 2008; MAGALHÃES et al., 2009).

Associado ao efeito químico, pode-se ter também o desafio mecânico, como a abrasão e atrição, causados pela escovação, por exemplo, e contato dentário patológico (bruxismo), respectivamente (ADDY; SHELLIS, 2006). Os desafios mecânicos são de grande impacto especialmente sobre superfícies erodidas, já que o ataque ácido leva à perda irreversível de estrutura dentária acompanhada por uma desmineralização na superfície. Dessa forma, forças mecânicas, como a escovação após uma refeição, poderiam remover a camada de esmalte ou dentina desmineralizada, dependendo da severidade dos desafios erosivos e abrasivos (JAEGGI; LUSSI, 1999, ATTIN et al., 2001a; ATTIN et al., 2001b; ATTIN et al., 2004; RIOS et al., 2006, MAGALHÃES et al., 2007).

Neste contexto, diversos tipos de agentes fluoretados têm sido pesquisados tanto prevenção e controle da cárie dentária (MARINHO et al., 2004; PETERSSON et al., 2004; SANTOS et al., 2009; LEE et al., 2010; CARVALHO et al., 2010;

WALSH et al., 2010; WONG et al., 2011; PESSAN et al., 2011) como da erosão dentária (GANSS et al., 2001; WIEGAND; ATTIN, 2003; GANSS et al., 2004; LAGERWEIJ et al., 2006; MAGALHAES et al., 2009; GANSS et al., 2010; MAGALHAES et al., 2011)

O mecanismo de ação do fluoreto convencional, como NaF e AmF, é atribuído a uma precipitação de fluoreto de cálcio (CaF_2) sobre a superfície dentária, que serve como uma barreira mecânica contra os ácidos, no caso da erosão dentária (GANSS et al., 2007). Já para a cárie dentária, o CaF_2 precipitado sobre a superfície do esmalte, em especial após a aplicação de produtos com alta concentração de fluoreto sobre uma superfície livre de biofilme, pode disponibilizá-lo ao biofilme que será formado (TENUTA et al., 2008; TENUTA et al., 2009), servindo como reservatório mineral de fluoreto. Por outro lado, na presença de biofilme dentário e baixa concentração de fluoreto, este íon pode ser incorporado diretamente a este reservatório biológico, na forma de CaF_2 ou aderido às bactérias (ROSE; SHELLIS; LEE, 1996; PESSAN et al., 2008; PESSAN et al., 2010; VOGEL et al., 2010; VOGEL, 2011).

A partir dos reservatórios, níveis adequados de fluoreto podem ser disponibilizados para o fluído do biofilme (TENUTA et al., 2009) ou fluído do esmalte, possibilitando que, durante as quedas de pH, haja a sua adsorção à hidroxiapatita, reduzindo a desmineralização (BUZALAF et al., 2011). Adicionalmente, o cálcio e fluoreto liberados na interface do esmalte dentário, durante as quedas de pH, podem remineralizar o tecido quando o pH retorna à neutralidade, induzindo incorporação de fluoreto à hidroxiapatita, o que pode tornar o substrato dentário mais resistente aos próximos desafios cariogênicos (TEN CATE, 1997; TEN CATE, 2004; LYNCH; NAVADA; WALIA, 2004; ROBINSON, 2009; BUZALAF et al., 2011). A remineralização da camada superficial do esmalte erodido pode ocorrer, porém não há evidências que a incorporação de fluoreto à apatita dentária possa torná-la mais resistente aos desafios erosivos (WEGEHAUPT et al., 2009).

Os fluoretos convencionais para aplicação profissional já foram testados nas formas de vernizes, géis e soluções. Em relação ao veículo, o verniz apresenta algumas vantagens, uma vez que se adere à superfície dentária, permitindo um longo tempo de contato entre o agente fluoretado e o esmalte, além de apresentar uma baixa toxicidade, ser bem tolerado e aceito pelo paciente (SORVARI et al., 1994; BELTRÁN-AGUILAR et al., 2000; SEPPA, 2004; VIEIRA et al., 2007).

Entretanto, não há evidência científica que mostre a superioridade do verniz em relação aos demais veículos para a prevenção da cárie (MARINHO et al., 2004; PETERSSON et al., 2004; SEPPA, 2004) e para a erosão dentária (MAGALHÃES et al., 2011).

Com base nas citações acima, a ação do fluoreto sobre a cárie dentária tem sido bem estabelecida por estudos clínicos e revisões sistemáticas. No entanto, para a erosão dentária, os estudos se restringem a delineamentos laboratoriais, nos quais os resultados obtidos mostram certo efeito preventivo do fluoreto, porém muito aquém daquele obtido para lesões cariosas. Esta diferença ocorre uma vez que os desafios erosivos envolvem ataques ácidos mais curtos, frequentes e mais agressivos (ácidos com pH mais baixo, entre 1,2-3,5), induzindo ao aparecimento de lesões mais superficiais com perda irreversível de estrutura dentária.

Como os fluoretos convencionais podem apresentar baixa eficácia na prevenção da cárie dentária considerando a polarização da doença e a presença de lesões não cavitadas que podem ser remineralizadas, assim como para erosão dentária, outros fluoretos não convencionais (fluoretos metálicos) têm sido testados. Destacam-se o SnF_2 que foi recentemente incorporado a um dentifrício comercial, e tem sido usado na Europa na forma de solução (Elmex Erosion Protection, SnCl_2 + AmF , 500 ppm F e 800 ppm Sn) (GANSS et al., 2010) e o TiF_4 que ainda não está comercialmente disponível no Brasil e está em processo de patente pelo nosso grupo de pesquisa.

Especificamente sobre o TiF_4 , vários estudos *in vitro* e *in situ* têm demonstrado que este sal incorporado à solução em uma concentração variando entre 1-4%, é mais eficaz que outros sais fluoretados sobre o processo de des-remineralização erosiva e cariogênica do esmalte dentário (MUNDORFF; LITTLE; BIBBY, 1972; SHRESTHA; MUNDORFF; BIBBY, 1972; REED; BIBBY, 1976; WEI; SOBOROFF; WEFEL, 1976; WEFEL, 1982; TVEIT et al., 1983; BÜYÜKYILMAZ; SKARTVEIT et al., 1991; VAN RIJKOM et al., 2003; CHEVITARESE et al., 2004; VIEIRA; RUBEN; HUYSMANS, 2005; HOVE et al., 2006; HOVE et al., 2007; EXTERKATE; TEN CATE, 2007; HOVE et al., 2008; WIEGAND et al., 2008; WIEGAND et al., 2009a; WIEGAND et al., 2009b; WIEGAND et al., 2010; WIEGAND; MAGALHÃES; ATTIN, 2010; HOVE et al., 2011).

Além dos efeitos descritos para o fluoreto convencional, a melhor eficácia do TiF_4 pode ser devido a um possível efeito adicional do titânio. Os íons titânio têm um

papel importante na prevenção da desmineralização, uma vez que podem substituir o cálcio da estrutura da apatita, mostrando potencial para se complexarem com os grupos fosfato formando uma camada tipo “glaze” sobre a superfície, a qual é ácido-resistente (MUNDORFF; LITTLE; BIBBY, 1972; BÜYÜKYILMAZ; OGAARD; RØLLA, 1997; TEZEL; ERGÜCÜ; ONAL, 2002; RIBEIRO; GIBSON; BARBOSA, 2006; YU et al., 2010). Além disso, é sugerido que a solução de TiF_4 interage com a superfície dentária, devido ao baixo pH do agente, levando a um aumento na incorporação de fluoreto pelo dente (MUNDORFF; LITTLE; BIBBY, 1972; GU; LI; SÖREMARK, 1996; WIEGAND; MAGALHÃES; ATTIN, 2010).

Esta ação poderia ser potencializada se este composto fosse aplicado na forma de verniz ao invés de solução, devido à adesão do verniz à superfície dentária, permitindo que o TiF_4 pudesse interagir com o esmalte por mais tempo. Além disso, a aplicação do verniz minimizaria possíveis efeitos deletérios do baixo pH do produto sobre a mucosa (MAGALHÃES et al., 2011), o que tem inviabilizado o uso deste agente como método de aplicação caseira como solução de bochecho, uma vez que a atuação do verniz seria localizada no dente em comparação à solução para bochecho.

Com base no exposto acima, esta tese teve como objetivo mostrar resultados de estudos realizados *in vitro* e *in situ* sobre o efeito do verniz experimental de TiF_4 a 4% sobre a desmineralização dentária. Sua estrutura será dividida em capítulos que correspondem a artigos publicados, aceitos ou em fase de elaboração seguindo a sequência abaixo:

(1) Cárie Dentária

- 1.1 Avaliação do efeito do verniz de TiF_4 sobre a desmineralização e remineralização do esmalte dentário, utilizando modelo abiótico para simular desafios cariogênicos (artigo publicado no J Dent. 2008 Feb;36(2):158-62., citado no ISI 5x, impacto 2,115).
- 1.2 Avaliação do efeito do verniz de TiF_4 sobre a desmineralização e remineralização do esmalte dentário, utilizando modelo *in situ* para simular desafios cariogênicos (artigo em fase de elaboração, revista a ser definida).

(2) Erosão dentária

- 2.1 Avaliação do efeito do verniz de TiF_4 sobre a erosão branda do esmalte *in vitro* (artigo publicado no Caries Res. 2008;42(4):269-74, citado 18x, impacto 2,926).
- 2.2 Avaliação do efeito do verniz de TiF_4 sobre a erosão severa do esmalte *in vitro* (artigo publicado no J Dent. 2007 Nov;35(11):858-61, citado 13x, impacto 2,115).
- 2.3 Avaliação do efeito do verniz de TiF_4 sobre a erosão e abrasão do esmalte *in vitro*, incluindo controle com soluções fluoretadas e os desafios abrasivos (artigo publicado no Int J Paediatr Dent. 2011 Jun 20, impacto 1,289).
- 2.4 Avaliação do efeito do verniz de TiF_4 sobre a erosão da dentina *in vitro* (artigo publicado no J Dent. 2010 Feb;38(2):153-7, citado 1x, impacto 2,115).
- 2.5 Avaliação do efeito do verniz de TiF_4 sobre a erosão e abrasão da dentina *in vitro* (artigo publicado no Acta Odontol Scand 2011 *in press*, impacto 1,13).
- 2.6 Avaliação do efeito do verniz de TiF_4 sobre a erosão e abrasão do esmalte e dentina *in situ* (artigo em fase de elaboração, revista a ser definida).

(3) Revisões de Literatura

- 3.1 Estratégias preventivas para erosão dentária (artigo publicado no J Appl Oral Sci. 2009 Mar-Apr;17(2):75-86, citado 16x, impacto 0,966).
- 3.2 O TiF_4 é efetivo em prevenir lesões cariosas e erosivas? (artigo publicado no Oral Health Prev Dent. 2010;8(2):159-64, sem citação).
- 3.3 Fluoretos na prevenção da erosão dentária (artigo recentemente publicado no Monogr Oral Sci. 2011;22:158-70).

Os estudos realizados *in situ* foram previamente aprovados pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia de Bauru-USP conforme Anexos A e B.

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2 Proposição Geral

2 PROPOSIÇÃO GERAL

O objetivo geral desta tese foi reunir os resultados de estudos realizados *in vitro* e *in situ*, os quais avaliaram o efeito de uma aplicação única de um verniz experimental de TiF_4 a 4%, comparando-o a vernizes à base de NaF, e às respectivas soluções fluoretadas, sobre os processos de desmineralização por desafios ácidos cariogênicos em esmalte (2 artigos) e erosivos associados ou não aos abrasivos em esmalte e dentina radicular (6 artigos). Em adição, ainda foi o objetivo deste trabalho incluir revisões de literatura publicadas pela pesquisadora (3 artigos), as quais abordam estratégias preventivas, em especial para a erosão dentária, com enfoque no efeito dos fluoretos, para dar o embasamento final para a conclusão desta tese e diretrizes para estudos futuros sobre este tópico.

3 Capítulos

3.1 Capítulo 1

Effect of 4% titanium tetrafluoride (TiF₄) varnish on demineralisation and remineralisation of bovine enamel in vitro

Short title: Effect of TiF₄ on enamel caries

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Keywords: dental caries; enamel; fluoride; titanium

Effect of 4% titanium tetrafluoride (TiF₄) varnish on demineralisation and remineralisation of bovine enamel in vitro

Summary

Objectives: This in vitro study assessed the effect of a 4% TiF₄ 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), TiF₄-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 TiF₄ 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 TiF₄ significantly improved the rehardening compared to the other varnishes up to 30-µm depths. *Conclusions:* The TiF₄ 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.

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 decades¹. 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 cavities¹.

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 variable⁷⁻¹¹. 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 demineralisation¹²⁻¹⁶. 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*.

Material and Methods

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 temperature¹⁹. The teeth were cut using ISOMET Low Speed Saw cutting machine (Bulher Ltda., Lake Bluff, IL, USA) and two diamond disks (Extex 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 Al₂O₃ 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-2; 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(NO₃)₂.4H₂O, 0.74 mmolL⁻¹ NaH₂PO₄.2 H₂O, 0.03 ppm F, pH 5.0, 37 °C], during 16 h²⁰. 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.

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, Joinville, Santa Catarina, Brazil, n=30), TiF₄ - T (2.45%F, pH 1.0, FGM, Joinville, Santa Catarina, Brazil, n=30) and no-fluoride – P (pH 5.0, FGM, Joinville, 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 acetone⁶. 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 [$2.0 \text{ mmolL}^{-1} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $2.0 \text{ mmolL}^{-1} \text{ NaH}_2\text{PO}_4 \cdot 2 \text{ H}_2\text{O}$, 0.075 mmolL^{-1} acetate buffer, 0.02 ppm F , pH 4.7] for 6 h (30 mL per block) and in remineralisation solution [$1.5 \text{ mmolL}^{-1} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $0.9 \text{ mmolL}^{-1} \text{ NaH}_2\text{PO}_4 \cdot 2 \text{ H}_2\text{O}$, $150 \text{ mmolL}^{-1} \text{ KCl}$, 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.

Hardness determination

Initially, enamel surface hardness was measured as described earlier (Knoop diamond, 25 g, 10 s, HMV-2; 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 = $100 \cdot (\text{SH lesion} - \text{SH}) / \text{SH}$].

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 = $100 \cdot (\text{SH}_1 - \text{SH}) / \text{SH}$ and %SHC carious enamel = $(\text{SH}_1 - \text{SH lesion}) / (\text{SH} - \text{SH lesion}) \cdot 100$].

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.

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%.

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 depths 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 depths. 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, $p < 0.001$).

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 recommended⁸. 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 studies⁷⁻¹¹. 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 gel²⁵.

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 enamel⁶.

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 conditions³. 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.

Conclusion

The TiF₄ 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.

Acknowledgements

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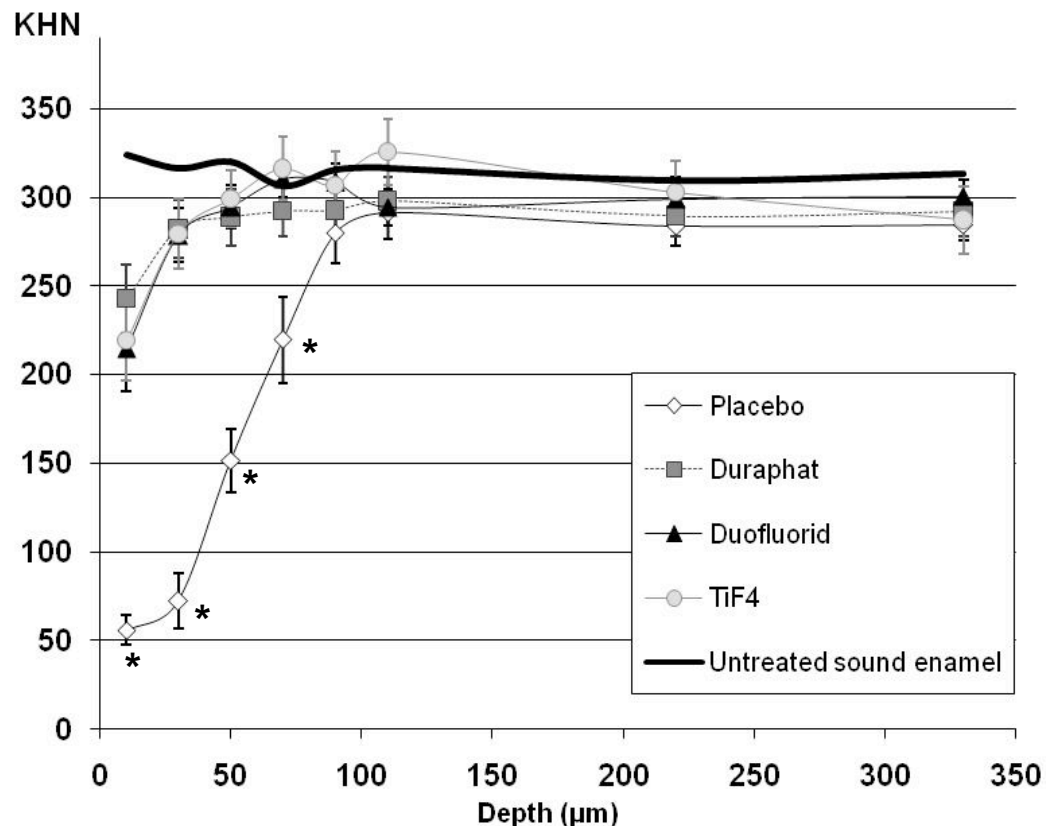
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Table and Figures

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

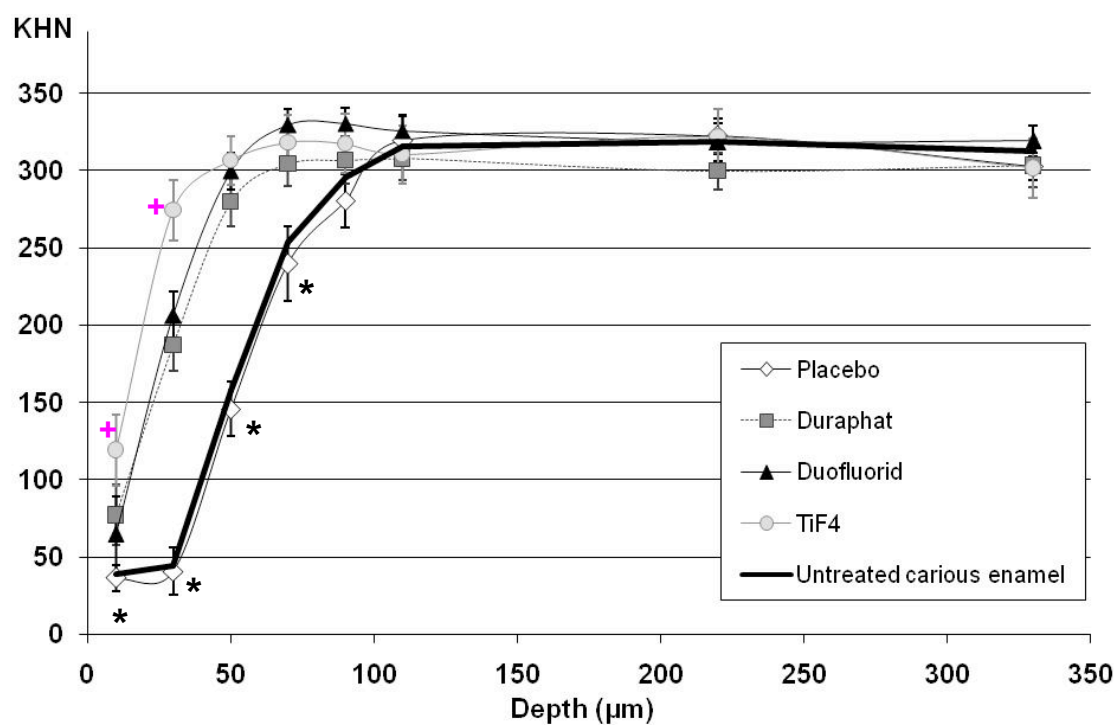
Treatment	Sound enamel	Carious enamel
Duraphat®-D	-27±4 ^a	+13±4 ^a
Duofluorid®-F	-27±6 ^a	+14±4 ^a
TiF ₄ -T	-38±8 ^b	+27±4 ^b
Placebo-P	-87±12 ^c	-10±4 ^c

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



* 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.

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. However, up to 30 μm depth, the TiF₄ treatment lead to significantly higher hardness values compared to the NaF vanishes (marked by +).

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

3.2 Capítulo 2

Effect of professional TiF_4 treatment on enamel de-/remineralization process *in situ*

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Running title: Effect of TiF_4 on enamel de-remineralization *in situ*

Key words: Topical fluorides; dental caries; titanium; tooth demineralization; tooth remineralization.

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Abstract

Objectives: The aim of this study was to evaluate the effect TiF_4 varnish and solution in comparison to NaF on prevention of enamel demineralization and increase of remineralization *in situ*. **Methods:** Eleven subjects took part in this crossover, split-mouth and double-blind study performed in 3 phases of each 14 days (washout of 7 days). Each 2 sound and 2 artificially demineralized (6 d, acidic buffer, pH 5.0) bovine enamel specimens (4x4 mm) were worn in palatal appliances. Each one sound and one demineralized specimens were treated with 5.42% NaF varnish or solution (2.45% F^- , pH 5.0, phase A); 4% TiF_4 varnish or solution (2.45% F^- , pH 1.0, phase B); placebo varnish, pH 5.0, or no-treatment (control. Phase C). The treatments were performed *in vitro* (varnish-6h and solution-1min). During the *in situ* phase, dental plaque accumulation was allowed. The initially sound enamel specimens were demineralized by dripping sucrose solution containing 20% weight/volume (w/v) sucrose for 5 min, 8 times a day, while the previous demineralized specimens were not, to allow remineralization. The response variables used were surface and cross-sectional hardness and transversal microradiography. The data were submitted to RM-ANOVA/ Tukey-Kramer, t test and 2-way ANOVA/ Bonferroni ($p < 0.05$). **Results:** TiF_4 varnish and solution were able to similarly reduce the surface and subsurface enamel demineralization compared to NaF varnish, while all formulations, with exception of NaF solution, performed significantly better than the controls. Only TiF_4 varnish and solution were able to increase the surface enamel remineralization significantly compared to control. However, the experimental protocol did not allow the subsurface remineralization *in situ*. **Conclusions:** TiF_4 varnish and solution were equally effective in prevention of enamel demineralization compared to NaF varnish *in situ*. TiF_4 seems to be promising to induce surface remineralization, but for the lesion repair in depth more studies are required.

Introduction

Fluoride is recognized as the main factor responsible for the dramatic decline in caries prevalence worldwide [1]. Fluoride controls caries mainly through its topical effect. The presence of low fluoride concentration in the oral fluids during an acidic challenge favors its absorption to the surface of the apatite crystals, inhibiting demineralization. When the pH is re-established, traces of fluoride in solution will make it highly supersaturated with respect to fluorhydroxyapatite, which will speed up the process of remineralization [1].

Therefore, it is justifiable to recommend the use of frequent application of low fluoride concentrated products such as dentifrices, to allow the maintenance of appropriate levels of fluoride in solution (plaque or enamel fluid). However, additional fluoride therapies, such as professional application of high fluoride concentrations products (varnish and gel), should also be targeted towards individuals at high caries risk [2,3]. Accordingly, the fluoride varnishes (NaF) were developed in Europe over 60's years in order to prolong the contact between the fluoride agent and the tooth surface, so increasing the fluoride uptake by enamel surface layers [4,5]. Clinical trials and several systematic reviews have provided strong evidence supporting the use of NaF varnishes, 2 times a year, for the control of caries progression [6-13].

The application of fluoride varnish allows the formation of CaF_2 -precipitates on dental hard tissues (mineral reservoir), which in turn can present a mechanical protection and also a chemical effect, releasing fluoride during the cariogenic challenge [14,15]. In order to improve the effect of fluoride varnishes on dental caries, TiF_4 instead of NaF was incorporated to an experimental varnish in 2005, and its effect was firstly tested using an pH cycling [16].

TiF_4 solution has gained increasing attention more than 30 years ago, as it is considered as a possible anti-cariogenic agent [17]. It might also be effective in reducing dentine hypersensitivity [18] and sealing of dentinal tubules of root canal dentine [19]. The protective capacity of TiF_4 is referred to both the formation of an acid-resistant surface coating, an increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice [20, 21].

Magalhães et al. [16] showed that the 4% TiF_4 varnish presented an similar effect than other commercial fluoride varnishes (NaF) in the prevention of enamel demineralization; however, it showed a better effect on the enamel remineralization

[16]. Prior the use of the TiF_4 varnish for the treatment of white spot lesions in the clinical situation, it is important to test this new product in an *in situ* model, which is more realist than *in vitro* models [22].

Based on the above considerations, the purpose of this study was to evaluate the effect of the experimental TiF_4 varnish and solution, compared to NaF, in preventing enamel demineralization and enhancing enamel remineralization *in situ*.

Materials and Methods

Ethical Aspects and experimental design

Sixteen healthy adult subjects (14 female, 2 male, aged 19-29 years) who fulfilled the inclusion criteria (physiological salivary flow rates: stimulated: >1 ml/min, unstimulated: >0.25 ml/min; good oral health: no frank cavities or significant gingivitis/periodontitis) without violating the exclusion criteria (systemic illness, pregnancy or breastfeeding, use of fixed or removable orthodontic appliances, use of fluoride mouthrinse or professional fluoride application in the last 2 months, hyposalivation) were enrolled following CONSORT guidelines. The sample size of 10 subject was previously calculated considering α -error level of 5% and β -error level of 20% (www.ddsresearch.com) according to the results of the previous *in vitro* study [16].

The study conformed to the Declaration of Helsinki and was performed to the guidelines of good clinical practice. Ethical approval for the study involving human subjects was granted by the local Ethics Committee (Ethics committee of the Bauru Dental School, University of Sao Paulo, Brazil, nº 058/2009). The study was planned as a prospective, crossover, split-mouth and double blind, performed in 3 phases of each 14 days (washout of 7 days). The subjects received written instructions and a schedule and were extensively trained for all procedures. Informed consent was obtained from all subjects prior to the study.

Preparation of the specimens

One enamel specimen (4 mm x 4 mm x 3 mm) was prepared from each bovine incisor (n = 192). The teeth were stored in 2% buffered formaldehyde solution (pH 7.0) at 4°C during this phase. The specimens were cut in the middle of the crown's surfaces using a ISOMET low-speed saw (Buehler Ltd., Lake Bluff, IL, USA) with 2

diamond disks (Extex Corp., Enfield, CT, USA) separated by a 4 mm-thick spacer. The specimens surfaces were ground flat with water-cooled silicon carbide discs (320-, 600-, and 1200-grade papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet with diamond spray (1 μm ; Buehler), resulting in removal of about 100 μm depth of the enamel. This was controlled with a micrometer. After polishing, the specimens were cleaned in an ultrasonic device with deionized water for 5 min and the initial surface hardness was measured (SH baseline).

Half of the specimens ($n = 96$) were then submitted to artificial caries lesion formation using a demineralizing solution containing 3 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Labsynth), 3 mM KH_2PO_4 (Sigma-Aldrich), 50 mM lactic acid (Sigma), 6 μM metilhidroxidiphospahte (Sigma-Aldrich) and traces of thymol was used (30 mL/specimen, pH 5.0, 6 days, 37°C) [23,24].

Specimens submitted for demineralization were covered partly by nail varnish (1/3 of the surface) in order to obtain a control surface. The surface hardness after the demineralization was measured (SH lesion). The other half of the specimens remained sound ($n=96$).

Treatment of the specimens

Prior to the *in vitro* treatment and *in situ* phase, the specimens were again disinfected by dipping in 70% alcohol solution for 30 minutes [25]. Thereafter, two thirds of the enamel surface of sound specimens (control areas) and the other one third of the enamel surface of previously demineralized specimens (demineralized area) were protected with nail varnish, in order to obtain a pre-treatment control surface.

Each one sound and one demineralized specimens were treated with 5.42% NaF varnish or solution (both 2.45% F^- , varnish prepared using a synthetic resin, FGM-Dentscare, pH 5.0, phase A); 4% TiF_4 varnish or solution (both 2.45% F^- , varnish prepared using a synthetic resin, FGM-Dentscare, pH 1.0, phase B); placebo varnish (varnish prepared using a synthetic resin, FGM-Dentscare, pH 5.0) or no-treatment (control. Phase C) *in vitro*.

The TiF_4 products presented native pH and the NaF formulations had their pH adjusted to 4.5 using a 5 M H_3PO_4 solution, in order to present similar pH as a commercial NaF varnish (Duraphat).

The varnishes were applied on the enamel surface using a microbrush, and then the specimens were stored in artificial saliva (0.2 mM glucose, 9.9 mM NaCl, 1.5 mM

CaCl₂, H₂O, 3 mM NH₄Cl, 17mM KCl, 2 mM NaSCN, 2.4 mM K₂HPO₄, 3.3 mM urea, 2.4 mM NaH₂PO₄ and traces of ascorbic acid, pH 6.8, v=30 mL/sample, 25°C, [26]. After 6h, the varnishes were removed with a scalpel and a cotton swab soaked in acetone diluted in water 1:1 [16,27].

The solutions were applied using a pipette (v=20 µL/ specimen) for 1 minute. The excess of solution was gently removed with a cotton swab [16,27]. Thereafter, the specimens were immersed in artificial saliva for 6h. The control specimens were only immersed into artificial saliva for 6h.

In situ protocol

Five days prior to and throughout the *in situ* phases, the subjects brushed their teeth with fluoride-free toothpaste (Crest, Procter & Gamble, USA), in order to avoid any residual effect of other fluoride sources on the enamel specimens.

Two cavities of 5X5X3 mm were made on each left and right sides of the acrylic palatal appliances. In one side, 2 sound enamel specimens were fixed with wax and at the other side, 2 previously demineralized enamel specimens (Figure 1). For this, a 4-mm-deep space was created in the acrylic appliance, leaving a 1.5-mm space for plaque accumulation [28]. For the formation of dental biofilm, the specimen was protected from mechanical disturbance by a plastic mesh fixed with wax and acrylic resin on the acrylic surface. The specimens were replaced after each phase.

All subjects participated in the 3 crossover, double-blind, split-mouth phases of 14 days each (washout of 7 days between them). The appliance was only removed for the main meals (4 times a day, maximum 1 hour duration each) and for the experiments. The interval between meals was maintained between 2 to 3 h. Immediately after the meals, before replacing the appliance into the mouth, the subjects were adviced to perform oral hygiene. The subjects were also adviced not to eat or to drink while the appliances were in place.

The subjects dripped a solution containing 20% weight/volume (w/v) sucrose on the sound enamel specimens (red side of the appliance) for 5 min, 8 times a day (interval of 1h between the challenges), *ex vivo*, to induce demineralization. Thereafter, the appliance was replaced into the mouth. The subjects were instructed to perform the experiment carefully, in order to avoid contamination of the previously demineralized specimens. The sucrose solution was renewed each 3 days of the experiment.

On the other hand, the previously demineralized specimens were not subjected to cariogenic challenges, in order to allow remineralization.

Surface and cross-sectional hardness analysis

Surface hardness was measured at baseline (SH baseline), after pre-demineralisation (pre-demineralised samples only, SH lesion) and at the end of the experiment (SH final). Five indentations were performed, 100 µm apart from each other, using 25g for 10s (Knoop, Microhardness tester Shimadzu HMV-2, kPa/mm²) to calculate the percentage of surface hardness change for sound specimens [% SHC = (SH final – SH baseline) / SH baseline) x100] and percentage of surface hardness recovery for the previously demineralized specimens [% SHR = (SH final – SH lesion) / (SH baseline – SH lesion) x100].

Thereafter, the specimens were longitudinally sectioned and each half was submitted to cross-sectional hardness or transverse microradiography (TMR).

For the cross-sectional hardness, the specimens were embedded in acrylic resin, thus polished as previously described in the Preparation of the Specimens section. Sequences of seven indentations at distances of 10, 30, 50, 70, 90, 110 and 220 µm from the outer surface of the enamel were made (Knoop, 25g/10s, kPa/mm²).

For the initially sound enamel specimens, 3 sequences of 7 indentations were performed on the *in situ* demineralized area (Figure 2a). For the previously demineralized specimens, 2 sequences were made on the *in vitro* demineralized area; while for the area demineralized and remineralized *in situ* we performed 3 sequences of indentations (Figure 2b).

The ΔKHN values (integrated mineral loss, kPa/mm².µm) were calculated for sound specimens as described in previous works [29,30] and the ΔΔKHN (ΔKHN lesion - ΔKHN effect [after *in situ* phase]) for the previously demineralized samples. The ΔKHN is defined as the product of the difference between the hardness of sound enamel and the hardness of demineralized or de and re-mineralized enamel considering the lesion depth (µm). The lesion depth was defined by the distance from the surface to the depth, in which the enamel presented a hardness value mean at least of 270 kPa/mm² corresponding to 90% of the minimal hardness values established in this study for sound enamel.

Transverse microradiography analysis

The other half of the specimen was further longitudinally sectioned (as described above) to obtain a fragment with a thickness of approximately 500 μm . The fragments were hand-polished plane-parallel from both cut sides with water-cooled silicon carbide discs (600-, and 1200-grade papers; Buehler, Lake Bluff, IL, USA) to a thickness of 127 ± 17 μm . A microradiograph of each section together with an aluminum calibration step wedge with 14 steps was taken. High-speed holographic films (SO 253; Kodak AG, Stuttgart, Germany) were exposed with Ni-filtered quasi-monochromatic Cu K α X-rays ($\lambda = 0.154$ nm) from a 1x10 mm focus X-ray tube (PW2233/20; Philips, Kassel, Germany) at 20 kV and 20 mA (PW 3830 generator; Philips) for 15 s. The film-focus distance was 40 cm. The developed film was analyzed using a transmitted light microscope with x20 objective (Axioplan; Zeiss, Oberkochen, Germany) with a CCD camera (XC-77CE, Sony, Tokyo, Japan) and a PC with framegrabber and data acquisition and calculation software (TMR 1.25e; Inspector Research BV, Amsterdam, The Netherlands). The analogue signal from the CCD camera was digitized with a framegrabber (Flashpoint 3D; Integral Technologies, Indianapolis, IN, USA). A detail of 400 x 315 μm of the original tooth section was displayed and imaged by using the described parameters.

The mineral content was calculated from the specimen grey levels using the formula of Angmar et al. [31], assuming the density of the mineral to be 3.15 kg/l. The mineral content of sound enamel was assumed to be 87 vol% [31]. The lesion depth was calculated using a threshold of 95% of the mineral content of sound enamel (82.7%). Integrated mineral loss (ΔZ), the average mineral loss over the depth of the lesion (R) and the mean thickness of the “relatively intact” surface layer (SL) were also calculated [32]. For the previously demineralized enamel, the $\Delta\Delta Z$ was calculated as the difference between the ΔZ lesion and ΔZ effect (after *in situ* phase).

Statistical analysis

Data were statistically analyzed using the softwares Graph Pad InStat and Graph Pad Prism version 5.0 for Windows and Mac Book (GraphPad Software, San Diego, CA, USA). For comparison between the groups (%SHC, %SHR, ΔZ , $\Delta\Delta Z$, ΔKHN , $\Delta\Delta Z$, lesion depth, surface layer and R value) the repeated measures ANOVA test followed by Tukey-Kramer were applied. For comparison within the same experimental group (ΔZ lesion x ΔZ effect, depth lesion x effect, surface layer lesion

x effect, R value lesion x effect), in case of the previous demineralized enamel specimens, we applied paired t test. For comparison between groups in the presence of two criteria (% min vol or cross-sectional hardness vs. depth), two-way ANOVA followed by Bonferroni test was applied. The number of subjects was adopted as the sample size and significance level for all tests was set at 5%.

Results

From 16 subjects included in this study, only 11 of them could finish all experimental phases (two subjects gave up of the study due to personal problems, other two could not follow the experiment schedule and one could not be adapted to the oral appliance). Considering the sample number of 11 and the response variables, the sample power for the statistical differentiation between the effect of NaF and TiF₄ varnishes was around 81%, in case of enamel demineralization. For the enamel remineralization, the sample power was 99.9% for the surface hardness measurement; however, for the cross-sectional analysis the value was low (9%).

Enamel demineralization

In general, TiF₄ varnish and solution were able to similarly reduce the surface and subsurface enamel demineralization compared to NaF varnish, which in turn were better than the placebo varnish and control (Tables 1 and 2, Figure 3). Placebo varnish, NaF solution and control did not differ from each other. From all studied variables, the surface layer was not detectable in the sound enamel specimens demineralized *in situ*.

Two-way ANOVA showed significant differences between the treatments and the depths. The interaction between them was also significant ($p < 0.05$) for both cross-sectional analyses (hardness and % mineral volume).

The specimens treated with TiF₄ varnish presented higher hardness values than NaF solution and control at 30 and 50 μm , and than placebo varnish at 30- μm depth. The same results were found for TiF₄ solution; however, it did not significantly differ from the NaF solution. There was no significant difference between the formulations (varnish or solution) regardless of the fluoride salt. The hardness of the specimens treated with NaF varnish was not significant different from placebo varnish and control-specimens along of the depth (Figure 3a).

Regarding to % mineral volume, specimens treated with TiF_4 varnish presented higher mineral content values than NaF solution up to 70 μm , and than placebo varnish and control at 10 and 30- μm depth. The same results were found for TiF_4 solution (NaF solution: from 10 to 50 μm , placebo varnish: 10 and 30 μm , and control: 10 μm). There was significant difference between the formulations (varnish or solution) in case of NaF. The specimens treated with NaF varnish presented higher mineral content than NaF solution specimens from 30 to 70 μm , than control at 10 μm and placebo varnish at 10 and 30 μm depth. The % mineral volume of the specimens treated with NaF solution, placebo varnish and control did not differ from each other along of the depth (Figura 3b).

Enamel remineralization

In general, TiF_4 varnish and solution were able to similarly increase the surface enamel remineralization compared to the other groups. However, there was no subsurface remineralization in any experimental group (Tables 3-6, Figures 4 and 5). Besides, there was a tendency for increasing demineralization for the placebo varnish and control, when ΔZ was analyzed.

Two-way ANOVA showed significant differences between the treatments and the depths, for the lesion and effect areas of the specimens, for both cross-sectional hardness and % mineral volume ($p < 0.05$). There was no significant interaction between the factors. However, when Bonferroni test was applied for the comparison between the groups, it was not observed difference in the mineral content or hardness of the specimens from the different groups along to the depth.

Discussion

Several *in vitro* studies showed that a single application of TiF_4 on sound enamel as solution [33,34] or as varnish [16] was effective in preventing the formation of artificial carious lesions. Thereby, TiF_4 was equally or more effective than NaF or AmF solutions or varnishes, respectively. The present *in situ* study confirmed the positive effect of TiF_4 , regardless of the formulation (varnish or solution) in reducing the surface and subsurface enamel demineralization. NaF solution but not NaF varnish was ineffective to reduce the enamel demineralization, which might be related to the low contact time of this formulation.

Topical fluoridation induces the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in case of conventional fluorides like NaF) or of metal-rich surface precipitates (in case of TiF_4) [35,36]. From the results of the present study, it can also be speculated that the CaF_2 -layer produced by NaF varnish was as effective as the metal-rich surface precipitates formed by the application of TiF_4 formulations.

In principle, we thought that the effect of TiF_4 would be better than NaF *in situ*, due to the presence of human saliva, which would minimize the impact of its low pH. It would be also expected a possible anti-bacterial effect of this fluoride salt. Although polyvalent cations might render the enamel surface more positively charged and hence influence the composition of the pellicle or plaque, TiF_4 treatment did not alter the properties of enamel and root surfaces with regard to plaque formation in a previous study [37]. Leonhardt et al. [38] also observed that the bacterial colonization and plaque composition on titanium surfaces was not significantly different to hydroxyapatite. Therefore, it might be assumed that titanium itself might be of minor impact on the plaque formation, being its effect mainly related to the mechanical protection [20,39,40] and high fluoride uptake [20,35], the later can be explained by the ability of the polyvalent metal ion to form strong fluoride complexes while simultaneously binding firmly to the enamel apatite crystals [41]. Wefel [42] affirmed that the effect of TiF_4 is more related to the "glaze" than the fluoride incorporation.

Previous study showed that 4% TiF_4 varnish was able to produce a glaze surface layer on bovine enamel [27]. Even 4% TiF_4 solution application produced a slight enamel demineralization in that *in vitro* study; both formulations were able to increase the F and Ti content of the enamel surface, which was analysed by SEM-EDX [27]. In further studies, the structurally bound and KOH-soluble fluoride produced by the experimental TiF_4 varnish on enamel should also be evaluated.

On the other hand, studies analysing a potential preventive effect of TiF_4 on lesion progression revealed conflicting results. While Wefel and Harless [40] found TiF_4 to be unable to reduce lesion progression, Magalhães et al. [16] showed that TiF_4 varnish led to an increased rehardening of demineralised enamel compared to NaF varnishes up to 30 μm depth *in vitro*. Despite TiF_4 varnish was not able to reharden the whole lesion depth in that *in vitro* study, the glaze might increase the resistance of the surface layer to further acid challenges. Exterkate and Ten Cate [43] also

showed that a TiF_4 derivative induced an arrestment of artificial carious lesions, but seems not suitable to enhance lesion repair.

In the present study, there was a significant remineralization of the surface enamel by the TiF_4 formulations compared to the other groups. This observation might be explained by the formation of the glaze layer and the fluoride incorporation. In order to avoid measuring the hardness of this glaze layer, the final indentations were performed close to the initial ones, in areas without surface alteration seen in the microscope (40x).

However, the protocol did not allow the subsurface enamel remineralization, even for the control group, which is related to the presence of dental plaque that could hinder the transport of calcium and phosphate from saliva throughout the lesion. This is an interesting results showing that the fluoride varnish might be not enough for the treatment of enamel white spot-lesions whether the patient still presenting a insatisfactory oral hygiene. Therefore, additional fluoride therapy should be accomplished of preventive measures such as oral hygiene instructions and diet counseling towards individuals at high caries risk. To better understanding of this context, further *in situ* studies should be done, to evaluate the effect of this experimental varnish on the enamel remineralization, in association with fluoride dentifrices and different levels of dental plaque.

Therefore, we still not know the effect of TiF_4 varnish regarding the arrestment of the carious lesion, especially the effect of the glaze layer on the subsurface remineralization, *in situ*.

Further studies about the effect of TiF_4 varnish should be encouraged due to two reasons; firstly, results of previous *in vivo* studies and clinical trial have shown good effect of TiF_4 solution compared to NaF solution and APF gel for caries control [44-46] and; secondly, the application of TiF_4 as varnish could enable the use of this fluoride salt in the clinical treatment. The low pH of TiF_4 solution does not allow self-application as mouth rinse by the patient, as it might induce adverse side effects on hard and soft tissues. Therefore, the varnish could be a good alternative for this inconvenient, as it is applied directly on the tooth surface, being safer for the patient compared to solution. The varnishes have some advantages compared to mouth rinses or gels: It is safe and well tolerated by infants, young children, and individuals with special needs and it is easy to apply.

Based on the results, it can be concluded that TiF_4 varnish and solution presented similar performance than NaF varnish to prevent enamel demineralization *in situ*. TiF_4 seems to be promising to induce surface remineralization, but for total lesion repair more studies are required.

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Figures

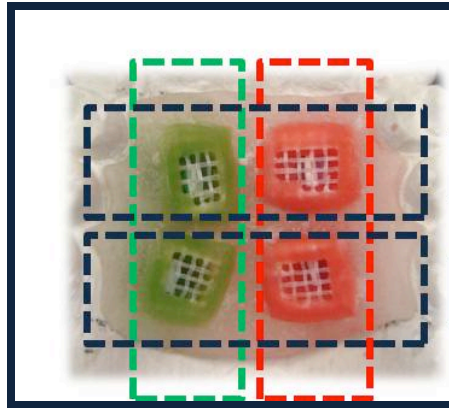


Figure 1 - The position of the specimens in the palatal appliance according to the treatment and the condition. Each black rectangle represents one treatment (varnish or solution); green represents the previously demineralized enamel specimens and the red one, the initially sound enamel specimens.

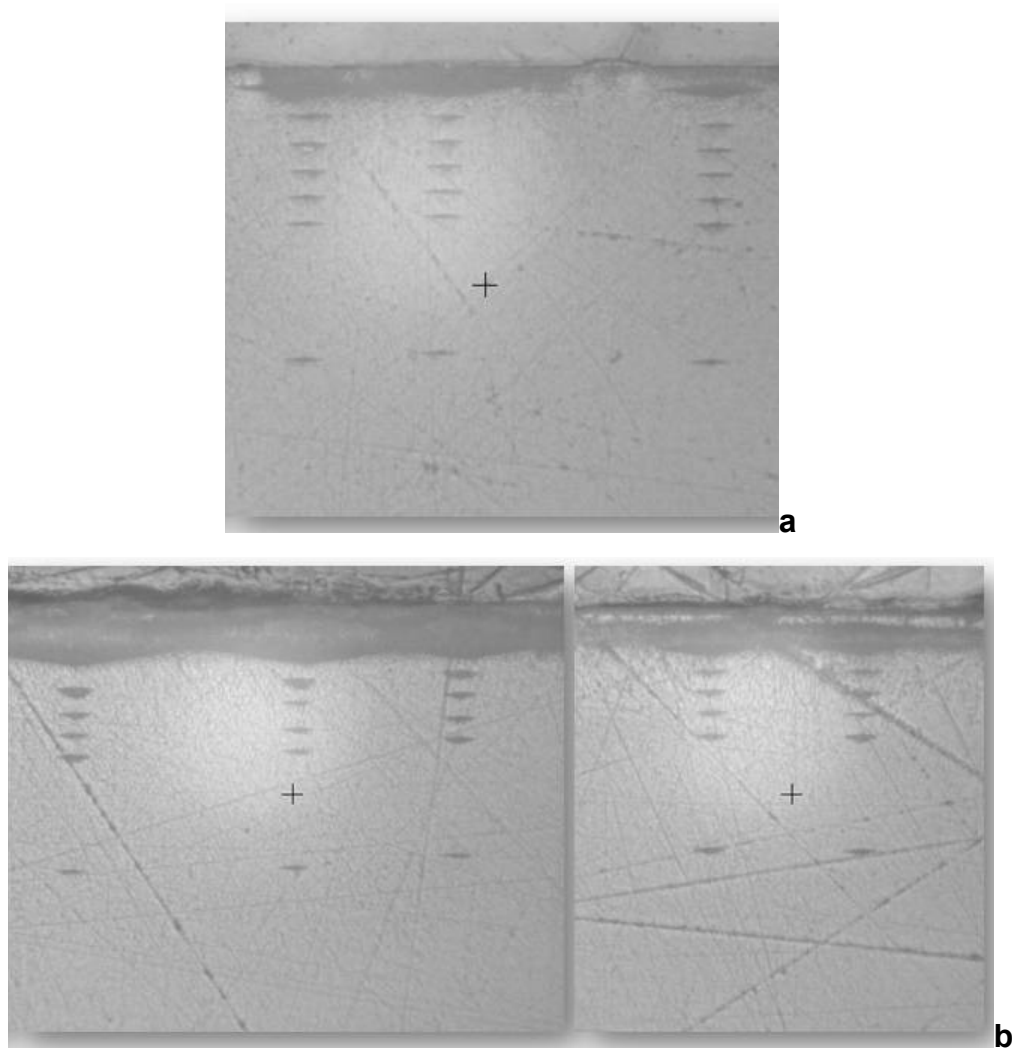


Figure 2 - a) Cross-sectional Hardness of initially sound enamel (3 sequences of indentation were performed in the center of the *in situ* demineralized area); **b)** Cross-sectional Hardness of initially demineralized enamel (3 sequences of indentation were performed in the center of the *in situ* de and re-mineralized area and 2 sequences on the *in vitro* demineralized area).

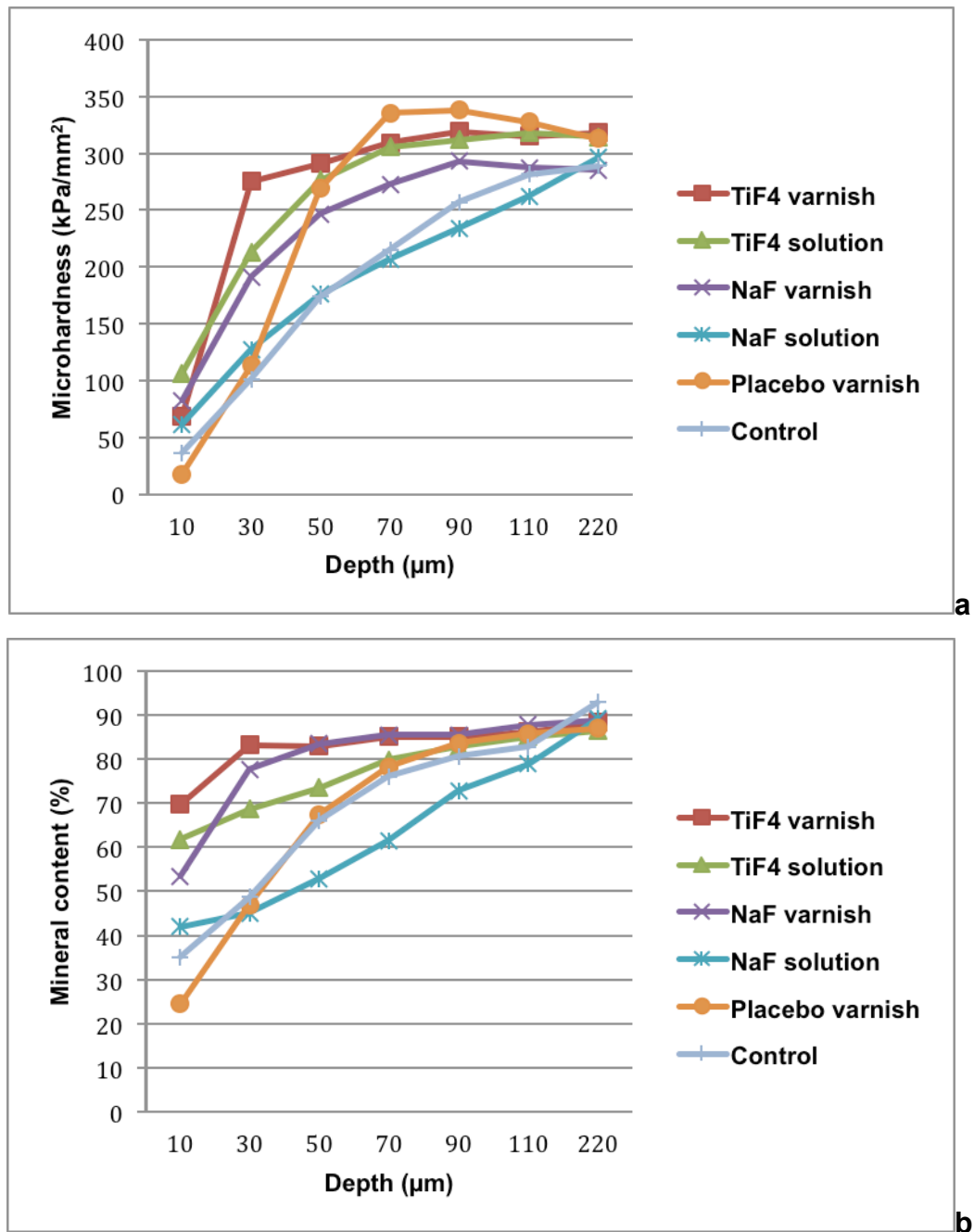


Figure 3 - a) Mean values of cross-sectional hardness in y-axes ($n=11$, kPa/mm^2) for each depth (μm) in x-axes. **b)** Mean values of % mineral volume in y-axes ($n=11$, %) for each depth (μm) in x-axes.

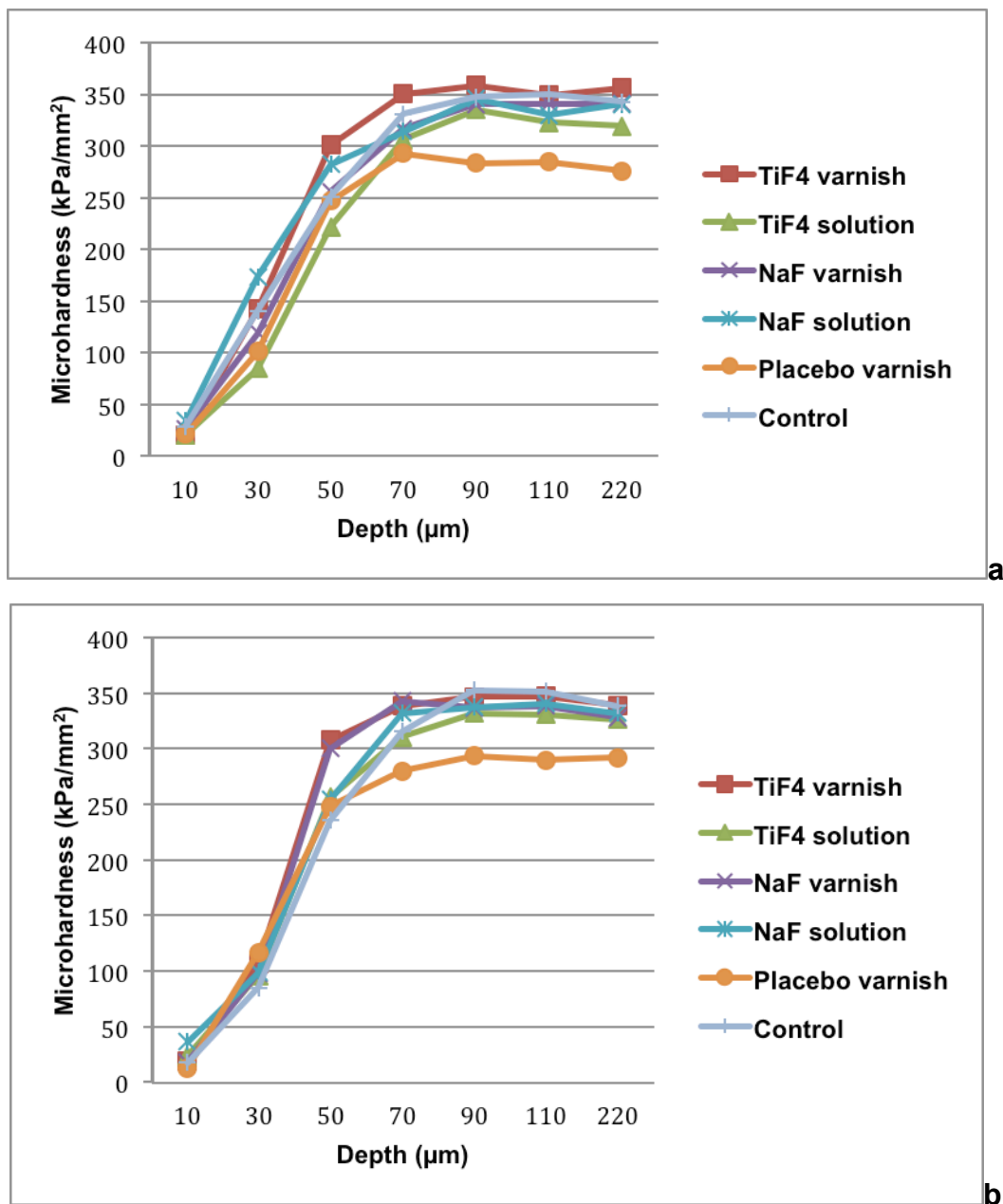


Figure 4 - a) Mean values of cross-sectional hardness in y-axes ($n=11$, kPa/mm^2) for each depth (μm) in x-axes in the lesion area. **b)** Mean values of cross-sectional hardness in y-axes ($n=11$, kPa/mm^2) for each depth (μm) in x-axes in the effect area.

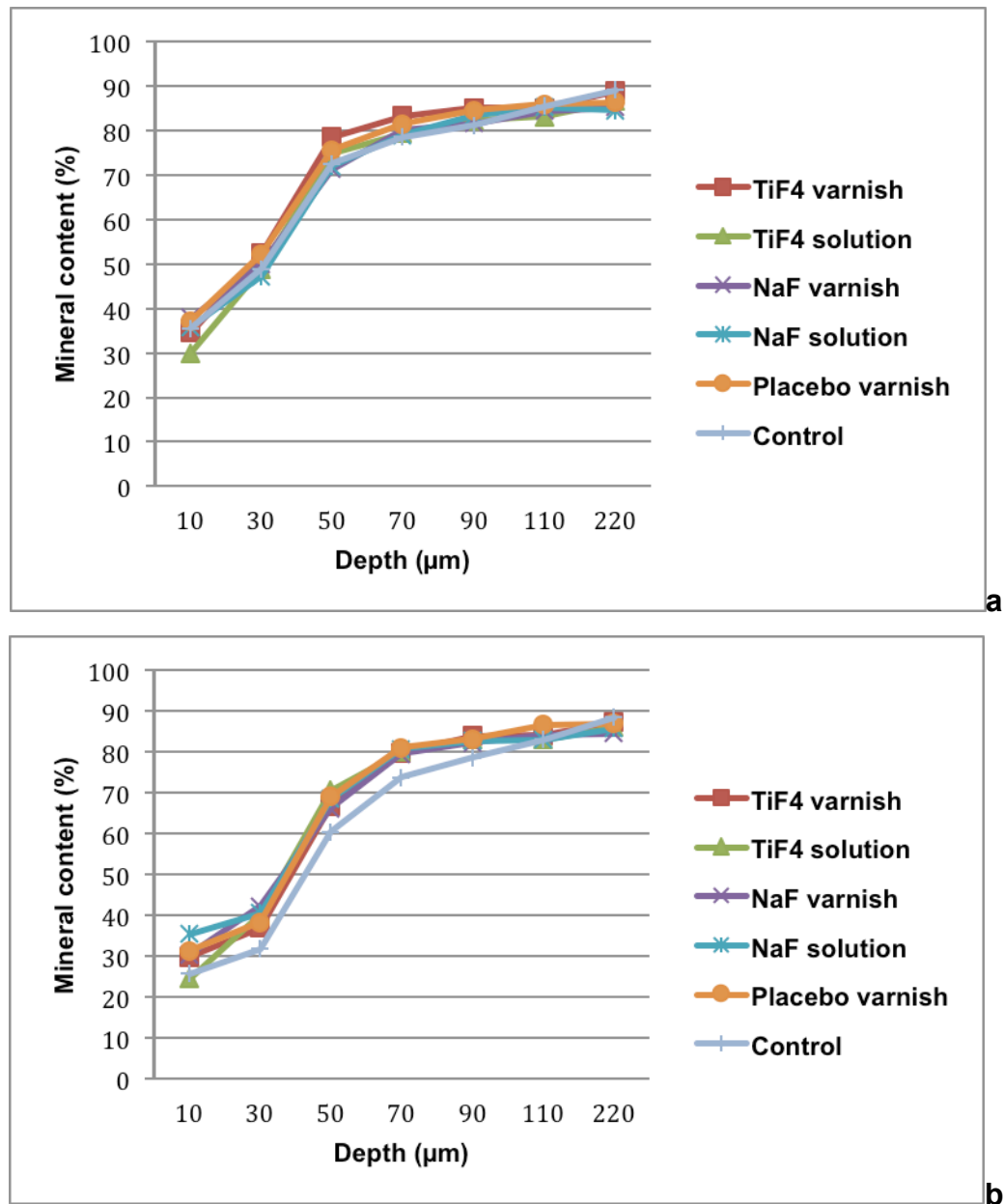


Figure 5 - a) Mean values of % mineral volume in y-axes (n=11, %) for each depth (μm) in x-axes in the lesion area. **b)** Mean values of % mineral volume in y-axes (n=11, %) for each depth (μm) in x-axes in the effect area.

Tables

Table 1 - Mean and standard deviation of SH baseline, SH final (kPa/mm²) and the %SHC, for the sound enamel specimens according to the treatment groups (n=11).

Group\Variable	SH baseline*	SH final	%SHC
TiF ₄ varnish	375±19	140±36 ^a	63±10 ^a
TiF ₄ solution	370±18	85±38 ^b	77±10 ^{a,b}
NaF varnish	370±19	90±61 ^{a,b}	75±17 ^{a,b}
NaF solution	371±20	65±64 ^{b,c}	82±18 ^{b,c}
Placebo varnish	372±17	22±16 ^c	95±4 ^c
Control	373±18	27±21 ^c	93±6 ^c

Values in the same column that have different superscript letters significantly differ from each other (ANOVA-RM, $p < 0.0001$, Tukey-Kramer test). *ANOVA ($p > 0.05$).

Table 2 - Mean and standard deviation of R value (% min.vol loss), TMR-lesion depth (μm), integrated mineral loss (ΔZ, % min.vol x μm) and integrated hardness loss (ΔKHN, kPa/mm² x μm), for the sound enamel specimens according to the treatment groups (n = 11).

Group\Variable	R (% min.vol)	Lesion depth (μm)	ΔZ (%min.vol x μm)	ΔKHN (kPa/mm ² x μm)
TiF ₄ varnish	28±6 ^{a,b}	14±7 ^a	449±232 ^a	3223±1086 ^a
TiF ₄ solution	25±6 ^a	56±25 ^{b,c}	1379±603 ^a	4370±1862 ^a
NaF varnish	32±4 ^{a,b,c}	31±13 ^{a,b}	954±379 ^a	4786±1908 ^a
NaF solution	41±8 ^d	105±25 ^d	3206±1399 ^b	67644±61162 ^b
Placebo varnish	39±5 ^{c,d}	67±31 ^c	2973±1193 ^b	9398±2675 ^a
Control	34±8 ^{b,c,d}	84±18 ^{c,d}	3289±1002 ^b	86069±63553 ^b

Values in the same column that have different superscript letters significantly differ (ANOVA-RM and Tukey-Kramer test, $p < 0.0001$).

Table 3 - Mean and standard deviation of the SH baseline, SH lesion, SH final (kPa/mm²) and the %SHR, for the previously demineralized enamel specimens according to the treatment groups (n=11).

Groups\Variables	SH baseline*	SH lesion*	SH final	%SHR
TiF ₄ varnish	374±19	19±9	77±24 ^a	18±6 ^a
TiF ₄ solution	377±23	22±11	70±29 ^{a,b}	14±7 ^a
NaF varnish	373±23	23±14	50±28 ^{a,b}	7±3 ^b
NaF solution	373±16	21±13	47±29 ^{a,b}	6±6 ^b
Placebo varnish	366±24	25±15	39±22 ^b	5±3 ^b
Control	369±24	24±14	49±32 ^{a,b}	6±2 ^b

Values in the same column that have different superscript letters significantly differ from each other (ANOVA-RM, SH final and %SHR, p=0.018 and p<0.0001, respectively, Tukey-Kramer test). *ANOVA (p>0,05).

Table 4 - Mean and standard deviation values of ΔKHN lesion, ΔKHN effect and ΔΔKHN (kPa/mm² x μm), for the previously demineralized enamel specimens according to the treatment groups (n=11).

Group\Variable	ΔKHN lesion (kPa/mm ² x μm)*	ΔKHN effect (kPa/mm ² x μm)**	Paired t- test P value	ΔΔKHN (kPa/mm ² x μm)**
TiF ₄ varnish	8214±2454	7800±1482	p=0.64	414±2859
TiF ₄ solution	10719±2731	9025±3188	p=0.008	1694±1716
NaF varnish	9101±2663	8393±1888	p=0.45	708±2985
NaF solution	7822±3275	7744±1872	p=0.91	78±2468
Placebo varnish	7402±1760	6964±2112	p=0.49	438±2033
Control	8433±2771	9282±1834	p=0.26	-849±2353

*There were no significant differences between the groups (ANOVA, p> 0.05).

**There were no significant differences between the groups (RM-ANOVA, p> 0.05). Positive values mean that there was some remineralization, and negative values, demineralization.

Table 5 - Mean and standard deviation values of ΔZ lesion, ΔZ effect and $\Delta\Delta Z$, for the previously demineralized enamel specimens according to treatment groups (n = 11).

Group/ Variable	ΔZ lesion*	ΔZ effect**	Paired t-test P value	$\Delta\Delta Z$ **
TiF ₄ varnish	2022±443	2496±758	p=0.06	-474±755
TiF ₄ solution	2452±594	2724±231	p=0.15	-272±586
NaF varnish	2383±534	2734±898	p=0.18	-351±813
NaF solution	2435±778	2810±1155	p=0.24	-375±992
Placebo varnish	2132±564	2684±544	p=0.005	-552±516
Control	2414±516	3360±515	p=0.0006	-946±642

*There were no significant differences between the groups (ANOVA, p> 0.05).

**There were no significant differences between the groups (RM-ANOVA, p> 0.05). Negative values mean that there was some demineralization.

Table 6 - Mean and standard deviation values of R values (% min. vol), depth (µm) and surface layer thickness (µm, SL) for the lesion and effect areas, for the previously demineralized enamel specimens according to treatment groups (n=11).

Group/ Variable	R lesion*	R effect*	Depth lesion*	Depth effect*	SL lesion*	SL effect*
TiF ₄ varnish	35±7	41±10	58±10	67±16	8±2	8±1
TiF ₄ solution	34±5	39±8**	75±22	75±17	7±2	7±2
NaF varnish	31±9	36±9	79±19	78±19	9±5	9±3
NaF solution	33±13	34±10	79±19	84±26	8±2	9±3
Placebo varnish	30±4	38±3**	72±20	70±11	9±3	9±3
Control	34±6	38±5**	73±16	90±17	8±2	7±2

* There were no significant differences between the groups (ANOVA or RM-ANOVA, p> 0.05).

** There was significant difference between the lesion and the effect areas, showing increase of the mineral loss (paired t-test, p<0.05)

3.3 Capítulo 3

The effect of an experimental 4% TiF₄ varnish compared to NaF varnishes and 4% TiF₄ solution on dental erosion in vitro

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Abstract

This in vitro study assessed the effect of an experimental 4%TiF₄ varnish compared to commercial NaF and NaF/CaF₂ varnishes and 4%TiF₄ solution on enamel erosion. For this, 72 bovine enamel specimens were randomly allocated to the following treatments: NaF varnish (2.26%F), NaF/CaF₂ varnish (5.63%F), 4% TiF₄ varnish (2.45% F), F-free placebo varnish, 4% TiF₄ 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 to the enamel surface for 1 min. Then, the specimens were alternately de- and re-mineralized (6 times/day) in an artificial mouth for 5 days at 37°C. Demineralization was performed with the beverage Sprite (1 min, 3 mL/min) and remineralization with artificial saliva (day: 59 min, 0.5 mL/min, during night: 0.1 mL/min). The mean daily increment of erosion and the cumulative erosion data were tested using ANOVA and ANCOVA, respectively, followed by Tukey's test ($\alpha = 0.05$). The mean daily erosion increments and cumulative erosion (μm) were significantly less for the TiF₄ varnish ($0.30 \pm 0.11/0.65 \pm 0.75$) than for NaF varnish ($0.58 \pm 0.11/1.47 \pm 1.07$) or NaF/CaF₂ varnish ($0.62 \pm 0.10/1.68 \pm 1.17$), which in turn showed significantly less erosion than placebo varnish ($0.78 \pm 0.12/2.05 \pm 1.43$), TiF₄ solution ($0.86 \pm 0.11/ 2.05 \pm 1.49$) and control ($0.77 \pm 0.16/2.06 \pm 1.49$). In conclusion, the TiF₄ varnish seems to be a promising treatment to reduce enamel loss under mild erosive conditions.

Introduction

Dental erosion is the loss of tooth tissue 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 dissolve readily 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 Rølla 1988]. Highly 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; Vieira et al. 2006]. Most 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, such as titanium tetrafluoride (TiF_4), have been investigated for erosion prevention [Tveit et al., 1983; Büyükyilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005, 2006; Hove et al., 2006, 2007; Schlueter et al., 2007; Magalhães et al., 2007a].

Several in vitro studies have shown an inhibitory effect of TiF_4 solution on dental erosion [Büyükyilmaz et al., 1997; van Rijkom et al., 2003; Hove et al., 2006, 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 [Mundorff et al., 1972; Büyükyilmaz et al., 1997; Tezel et al., 2002; Ribeiro et al., 2006]. Moreover, it is suggested that titanium interacts with the enamel surface, because of the low pH of the agent, thus leading to an increased fluoride uptake by enamel [Mundorff et al., 1972; Gu et al., 1996]. However, other studies did not find a protective effect of TiF_4 against erosion or combined erosion and abrasion [Vieira et al., 2005, 2006; Magalhães et al., 2007a].

Because of their capacity to adhere to 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, the use of an experimental TiF_4 varnish for the prevention of erosive wear has been proposed. In a previous study, TiF_4 varnish did not reduce enamel loss under severe erosive conditions [Magalhães et al., 2007b]. Thus, the present study aimed to compare the same TiF_4 varnish with a TiF_4 solution (the most investigated form of TiF_4 application) and commercial NaF and NaF/ CaF_2 varnishes (as positive controls) in an in vitro model characterized by a milder erosive challenge.

Material and Methods

Specimen 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, waterproof silicon carbide paper; Struers, Erkrath, Germany), approximately 200 μm of the outer enamel being removed. Surface hardness of enamel specimens was used as a criterion for stratified allocation of the specimens among 6 groups of 12. Prior to the experiment, baseline scans were obtained from the specimens with a contact profilometer (Mahr Perthometer, Göttingen, Germany). Reference areas on the polished enamel surface were covered with parallel strips of adhesive tape (Tesa, Beiersdorf, Hamburg, Germany), 1.5 mm apart. After preparation, the specimens were stored in water until used for the experiment to avoid dehydration.

Fluoride pre-treatment

Prior to acid exposure, the specimens were pre-treated as follows: (1) NaF varnish (2.26% F, pH 4.5; Duraphat, Colgate-Brazil); (2) NaF/CaF₂ varnish NaF, (2.71% F as NaF, 2.92% F as CaF₂, pH 8.0; Duofluorid, FGM-Brazil); (3) 4% TiF₄ varnish (2.45% F, pH 1.0; FGM-Brazil); (4) F-free placebo varnish (pH 5.0; FGM-Brazil); (5) 4% TiF₄ solution (2.45% F, pH 1.0); (6) no treatment (control). While Duofluorid is transparent; the other varnishes are yellow. According to the manufacturers, the Brazilian varnishes (FGM) contain colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol, while Duraphat contains 2.26% NaF, 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. To prepare the 4% TiF₄ solution, solid TiF₄ (Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in deionized water. The pH of all solution/varnishes was measured by indicator paper (± 0.5 units).

The TiF₄ solution was applied with a microbrush and left on the surface undisturbed until the surface appeared dry. Additional drops were applied in the same manner over the course of 1 min [van Rijkom et al., 2003]. Excess solution was removed from the surface by a cotton roll and the specimens were stored in artificial saliva [Klimek et al., 1982].

The varnishes were applied in a thin layer using a microbrush and the specimens 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. Completeness of removal layer was checked microscopically. Prior to cycling, surface loss due to application of the varnishes or solution was determined by profilometry.

pH-cycling

For pH cycling, the specimens were mounted in an artificial mouth [Attin et al., 2003] for 5 days allowing for alternating de- and remineralization treatment. The artificial mouth consisted of 12 chambers, 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 specially written software. Only one specimen was placed in each chamber. The specimens were randomly distributed to the 12 chambers in each pH

cycle, 2 specimens from each group being randomly selected and submitted to the 5-day cycle in the artificial mouth.

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. Between 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 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3 mM NH_4Cl , 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4 , 3.3 mM urea, 2.4 mM NaH_2PO_4 and ascorbic acid. After 6 de- and re-mineralization treatments, enamel surface loss was determined by profilometry, and the specimens were rinsed with artificial saliva overnight (0.1 mL/min, 18 h).

Profilometry

Surface profiles of the enamel specimens were obtained with a contact profilometer (Mahr Perthometer, Göttingen, Germany) prior to the experiment, after the respective fluoride pre-treatment and after every 5 daily de- and re-mineralization 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 specimens were provided with identification marks, which allowed the stylus to be re-positioned accurately 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.

Scanning Electron Microscopy (SEM)

To show the interaction between the TiF_4 varnish and the solution with enamel, pairs of polished bovine enamel specimens (4 x 4 mm) were freshly treated with TiF_4 varnish, TiF_4 solution or remained untreated (control). For this, three specimens were obtained from each of two teeth, and one was allocated to each

treatment group. The samples were treated for 1 min with the TiF_4 solution and for 6 h with TiF_4 varnish in exactly the same way as for the main experiment, the varnish again being removed after treatment using acetone and a scalpel blade. The two control samples remained untreated.

After treatment, the specimens were carefully dried with paper, coated with gold/palladium using sputter coating, dried by vacuum and examined with a scanning electron microscope (XL 30 FEG SEM; Philips, The Netherlands, with field emission gun at 20KV) in both secondary electron and backscattered electron modes. Elemental surface composition of the whole enamel surface was obtained using x-ray energy dispersive spectroscopy (EDS) using an ISIS 300 spectrometer (Link; Oxford, U.K.) coupled to the SEM. In the samples treated with the TiF_4 varnish, the whole surface was analyzed, including the surface layer and the enamel below this layer. Determinations were made using quantitative analysis with ZAF correction.

Statistical analysis

To test whether the treatments caused any significant alteration of the enamel surface, the profiles obtained before and after application of the agent were compared within each group by paired t-tests. Mean daily erosion increments were calculated for each treatment. Linear regressions for cumulative erosion on number of treatments were calculated for each treatment (days 0-5; day 0 being the profile after treatment). 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, differences in the mean daily erosion increment were tested using one-way ANOVA and the cumulative erosion regressions were compared by ANCOVA. Tukey's test was used for post-hoc pairwise testing. Graph Pad InStat version 3.0 for Windows, Graph Pad Software (San Diego, CA, USA) and Statistica v.5.1, StatSoft Inc. (Tulsa, USA) software were used for statistical analysis. The significance level was set at 5%.

Results

The application of the TiF_4 solution produced significant enamel loss ($p < 0.001$) when compared to the baseline profile after the polishing. In contrast, the TiF_4 varnish provoked a significant growth of the surface ($p = 0.043$). The other groups did

not present any significant difference compared to baseline profile after polishing (Table 1).

Figure 1 shows the linear regression lines and the cumulative erosion for the treatments at each day, considering the baseline after the application of the agents as day 0 (Days 0-5). ANCOVA of the linear regressions (days 0-5) showed that mean daily cumulative erosion (μm) was significantly less for the TiF_4 varnish (0.65 ± 0.75) than for NaF varnish (1.47 ± 1.07) or NaF/ CaF_2 varnish (1.68 ± 1.17) which did not differ significantly from each other. However, both showed significantly less erosion ($p < 0.001$) than placebo varnish (2.05 ± 1.43), TiF_4 solution (2.05 ± 1.49) and control (2.06 ± 1.49), which did not differ significantly.

Table 1 shows the daily increments of enamel erosion. ANOVA showed that the mean daily increment (μm) was significantly less for TiF_4 varnish (0.30 ± 0.11) than for NaF varnish Duraphat (0.58 ± 0.11) or NaF/ CaF_2 varnish (0.62 ± 0.10), both of which showed significantly less erosion ($p < 0.001$) than placebo varnish (0.78 ± 0.12), TiF_4 solution (0.86 ± 0.11) and control (0.77 ± 0.16), which did not differ significantly.

The SEM image of the untreated enamel sample presented a polished surface (Figure 2). Enamel treated with TiF_4 varnish presented a thin smooth surface layer in which microcracks were visible (Figure 3). In contrast, enamel treated with TiF_4 solution showed an etched surface (Figure 4). For TiF_4 solution treated samples, at low power a honeycomb appearance reflecting the prism structure was visible (not shown). Ti and F were not detectable at the untreated enamel surfaces, but enamel treated with TiF_4 varnish exhibited mean surface concentrations of $3.5 \pm 1.0\%$ Ti and $2.0 \pm 0.80\%$ F. Enamel treated with the TiF_4 solution showed mean surface concentrations of $0.96 \pm 0.06\%$ Ti and $1.75 \pm 0.90\%$ F.

Discussion

The conflicting results for TiF_4 in previous studies [Büyükyilmaz et al., 1997; van Rijkom et al., 2003; Vieira et al., 2005, 2006; Hove et al., 2006, 2007; Schlueter et al., 2007; Magalhães et al., 2007a] can be explained by the different protocol used, as well as the different response variables analyzed.

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 present study in the

form of a varnish. The varnishes and the solution were applied only once in order to simulate the clinical situation with a single professional application by a dentist. In contrast to most of the studies showing a protective effect of fluoride varnishes against dental erosion [Vieira et al., 2005, 2006, 2007], the varnishes were completely removed after 6 h in the present study. This was done to focus on the chemical effect of the varnishes (NaF or CaF_2 versus 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 especially important for eroded surfaces, which are usually more susceptible to mechanical forces.

A mild erosive challenge of the specimens was conducted during 5 days to simulate a frequent acid contact in the in vivo situation. Enamel erosion was assessed by contact profilometry. It must be remembered that the stylus might be able to scratch the acid softened surface [Barbour and Rees, 2004] but this would occur in all the groups, so should not bias the results.

Regarding the positive controls, most previous studies tested the effect of commercial varnishes containing NaF, and found a reduction of erosion in vitro and in situ [Sorvari et al., 1994; Vieira et al., 2005, 2006, 2007]. In the present study, a NaF varnish and a NaF/ CaF_2 varnish reduces enamel loss significantly when compared to the placebo varnish, TiF_4 solution and control, and they did not differ from each other.

The experimental TiF_4 varnish showed the best protective effect on erosion in this mild 5-days erosive protocol. On the other hand, the TiF_4 solution did not provide a preventive effect against enamel erosion during 5 days of de- and remineralisation. The SEM and EDS results might help to explain this difference in behavior of the TiF_4 when employed as a varnish or as a solution. Enamel treated with TiF_4 solution showed an etched appearance which is consistent with the small profilometric loss after treatment observed both in this study (Table 1) and also by Magalhães et al. [2007a]. This contrasts with the layer produced by treatment with TiF_4 varnish, which probably provides a mechanical and chemical barrier, even though it has cracks [Tveit et al., 1983; Büyükyilmaz et al., 1997; Vieira et al., 2006; Hove et al., 2006]. Besides the layer on the enamel surface, TiF_4 varnish-treated enamel had a higher surface concentration of both Ti and F than the TiF_4 solution-treated surface. The different surface effects of TiF_4 formulations seem to be related

to the better ability of the varnish to adhere to the tooth surface, which allows an increased contact time with the enamel and hence prolongs the reaction between the TiF_4 and enamel [Vieira et al., 2006, 2007]. The lack of erosion after TiF_4 varnish (Table 1) suggests that the varnish might reduce the effects of the low pH on the enamel.

However, because TiF_4 varnish was more protective of enamel against erosion by 30 min treatment with Sprite than against erosion 240 min exposure to cola [Magalhães et al, 2007b], it is possible that the protective layer would be removed over time with erosive challenges. In the clinical situation this experimental varnish might be less effective in preventing dental erosion for a long time, especially in patients at high risk for erosion. The persistence of the protective effect of TiF_4 varnish and the frequency of application have to be evaluated in further studies, using experimental times higher than 5 days.

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 the efficacy of this experimental varnish against abrasive forces. Moreover, the effect of this varnish on human enamel should be evaluated. Hove et al. [2007] showed that the protective effect of a 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 TiF_4 varnish, but not TiF_4 solution, reduced erosive bovine enamel erosion. Moreover, TiF_4 varnish showed better results than two commercial NaF varnishes. However, more studies must be conducted in situ and in vivo, before TiF_4 varnish can be widely used in the clinical situation.

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Table 1 - Daily increment of erosion (μm) for each experimental group. Negative numbers signify growth of the surface. Means \pm SD.

	Duraphat	Duofluorid	TiF ₄ varnish	Placebo Varnish	TiF ₄ solution	Control
Days Treatment	-0.01 \pm 0.10	0.01 \pm 0.10	-0.12 \pm 0.18	0.02 \pm 0.10	0.24 \pm 0.14*	_____
1	0.68 \pm 0.27	0.68 \pm 0.23	0.10 \pm 0.25	0.91 \pm 0.32	0.73 \pm 0.17	0.90 \pm 0.23
2	0.59 \pm 0.18	0.83 \pm 0.27	0.27 \pm 0.16	0.88 \pm 0.21	0.78 \pm 0.20	0.94 \pm 0.34
3	0.57 \pm 0.18	0.45 \pm 0.12	0.27 \pm 0.13	0.78 \pm 0.14	0.92 \pm 0.22	0.91 \pm 0.21
4	0.49 \pm 0.17	0.55 \pm 0.21	0.41 \pm 0.17	0.85 \pm 0.24	1.02 \pm 0.21	0.61 \pm 0.15
5	0.58 \pm 0.10	0.59 \pm 0.24	0.49 \pm 0.28	0.48 \pm 0.14	0.86 \pm 0.21	0.58 \pm 0.35

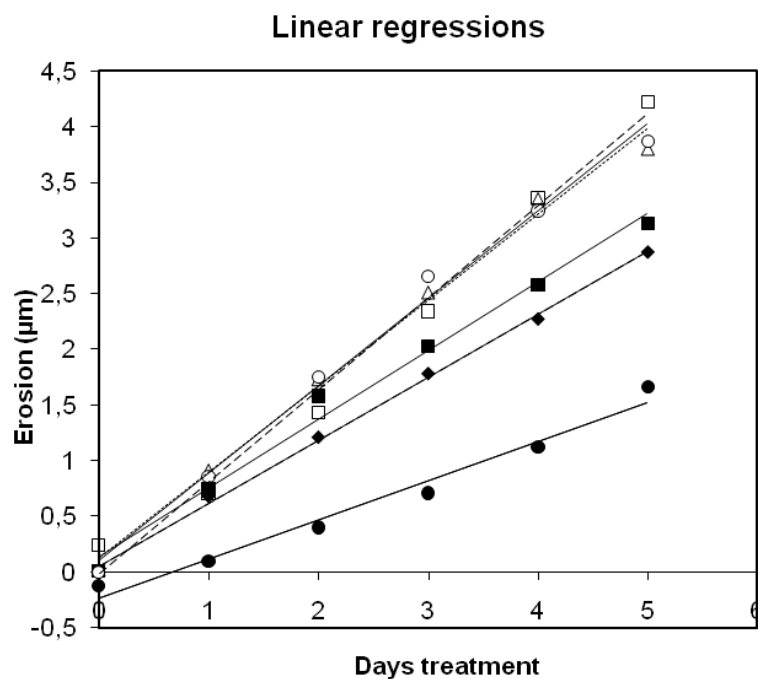
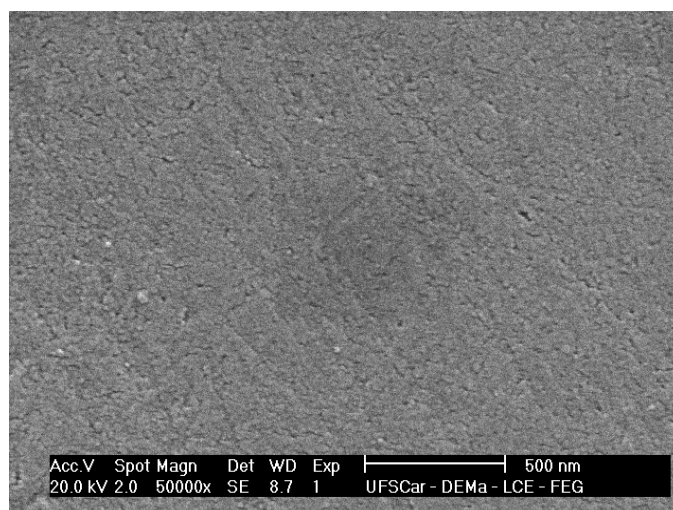
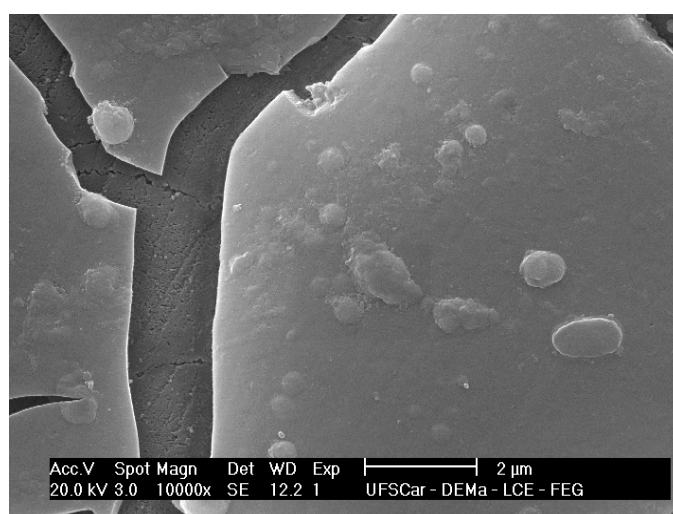


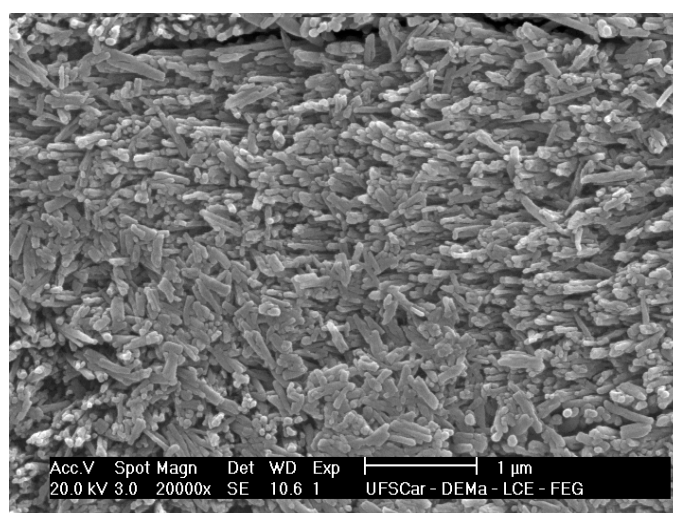
Figure 1 - The linear regression line and the cumulative erosion points for the treatments at each day, considering baseline (zero) as profile after the treatment. Open circle = control ($r^2=0.81$); open triangle = placebo varnish ($r^2=0.87$); open square = TiF₄ solution ($r^2=0.90$); solid diamond = Duraphat ($r^2=0.83$); solid square = Duofluorid ($r^2=0.82$); solid circle = TiF₄ varnish ($r^2=0.62$).



2



3



4

Figure 2 - SEM image of untreated enamel (control).

Figure 3 - SEM images of enamel treated with 4% TiF_4 varnish, showing a continuous surface layer with microcracks.

Figure 4 - SEM image of enamel treated with 4 % TiF_4 solution, showing an etched appearance.

3.4 Capítulo 4

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

Short title: Effect of TiF₄ 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

Summary

Objectives: This *in vitro* study assessed the effect of an experimental 4% TiF₄ 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), TiF₄-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 (\pm 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 $-92.04 \pm 2.54^{A,B}$) and P (-87.96 ± 2.23^b and -94.15 ± 1.14^B). The mean wear (μ m, \pm SD) at the 2nd and 4th day was, respectively: D (3.16 ± 0.32^a and 7.56 ± 0.90^A), F ($3.35 \pm 0.78^{a,b}$ and 7.92 ± 0.98^A), T (3.81 ± 0.43^b and 7.69 ± 0.76^A) and P ($3.43 \pm 1.13^{a,b}$ and 7.31 ± 0.53^A). **Conclusions:** The NaF varnishes reduced the softening, but had no effect on the reduction of the wear. The TiF₄ varnish was not able to reduce the softening and wear.

Introduction

With the decline in the prevalence of dental caries in most developed countries¹, as well as in Brazil², an increasing interest in other dental disorders including tooth wear has arisen³. The term tooth wear is used to describe the processes of erosion, attrition and abrasion⁴⁻⁶.

Dental attrition and abrasion are mechanical wear by tooth to tooth contact⁴⁻⁶ and by abrasive substances⁴⁻⁶, 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 drinks¹⁶. 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 enamel¹⁷.

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 (SnF₂) or amine fluoride (AmF). More recently, other agents such as titanium tetrafluoride (TiF₄) have been investigated for erosion prevention¹⁸⁻²⁵. With respect to TiF₄ 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 defects¹⁶ due to their better capability to adhere on the tooth surface and create a calcium fluoride reservoir²⁶, it seems to be interesting to analyze the effect of an experimental TiF₄ 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*.

Material and Methods

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, Joinville, Santa Catarina, Brazil, n=15), TiF_4 - T (2.45%F, pH 1.0, FGM, Joinville, Santa Catarina, Brazil, n=15) and no-fluoride - P (pH 5.0, FGM, Joinville, 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.

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 (Extex 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-2; 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.

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 [$1.5 \text{ mmolL}^{-1} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $0.9 \text{ mmolL}^{-1} \text{ NaH}_2\text{PO}_4 \cdot 2 \text{ H}_2\text{O}$, $150 \text{ mmolL}^{-1} \text{ KCl}$, $0.1 \text{ molL}^{-1} \text{ Tris buffer}$, 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).

Microhardness and wear determinations

Initially, enamel surface microhardness was measured as described above (Knoop diamond, 25 g, 10 s, HMV-2; 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 wool²⁹ and final microhardness test (SMH₁) was made. The percentage of surface microhardness change was calculated for both days as follows: $\% \text{SMHC} = 100(\text{SMH}_1 - \text{SMH})/\text{SMH}$.

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.

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%.

Results

Tables 1 and 2 show the mean %SMHC (\pm SD) and the mean wear (μm , \pm SD) at the 2nd and 4th day, respectively. The mean %SMHC (\pm 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 $-92.04 \pm 2.54^{A,B}$) and P (-87.96 ± 2.23^b and -94.15 ± 1.14^B). The mean wear (μm , \pm SD) at the 2nd and 4th day was: D (3.16 ± 0.32^a and 7.56 ± 0.90^A), F ($3.35 \pm 0.78^{a,b}$ and 7.92 ± 0.98^A), T (3.81 ± 0.43^b and 7.69 ± 0.76^A) and P ($3.43 \pm 1.13^{a,b}$ 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 ($p > 0.05$).

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 wear¹⁶. This is the first study that evaluated the effect of a TiF₄ 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 TiF₄ varnish was similar to the placebo varnish. As previously mentioned, we are unaware of studies testing TiF₄ varnish, which makes the discussion of the results difficult. According to the literature, the beneficial action of TiF₄ 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.

Conclusion

In this *in vitro* protocol, the experimental TiF₄ 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.

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Tables

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

%SMHC mean* \pm SD				
Day	Duraphat	Duofluorid	TiF ₄	No-F
2 nd	-77.26 \pm 5.05 ^a	-76.79 \pm 7.82 ^a	-88.28 \pm 3.20 ^b	- 87.96 \pm 2.23 ^b
4 th	-88.59 \pm 5.11 ^A	-88.78 \pm 6.11 ^A	-92.04 \pm 2.55 ^{A,B}	-94.15 \pm 1.14 ^B

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

Table 2 - Mean and standard deviation of wear (μ m) for each experimental group.

Wear (μ m) mean* \pm SD				
Day	Duraphat	Duofluorid	TiF ₄	No-F
2 nd	3.16 \pm 0.32 ^a	3.35 \pm 0.78 ^{a,b}	3.81 \pm 0.43 ^b	3.43 \pm 1.13 ^{a,b}
4 th	7.56 \pm 0.90 ^A	7.92 \pm 0.98 ^A	7.69 \pm 0.76 ^A	7.31 \pm 0.53 ^A

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

3.5 Capítulo 5

The erosion and abrasion-inhibiting effect of TiF_4 and NaF varnishes and solutions on enamel in vitro

Key words: Abrasion; Enamel; Erosion; Fluoride

Word count: 3,595

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The erosion and abrasion-inhibiting effect of TiF_4 and NaF varnishes and solutions on enamel in vitro

Summary

Objective: Previous in vitro study has shown that TiF_4 varnish might reduce enamel erosion. However, no data regarding the effect of this experimental varnish on enamel erosion plus abrasion is available so far. Thus, this in vitro study aimed to analyse the effect of TiF_4 compared to NaF varnishes and solutions, to protect against enamel erosion with or without abrasion.

Methods: Enamel specimens were pre-treated with experimental- TiF_4 (2.45%F), experimental-NaF (2.45%F), NaF-Duraphat (2.26%F) and placebo varnishes; NaF (2.26%F) and TiF_4 (2.45%F) solutions. Controls remained untreated. The erosive challenge was performed using a soft drink (pH 2.6) 4x90s/day (ERO), and the toothbrushing-abrasion (ERO+ABR) 2x10s/day, for 5 days. Between the challenges, the specimens were exposed to artificial saliva. Enamel loss was measured profilometrically (μm).

Results: Kruskal-Wallis/Dunn tests showed that all fluoridated varnishes (TiF_4 -ERO:0.53 \pm 0.20, ERO+ABR:0.65 \pm 0.19/ NaF-ERO:0.94 \pm 0.18, ERO+ABR:1.74 \pm 0.37/ Duraphat-ERO:1.00 \pm 0.37, ERO+ABR:1.72 \pm 0.58) were able to significantly reduce enamel loss when compared to placebo varnish (ERO:3.45 \pm 0.41/ ERO+ABR:3.20 \pm 0.66) ($p < 0.0001$). Placebo varnish, control (ERO:2.68 \pm 0.53/ ERO+ABR:3.01 \pm 0.34) and fluoridated (NaF-ERO:2.84 \pm 0.09/ ERO+ABR:2.40 \pm 0.21/ TiF_4 -ERO:3.55 \pm 0.59/ ERO+ABR:4.10 \pm 0.38) solutions did not significantly differ from each other.

Conclusion: Based on the results, it can be concluded that the TiF_4 varnish seems to be a promising treatment to reduce enamel loss under mild erosive and abrasive conditions in vitro.

Introduction

Dental erosion is defined as substance loss by exogenous or endogenous acids without bacterial involvement. The most important sources are dietary acids [1] and one originated from the stomach, in cases of regurgitation and reflux disorders [2]. The acids cause dissolution of prism and interprismatic enamel; if the erosive challenge is ongoing, the dissolution process results in surface loss by a progressive softening of the surface. The softening surface is susceptible to mechanical forces such as toothbrush-abrasion [3,4], which can adversely affect the progression of enamel loss.

Conventional fluorides whose beneficial effect against caries is well known [5] have been tested for prevention or control of dental erosion [6]. The potential of conventional fluorides, such as NaF, to prevent erosive demineralisation is mainly related to the formation of a calcium fluoride (CaF_2)-layer [7,8]. This layer is assumed to behave as a physical barrier hampering the contact of the acid with the underlying enamel or to act as a mineral reservoir, which is attacked by the erosive challenge. Thereafter, calcium and fluoride released might increase the saturation level with respect to dental hard tissue in the liquid adjacent to the surface thus promoting remineralisation.

As the anti-erosive effect of conventional fluorides requires a very intensive fluoridation regime [9], current studies have focused on fluoride compounds which might have a higher efficacy. In this context, compounds containing polyvalent metal ions such as stannous fluoride or titanium tetrafluoride were tested.

The potential of TiF_4 to prevent dental erosive demineralisation has been investigated since 1997 [10]. Its protective effect is related to the formation of an acid-resistant surface coating, the increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice. The glaze-like surface layer observed after the application of TiF_4 is assumed to be formed from a new compound (hydrated hydrogen titanium phosphate) or organometallic complexes that might primarily act as a diffusion barrier [10,11].

Several in vitro studies have shown an inhibitory effect of 0.4-10% TiF_4 solution on dental erosion [10,12-15]. In situ studies showed TiF_4 to be as effective as SnF_2 or AmF solution in the prevention of erosion or combined erosion/abrasion [16,17], while others in situ and in vitro studies did not show any protective effect [18-

20]. The efficacy of TiF_4 is highly dependent on the pH of the agent, since it was shown that enamel erosion can be significantly reduced by TiF_4 (0.5 M F) at native pH (pH 1.2) but not at a pH buffered to 3.5 [21], but the low pH of TiF_4 products does not allow self-application by the patient. Therefore, a recent study showed that TiF_4 incorporated in an experimental varnish, indicated for the clinical application, might be of higher efficacy than a solution [22], however, no data regarding the effect of this experimental varnish on enamel erosion plus abrasion is available so far. It should be highlighted that dental loss generally involves both chemical (erosion) and mechanical (abrasion, for example) factors [23]. Thus, the aim of this in vitro study was to analyse the effect of a single application of TiF_4 varnish/solution compared to NaF varnishes/solution, to protect against enamel erosion with or without abrasion.

Materials and Methods

Preparation of the specimens

One hundred and forty enamel specimens (4 mm x 4 mm x 3 mm) were prepared from labial surfaces of bovine incisors crowns, which were storage in 0.1% buffered thymol solution (pH 7.0) during the preparation of the specimens at 4°C. The specimens were cut using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extex Corp., Enfield, CT, USA), which were separated by a 4-mm thickness spacer. The specimens surfaces were ground flat with water-cooled silicon carbide 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). After the polish, the specimens were cleaned in ultrasonic device with deionized water for 2 min. Prior to the experiment, two layers of nail varnish were applied on 2/3 of the surface of each specimen to maintain reference surfaces for the loss determination after the experiment, leaving 1mm of exposed enamel.

Each 20 enamel specimens were randomly allocated to one control (untreated) and 6 test groups: experimental- TiF_4 varnish (FGM-Dentscare – Brazil, 2.45%F, pH 1.2), experimental-NaF varnish (FGM-Dentscare – Brazil, 2.45%F, pH 4.5), NaF-Duraphat varnish (Colgate - Brazil, 2.26%F, pH 4.5), NaF solution (2.26%F, pH 4.5), TiF_4 solution (2.45%F, pH 1.2) and placebo varnish (FGM-

Dentscare – Brazil, pH 5.0, no-F). In each group, half of the specimens were subdivided into erosion only (n = 10) and erosion plus abrasion (n = 10).

Treatment

The treatments were performed at the beginning of the experiment. For this, the NaF (5g/100 mL) and TiF_4 (4g/100 mL) powders (Sigma-Aldrich, USA) were dissolved in deionized water immediately before the application. The NaF solution was adjusted to pH 4.5 by adding 12.6 g 5 M H_3PO_4 /100 mL. The pH of 1.2 was native for TiF_4 . These F solutions were applied once using a microbrush, and were left on the surface for 1 min [12,22,24]. Excess of the solution was removed from the surface by a cotton roll. After that, the specimens were immersed in artificial saliva for 6 h [22,25].

According to the manufacturers, the varnishes from FGM-Dentscare include colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol (with or without F); while Duraphat contains 2.26% NaF, 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. The pH of all solution/varnishes was measured by indicator paper (± 0.5 units) and by electrode (± 0.01 unit). The pH of the solutions was checked only after the preparation, due to the fact that the solutions were immediately applied on enamel surface. On the other hand, the pH of varnishes was checked several times and remained constant over the experiment.

The varnishes were applied once in a thin layer using a microbrush. The specimens remained in artificial saliva for 6 h [22,25]. After this period, the varnishes were carefully removed from the surface using acetone and a scalpel blade, taking care to avoid touching of the dental surface. Complete removal of the layer was checked microscopically (40x) [22,25].

Erosive and abrasive challenges

All specimens were submitted to a 5-day erosive de- and remineralisation cycling. Erosion was performed with freshly opened bottle of Sprite Zero drink (Coca-Cola Company SpA, Porto Real, RJ, Brazil, pH 2.6, 30 ml/specimen, unstirred, 25°C) four times daily for 90s each. After each demineralisation, the specimens were rinsed with deionized water (5 s) and transferred into artificial saliva (pH 6.8, 30 ml/specimens, unstirred, 25°C) for 2 h. After the last daily erosive treatment, the

specimens were also stored in artificial saliva overnight. The artificial saliva was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3 mM NH_4Cl , 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4 , 3.3 mM urea, 2.4 mM NaH_2PO_4 and ascorbic acid (pH 6.8) [26].

Half of the specimens were also abraded using an electrical toothbrush (Colgate Motions Multi-Action, Brazil) and freshly slurries of an experimental non fluoridated toothpaste (0,5 mL/specimen, 25° C, ratio: 1 toothpaste: 3 water, silica as abrasive: Crest[®], Procter & Gamble, USA) for 10 s (166 oscillations/s). The abrasion was performed 2 times daily, after the first and the last erosive challenges. The toothbrushes were fixed in the constructed device that allowed the heads of the toothbrushes to be aligned parallel to the surface of the specimens. The toothbrushing head was weighted by a precision scale (Pesola, Switzerland) and the weight converted to power (1Kg~9,80665 N, $F=1.5$ N) [27]. The toothbrush heads were replaced per group daily.

After the toothbrushing, the specimens were rinsed in water for 5 s, before being immersed in artificial saliva.

Profilometric measurement

Enamel loss (μm) was quantitatively determined by a contact profilometry (Hommel Tester T1000, VS, Schwenningen, Germany), which presents an accuracy around 0.5 μm . For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water) and the specimens were dried. The diamond stylus moved 2.5 mm from the first reference across the exposed area and on to the other reference area. Four profile measurements were randomly performed in the center of each specimen. The vertical distance between the midpoints of regression lines on the reference and experimental areas was defined as tissue loss, using the software of the device. The values were averaged (μm) and submitted for statistical analysis. The standard deviation of repeated analysis of a given sample was 0.4 μm (Enamel loss mean = 3.5 μm).

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, USA) was 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 equality of variances was not satisfied, the differences among the treatments were analysed by Kruskal-Wallis followed by Dunn's post-hoc tests, separately for the conditions: enamel erosion (ERO) and enamel erosion+abrasion (ERO+ABR). Regarding the difference between the conditions, unpaired t test (TiF₄ varnish, Duraphat, TiF₄ solution, control, placebo varnish) or Mann-Whitney test (NaF varnish and NaF solution) was applied separately for each treatment. The level of significance was set at 5%.

Results

A sample size of ten specimens was calculated considering α -error level of 5% and β -error level of 20% based on previous data [22]. Kruskal-Wallis showed that all fluoridated varnishes were able to significantly reduce enamel ERO and ERO+ABR when compared to placebo varnish ($p < 0.0001$). However, only TiF₄ varnish significantly differed from control for both conditions ($p < 0.0001$). Control, placebo varnish and fluoridated solutions did not significantly differ from each other for all conditions (Table 1, $p > 0.05$).

Generally, there was no significant difference between the conditions ERO vs. ERO+ABR (control and placebo varnish, $p > 0.05$). When the treatments were performed, TiF₄ varnish and NaF solution presented similar results to the control and placebo groups (ERO=ERO+ABR, $p > 0.05$). However, for NaF varnish, Duraphat and TiF₄ solution, the enamel loss by erosion plus abrasion was significantly higher than erosion only ($p < 0.001$, $p = 0.004$ and $p = 0.02$, respectively).

Discussion

The present study gives additional data to the paper by Magalhães et al.[22], since it included a group with an experimental NaF varnish (with the same base and F concentration as TiF₄ varnish) and a group with NaF solution. Besides, in the present study TiF₄ varnish and solution were tested also for the prevention of enamel erosion plus abrasion. However, the low pH required for the efficacy of the titanium tetrafluoride solution does not allow a self-application of the patient, but only a clinical use. Thus, the present study focused mainly on the possibility of using the TiF₄ varnish in the clinical situation. Fluoride varnish is considered a good vehicle for

topical application due to the formation of a mechanical barrier, in comparison with other fluoride vehicles such as solutions and gels [18,19]. Varnish has other advantages, as it is easy to apply and safe and well tolerated by infants, young children, and individuals with special needs.

In the present study, all fluoridated varnishes were able to significantly to reduce enamel erosion and erosion plus abrasion compared to placebo varnish, however, only TiF_4 varnish was better than control. When both NaF varnishes are compared, it should be consider that the small differences in the fluoride concentration and the base might not have influenced the effect of the products on the enamel loss. In this case the contact time seems also not to be an important factor, as NaF varnishes and solutions presented similar effect on enamel loss. On the other hand, TiF_4 varnish was the best treatment, while the TiF_4 solution was the worst treatment option against enamel loss.

Topical fluoridation induces the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in case of conventional fluorides like NaF) or of metal-rich surface precipitates (in case of TiF_4). From the results of the present study, it can also be speculated that the CaF_2 -layer is less resistant to the erosive and abrasive challenges compared to the metal-rich surface precipitates formed by the application of TiF_4 varnish on enamel *in vitro*, in accordance with Magalhães et al.[22]. However, the protective effect of TiF_4 varnish on enamel seems to be over after 120 minutes of erosive challenges *in vitro* [28].

On the other hand, TiF_4 solution, with similar pH and fluoride concentration as TiF_4 varnish, was unable to prevent enamel erosion [22] and erosion plus abrasion in the present study. This result was expected, as TiF_4 agents present a low pH that might cause some damage of the enamel surface during the application [22]. The different surface effect of TiF_4 formulations seems to be related to the better ability of the varnish to adhere to the tooth surface, which allows an increased contact time and hence prolongs the reaction between the TiF_4 and dental surface. It suggests that TiF_4 incorporated into a varnish might reduce the effects of its low pH on the enamel, when in contact with saliva for 6h in comparison to TiF_4 solution. Magalhães et al.[22] showed that enamel treated with TiF_4 varnish presented a thin smooth surface layer in which microcracks were visible. In contrast, enamel treated with TiF_4 solution showed an etched surface.

In situ and in vitro studies also did not show any protective effect of TiF_4 solution [18-20]. On the other hand, other studies have shown an inhibitory effect of TiF_4 solution on dental erosion [10,12-17]. The most plausible explanation for the contrasting results is the difference between the methodologies used in those studies, as well as the response variables. In most of the studies, a less concentrated TiF_4 solution (0.5M F) [13-17,29] is applied more than once [15,16] or the experimental period is too short [13,14,17], which might not adequately simulate the clinical condition.

Generally, ERO+ABR condition showed slight differences in enamel loss values compared to the ERO only. This result may be a consequence of the experimental protocol, in which the abrasion procedure (2x10s) was less aggressive than the erosive challenges (4x90s). Using this protocol, the toothbrushing abrasion could not have adversely affected the progression of enamel erosive loss. Only for both NaF varnishes and TiF_4 solution, the ERO+ABR induced a higher enamel loss in comparison to the ERO. A hypothesis to explain this finding for NaF varnishes is that the F incorporated into enamel by the application of the products might be not resistant even to the mild abrasive forces. The same is valid for TiF_4 solution that has shown be unable to reduce enamel loss. However, the relevance of this result should be proved in further studies. For the other treatments, no significant differences were found between both conditions.

Under the conditions of the present study it can be concluded that the TiF_4 varnish seems to be a promising treatment to reduce enamel loss under mild erosive and abrasive conditions in vitro. Therefore, before F varnishes start to be frequently used in the clinical situation, it is advised to test the effect of these treatments on human enamel and dentin erosion and abrasion in situ, a condition that more closely resembles the clinical situation.

Bullet Points

This paper showed that 1) the experimental TiF_4 varnish is able not only to reduce enamel erosion, but also erosion associated with abrasion, situation more close to the clinical condition; 2) the NaF varnishes and solution presented the same effect on the prevention of enamel loss by erosive and abrasive challenges and; 3) TiF_4 solution is unable to reduce enamel loss and it should be not use in the clinical situation as mouthrinse since it has low pH.

This paper is important to pediatric dentists since 1) there is evidence that the prevalence of erosion is steadily increasing especially in children and adolescents due to the changes in the modern diet; 2) The experimental TiF_4 varnish seems to be a promising treatment to reduce enamel erosion and abrasion and; 3) The fluoride varnishes have some advantages compared to mouthrinses or gels: It is safe and well tolerated by infants, young children, and individuals with special needs and it is easy to apply.

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Table 1 - Mean enamel ERO and ERO+ABR (μm) \pm S.D. in the different groups (n=10).

Treatment	ERO	ERO+ABR
TiF ₄ varnish	0.53 \pm 0.20 ^a	0.65 \pm 0.19 ^a
NaF varnish	0.94 \pm 0.18 ^{ab}	1.74 \pm 0.37 ^{ab*}
Duraphat	1.00 \pm 0.37 ^{ab}	1.72 \pm 0.58 ^{ab*}
NaF solution	2.84 \pm 0.09 ^{bc}	2.40 \pm 0.21 ^{bc}
TiF ₄ solution	3.55 \pm 0.59 ^c	4.10 \pm 0.38 ^{d*}
Placebo varnish	3.45 \pm 0.41 ^c	3.20 \pm 0.66 ^{cd}
Untreated (Control)	2.68 \pm 0.53 ^{bc}	3.01 \pm 0.34 ^{bcd}

Distinct lower case letters in the same column indicate significant differences among the fluoride solutions/varnishes (Kruskal-Wallis, $p < 0.0001$).

* indicates significant difference between the conditions ERO and ERO+ABR for each group separately (Unpaired t test or Mann Whitney, $p < 0.05$).

3.6 Capítulo 6

Effect of a single application of TiF_4 and NaF varnishes and solutions on dentin erosion *in vitro*

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Key words: Dentin; Erosion; Fluoride

Effect of a single application of TiF_4 and NaF varnishes and solutions on dentin erosion *in vitro*

Summary

Objectives: This *in vitro* study aimed to analyse the effect of a single application of TiF_4 and NaF varnishes and solutions to protect against dentin erosion. **Methods:** Bovine root dentin samples were pre-treated with NaF-Duraphat varnish (2.26%F, pH 4.5), NaF/ CaF_2 -Duofluorid varnish (5.63%F, pH 8.0), NaF-experimental varnish (2.45%F, pH 4.5), TiF_4 -experimental varnish (2.45%F, pH 1.2), NaF solution (2.26%F, pH 4.5), TiF_4 solution (2.45%F, pH 1.2) and placebo varnish (pH 5.0, no-F varnish control). Controls remained untreated. Ten samples in each group were then subjected to an erosive demineralization (Sprite Zero, 4x90s/day) and remineralization (artificial saliva, between the erosive cycles) cycling for 5 days. Dentin loss was measured profilometrically after pre-treatment and after 1, 3 and 5 days of de-remineralization cycling. The data were statistically analysed by two way ANOVA and Bonferroni's *post-hoc* test ($p < 0.05$). **Results:** After pre-treatment, TiF_4 solution significantly induced surface loss ($1.08 \pm 0.53 \mu\text{m}$). Only Duraphat reduced the dentin loss overtime, but it did not significantly differ from placebo varnish (at 3rd and 5th days) and TiF_4 varnish (at 3rd day). **Conclusions:** Duraphat varnish seems to be the best option to partially reduce dentin erosion. However, the maintenance of the effects of this treatment after successive erosive challenges is limited.

Introduction

The potential of conventional fluorides, such as sodium fluoride, to prevent dental erosive demineralisation is related to the formation of a CaF_2 -layer which is assumed to act as a physical barrier hampering the contact of the acid with the underlying dental structure or as acting as a mineral reservoir, which is attacked by the erosive challenge, thus buffering the acids or promoting the remineralisation^{1,2}. However, CaF_2 -precipitates are readily soluble in acids and, thus, the erosion-protective capability of sodium fluoride is probably limited³. Therefore, current research is focusing on other fluoride compounds, containing polyvalent metal ions, such as titanium tetrafluoride (TiF_4), which might have a higher capacity to prevent dental erosion⁴.

The potential of TiF_4 to prevent dental erosive demineralisation has been investigated since 1997⁵. Its protective effect is attributed to the formation of an acid-resistant surface coating, the increase of fluoride uptake and the titanium incorporation in the hydroxyapatite lattice⁴⁻⁸. The glaze-like surface layer observed after the application of TiF_4 is assumed to be formed from TiO_2 or organometallic complexes that might primarily act as a diffusion barrier⁴⁻⁸. As the layer is rich of titanium and fluoride it is discussed that the coating might also act as reservoir for fluoride ions which in turn might retard acid dissolution or increase remineralisation of the underlying dental hard tissue. The increased fluoride uptake found after application of TiF_4 can be explained by the ability of the polyvalent metal ion to form strong fluoride complexes firmly bound to the apatite crystals and to organic content^{4,7}.

Even though TiF_4 solution was shown to be more effective to prevent dental erosive demineralisation than sodium, stannous or amine fluoride⁹⁻¹², some studies found that TiF_4 solution is not able to completely protect against dental erosion, especially due the fact that the low pH of the agent might cause some dental loss during the application¹²⁻¹⁶. However, it might be assumed that the preventive effect of TiF_4 against demineralisation is also dependent on the agent used, as it was recently shown in vitro that a TiF_4 varnish exhibited a higher protective potential than a TiF_4 solution on enamel erosion¹⁶.

On the other hand, to prevent dentin erosion, although a study had shown that TiF_4 was more effective than NaF ¹⁴, the lack of data requires further studies dealing

with the impact of TiF_4 solution on dentin erosion. Additionally, there is no study testing the impact of TiF_4 varnish on dentin erosion so far.

Thus, the aim of this in vitro study was to analyse the effect of a single application of TiF_4 and NaF varnishes and solutions to protect against dentin erosion.

Materials and methods

Samples preparation

Eighty dentin samples (4 mm x 4 mm x 3 mm) were prepared from the labial surfaces of bovine root. The samples were cut using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extex Corp., Enfield, CT, USA), which were separated by a 4-mm thickness spacer. The sample surfaces 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). Prior to the experiment, two layers of nail varnish were applied on half of the surface of each sample to maintain a reference surface for wear determination after the experiment.

Each 10 dentin samples were randomly allocated to one control (untreated) and 7 test groups: NaF-Duraphat varnish (Colgate - Brazil, 2.26% F, pH 4.5), NaF/ CaF_2 -Duofluorid varnish (Dentscare - Brazil, 5.63%F, pH 8.0), NaF-experimental varnish (Dentscare - Brazil, 2.45% F, pH 4.5), TiF_4 - experimental varnish (Dentscare - Brazil, 2.45% F, pH 1.2), NaF solution (2.26% F, pH 4.5), TiF_4 solution (2.45% F, pH 1.2) and placebo varnish (Dentscare - Brazil, pH 5.0, no-F varnish control).

Treatment

The NaF (5 g/100 mL) and TiF_4 (4 g/100 mL) powders (Sigma-Aldrich, USA) were dissolved in water immediately before the application. These F solutions were applied once using a microbrush, and were left on the surface for 1 min^{9,10,14}. Excess of the solution was removed from the surface by a cotton roll. After that, the samples were immersed into artificial saliva for 6 h.

Regarding the varnishes, Duofluorid was transparent, while the other varnishes were yellow. According to the manufacturers, the varnishes from Dentscare contain colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and

ethanol (with or without F), while Duraphat contains 2.26% NaF, 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. The pH of all solution/varnishes was measured by indicator paper (± 0.5 units) and by electrode (± 0.01 unit). The pH of the solutions was checked only after the preparation, due to the fact that the solutions were immediately applied on dentin surface. On the other hand, the pH of varnishes was checked several times and remained constant over the experiment.

The varnishes were applied once in a thin layer using a microbrush. The samples remained in artificial saliva for 6 h¹⁶. After this period, the varnishes were carefully removed from the surface using acetone and a scalpel blade, taking care to avoid touching of the dental surface. Complete removal of the layer was checked microscopically (40x)¹⁶.

Erosive cycling

The samples were submitted to a 5-day de- and remineralisation cycling. Erosion was performed with Sprite Zero (pH 2.6, 30 ml/sample, unstirred, 25° C, Coca-Cola, Brazil) four times daily for 90 s. After demineralisation, the samples were rinsed with tap water and transferred to artificial saliva (30 ml/sample, unstirred, 25° C) for 2 h. After the last daily erosive treatment, the samples were stored in artificial saliva overnight. The artificial saliva (pH 7.0) was renewed daily and consisted of 1.5 mmolL⁻¹ Ca(NO₃)₂·4H₂O, 0.9 mmolL⁻¹ NaH₂PO₄·2 H₂O, 150 mmolL⁻¹ KCl, 0.1 molL⁻¹ Tris buffer, 0.03 ppm F, pH 7.0¹⁷.

Profilometric measurement

Dentin losses (μm) were quantitatively determined by profilometry (Hommel Tester T1000, VS, Schwenningen, Germany) after fluoride application as well as after 1, 3 and 5 days of de-remineralisation cycling. As no pre-treatment was performed in the control group, these samples were measured only after pH cycling days.

The dentin samples were maintained wet until the analysis to avoid shrinkage. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water)¹⁶. The samples were slightly dried, which means that only the excess of water was gently removed with paper, and immediately analysed. The diamond stylus moved from the reference to the exposed area (2 mm length). Three profile measurements were performed in the center of

each specimen and averaged. After the treatment measurement, the reference area of the specimens was again covered with nail varnish. To assure that the nail varnish was placed over the original reference area, the position of the nail varnish was marked by carving with a scalpel at the borders of the sample.

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, USA) was 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, the data were analysed by 2-way ANOVA followed by Bonferroni's post-hoc tests. Thereby, the F solutions/varnishes were considered as dependent and the time points (1, 3 and 5 day of pH cycling) as independent variables. The level of significance was set at 5%.

Results

After pre-treatment, only TiF_4 solution significantly induced surface loss ($1.08 \pm 0.53 \mu\text{m}$). For the other groups, it was not possible to measure any surface alteration by profilometry, which has an accuracy of $0.5 \mu\text{m}$.

Dentin losses (mean \pm standard deviation [μm]) after 1, 3 and 5 days of de-mineralisation cycles are presented in Table 1. Two-way ANOVA revealed significant differences among the F solutions/varnishes ($F=77.30$, $p<0.0001$), time points ($F=6.05$, $p<0.0001$) and interaction between these variables ($F=2.57$, $p=0.0007$).

On the first day, only Duraphat varnish was able to significantly reduce the loss compared to untreated group. On the other hand, TiF_4 solution presented higher wear than untreated group. On the third day, Duraphat, TiF_4 and placebo varnishes resulted in less dentin wear than untreated group. On the fifth day, only Duraphat varnish was able to significantly reduce dentin loss when compared to untreated group, but it did not significantly differ from NaF varnish, NaF solution, placebo varnish and TiF_4 varnish, which in turn did not significantly differ from the untreated group.

Discussion

In the present study, the varnishes and solutions were applied only once in order to simulate the clinical situation with a single professional application. In contrast to Vieira et al.¹⁸⁻²⁰, the varnishes were removed after 6 h of application in the present study, before the de-remineralisation cycling. This was done to focus on the chemical effect of the varnishes rather than on the mechanical protection. Thus, the varnishes were removed to simulate the clinical situation in which the varnishes might be removed after some hours by toothbrushing or mastication.

Regarding F solutions, the time of application ranges from 1 up to 4 minutes in the Literature^{5,9-16,18-20}. In the present study, the time of 1 minute was chosen based on the results of previous study that showed a glaze layer on enamel surface after 1 min of application of 4% TiF_4 solution *in situ* by SEM¹⁴. This protocol was also followed by other authors^{5,9,14,16}.

As a gold-standard control, a commercial NaF varnish (Duraphat, 2.26% F, pH 4.5) which is frequently used in the clinic was chosen. The other varnishes were prepared by the same company and presented the same base composition. Duofluorid (positive control, 5.63% F, pH 8.0) is a commercial product while the NaF-experimental varnish/solution were prepared in order to present the same F concentration as TiF_4 varnish/solution and the same pH as the gold-standard control.

The fluoride concentration of the TiF_4 varnish/solution (2.45% F, pH 1.2) was chosen in accordance to a previous study, showing favorable results for this TiF_4 varnish to prevent enamel erosion¹⁶. The TiF_4 agents show a natural lower pH compared to the NaF product. It was previously shown that the erosion inhibiting effect of TiF_4 is pH-dependent and that TiF_4 is less effective at higher pH (when it is buffered) for both enamel and dentin^{21,22}.

On the other hand, NaF commercial varnishes present a high pH range, varying from 4.5 to 8.0. However, it is not advisable to reduce the pH of NaF products below 3.5, considering the clinical application, since the same study cited above showed that NaF solution at pH 1.2 was not able to significantly reduce enamel erosion compared to TiF_4 solution at the same pH²¹.

In the present study, only TiF_4 solution provoked dentin loss by the application. This result was expected, as TiF_4 agents present a low pH. This is agreement with previous studies in which the application of the 4% TiF_4 solution (1 min) produced

significant enamel loss^{14,16}. However, Magalhães et al.¹⁶ showed that TiF₄ varnish led to a significant growth of enamel surface after 6 h of contact, which could not be seen in the present study. The different surface effect of TiF₄ formulations seems to be related to the better ability of the varnish to adhere to the tooth surface, which allow an increased contact time with the enamel and hence prolongs the reaction between the TiF₄ and enamel. The lack of erosion after TiF₄ varnish suggests that the varnish might reduce the effects of the low pH on the enamel, when in contact with saliva. The same hypothesis can be used in the present study, to justify the differences between TiF₄ agents.

The results showed that only on the first day Duraphat significantly reduced the dentin loss compared to placebo varnish and untreated group. After that, Duraphat was similar to placebo varnish, even the former was significantly better than untreated group. These findings showed that despite NaF (especially from Duraphat varnish) might have decreased the erosive dentine loss, this effect was only present on the first day. After that, it can be speculated that the CaF₂-layer formed might have not been more resistant to acid challenges than the varnishes' base that could have remained on the dental surface. This assumption is based on the fact that in the present study even the F free- varnish itself showed an effect against dentin erosion. It is possible that some base of the varnishes could have been not detected by the microscope (40x) and thus remained on the surface. However it is important to point out that the base and F content of the varnishes were not more able to resist the erosive challenges on the 5th day.

Generally, the best effect of Duraphat might be related to both chemical and mechanical impact of the varnish, which is contrasting to the results found with enamel erosion in a previous study that showed better results with TiF₄ varnish¹⁶. The explanation for that opposite result might be related to the kind of dental substrate and erosive protocol.

The impact of F varnishes seems to be higher on enamel¹⁶ than on dentin, both exposed to the same total erosion time (6 min/day, total 30 min), which might be related to the composition of these dental substrates and their interaction with the components of the varnishes. Consider the different results for the NaF-based varnishes in the present study, it is important to point out that the base of the Duraphat varnish and Dentscare's varnishes are different. This might explain the

different results between NaF varnishes. Based on this fact, it might be speculated that base of the varnishes might have reacted with the dentin organic content.

Other important aspect is the presence of smear layer and its influence on the fluoride uptake^{23,24}. In the present study, the smear layer was not removed using EDTA or NaOCl, in order to simulate the clinical situation. The presence of smear layer have shown to produce a massive structure when TiF₄ was applied²³ and to increase KOH-soluble and structurally bound fluoride uptake into superficial and deeper layers compared to smear layer free surface, when NaF was used²⁴.

As dentin presents a lower mineral content than enamel, this tissue is more susceptible to erosion during the initial challenges. With the erosion time, the organic content of dentin is exposed, which might act as a barrier to the acid diffusion, reducing the progression of dentin loss^{25,26}. As it can be seen in Table 1, the rate of dentin erosion progression was much lower from day 3 to day 5 as compared to that observed between days 1 and 3. Therefore, the effect and the differences among the F treatments might be reduced overtime for this dental substrate.

In relation to the erosive protocol, in the above mentioned study¹⁶ the erosive challenges were performed in an artificial mouth, with a controlled acid and saliva flow, which might have removed the varnishes' base from the enamel surface, reducing the impact of the mechanical barrier and allowing the chemical effect of the products to be distinguished. In agreement to this hypothesis, the first study published about the effect of TiF₄ and NaF varnishes on enamel erosion, using a similar in vitro protocol as the present study, with higher erosive challenges, also did not show good results²⁷.

Therefore, in further studies it would be interesting to test and compare the efficacy of F products against enamel and dentin erosion, using an artificial mouth, also associated to abrasive forces, overtime (> 5 days). Moreover, the effect of these treatments on human enamel and dentin should be evaluated *in situ*, a condition that more closely resembles the clinical situation.

Under the conditions of the present study it can be concluded that Duraphat varnish seems to be the best option to partially reduce dentin erosion. However, the maintenance of the effects of this treatment after successive erosive challenges is limited.

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Table 1 - Mean dentin loss (μm) \pm S.D. in the different groups (n=10).**Table 1**

Treatments	Days		
	1 st	3 rd	5 th
Duraphat	0.65 \pm 0.37a	1.99 \pm 0.26a	2.71 \pm 0.41 ^a
Duofluorid	1.40 \pm 0.53b,c	2.73 \pm 0.32c	3.54 \pm 0.28c
NaF varnish	1.00 \pm 0.31a,b	2.95 \pm 0.31c	2.97 \pm 0.15a,b
TiF₄ varnish	1.21 \pm 0.41b,c	2.22 \pm 0.36a,b	2.95 \pm 0.33a,b
NaF solution	1.18 \pm 0.42b,c	2.82 \pm 0.37c	3.12 \pm 0.62a,b
TiF₄ solution	1.56 \pm 0.29c	2.62 \pm 0.37b,c	3.25 \pm 0.23b,c
Placebo varnish	1.07 \pm 0.23b	2.19 \pm 0.18a	3.16 \pm 0.65a,b
Untreated	1.15 \pm 0.15b	2.79 \pm 0.35c	3.25 \pm 0.50b,c

Distinct lower case letters indicate significant differences among the F solutions/varnishes for each day of pH- cycling.

3.7 Capítulo 7

Effect of NaF and TiF₄ varnish and solution on bovine dentin erosion plus abrasion in vitro

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Short title: NaF and TiF₄ on dentin erosion/abrasion

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Abstract

Objectives: This in vitro study aimed to analyze the effect of TiF_4 compared to NaF varnishes and solutions, to protect against dentin erosion associated with abrasion.

Material and Methods: Bovine dentin specimens were pre-treated with NaF-Duraphat (2.26% F), NaF/ CaF_2 -Duofluorid (5.63% F), experimental-NaF (2.45% F), experimental- TiF_4 (2.45% F) and placebo varnishes; NaF (2.26% F) and TiF_4 (2.45% F) solutions. Controls remained untreated. The erosive pH cycling was performed using a soft drink (pH 2.6) 4x 90 s/day, and the toothbrushing-abrasion 2x10 s/day, in vitro for 5 days. Between the challenges, the specimens were exposed to artificial saliva. Dentin tissue loss was measured profilometrically (μm). **Results:** ANOVA/Tukey's test showed that all fluoridated varnishes (Duraphat- 7.5 ± 1.1 / Duofluorid- 6.8 ± 1.1 / NaF- 7.2 ± 1.9 / TiF_4 - 6.5 ± 1.0) were able to significantly reduce dentin tissue loss (40.7% reduction compared to control) when compared to placebo varnish (11.2 ± 1.3), control (11.8 ± 1.7) and fluoridated (NaF- 9.9 ± 1.8 / TiF_4 - 10.3 ± 2.1) solutions ($p < 0.0001$), which in turn did not significantly differ from each other. **Conclusion:** All fluoridated varnishes, but not the solutions, had a similar performance and a good potential to reduce dentin tissue loss under mild erosive and abrasive conditions in vitro. Risk patients for erosion and abrasion, especially those with exposed dentin, should benefit from this clinical preventive measure. Further research has to confirm this promising result in the clinical situation.

Key-words: Abrasion; Dentin; Erosion; Fluoride

Introduction

Dental erosion is defined as substance loss by exogenous or endogenous acids without bacterial involvement. The most important sources are dietary acids [1] and one acid originated from the stomach, in cases of regurgitation and reflux disorders [2]. In dentin, the erosive demineralisation is mostly diffusion controlled, as the increasing exposure of organic matrix hampers ion diffusion and, thus, reduces further progression of dentin erosion [3,4]. The demineralized collagen layer might be affected by enzymatic and chemical degradation [5] as well as by abrasive influences [6].

The exposed root dentin, in case of gingival trauma or periodontal disease, might be accompanied by an increased risk for dental hard tissue loss by different chemical (erosion) and physical (toothbrushing abrasion) processes. In order to prevent dentin tissue loss, fluoride application is recommended. However, considering the severe and chronic acid exposure in patients suffering from dental erosion, the effect of conventional fluoride, such as NaF, by the formation of a protective CaF_2 layer, is probably limited over time [7]. Therefore, fluoride compounds with a distinct potential to resist to erosive challenges are required.

The potential of TiF_4 to prevent dental erosive demineralization has been investigated since 1997 [8]. Its protective effect is related to the formation of an acid-resistant surface coating, the increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice [8-11]. The glaze-like surface layer observed after the application of TiF_4 is assumed to be formed from a new compound (hydrated hydrogen titanium phosphate) or organometallic complexes that might primarily act as a diffusion barrier [8-11].

Previous in vitro studies have shown that TiF_4 was as effective as or better than NaF solution on prevention of dentin erosion [12,13]; however, other works indicated a similar or lower effect of TiF_4 compared to AmF solution against dentin erosion [14,15]. The different results might be explained by the F concentration, pH and erosive challenges. It might be assumed that the preventive effect of TiF_4 against erosive demineralization is also dependent on the agent used, as it was recently shown in vitro that a 4% TiF_4 varnish exhibited a higher protective potential than a 4% TiF_4 solution on enamel erosion [16].

Recently, Magalhães et al. [17] showed that only NaF, but not TiF_4 , varnish seems to be able to partially reduce dentin erosion. However, no data regarding the effect of this experimental varnish on dentin erosion plus abrasion is available so far. Thus, the aim of this in vitro study was to analyze the effect of a single application of TiF_4 varnish/solution compared to NaF varnishes/solution, to protect against dentin erosion and abrasion.

Materials and Methods

Preparation of the dentin specimens

Eighty dentin specimens (4 mm x 4 mm x 3 mm) were prepared from forty bovine dental roots, which were stored in 0.1% buffered thymol solution (pH 7.0) at 4°C. The specimens were cut from the cervical region of the root (labial and lingual surfaces), using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extex Corp., Enfield, CT, USA), which were separated by a 4-mm thickness spacer. The specimens surfaces were ground flat with water-cooled silicon carbide discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and finally polished with felt paper wet by a diamond solution (1 μm thickness; Buehler). After the polish, the specimens were cleaned in ultrasonic device with deionized water for 2 min. Prior to the experiment, two layers of nail varnish were applied on 2/3 of the surface of each specimen to maintain reference surfaces for the dentin tissue loss determination after the experiment (1mm of the exposed dentin area).

A sample size of ten specimens was calculated considering α -error level of 5% and β -error level of 20% based on previous data [17]. Ten dentin specimens were randomly allocated to one control (untreated) and to each of the 7 test groups: NaF-Duraphat varnish (Colgate - Brazil, 2.26%F, pH 4.5), NaF/ CaF_2 -Duofluorid varnish (FGM-Dentscare – Brazil, 5.63%F, pH 8.0), experimental- NaF varnish (FGM-Dentscare – Brazil, 2.45%F, pH 4.5), experimental- TiF_4 varnish (FGM-Dentscare – Brazil, 2.45%F, pH 1.2), NaF solution (2.26%F, pH 4.5), TiF_4 solution (2.45%F, pH 1.2) and placebo varnish (FGM-Dentscare – Brazil, pH 5.0, no-F varnish control).

Treatment

The treatments were performed at the beginning of the experiment. For this, the NaF (5 g/100 mL) and TiF_4 (4 g/100 mL) powders (Sigma-Aldrich, USA) were dissolved in deionized water immediately before the application. The NaF solution was adjusted to pH 4.5 by adding 12.6 g 5 M H_3PO_4 /100 mL. The pH of 1.2 was native for TiF_4 . These F solutions were applied once using a microbrush, and were left on the surface for 1 min [16-19]. Excess of the solution was removed from the surface by a cotton roll. After that, the specimens were immersed in artificial saliva for 6 h [16,17].

Regarding the varnishes, Duofluorid was transparent, while the other varnishes were yellow. According to the manufacturers, the varnishes from FGM-Dentscare include colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol (with or without F); while Duraphat contains 5% NaF (2.26% F), 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. All varnishes had a soft consistency. The pH values of all varnishes and solutions were measured by indicator paper (± 0.5 units) and by electrode (± 0.01 unit), respectively. The pH of the solutions was checked only after the preparation, due to the fact that the solutions were immediately applied on dentin surface. On the other hand, the pH of varnishes was checked several times (after the preparation, before the application and after the experiment) and remained constant over the study.

The varnishes were applied once in a thin layer using a microbrush. The specimens remained in artificial saliva for 6 h [16,17]. The difference between the application times of the solutions and varnishes was purposely done in order to simulate the clinical condition. After this period, the varnishes were carefully removed from the surface using acetone solution (1:1 water) and a scalpel blade, taking care to avoid touching of the dental surface. Complete removal of the layer was checked microscopically (40x) [16,17]. After that, all specimens were immersed in artificial saliva for 6 h up to the next day when the erosive and abrasive challenges started [16,17].

Erosive and abrasive challenges

All specimens were submitted to a 5-day erosive de- and remineralization cycling. Erosion was performed with freshly opened bottle of Sprite Zero drink (Coca-Cola Company SpA, Porto Real, RJ, Brazil, pH 2.6, 30 ml/specimen, unstirred, 25°C)

four times daily for 90 s each. After each erosive challenge, the specimens were rinsed with deionized water (5 s) and transferred to artificial saliva (pH 6.8, 30 ml/specimens, unstirred, 25° C) for 2 h. The artificial saliva was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl₂·2H₂O, 3 mM NH₄Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K₂HPO₄, 3.3 mM urea, 2.4 mM NaH₂PO₄ and ascorbic acid (pH 6.8) [20].

All specimens were also exposed to freshly made slurries of an experimental non-fluoridated toothpaste (ratio: 1 toothpaste: 3 water, silica as abrasive: Crest[®], Procter & Gamble, USA) 2 times daily (0.5 mL/specimen, 25° C), after the first and the last erosive challenges and then, abraded using an electrical toothbrush for 10 s (166 oscillations/s, Colgate Motions Multi-Action, Brazil). The toothbrushes were mounted in a device that was constructed to allow the heads of the toothbrushes to be aligned parallel to the specimen surface. The power of toothbrushing was standardized at 1.5 N for all specimens (1Kg~9,80665 N, F=1.5 N) [21], by weighing the toothbrushing head using a precision scale (Pesola, Switzerland) and adjusting with elastic bands. The toothbrush heads were replaced per group daily.

After the toothbrushing, the specimens were rinsed in water for 5 s, before being re-immersed in artificial saliva. At the end of the day, the specimens were also stored in artificial saliva overnight

Profilometric measurement

After 5 days, dentin tissue loss (µm) was quantitatively determined by a contact profilometer (Mahr Perthometer, Göttingen, Germany). The dentin specimens were maintained wet until the analysis to avoid shrinkage. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). The specimens were slightly dried, which means that only the excess of water was gently removed with filter paper, and immediately analyzed [17]. The diamond stylus moved from the first reference across the exposed area and on to the other reference area (2.5 mm length and 2.0 mm width). Five profile measurements were performed in the center of each specimen at intervals of 0.5 mm. The vertical distance between the midpoints of regression lines on the reference and experimental areas was defined as tissue loss (µm) (Software Marh Surf XR20, 2009). The accuracy of the method is around 0.5 µm. The standard deviation of 5 repeated analyses of a given sample was 0.3 µm.

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, USA) was 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, the data were analysed by ANOVA followed by Tukey's post-hoc tests. The level of significance was set at 5%.

Results

ANOVA showed that all fluoridated varnishes were able to significantly reduce dentin tissue loss (40.7% reduction in dentin tissue loss compared to control) when compared to placebo varnish, control and fluoridated solutions ($p < 0.0001$). There were no significant differences among the fluoridated varnishes (from 36.4 to 44.9% reduction). The placebo varnish, control and fluoridated solutions -treated specimens presented the highest dentin tissue loss means, which in turn did not significantly differ from each other (Table 1).

Discussion

In general, the present study showed that fluoridated varnishes, regardless of the F salt and pH, were able to significantly reduce dentin tissue loss, while the fluoridated solutions were not effective. In the present study, the varnishes and solutions were applied only once in order to simulate the clinical situation with a single professional application, according to previous studies [16,17]. The varnishes were removed after 6h, reproducing the *in vivo* situation in which the varnishes might be removed after some hours by toothbrushing or mastication. Thus, the present study focuses more on the chemical rather than on the mechanical effect of the varnishes. Regarding the solutions, they were applied for 1 minute, since the time of application ranges from 1 up to 4 minutes in the literature [8,12,16-19].

Two NaF varnishes that are frequently used in the clinic were chosen as controls. An experimental NaF varnish was prepared by the same company and presented the same base composition and F concentration as the experimental TiF_4 varnish. The F concentration of the experimental TiF_4 varnish/solution was chosen in

accordance to a previous study, showing favorable results for this TiF_4 varnish to prevent enamel erosion [16]. The TiF_4 agents show naturally lower pH compared to the NaF products, which in turn present a high pH range, varying from 4.5 to 8.0. The efficacy of TiF_4 is highly dependent on the pH of the agent, since it was shown that dentin erosion can be significantly reduced by TiF_4 (0.5 M F) at native pH (pH 1.2) but not at a pH buffered to 3.5 [13]. NaF solution with pH 1.2 was also able to significantly reduce dentin erosion [13]. However, NaF solution, in the present study, was used as vehicle control of the Duraphat varnish, because of this the pH and F concentration were not adjusted to be similar to TiF_4 . A limitation of using experimental-NaF varnish with low pH (1.2) is the stability of the product over time.

In the present study, TiF_4 solution was unable to prevent dentin erosion-abrasion according to the previous data [15,17]. It was able to open dentinal tubules after the application, working as a smear layer remover (data unpublished). This result was expected, as TiF_4 agents present a low pH. However, a surface layer covering the dentinal tubules was seen after the application of TiF_4 varnish (data unpublished). The different surface effect of TiF_4 formulations seems to be related to the better ability of the varnish to adhere to the tooth surface, which allows an increased contact time and hence prolongs the reaction between the TiF_4 and dental surface. It suggests that TiF_4 incorporated into a varnish might reduce the effects of its low pH on the dentin, when in contact with saliva for 6h.

Wiegand et al.[15] showed that titanium tetrafluoride solution induced some granular coating on dentin surfaces, which partly covered dentinal tubules. However, its protective potential did not exceed the efficacy of NaF or AmF [12,15,17], in accordance with the present study.

All F varnishes were able to significantly reduce dentin erosion-abrasion. Thus, the F concentration, type of F salt and base as well as pH might not have influenced the effect of the products on the dentin tissue loss in this case. The contact time seems to be the most important factor, as fluoridated varnishes worked better than the corresponding solutions, regardless of the other factors. Topical fluoridation induces the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in case of conventional fluorides like NaF) or of metal-rich surface precipitates (in case of TiF_4). It can also be speculated that the CaF_2 -layer or organometallic glaze like-surface formed by the application of NaF and TiF_4

varnishes, respectively, might have had the same impact on the prevention of dentin erosion and abrasion.

In a previous study, Duraphat was the only treatment able to reduce dentin erosion [17]. The differences between the previous data and the present study might be related to the profilometric analyses. Besides, it is speculated if the abrasion could have removed some particles of the varnishes that might be inside of the tubules, allowing a better differentiation among the fluoridated varnishes and placebo varnish/control.

The preventive effect of fluorides on dentin erosion is highly dependent on the presence of the organic matrix [5,22]. Initial studies showed that a very intensive fluoridation was most effective in the prevention of dentin erosion [23,24]. However, after enzymatic removal of the organic matrix fluoride was ineffective [3,5]. It was assumed that the demineralized organic dentin matrix has a buffering capacity sufficient to prevent further dentin demineralization especially in the presence of high amounts of fluoride [3]. Moreover, the exposed organic matrix of etched dentin involves an increased surface area and increased diffusion pathways, enhancing the amount of structurally bound or KOH-soluble fluoride compared to sound dentin [25]. However, it remains unclear to what extent the organic material is retained under clinical conditions, when the collagen layer might be affected by enzymatic and chemical degradation [5] as well as by abrasive forces [6]. From the clinical appearance of dentin erosive lesions it seems likely that the collagenous layer is at least partly removed.

Therefore, before F varnishes start to be frequently used in the clinical situation, the next step is to test the effect of these treatments on human enamel and dentin erosion and abrasion *in situ*, a condition that more closely resembles the clinical situation.

Under the conditions of the present study it can be concluded that all fluoridated varnishes, but not the solutions, had a similar performance and seem to have a good potential to reduce dentin tissue loss under mild erosive and abrasive conditions *in vitro*.

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Table 1 - Mean dentin erosive-abrasive loss (μm) \pm S.D. in the different groups (n=10).

Treatment	Dentin tissue loss
Duraphat	7.5 \pm 1.1 ^a
Duofluorid	6.8 \pm 1.1 ^a
NaF varnish	7.2 \pm 1.9 ^a
TiF ₄ varnish	6.5 \pm 1.0 ^a
NaF solution	9.9 \pm 1.8 ^b
TiF ₄ solution	10.3 \pm 2.1 ^b
Placebo varnish	11.2 \pm 1.3 ^b
Untreated (control)	11.8 \pm 1.7 ^b

Distinct lower case letters indicate significant differences among the F solutions/varnishes (ANOVA and Tukey's test, $p < 0.0001$).

3.8 Capítulo 8

Efficacy of an experimental TiF_4 varnish: a randomized *in situ* trial on erosion-abrasion

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Abstract

Objectives: This *in situ/ex vivo* study aimed to analyse the impact of TiF_4 varnish on human enamel and dentine wear induced by erosion and erosion plus abrasion.

Methods: Twelve subjects took part in this crossover and double-blind study performed in 3 phases of 5 days each. Each two human enamel and dentine samples were pre-treated with experimental-NaF varnish or NaF solution (both 2.45%F, pH 4.5) in phase A; experimental- TiF_4 varnish or TiF_4 solution (both 2.45%F, pH 1.2) in phase B; placebo varnish or control (untreated) in phase C. The samples were used in palatal appliances and one enamel and dentine sample, treated with one of the agents, was subjected to extraoral erosion (4 times/day, Coca-Cola, 90s, ERO) or erosion plus abrasion (2 times/day, fluoride-free toothpaste and electrical toothbrush, 10s/sample, ERO+ABR). The dental tissue loss was quantified by a contact profilometry (μm). The data were analysed by two-way repeated measures ANOVA and Bonferroni *post hoc* test ($p < 0.05$).

Results: All F varnishes and solutions reduced the enamel and dentine wear significantly when compared to the control and placebo varnish ($p < 0.001$). Generally, there were no significant differences among the F formulations and the conditions ERO and ERO+ABR.

Conclusions: Differently from the previous *in vitro* results, TiF_4 varnish has the same protective potential as the solution and NaF products to reduce human enamel and dentine erosion with or without abrasion *in situ*.

Introduction

Conventional fluorides whose beneficial effect against caries is well known [1] have been tested for prevention or control of dental erosion [2,3]. The potential of conventional fluorides, such as NaF, to prevent erosive demineralisation is mainly related to the formation of a calcium fluoride (CaF_2)-layer [4,5]. This layer is assumed to behave as a physical barrier hampering the contact of the acid with the underlying enamel or to act as a mineral reservoir, which is attacked by the erosive challenge. Thereafter, calcium and fluoride released might increase the saturation level with respect to dental hard tissue in the liquid adjacent to the surface thus promoting remineralisation.

As the anti-erosive effect of conventional fluorides requires a very intensive fluoridation regime [6,7], current studies focused on fluoride compounds that might have a higher efficacy. In this context, compounds containing polyvalent metal ions such as stannous fluoride or titanium tetrafluoride were tested.

The potential of TiF_4 to prevent dental erosive demineralisation has been investigated since 1997 [8]. Its protective effect is related to the formation of an acid-resistant surface coating, the increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice. The glaze-like surface layer observed after the application of TiF_4 is formed from a new compound (hydrated hydrogen titanium phosphate) or organometallic complexes that might primarily act as a diffusion barrier [8,9].

In situ studies showed TiF_4 solution to be as effective as SnF_2 or AmF solution in the prevention of erosion or combined erosion/abrasion [10,11], while another in situ study did not show any protective effect [12]. The efficacy of TiF_4 is highly dependent on the pH of the agent, since it was shown that enamel and dentine erosion can significantly reduced by TiF_4 (0.5 M F) at its native pH (pH 1.2) but not at a pH buffered to 3.5 [13,14]. However, the low pH of TiF_4 solution does not allow self-application as mouth rinse by the patient. Therefore, recent studies showed that TiF_4 incorporated in an experimental varnish, indicated for the clinical application, might be of higher efficacy than a solution on enamel erosion and abrasion [15,16] and dentine erosion plus abrasion [17]. Besides, it would be safer for the patient, as it is directly applied on the tooth surface. However, further properly designed in situ or clinical studies are recommended in order to better understand the relative differences in performance of the various formulations.

Thus, the aim of this *in situ* study was to analyse the effect of a single application of TiF_4 varnish/solution compared to NaF varnish/solution, to protect against enamel and dentine erosion with or without abrasion.

Materials and Methods

Ethical Aspects

Twelve healthy adult subjects (11 female, 1 male, aged 23-35 years) who fulfilled the inclusion criteria (physiological salivary flow rates: stimulated: >1 ml/min, unstimulated: >0.25 ml/min; good oral health: no frank cavities or significant gingivitis/periodontitis) without violating the exclusion criteria (systemic illness, pregnancy or breastfeeding, use of fixed or removable orthodontic appliances, use of fluoride mouthrinse or professional fluoride application in the last 2 months, hyposalivation) were enrolled following CONSORT guidelines. Sample size calculation was based on previous *in situ* study [12]. A sample size of twelve subjects was calculated considering α -error level of 5% and β -error level of 20% (www.ddsresearch.com).

The study was conformed to the Declaration of Helsinki and was performed to the guidelines of good clinical practice. Ethic approval for the study involving human subjects was granted by the local Ethics Committee (Ethics committee of the Bauru Dental School, University of Sao Paulo, Brazil, n° 083/2008). The study was planned as a prospective, single-center, double blind, and six-cell study in crossover design and split-mouth with an overall experimental period of 3 x 5 days (washout period of 5 days). The subjects received written instructions and a schedule and were extensively trained for all procedures. Informed consent was obtained from all subjects prior to the study.

Preparation of specimens

Enamel and root dentine specimens (4 mm x 4 mm x 3 mm) were prepared from human impacted 3rd molars. The teeth were stored in 2% buffered formaldehyde solution (pH 7.0) at 4°C during this phase. The specimens were cut in the middle of the surfaces using an ISOMET low-speed saw (Buehler Ltd., Lake Bluff, IL, USA) with 2 diamond disks (Extex Corp., Enfield, CT, USA) separated by a 4 mm-thick spacer. The specimens' surfaces were ground flat with water-cooled silicon carbide discs

(320-, 600-, and 1200-grade papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet with diamond spray (1 μm ; Buehler).

After polishing, the specimens were cleaned in an ultrasonic device with deionized water for 2 min. Prior to the experiment, 2 layers of nail varnish were applied on two-thirds of the surface of each specimen to maintain reference surfaces for determining loss after the experiment, leaving 1 mm of exposed area. The specimens were maintained in 100% humidity until the *in situ* experiment was conducted.

Twenty-four enamel and dentine samples were randomly allocated to each group: Phase A (experimental NaF varnish and NaF solution, FGM-Dentscare – Brazil, 2.45%F, pH 4.5); Phase B (experimental TiF_4 varnish and TiF_4 solution, FGM-Dentscare – Brazil, 2.45%F, pH 1.2) and Phase C (placebo varnish FGM-Dentscare – Brazil, pH 5.0 and control - untreated). In each group, half of the specimens were subdivided into erosion only (ERO, n = 12) and erosion plus abrasion (ERO+ABR, n = 12).

Each 4 enamel and 4 dentine samples were fixed with wax into the recesses of the individual acrylic palatal appliances. The treatment (solution or varnish) was randomly divided in the rows, and the conditions (ERO or ERO+ABR) were randomly divided in line for each volunteer (Figure 1).

In situ experiment

Five days prior to and throughout the entire experiment, the subjects brushed their teeth with fluoride-free toothpaste (Crest, Procter & Gamble, USA), in order to allow the protective effect of F to be only due to the solution/varnish treatments. The subjects used the appliance for 2 h prior to the start of each phase to allow the formation of a salivary pellicle. After the 2 h lead-in period, the treatments were performed. The F solutions were applied once using a microbrush, and were left on the surface for 1 min [15-19]. Excess of the solution was removed from the surface by a cotton roll. The varnishes were similarly applied as the solutions; however, they were removed 6h later according to previous studies using scalpel and acetone solution (1:1 water) [15-17]. During the period of 6h, the appliances were placed in the subjects' mouth.

At the next day, the erosive regimens were performed four times daily (morning, midday, afternoon and evening) with at least 4 h apart. For erosion, the subjects were instructed to immerse the appliance in a cup containing 150 ml Coca-Cola (pH 2.6,

0.32 ppm F, Coca-Cola Company, Brazil) at room temperature for 90s. Immediately after erosion, the appliances were washed and reinserted into the mouth.

Two times a day, after the first and last erosive challenges, abrasion was performed in one line of each row. Each sample was brushed extraorally with an electrical toothbrush (Colgate® Motions Multi-action, Brazil) for 10 s (166 oscillations/s) using one drop (around 35 µl) of a fluoride-free dentifrice slurry (Crest, Procter & Gamble, USA, Ratio dentifrice: water = 1:3, pH 6.8, 1.5 N) [16,17].

The appliances were worn day and night and were stored in humidity during meals and oral hygiene (4 times daily, 1 h each) procedures, for 5 days. The subjects were advised not to eat or to drink while the appliances were in place. A minimum of 30 min elapsed between individual oral hygiene and the experiment.

Wear analysis

Dental hard tissue loss (µm) was defined as outcome parameter. After 5 days, the enamel and dentine samples were removed from the appliances and the nail varnish on the reference surfaces was carefully removed with acetone/water-soaked cotton wool [15-17]. The samples were stored in humidity until the analysis to avoid shrinkage of dentine. The loss was quantitatively determined by a contact profilometry (Hommel Tester T1000, VS, Schwenningen, Germany), which presents accuracy around 0.5µm. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). The diamond stylus moved 2.5 mm from the first reference across the exposed area onto the other reference area. Four profile measurements were randomly performed in the center of each sample. The vertical distance between the midpoints of regression lines on the reference and experimental areas was defined as tissue loss, using the software of the device. The values were averaged (µm) and submitted for statistical analysis. The standard deviation of repeated analysis of a given sample was 0.35µm.

Statistical analysis

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

Software, San Diego, CA, USA). Thereby, the different treatments were considered as dependent and the conditions (ERO and ERO+ABR) as independent variables. The significance level was set at 5%.

Results

All participants completed the study, and all samples could be measured profilometrically. There was no significant difference between the conditions ERO and ERO+ABR for enamel ($p=0.19$, $F=1.79$) and dentine ($p=0.30$, $F=1.12$). There was also no interaction between the factors. All F varnishes and solutions reduced the enamel and dentine wear significantly when compared to the control and placebo varnish ($p<0.001$, Tables 1 and 2). There were no significant differences among the F formulations, except between NaF varnish and TiF_4 solution on dentine erosion.

Discussion

In the present study, all fluoridated varnishes and solutions were able to significantly to reduce enamel and dentine erosion and erosion plus abrasion compared to placebo varnish and control. Differently from previous results obtained from *in vitro* studies [15-17], TiF_4 varnish has the same protective potential as the solution and NaF products to reduce human enamel and dentine erosion with or without abrasion *in situ*. Besides, there was no significant difference between varnish and solution, showing that the contact time seems not to be an important factor, as varnishes and solutions presented similar effect on enamel and dentine loss.

Topical fluoridation induces the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in case of conventional fluorides like NaF) or of metal-rich surface precipitates (in case of TiF_4). From the results of the present study, it can also be speculated that the CaF_2 -layer was as resistant to the erosive and abrasive challenges as the metal-rich surface precipitates formed by the application of TiF_4 on human enamel and dentine *in situ*.

The present results are also in disagreement with an *in situ* study, in that 4% TiF_4 solution was unable to reduce human permanent and deciduous enamel erosion [12]. However, in the study of Magalhães et al. [12] the samples were not covered by

salivary pellicle before the treatments. It should be highlight that the present study is significantly different from the previous *in vitro* study, since human saliva might allow the formation of salivary pellicle, which in turn might have a significant impact on the reaction between F and tooth. The salivary pellicle and the human saliva might allow better stability to the CaF_2 layer formed by the conventional fluoride compared to the artificial saliva in *in vitro* models [5] The same hypothesis can be given for the metal fluorides.

Wiegand et al. [20] showed that the efficacy of the tetrafluorides was influenced by the presence of the pellicle layer, in which the protection against bovine dentine erosion by TiF_4 was greater on pellicle-covered specimens *in vitro*. On the other hand, two studies performed by Hove et al. [21,22] pointed out that TiF_4 solution provide protection for the enamel against acid attack, regardless of the presence of the 2h-pellicle.

Hove et al. [22] also showed that TiF_4 solution might better react with bovine than human enamel, since it was more effective on the prevention of bovine enamel erosion. Our study does not allow such comparison, but from the previous *in vitro* studies using bovine enamel, at least for TiF_4 solution, it seems this product reacts better with human than bovine enamel, contradicting previous findings.

Regarding dentine, the preventive effect of fluorides on erosion is highly dependent on the presence of the organic matrix [23,24]. It was assumed that the demineralized organic dentin matrix has a buffering capacity sufficient to prevent further dentin demineralization especially in the presence of high amounts of fluoride [23,24]. However, it remains unclear to what extent the organic material is retained under clinical conditions, when the collagen layer might be affected by enzymatic and chemical degradation [23]. From the clinical appearance of dentin erosive lesions it seems likely that the collagenous layer is at least partly removed. However, in this *in situ* protocol, if the demineralized organic dentin was affected by enzymes or mechanical forces, the alterations were not enough to have influence on the effect of fluoride. Thereby, the F treatments were as effective on enamel as on dentine samples.

Generally, ERO+ABR condition showed similar enamel and dentine loss values compared to the ERO only. The remineralizing potential of human saliva as well as the presence of the salivary pellicle might have reduced the progression of dental loss. Besides, this result may be a consequence of the experimental protocol, in

which the abrasion procedure (2x10s/day) was less aggressive than the erosive challenges (4x90s/day). Using this protocol, the toothbrushing abrasion could not have adversely affected the progression of dental erosive loss, differently from the results of Wiegand et al. [11] in that abrasion was performed 2x30s a day.

Under the conditions of the present study it can be concluded that TiF₄ varnish has the same protective potential as the solution and NaF products to reduce human enamel and dentine erosion with or without abrasion *in situ*.

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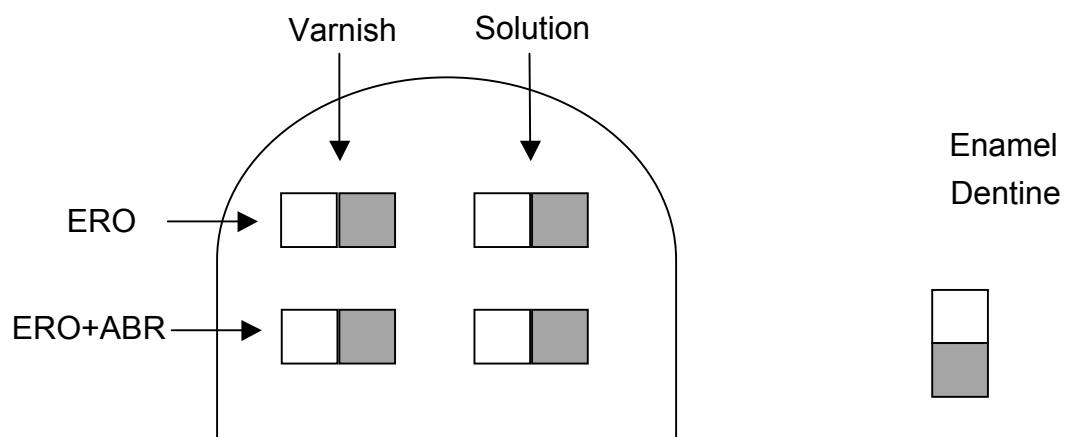


Figure 1 - Position of the specimens in the palatal appliance according to the treatment and the condition.

Table 1 - Mean enamel loss (μm) \pm S.D. in the different groups.

	NaF varnish^a	NaF solution^a	TiF₄ varnish^a	TiF₄ solution^a	Placebo^b	Control^b
ERO^A	1.1 \pm 0.5	1.3 \pm 0.4	1.2 \pm 0.5	1.2 \pm 0.7	1.8 \pm 0.8	1.8 \pm 0.8
ERO+ABR^A	1.5 \pm 0.6	1.6 \pm 0.6	1.2 \pm 0.5	1.5 \pm 0.7	2.1 \pm 0.6	2.2 \pm 0.8

Distinct upper case letters indicate significant difference between the conditions. Distinct lower case letters indicate significant differences among the F products.

Table 2 - Mean dentine loss (μm) \pm S.D. in the different groups.

	NaF varnish	NaF solution	TiF₄ varnish	TiF₄ solution	Placebo	Control
ERO^A	1.3 \pm 0.4 ^a	1.3 \pm 0.4 ^{ab}	1.3 \pm 0.4 ^{ab}	1.6 \pm 0.4 ^b	2.0 \pm 0.6 ^c	1.9 \pm 0.6 ^c
ERO+ABR^A	1.4 \pm 0.4 ^a	1.6 \pm 0.5 ^a	1.4 \pm 0.4 ^a	1.6 \pm 0.3 ^a	2.1 \pm 0.6 ^b	2.2 \pm 0.6 ^b

Distinct upper case letters indicate significant difference between the conditions. Distinct lower case letters indicate significant differences among the F products (lines).

3.9 Capítulo 9

INSIGHTS INTO PREVENTIVE MEASURES FOR DENTAL EROSION **CONCEITOS ATUAIS SOBRE ESTRATÉGIAS PREVENTIVAS PARA EROSÃO** **DENTÁRIA**

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INSIGHTS INTO PREVENTIVE MEASURES FOR DENTAL EROSION

CONCEITOS ATUAIS SOBRE ESTRATÉGIAS PREVENTIVAS PARA EROSÃO DENTÁRIA

Abstract

Introduction: Dental erosion is defined as the loss of tooth substance by acid exposure not involving bacteria. The etiology of erosion is related to different behavioral, biological and chemical factors. *Objective:* Based on an overview of the current literature the paper presents a summary of the preventive strategies relevant for patients suffering from dental erosion. *Review of Literature:* Behavioral factors, such as special drinking habits, unhealthy lifestyle factors or occupational acid exposure, might modify the extent of dental erosion. Thus, preventive strategies have to include measures to reduce the frequency and duration of acid exposure as well as adequate oral hygiene measures, as it is known that eroded surfaces are more susceptible to abrasion. Biological factors, such as saliva or acquired pellicle, act protectively against erosive demineralisation. Therefore, the production of saliva should be enhanced, especially in patient with hyposalivation or xerostomia. With regard to chemical factors, the modification of acidic solutions with ions, especially calcium, was shown to reduce the demineralisation, but the efficacy depends on the other chemical factors, such as the type of acid. To enhance the remineralisation of eroded surfaces and to prevent further progression of dental wear, high-concentrated fluoride applications are recommended. Currently, little information about the efficacy of other preventive strategies, such as calcium and laser application, as well as use of inhibitors for matrix metalloproteinases, is available. Further studies considering these factors are required. *Conclusion:* Preventive strategies for patients suffering from erosion are mainly obtained from in vitro and in situ studies and include dietary advice, stimulation of salivary flow, optimization of fluoride regimens, modification of erosive beverages and adequate oral hygiene measures.

Uniterms: Dental erosion; Prevention; Tooth wear

1 Introduction

The term tooth wear is defined as loss of dental hard tissues due to the processes of dental erosion, attrition and abrasion⁵⁷. Dental attrition is the wear of tooth resulting from tooth to tooth contact⁵⁷, while abrasion is caused by oral habits or abrasive substances, such as highly abrasive toothpastes⁵⁷. Dental erosion is defined as the loss of tooth substance by chemical processes (acid exposure) not involving bacteria⁶⁰. The acidic attack leads to an irreversible loss of dental hard tissue, which is accompanied by a progressive softening of the surface⁶⁰. This softened zone is more susceptible to mechanical forces, such as abrasion⁸², which in turn have little or no effect on sound dental hard tissues¹. The chemical and mechanical processes can occur individually or together, although the effect of erosion is often dominant².

Clinically, early enamel erosion appears as a smooth silky-shining glazed surface (FIGURE 1). Typical for erosions of the facial aspects of teeth is a ridge of enamel that separates the defect from the marginal gingiva. Occlusal erosion is characterized by rounded cusps and concavities. Further progression of occlusal erosion leads to a distinct grooving of the cusps (FIGURE 2), and restorations are rising above the level of the adjacent tooth surface. In cases of severe erosion, the whole occlusal or facial morphology disappears. Erosion can be distinguished from wedge shaped lesions, which present a sharp margin in the coronal part and cuts at right angles into the enamel surface as well as from attrition. Attrition appears often glossy and has distinct margins and corresponding features at the antagonistic teeth. In cases of occlusal tooth wear, the distinction between erosion and abrasion is often difficult, as both are of similar shape²⁹.



Figure 1 - Clinical appearance of tooth wear in enamel: Enamel erosion in a 36 year old female patient caused by the frequent consumption of Coke. Erosion can be distinguished from a wedge-shaped defect, which shows a sharp margin of the coronal part.



Figure 2 - Clinical appearance of tooth wear in dentin in a 25 years old athlete due to frequent consumption of acidic sport drinks.

The etiology of erosion is multifactorial and not fully understood. The most important sources of acids are those found in the diet, such as acidic foods and drinks⁵⁹ and those originated from the stomach, like gastric acids from regurgitation and reflux disorders¹⁶. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor for the development of erosive wear⁶⁰.

As erosive tooth wear is a multifactorial condition (TABLE 1), preventive strategies has to be applied which account for chemical, biological and behavioral factors involved in the etiology and pathogenesis of erosion⁶⁰ (FIGURE 3). However, the informations about the all possible preventive measures for erosion including those already known and the new ones are missing. Thus, the objective of this paper is to present an overview of the current literature and to summarize the preventive strategies relevant for patients suffering from dental erosion.

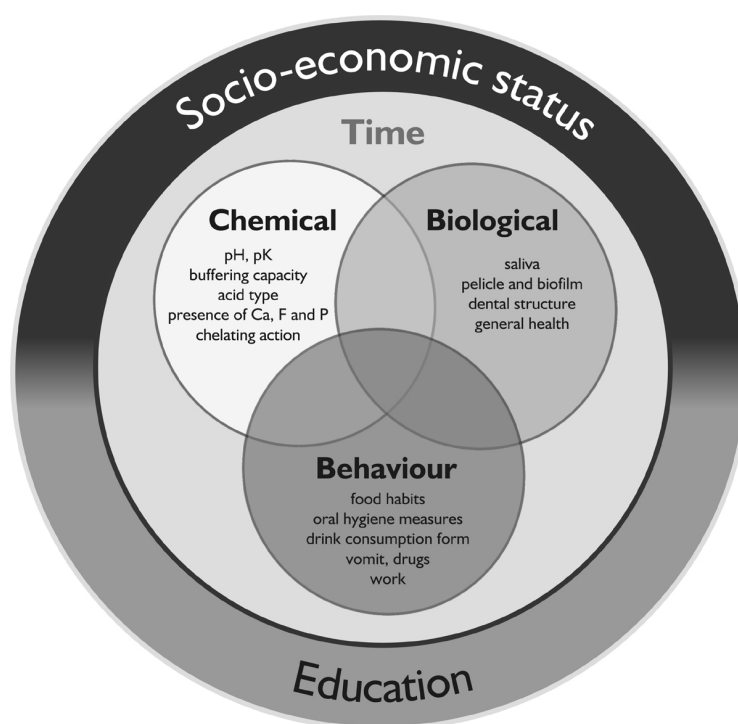


Figure 3 - Diagram proposed by Lussi⁶⁰ (2006) modified.

Table 1 - Anamnestic factors indicating an increased risk for dental erosion.

Diet	Frequency of the consumption of acid drinks (soft drinks, fruit juices, sport drinks) and foods (citrus fruits, salad dressing), eventually diet diary
General diseases	Gastrointestinal diseases (reflux) Eating disorders Alcohol abuse General diseases affecting salivary flow rate Diseases of salivary glands Radiation of the Head and Neck Sjögren-Syndrome Diabetes mellitus Chronic renal failure
Medication	Acidic medicaments (acetylsalicylic acid, vitamin C) Reduction of saliva secretion as side effect of Psychotropic drugs Anticholinergics Antihistaminics Antiemetics Parkinson medication Drugs abuse
Occupation/Sports	Occupational acid exposure Sports (swimming pool, increased consumption of acidic sport drinks)

2 Review of Literature and Discussion

2.1 Preventive strategies for behavioral factors

The behavioral factors have a decisive influence on the appearance and the progression of dental erosion¹⁰⁶. The frequent and excessive consumption of acids is associated with an increased risk for dental erosion. Special drinking habits, such as nipping from a bottle, might enhance the acid contact time and, thus, increase the erosive attack. Oral hygiene measures might also influence the progression of erosive lesions. Abrasive influences, such as toothbrushing, are known to remove the

fragile surface of demineralised dental hard tissues. Thus, the time point of toothbrushing after an erosive attack^{8,10} as well as the kind of toothbrush and toothpaste used might influence the progression of dental wear⁴⁰.

2.1.1 Measures to reduce the acid exposure

Of major importance for the prevention of dental erosion is the reduction of the acid exposure. The frequency and duration of acid contact might be important variables for the development of erosive lesions^{25,94}. Moreover, the adhesiveness and displacement of liquids might influence the erosive process, as an increased adherence of an acidic substance is associated with a longer contact time on the tooth. The ability of beverages to adhere on enamel is based on their thermodynamic properties⁴⁵.

Extrinsic acid sources of erosion are mainly dietary acids, but also lifestyle factors (e.g. drugs) or occupational acid exposure (TABLE 1). To decrease the risk of dietary induced erosive lesions, patients should be advised to refuse from acidic snacks between the principal meals to allow the saliva to reharder eroded tooth surfaces. Special drinking habits, such as holding or moving the liquid in the mouth prior to swallowing, sucking from a straw or nipping from a bottle, lead to an increased acid contact time in the oral cavity and, thus, to prolonged duration of an acidic pH-value in the environment of the teeth¹⁷. Therefore, it seems advisable to avoid these drinking habits to reduce the duration of the erosive attack (TABLE 2). Besides the dietary acids, patients should be aware of unhealthy lifestyle factors, such as consumption of drugs, alcohol abuse and lactovegetarian diet, which might increase the risk for erosion¹⁰⁶ (TABLE 1). However, as it is difficult to control possible etiological factors, such as the intake of acidic beverages or special drinking habits, other strategies have been developed for the prevention of dental erosion.

Table 2 - Preventive measures for patients with increased risk for erosion.

Aim	Recommendation/Measure
Reduction of acid exposure	
Extrinsic factors (e.g. diet)	<ul style="list-style-type: none"> • Reduction of the intake of acidic drinks and snacks • Acidic beverages should be drunken quickly and cooled • Consumption of acidic drinks with a high content of calcium, phosphate, fluoride and xylitol
Intrinsic factors	<ul style="list-style-type: none"> • Evaluation of the aetiology of acid exposure, therapy of organic (e.g. reflux, xerostomia) or psychosomatic (e.g. bulimia nervosa) disorders
Reduction of demineralisation, Enhancement of remineralisation	<ul style="list-style-type: none"> • Increase of salivary flow <ul style="list-style-type: none"> ○ Chewing of sugar-free gums ○ Xerostomia patients: systemic medication (cholinergic drugs), use of saliva substitutes • Behavior after acid contact <ul style="list-style-type: none"> ○ Rinsing of the oral cavity with water, milk or low concentrated fluoride solutions ○ Consumptions of neutralizing food (cheese, milk) • Frequent fluoridation <ul style="list-style-type: none"> ○ Use of fluoridated toothpaste, solution and gel
Reduction of abrasion	<ul style="list-style-type: none"> • No toothbrushing immediately after acid consumption • Use of manual toothbrushes or electric toothbrushes applied with gentle pressure • Use of fluoridated toothpastes with low REA/RDA-value

With regard to an environmental acid exposure, an increased risk for dental erosion is reported for battery, charging and galvanizing workers, which are commonly exposed to sulphuric or hydrochloric acid (TABLE 1). Thereby, the risk for erosive tooth wear and the severity of erosion increase with increasing concentration of the acid or the acidic fumes, increasing exposure time and duration of employment. Personal protective equipments (respiratory masks) and adherence to

threshold limit values recommended by occupational health legislations are considered as important preventive strategies to decrease occupational erosion⁹⁶.

The intrinsic aetiology factors of erosion include disorders which are associated with the occurrence of gastric acid in the oral cavity, such as vomiting or gastroesophageal reflux (TABLE 1). Therefore, erosive tooth wear is a common manifestation in patients suffering from organic or psychosomatic disorders such as anorexia or bulimia nervosa or alcohol abuse. These disorders require a causal therapy (general medicine, psychological therapy) for a permanent reduction of the intrinsic acid exposure. However, dental professionals are often the first to discover and diagnose eating disorders by detecting structural changes of dental hard tissues and, thus, to induce general diagnostics and therapeutics (TABLE 2).

2.1.2 Measures to reduce the mechanical impact

From in vitro⁹¹ and in situ studies^{31,98} it is concluded that the mechanical stress of eroded surfaces may be mainly induced by toothbrushing but also by attrition due to tooth-tooth-contact, tongue friction or abrasion of the surrounding soft tissues in the clinical situation.

Attin et al.^{8,10} showed that the resistance of eroded enamel and dentin to toothbrushing abrasion was significantly decreased after erosion, but was enhanced with increasing remineralisation time. However, even after a remineralization period of 60 min the wear of enamel samples was significantly increased as compared to the demineralized, but not brushed control⁸. In contrast, dentin wear was not significantly higher than in unbrushed controls after intra-oral periods of 30 and 60 min¹⁰. Thus, patients who present high risk for dental wear should be recommended to avoid toothbrushing immediately after an acidic attack, but wait at least 30-60 min (TABLE 2).

Besides the time point of toothbrushing, abrasion of eroded enamel and dentin is dependent on the kind of toothbrush, the applied brushing force and several toothpaste factors^{40,97,99,101,103,105}.

In previous studies it was shown that powered and manual toothbrushes as well as manual toothbrushes applied with different brushing loads vary in their ability to remove the fragile surface of demineralised enamel and dentin^{97,99,101}. On the basis of the observation that enamel and dentin wear increased with increasing

toothbrushing force⁹⁹, patients with erosive lesions should apply their toothbrushes with slight pressure to minimize loss of dental hard tissues (TABLE 2).

The hardness of the toothbrush seems to be of minor importance for the abrasion of eroded dental hard tissues. Wiegand et al.¹⁰³ showed that the ability of toothbrushes with filament diameters of 0.15, 0.20 and 0.25 mm to remove eroded enamel did not differ significantly when toothpaste slurries of REA 2, 6 and 9 were used.

In contrast, toothbrushing abrasion is mainly influenced by the toothpaste used¹⁰³. The abrasivity of the toothpaste is determined by the size and amount of abrasives, the pH, buffering capacity and fluoride concentration. Generally, the enamel and dentin loss increase with increasing abrasivity (determined by the REA and RDA-value of the toothpaste)^{40,103}. Fluoridated toothpastes might not only reduce the erosive demineralisation, but might also reduce the abrasion of eroded tissues^{64,67}. Therefore, patients with erosive lesions should use fluoridated toothpastes with low abrasivity for their oral hygiene measures.

2.2 Preventive strategies for biological factors

With regard to the biological factors, the quality of dental tissues, the properties of saliva, the tooth position and the anatomy of the soft tissues might affect the development of dental erosion. Severe erosive lesions affect not only the enamel surface but might also lead to the exposure of coronal or radicular dentin and, thus, to painful hypersensitivity. Moreover, erosive tooth wear is not only found in permanent teeth, but also is increasingly reported in the primary dentition³⁹.

2.2.1 Progression of erosion in different tissues

The interaction between erosive agents and dental tissues is different for enamel and dentin, and for deciduous and permanent teeth. Basically, permanent enamel is composed by mineral (85% volume), in the form of (hydroxy or fluor) apatite crystals organized in prisms. At a pH less than 4.5, the apatite crystals are easily dissolved by acids, generating a surface lesion (FIGURE 4) with concave clinical appearance (FIGURES 1 and 2). Permanent dentin contains inorganic-47% (apatite), organic-33% (collagen) components and water-20%. Studies have shown that demineralisation of dentin is firstly apparent at the interface between inter- and

peritubular dentin. With increasing exposure time the erosive attack results in a hollowing and funneling of the tubules. Finally, the peritubular dentin is completely dissolved. The erosive demineralisation results in the exposure of an outer layer of fully demineralised organic matrix followed by a partly demineralised zone until the sound inner dentin is reached⁵⁰ (FIGURE 5). The dentin demineralisation rate decreases when the amount of degradable collagen increases, whereby the demineralised matrix is attributed to hamper ionic diffusion into and out of the demineralising area^{27, 28}. On the other hand, the erosion time of enamel is linear over time²⁶.

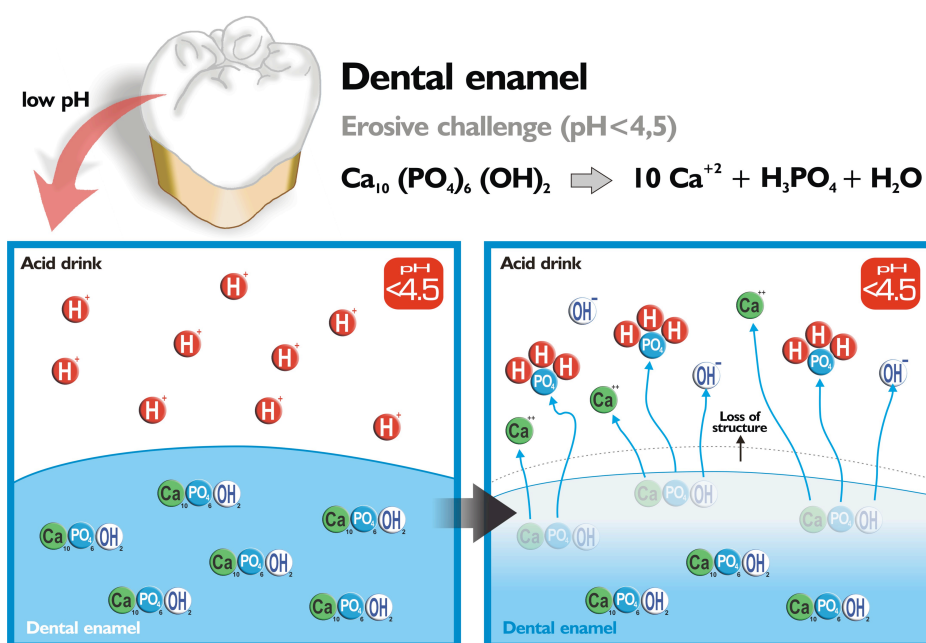


Figure 4 - The erosive demineralisation of dental enamel (pH<4.5).

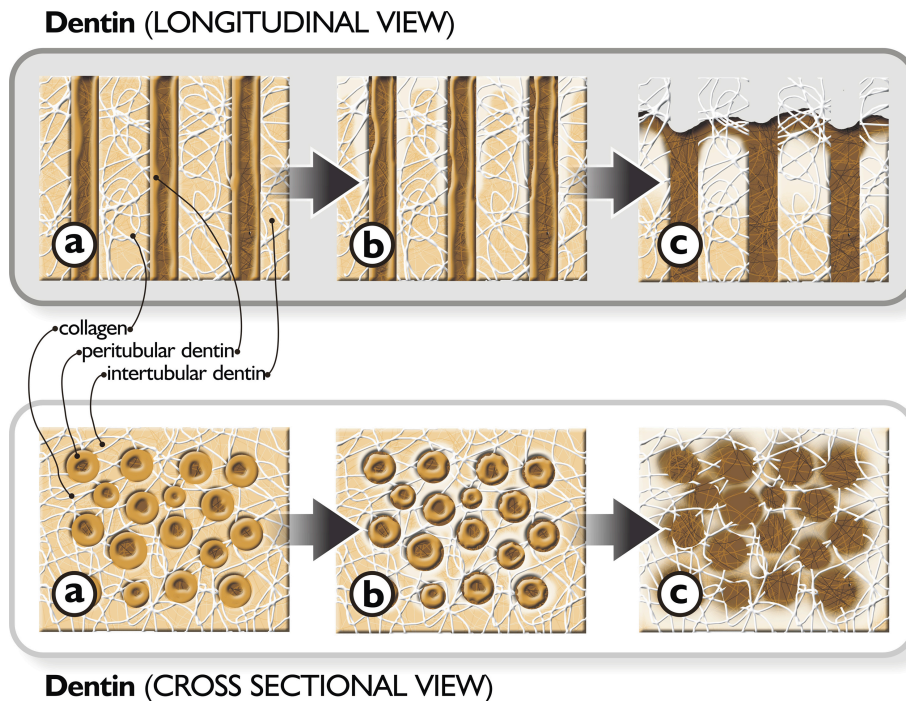


Figure 5 - The erosive demineralisation of dentin (longitudinal and cross sectional view).

In addition, deciduous enamel and dentin are thinner than permanent. Therefore, the erosive process reaches the dentin earlier and leads to an advanced lesion after a shorter exposure period to acids, compared with permanent teeth⁴. However, studies about the susceptibility of these teeth to erosive softening have revealed conflicting results. While several authors found an increased susceptibility to erosion in deciduous teeth, others found no difference between deciduous and permanent dental hard tissues^{4,44,56}.

2.2.2 Measures to increase the quality and the quantity of saliva and pellicle

Saliva seems to play an important role in minimizing enamel and dentin wear in erosive/abrasive attacks due to its buffering and remineralising capacities as well as the ability to form a protective pellicle layer on dental hard tissues^{35,69}. Xerostomia or hyposalivation is a condition frequently observed in patients undergoing a radiation treatment of the head and neck, but is also common in patients suffering from diseases of the salivary glands (Sjögren syndrome) or can be induced by several systemic medications (TABLE 1). In these patients, the decreased salivary flow rate is associated with a low pH of the saliva and a decreased buffering capacity⁷⁰. It has been shown that low salivary flow rate and low buffering capacity are strongly associated with dental erosion⁷⁵.

Salivary flow stimulation can yield an increase in bicarbonate buffer and in salivary mineral content, which can facilitate calcium and phosphate redeposition onto the enamel and dentin surface and reduction of dental tissues loss²⁴ (TABLE 2). Rios et al.⁸³ showed that saliva stimulated by the use of sugar-free chewing gum promoted a remineralising action in the erosive/abrasive phenomena. In contrast, sucking of acidic candies might change the whole-mouth saliva composition so that it may have erosive potential⁴⁶.

This remineralizing effect might be increased by rinsing with milk or eating cheese, which are of interest as they contain higher levels of calcium and phosphate than water or saliva and, therefore, may act as donor of calcium and phosphate for remineralisation³³ (TABLE 2). Although the consumption of milk or cheese is frequently recommended to enhance the rehardening of demineralised dental hard tissues, only few investigated their effects on enamel demineralisation as yet³³.

Besides local saliva stimulators like chewing gum, the salivary flow rate can also be increased systemically. Thus, patients suffering from xerostomia are often treated by cholinergic drugs, such as pilocarpine. Moreover, saliva substitutes might provide relief of the oral symptoms. Saliva substitutes should be of neutral pH to prevent demineralisation of the dental hard tissues and should be saturated with respect to calcium and phosphate to gain remineralising potential⁷¹ (TABLE 2).

Saliva is also responsible for the formation of the acquired pellicle, which is a physical barrier that protects the tooth against erosive attacks. It is composed of a protein layer formed on the tooth surface, acting as a diffusion barrier or permeability membrane^{39,69}. This selective barrier prevents the direct contact between acids and the tooth surface, thus reducing the dissolution of hydroxyapatite. Protection of the tooth surface by the acquired pellicle is well established in the literature and has been demonstrated by several studies^{37,38}.

Pellicle thickness varies within the dental arches and among individuals³. An inverse relationship was observed between the degree of erosion and pellicle thickness. This relationship suggests that the thickness of the acquired salivary pellicle may be an important factor for site-specificity of dental erosion^{3,6}. Conflicting results have been published regarding the impact of the formation time on the protective properties of the pellicle layer³⁸. However, it is generally accepted that the function of the pellicle as a diffusion barrier to ionic conductivity on the enamel surface is improved with the process of pellicle maturation³⁷.

It is important to point out that the pellicle is not dissolved in total from the enamel surface, but rather gradually from its external to basal components; this fact suggests a partial acid resistance of the *in vivo* formed pellicle layers³⁷. As toothbrushing can remove parts of the salivary pellicle⁴⁷, patients at risk of dental erosion should diminish the frequency of toothbrushing and use dentifrice with low abrasivity to avoid damaging of the acquired pellicle (TABLE 2).

2.3 Preventive strategies for chemical factors

The chemical factors relevant for the erosivity of an acid solution are the type and quantity of the acid, the pH, buffering capacity and temperature as well as the presence of chelating agents and the concentration of phosphate, calcium and fluoride^{58,94}. Fruit juices, soft drinks, vinegar and ice tea are known as highly erosive, as they are composed of acids (citric, phosphoric, acetic acids) with a pH lower than 4.5. Usually they are unsaturated regarding to apatite and present a high buffer capacity⁵⁹.

2.3.1 Fluoride and metal fluoride application

The impact of fluoride treatment on the progression of enamel and dentin erosion has been analyzed in several studies. The action of fluoride is mainly attributed to a precipitation of CaF_2 -like material on eroded dental surfaces^{26,27}. The formation of the CaF_2 -like layer and its protective effect on demineralisation depend on the pH, F concentration and type of F salt of the agent⁸⁶. However, the role of fluoride application on the prevention of dental erosion is still controversially discussed⁹⁵, since the deposited calcium fluoride-like material from topical fluoride application is supposed to be readily dissolved in most acidic drinks³⁰.

High-concentrated fluoride agents, such as oral rinses, gels or varnishes, have been demonstrated to increase abrasion resistance and decrease the development of enamel and dentin erosion *in vitro* and *in situ*^{26,51}. Most 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) (12.300 to 22.600 ppm F, pH 1.0 to 7.0). Although the results of an *in vitro* study by Ganss et al.³² suggest considerable differences between NaF, AmF and SnF_2 , the impact of different fluoride compounds on erosion

was not analysed under clinical conditions as yet. The efficacy of fluorides to affect de- and remineralisation is related to its concentration and depends on the pH of the fluoride agent. It is known that the formation of a CaF_2 reservoir is increased under acidic compared to neutral conditions⁸⁸. Depending on the design of the study, the application of high-concentrated fluoride agents might lead to a nearly complete reduction of dental erosion.

In contrast to the application of highly fluoridated agents, a 1,000 ppm F dentifrice was shown to have a limited beneficial effect compared to non-fluoridated dentifrices on abrasion of eroded dentin and enamel^{64,78}. However, in a recent *in situ* study, it was shown that a 5,000 ppm F dentifrice had the same effect as a 1,100 ppm F dentifrice on eroded and eroded and abraded dentin⁶⁷. Also for enamel wear, no significant differences were found among 1,100 and 5,000 ppm F dentifrices⁸⁴. Overall, the efficacy of a fluoridated dentifrice is not increasing along with the F concentration in dentifrices containing more than 1,000 ppm F and the reduction of wear seems to be less than 30% for this fluoride vehicle compared to placebo^{67,84}.

More recently, other agents, such as tetrafluorides (TiF_4 , ZrF_4 , HfF_4 in a concentration between 0.4 to 10%, pH 1-2), especially titanium tetrafluoride, have been investigated for erosion prevention^{41,42,65,87,91,92,100,102}. With regard to TiF_4 solution, several *in vitro* studies have shown an inhibitory effect on dental erosion^{41,42,87}, which is attributed not only to the effect of fluoride, but also to the action of titanium. 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 a stable titanium dioxide layer^{72,81}. Moreover, it is suggested that titanium interacts with the enamel surface, thus leading to an increased fluoride uptake by enamel⁷². However, other studies have not found a protective effect of TiF_4 against erosion or combined erosion and abrasion^{65,91,92}. Recently, an *in vitro* study comparing the efficacy of a 4 % TiF_4 solution, an experimental 4 % TiF_4 varnish and commercial NaF varnishes on the progression of enamel erosion was performed. The experimental TiF_4 varnish showed the best protective effect when compared to commercial NaF varnishes, while TiF_4 solution was not effective to reduce the enamel wear⁶². However, TiF_4 agents have a very acidic pH (pH 1-2), which do not allow for self-application of the patient.

Overall, the protective impact of high-concentrated fluoride applications on the progression of erosive lesions has been shown both *in vitro* and *in situ* studies, but

clinical studies giving support for this observation are not available yet. It is interesting that the fluoridation effects might be more enhanced in dentin than in enamel²⁶. The buffering effect of the demineralised matrix reduces the pH fall within this layer. Combined with the presence of high concentrations of fluoride, this might reduce further dentin demineralisation²⁷. Also, metal fluorides might be more effective in dentin than in enamel^{87,102}, since it is assumed that the metals (titanium ion) might play an essential role because of its complexing ability and protein-binding properties⁷². Based on the findings of in vitro and in situ studies it seems valid to recommend high-concentrated fluoride applications for prevention of dental erosion (TABLE 2). The application of high-concentrated agents has to be done by dentist, taking care for the quantity and for avoiding that the patient swallows the product. If these cares are taken, even the frequent application of high-concentrated agents seems to be safe. However, clinical and epidemiological studies are required to confirm the promising results found in vitro and in situ.

2.3.2 *Modification of acid solutions and beverages*

In the daily life situation, preventive strategies influencing biological and behavioral factors might be of limited impact as they are highly dependent on the patient's compliance. Thus, it seems to be of great interest to develop preventive strategies, which are less dependent on the patient's behavior.

One preventive strategy might be the reduction of the erosive potential of acidic beverages by ions supplementation (calcium, phosphate and fluoride). The addition of calcium has been shown to reduce the erosive potential of pure acids and acidic drinks, especially on enamel erosion^{14,15,43} (TABLE 2).

Orange juice (pH 4.0) supplemented with 40 mmol/l calcium and 30 mmol/l phosphate did not erode the enamel as the calcium and phosphate saturated the drink with respect to apatite⁵³. Attin et al.⁹ showed that Ca supplementation of 0.5-1.5 mmol/L was effective in reducing the erosive potential of citric acid, while the addition of F and P failed to reduce erosivity. Saturation with CaF₂ reduced the in vitro development of erosions by 28% induced by drinks with pH above 3; in drinks with pH below 3, erosions were not affected by fluoride concentrations up to 20 ppm⁵⁴.

Larsen and Nyvad⁵³ and Larsen and Richards⁵⁴ showed that fluoride admixtures in a concentration excluding toxicological side effects seem unable to reduce erosive lesions. The supplementation of low levels of calcium, phosphate and

fluoride was not effective in decreasing the erosive potential of solutions with a pH below 4.0 in the above-mentioned studies. Amaechi et al.⁵ found that the supplementation of an orange juice with xylitol (25% w/v) and fluoride (0.5 ppm) had an additive effect on the reducing dental erosion *in vitro*. Xylitol might form complexes with calcium, penetrate into demineralised enamel and interfere with the transport of dissolved ions from the lesion to the demineralising solution by lowering the diffusion coefficient of calcium and phosphate ions from the lesion into the solution^{7,68}.

Alternatively, acidic solutions can be supplemented with metal ions, such as iron, which seems to decrease the erosive potential of acidic solutions^{18,48}. Iron can participate in the remineralisation of human enamel, in the nucleation of apatite, in the substitution of calcium in apatite and in inhibition of demineralisation¹². In addition, rinsing with an iron solution after an erosive attack can significantly reduce dentin wear by erosion or combined erosion-abrasion⁸⁵. It is important to highlight that studies analyzing the effect of iron supplementation to soft drinks or acid solutions, used high concentrations of iron, which might exhibit toxic effects^{18,48}. Buzalaf et al.¹⁸ 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.⁴⁸ 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 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). On the other hand, the admixture of low concentrations of Fe (1mM) into a soft drink (sprite zero) were not able to reduce the enamel loss⁶³. Besides, the supplementation of iron may lead to a metallic taste of the soft drink and might affect the tooth color and the taste of other foods.

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^{9,11}. Attin et al.¹¹ 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. They 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. On

the other hand, the admixture 1 mM Ca, 1mM Fe, 1 mM P and 0.047 mM F to a soft drink (sprite zero) was not effective to decrease the erosive potential⁶³.

The erosive potential of soft drinks and acidic beverages can be also decreased by replacing highly erosive acids by acids with lower erosivity. Citric acid is for instance known to exhibit a greater erosivity than hydrochloric and phosphoric acids^{36,94,104}. The greater erosive potential of citric acid might be related to its ability to form chelating complexes with calcium. Moreover, differences in their specific interaction with hydroxyapatite might influence the erosive potential of different mono-, di- and tri-carboxylic acids¹⁰⁴.

To summarize the above mentioned-studies critically, the efficacy of the ions supplementation depends not only on the mineral content, but on various factors, such as acid type, pH, amount of titratable acid and buffering capacity of the acid solution^{53,58}. Thus, more studies taking into account these factors have to be performed, with special emphasis on the consequences of the modification regarding taste, stability of the solution and systemic effects for the patients. Additionally, studies evaluating the effects of soft drink modification on dentin erosion have to be performed.

2.4 Further studies

2.4.1 Calcium application

Studies involving dental caries suggest that increased salivary and plaque calcium concentrations might enhance fluoride uptake and retention and, thus, increase the action of fluoride in the demineralisation and remineralisation process. For increasing the saliva or plaque calcium content, several calcium compounds in form of rinses, dentifrices or chewing gums have been investigated^{20,61,76,93}.

Regarding dental erosion, it might be reasonable to increase of the salivary concentration of calcium, which might enhance the deposition of fluoride on the dental tissues by formation of a CaF_2 -like reservoir.

Nowadays, there are only few studies about the effect of calcium-rich toothpastes on dental erosion. Lennon et al.⁵⁵ analyzed the effect of a casein/calcium phosphate-containing tooth cream (Topacal) on enamel erosion *in vitro*. Thereby, Topacal or a combination of Topacal and a 250 ppm fluoride solution provided only little protection against erosion and were significantly less effective than a highly

fluoridated amine fluoride gel. In contrast, Rees et al.⁷⁹ and Piekarczyk et al.⁷⁷ found that Tooth Mousse (CPP-ACP: Casein phosphopeptide - amorphous calcium phosphate) significantly reduced enamel erosion *in vitro*. Due to the few data, available final conclusions about the efficacy of calcium-rich products on dental erosion cannot be drawn so far. Further studies must be performed testing the preventive effect of calcium solutions and calcium-rich dentifrices on enamel and dentin erosion.

2.4.2 Laser application

The protective effects of laser application on enamel and dentin demineralisation have gained increasing attention in the last years. Several types of lasers, such as ruby, CO₂, Nd:YAG and argon with different operative modes and energy outputs have been investigated. The laser treatment causes several chemical changes on the tooth surface, such as the reduction of the carbonate content and the exchange of hydroxyapatite to fluorapatite when applied with fluoride vehicles⁷⁴. Besides, it melts and solidifies the dental surface, creating a smoother new surface^{52,74}. The melted enamel surface can show a crystal growth that can reduce the interprismatic spaces and consequently, the diffusion of acids during an acid challenge²². All these chemical and morphological changes of the dental surface might lead to a decreased susceptibility to erosive demineralisation.

However, there are few studies available testing the effect of the laser application on the prevention of erosive demineralisation and most of them are related to carious and not erosive demineralisation. Tsai et al.⁹⁰ compared the effectiveness of laser treatment (pulsed CO₂ and pulsed Nd:YAG – 83.33 J/cm²) on the acid resistance of human enamel *in vitro*. The Nd:YAG laser was not able to increase the enamel resistance to an acid challenge (lactate buffer solution, pH 4.5, 24 and 72 h). In contrast, the application of Nd:YAG laser (0.5, 0.75 and 1 W) combined or not to fluoride application (fluoride gel and varnish) significantly reduced the enamel erosive wear in a 5-day-*in vitro* study¹⁹. Additionally, when the erosive challenge was extended to 10 days, the combined application of Nd:YAG laser and fluoridated gel was still effective on the reduction of the enamel wear, which could be attributed to the low pH of the fluoride agent¹⁹.

Regarding dentin, Naylor et al.⁷³ showed that irradiation with Nd:YAG laser produces obliteration of dentinal tubules as well as a melting and resolidification, with the formation of recrystallization granules. The authors suggested that dentin

irradiated with 0.6 W Nd:YAG laser presented a higher resistance to acidic beverages such as cola soft drink and passion fruit juice. On the other hand, Magalhães et al.⁶⁶ showed that the application of Nd:YAG laser (0.5, 0.75 and 1 W) was unable to reduce the dentin erosive wear.

Due to the few data available so far, final conclusions about the efficacy of laser application on dental erosion cannot be drawn as yet. Further studies are necessary to clarify this topic.

2.4.3 MMPs (matrix metalloproteinases) inhibitors agents

Matrix metalloproteinases (MMPs) are responsible for hydrolyzing the components of the extracellular matrix (ECM) during the remodeling and degradation processes in the oral environment. Thus, the organic matrix of dentin (collagen) can be degraded by MMPs present in dentin and saliva. The balance between activated MMPs and tissue inhibitors of metalloproteinases (TIMPs) controls the extent of ECM remodeling/degradation⁸⁰. The activation of MMPs seems to play a part in dentinal caries progression, since they have a crucial role in the collagen breakdown in caries lesions. Individuals with a high concentration of MMPs in saliva present an increased susceptibility to dental caries²³. MMPs related to the degradation of collagen present in dentin are MMPs 2, 8, and 9⁸⁹. Besides, the phosphorylated proteins released during the dentin matrix demineralisation could interact with TIMP-inhibited host MMPs within the lesion and reactivate them, thus enhancing the degrading activity. Despite a lack in studies investigating the role of MMPs in dental erosion, processes similar to the caries process can be assumed for erosive lesions.

Tjäderhane et al.⁸⁹ found that the latent forms of MMP 2 and MMP 9 can be activated in acidic conditions followed by neutralization, as it occurs during the carious process when the pH in dental plaque drops within minutes after sugar ingestion until neutralized by salivary buffers. The exposed demineralised dentin matrix is assumed to hamper ionic diffusion into and out of the demineralising area. Therefore, a destruction of the collagen layer by host MMPs of dentin is expected to increase the progression of dental caries in human teeth⁸⁹.

Due to the connection of host MMPs to the progression of dental caries in human teeth, it might be interesting to find MMP inhibitors for patients with high risk for caries but also for erosion¹³. Green tea polyphenols, especially epigallocatechin gallate (EGCG), were found to have distinct inhibitory activities against MMPs²³.

Recently, one study about the preventive effect of green tea on dentin wear was performed, showing that the rinse with green tea reduced dentin erosion and abrasion *in situ*⁴⁹.

Other potential MMP inhibitors are chlorhexidine (CHX), an antibacterial agent, which was found to inhibit the activity of MMPs 2, 8 and 9³⁴, as well as natural products such as avocado, soya bean and oleic acid²³. CHX presents beneficial effects on the preservation of dentin bond strength *in vivo*, as an MMP inhibitor²¹, when applied between the acid attack and the bonding. However, the mechanisms of action of these agents and their impact on dental erosion have not been investigated yet. Therefore, this topic might be of interest in further researches for the prevention of dental erosion.

3 Conclusion

From the available data of *in vitro* and *in situ* studies, preventive strategies for patients suffering from erosion include dietary advice, stimulation of salivary flow, optimization of fluoride regimens, modification of erosive beverages and adequate oral hygiene measures. However, clinical trials are required to confirm the relevance of these measures. As erosive tooth wear can not be prevented totally with the recommended strategies, further research is necessary to develop new measures with higher protective capabilities and good clinical acceptance.

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3.10 Capítulo 10

Is titanium tetrafluoride (TiF₄) effective to prevent carious and erosive lesions? A review of the literature

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Abstract

This paper summarizes the effects of titanium tetrafluoride (TiF_4) on the development and progression of carious and erosive lesions. The mode of action of TiF_4 is attributed to the formation of an acid-stable surface layer, which provides mechanical protection of the surface, and to an increased fluoride uptake, which might reduce demineralization of dental hard tissues chemically. In vitro studies mostly showed that TiF_4 is effective in reducing the formation of carious and erosive enamel and dentine lesions. Thereby, TiF_4 was equally or more effective than NaF , AmF or SnF_2 . While the caries-preventive effect was also confirmed by clinical data, clinical trials analysing the anti-erosive effect of TiF_4 are lacking. Few data available from in situ studies revealed conflicting results by showing either no or a beneficial effect of TiF_4 on enamel erosion. Even though research focused on TiF_4 , there is also evidence for other metal fluorides, such as zirconium and hafnium tetrafluorides, to affect enamel and dentine demineralisation.

In conclusion, the potential of TiF_4 to prevent acid demineralisation requires further research to confirm the promising in vitro results by in situ studies and clinical trials.

Introduction

Topical fluoridation in form of toothpastes, gels, solutions and varnishes of sodium fluoride, stannous fluoride, amine fluorides, acidulated phosphate fluoride and monofluorophosphate is considered as main key to caries prevention for many decades (Hellwig and Lennon, 2004). The mode of action of fluoride is mainly attributed to its influence on de- and remineralization kinetics of dental hard tissues, but also to its interference with the acid production of cariogenic bacteria (Ten Cate, 1999).

The formation of CaF_2 -precipitates induced by application of topical fluorides is regarded as decisive for the caries inhibiting effect, as they act as a source of free fluoride ions available during the cariogenic challenge. These are subsequently incorporated into the enamel as hydroxyfluorapatite or fluorapatite, resulting in a decreased susceptibility to further dissolution (Ten Cate, 1997). The same mode of action is assumed for the anti-erosive capability of fluorides. Additionally, the CaF_2 -layer might act as a physical barrier hampering the contact of the acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. Fluoride compounds that have been used over years for caries prevention were shown to be also effective in reducing erosive demineralisation by formation of a CaF_2 layer. As high concentrated fluoride agents or a prolonged application time might lead to a thicker and more stable CaF_2 -precipitate, an intensive fluoridation is considered as most effective for prevention of erosive mineral loss (Lagerweij et al, 2006; Wiegand and Attin, 2003). However, as the protective efficacy of common fluorides, such as sodium or amine fluorides, on erosion is limited, current research focuses on the efficacy of other fluoride compounds, such as titanium tetrafluoride (TiF_4), for prevention of erosive loss. TiF_4 has gained increasing attention more than 30 years ago, as it is considered as a possible anti-cariogenic agent (Shrestha et al, 1972). It might also be effective in reducing dentine hypersensitivity (Charvat et al, 1995; Kazemi et al, 1999) and sealing of dentinal tubules of root canal dentine (Sen and Büyükyilmaz, 1998).

This review aimed to summarize the effects of TiF_4 on prevention, development and progression of carious and erosive lesions. Final considerations will also discuss the potential of other metal fluorides, namely zirconium (ZrF_4) and hafnium (HfF_4)

tetrafluorides, to prevent demineralisation of dental hard tissues. Due to the paucity of clinical studies, this review was not performed as systematic review.

TiF₄ - mode of action

Titanium itself is a non-toxic element, and no adverse side effects have been reported with TiF₄ as yet. However, the dissolution of TiF₄ in water results in a highly acidic solution, which might account for the higher cytotoxic potential on L929-fibroblasts compared to NaF in vitro (Sen et al, 1998).

In most studies, TiF₄ is applied in form of solutions with a concentration of 1% to 4%, but gels or varnishes were also used as vehicle. While the demineralisation-reducing effect of sodium or amine fluoride is mainly attributed to the deposition of CaF₂-precipitates, the protective capacity of TiF₄ is referred to both the formation of an acid-resistant surface coating, an increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice.

By SEM analysis it could be shown, that the application of TiF₄ solution induced a glaze-like non-globular coating which is assumed to be formed from TiO₂ and/or from organometallic complexes of titanium and the organic dental matrix (Gu et al, 1996; Wefel and Harless, 1981; Wei et al, 1976). The acid-resistant coating might consist of TiO₂ produced by the reaction of titanium with oxygen groups from water or phosphate bound oxygen (Tveit et al, 1988).

Alternatively, it is assumed that the coating is composed of organometallic complexes. This hypothesis is confirmed by the observation of Mundorff et al. (1972), who showed that the formation of the glaze-like layer was distinctly decreased on organic reduced enamel. However, the glaze-like layer might be also affected by the presence of fluoride, as TiCl₃ failed to produce a glaze. The protective effect of the amorphous coating might be mainly related to its ability to act as diffusion barrier. Moreover, as the layer is rich of titanium and fluoride it is discussed that the coating might also act as reservoir for fluoride ions which in turn might retard acid dissolution or increase remineralisation of the underlying dental hard tissue.

The increased enamel fluoride uptake after application of TiF₄ can be explained by the ability of the polyvalent metal ion to form strong fluoride complexes while simultaneously binding firmly to the enamel apatite crystals (McCann, 1969). Neither KOH nor inorganic solutions were able to substantially remove the fluoride after a

TiF₄ treatment, indicating that a higher level of permanently bound fluoride is present (Wefel and Harless, 1981; Wefel and Harless, 1982). Moreover, the TiF₄ solution in itself is very acidic and might induce a demineralization of the enamel surface and the formation of HF. This might enhance the depth of penetration of fluoride ions into the apatite lattice. The fluoride uptake from TiF₄ might also be influenced by the organic matrix. Gu et al. (1996) showed that the enamel fluoride uptake was nearly completely reduced when enamel was pretreated with sodium hypochlorite, which deprived organic components from dental hard tissue. In contrast, fluoride uptake was significantly increased, when the organic matrix of enamel was exposed by prior etching with phosphoric acid (Gu et al, 1996).

Additionally, the efficacy of TiF₄ might be also attributed to its ability to substitute calcium in the apatite lattice or to formation of a titanium phosphate compound (Leadley et al, 1997; Ribeiro et al, 2006) leading to a higher acid resistance of dental hard tissues. Surprisingly, the penetration depth of titanium was shown to be higher in sound than in demineralised enamel, although it was assumed that the penetration depth would be larger in demineralised lesions with a higher porosity (Chevitere et al, 2004).

TiF₄ has not only a local effect, but might also influence fluoride uptake of enamel during maturation when applied systemically. Shrestha (1983) showed that developing rat enamel exhibited significantly higher fluoride concentrations after systemic treatment with equimolar solutions of 0.12 % TiF₄ than with 0.17 % NaF (given at a daily dose level of 7.5 mg F/kg body weight).

In dentine, the exposure of TiF₄ (1.1 M F, 1-4 min) resulted in the formation of a 0.1 µm thick electron dense coating, covering a partly demineralised zone of 5 - 27 µm depth (Skartveit et al, 1991a). Sen and Büyükyılmaz (1998) showed that dentine specimens treated with TiF₄ after smear layer removal showed a granular coating on intertubular and intratubular dentine.

Similarly to enamel, TiF₄ promoted a higher fluoride uptake as well as a greater depth of fluoride penetration in dentine when compared to NaF (Tveit et al, 1985). In vivo, it could be shown that TiF₄ not only reacts very rapidly with dentine, but the increased fluoride concentration was also retained for up to several months (Skartveit et al, 1989b; Skartveit et al, 1989a; Tveit et al, 1988).

In addition to the cariostatic action of fluoride by affecting de- and remineralisation of dental hard tissues, various fluoride salts and polyvalent cations might also exhibit a

direct antimicrobial effect (Skartveit et al, 1990). While TiF_4 treatment reduced the bacterial growth of *S. mutans* and *B. gingivalis* in vitro, it failed to influence the bacterial colonisation under clinical conditions. Although polyvalent cations might render the enamel surface more positively charged and hence influence the composition of the pellicle or plaque, TiF_4 treatment did not alter the properties of enamel and root surfaces with regard to plaque formation (Skartveit et al, 1990). Due to the observation that the bacterial colonization and plaque composition on titanium surfaces was not significantly different to hydroxyapatite (Leonhardt et al, 1995), it might be assumed that titanium itself might be of minor impact on the plaque formation. Moreover, although fluorides might affect initial plaque formation, the observed lack of effect was explained by a firm binding of fluoride in dental hard tissues which impede the release of fluoride into plaque nearly completely (Skartveit et al, 1990).

Caries preventive effect of TiF_4

Several in vitro studies showed that the single treatment of sound enamel with TiF_4 solutions (Tezel et al, 2002; Wefel and Harless, 1982) or varnishes (Magalhães et al, 2008a) was effective in preventing the formation of artificial carious lesions. Thereby, TiF_4 was equally or more effective than NaF or AmF solutions or varnishes, respectively. Exterkate and Ten Cate (2007) showed that the protective effect of a TiF derivative could be increased by frequent re-application of the agent. Studies analysing a potential preventive effect of TiF_4 on lesion progression revealed conflicting results. While Wefel and Harless (1984) found TiF_4 to be unable to reduce lesion progression, Magalhães et al. (2008a) showed that TiF_4 led to an increased rehardening of demineralised enamel compared to NaF varnishes. Exterkate and Ten Cate (2007) showed that a TiF derivative induced an arrestment of artificial carious lesions, but seems not suitable to enhance lesion repair. This observation was explained by the formation of the glaze layer that might hinder the transport of calcium and phosphate throughout the lesion.

As for enamel, TiF_4 solutions were also shown to reduce the development of carious-like lesions on dentine root surfaces in vitro (Derand et al, 1989) and in situ (Büyükyilmaz et al, 1997a).

Currently, only few studies assessed the caries-inhibitory effect of TiF_4 in vivo. Skartveit et al. (Skartveit et al, 1991b) evaluated caries scores in rats, which were treated with 1% TiF_4 (1 min, day 1 and day 17) and subjected to *S. mutans* inoculation (twice weekly) and a cariogenic diet. After 55 days, rats treated with TiF_4 showed significantly less caries than animals of the control group, but TiF_4 was not more effective than NaF. In contrast, TiF_4 was significantly more effective in preventing root surface caries of hamsters than acidulated phosphate fluoride (APF), stannous fluoride (SnF_2) or copper fluoride (CuF_2) (Oliveira Cordeiro, 1995). Büyükyilmaz et al. (1994) evaluated the cariostatic potential of 1% TiF_4 application around orthodontic brackets. After 4 weeks, the premolars were extracted for orthodontic reasons and analysed by microradiography, which showed that the TiF_4 solution reduced lesion depth and total mineral loss significantly.

Finally, Reed and Bibby (1976) investigated the caries inhibitory effect of TiF_4 and APF in a split mouth design in 110 children. The fluoride compounds were applied annually for 1 min (TiF_4) or 4 min (APF), respectively. The caries incidence over 3 years was significantly lower in teeth treated with TiF_4 than in teeth treated with APF.

Erosion preventive effect of TiF_4

Most 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 APF. Thus, also TiF_4 was considered as potential agent for the prevention of erosive lesions.

Most in vitro studies using TiF_4 solutions in a concentration of 1-4% demonstrated that TiF_4 decreased the formation of erosive enamel lesions (Büyükyilmaz et al, 1997b; Hove et al, 2007b; Wiegand et al, 2008b) and was more effective than NaF, AmF and SnF_2 (Hove et al, 2006; Hove et al, 2007a; Schlueter et al, 2007; van Rijkom et al, 2003). In contrast, Vieira et al. (2005; 2006) and Magalhães et al. (2008b) showed no beneficial effect of 1-4% TiF_4 solutions and gels on erosion or combined erosion and abrasion. Studies concerning the protective efficacy of a TiF_4 varnish on enamel erosion are conflicting by showing either no effect (Magalhães et al, 2007) or a significant reduction of enamel loss (Magalhães et al, 2008b) depending on the intensity of the erosive challenge. With regard to dentine, only two studies evaluated the impact of TiF_4 solutions in vitro (Schlueter et al, 2007; Wiegand

et al, 2008b). Thereby, TiF_4 was more effective than NaF (Schlueter et al, 2007) or AmF (Wiegand et al, 2008b) in reducing erosive dentine loss.

The few data available from in situ studies found contradictory results. Magalhães et al. (2008c) showed that the single application of 4% TiF_4 solution was not able to reduce erosive enamel wear and softening significantly. In contrast, Hove et al. (2008) showed that the repeated application of 1.5% TiF_4 reduced enamel erosion significantly and was more effective than NaF and SnF_2 . However, as both studies focused on the effects of erosion only and excluded abrasive influences, such as brushing, further studies have to evaluate whether the glaze layer formed after TiF_4 application withstands an abrasive challenge. As TiF_4 was not able to reduce erosion followed by brushing abrasion in vitro, it was assumed that the glaze like layer is not stable under abrasive influences (Vieira et al, 2006).

Effects of ZrF_4 and HfF_4 on demineralisation of dental hard tissues

Early studies in caries prevention suggested that not only TiF_4 but also other tetrafluoride compounds might reduce the formation of artificial caries lesions. Shrestha et al. (1972) demonstrated that TiF_4 and also zirconium (ZrF_4) and hafnium tetrafluoride (HfF_4) reduced enamel dissolution in an acetic acid buffer (pH 4) significantly. Similarly, Mühlemann et al. (1957) found that enamel pretreatment with ZrF_4 lead to 53% reduction of calcium and phosphate loss induced by an artificial caries solution. Recently, it could be shown that HfF_4 and ZrF_4 solutions were also able to decrease erosive enamel and dentine loss (Wiegand et al, 2008a; Wiegand et al, 2008b).

Although Mundorff et al. (1972) suggested that HfF_4 and ZrF_4 might be unable to form an acid-stable surface glaze, SEM analysis revealed that zirconium applied in form of ZrCl might be able to induce an amorphous surface coating on enamel surfaces treated with APF (Clarkson et al, 1984). Thereby, ZrCl lead to the formation of a relatively thick surface coating, while titaniumchloride pretreatment showed a thinner and fragile surface coating (Clarkson et al, 1984). Thus, it can be speculated that HfF_4 and ZrF_4 application might also lead to a mechanical protection of dental hard tissues the enamel surface, which shows a similar mode of action as the TiF_4 glaze-like layer (Wiegand et al, 2008a). Moreover, it is suggested that the protective effect of HfF_4 and ZrF_4 might also due to act chemical interaction with the apatite, as

it is known that metal ions lead to an increased fluoride retention due to their ability to complex with fluoride.

Discussion

Although several in vitro studies suggest a caries and erosion preventive effect of TiF_4 , clinical studies analysing the efficacy of TiF_4 or other tetrafluoride compounds are lacking. Actually, only few clinical data about the caries preventive effect of TiF_4 and no clinical studies about the protection of erosion by TiF_4 are available, underlining the demand for further clinical trials.

Due to the lack of biocompatibility data, further studies have to focus also on safety issues of TiF_4 , not least as TiF_4 varnishes and solutions exhibit a very low pH. This low pH might induce adverse side effects on hard and soft tissues. Even though the depth of penetration of fluoride ions into the apatite lattice might be enhanced under acidic conditions, the low pH of TiF_4 agents might also provoke a demineralisation of the surface during application. Depending on the study protocol, some authors reported a loss of enamel (Magalhães et al, 2008) or a distinct demineralised subsurface layer (Skartveit et al, 1991a) after application of TiF_4 agents.

Although the precipitates formed after application of TiF_4 might be resistant to an acidic attack, this glaze might be less wear resistant and the subsurface zone might be more prone to wear. Büyükyilmaz et al. (Büyükyilmaz et al, 1997c) reported that TiF_4 is already worn off on cusps after three to six months. After 1 year the glaze was only present in pits and fissures. The abrasion resistance of TiF_4 -treated enamel was analysed only in one study as yet (Vieira et al, 2006). Thereby, brushing abrasion led to slightly, but not significant, higher wear in TiF_4 -treated enamel compared to untreated controls. These findings ask for an evaluation of the benefit of TiF_4 fluoridation on the cost of making the teeth more prone to wear.

Moreover, due to the very low pH and the potential adverse side effects, TiF_4 agents are currently not considered for a self-application by a patient. For a potential home use of TiF_4 , products at higher pH which are equally effective to the agents at low pH would be desirable and should be analysed in further studies. The potential of TiF_4 to be used as oral hygiene product is of specially interest, as several studies found a distinct protective effect on carious and erosive lesions in vitro (Exterkate and ten

Cate, 2007; Hove et al, 2008; Schlueter et al, 2007) when the TiF_4 agents were applied frequently.

Finally, the exact mode of action and the efficacy of ZrF_4 and HfF_4 to impede demineralization of dental hard tissues should be evaluated in further studies.

Conclusion

The results available from in vitro and in situ studies suggest that TiF_4 is an effective agent for the prevention of carious and erosive lesions. However, only few clinical studies analysed the caries preventive effect of TiF_4 in vivo, and clinical data evaluating the potential on dental erosion are lacking as yet. Thus, further research has to confirm the promising results found for artificial lesions in the clinical situation. Moreover, zirconium and hafnium tetrafluoride should be considered and, thus, analysed as alternative agent.

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3.11 Capítulo 11

Fluoride in Dental Erosion

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Short Title: Fluorides and Erosion

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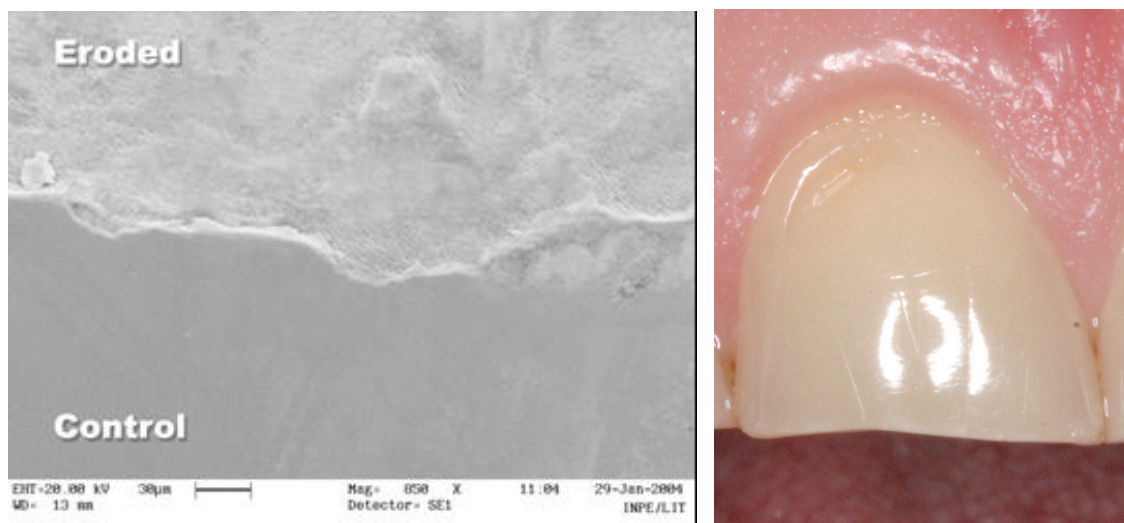
Abstract

Dental erosion develops under the chronic exposure to extrinsic/intrinsic acids with a low pH. Enamel erosion is characterised by a centripetal dissolution leaving a small demineralised zone behind. In contrast, erosive demineralisation in dentine is more complex as the acid-induced mineral dissolution leads to the exposure of collagenous organic matrix, which hampers ion diffusion and, thus, reduces further progression of the lesion. Topical fluoridation inducing the formation of a protective layer on dental hard tissue, which is composed of CaF_2 (in case of conventional fluorides like amine fluoride or sodium fluoride) or of metal-rich surface precipitates (in case of titanium tetrafluoride or tin-containing fluoride products), appears to be most effective on enamel. In dentine, the preventive effect of fluorides is highly dependent on the presence of the organic matrix. *In situ* studies showed a higher protective potential of fluoride in enamel compared to dentine, probably as the organic matrix is affected by enzymatical and chemical degradation as well as by abrasive influences in the clinical situation. There is convincing evidence that fluoride, in general, can strengthen tooth against erosive acid damage, and high concentration fluoride agents and/or frequent applications are considered potentially effective approaches to prevent dental erosion. The use of tin-containing fluoride products might provide the best approach for effective prevention of dental erosion. Further properly designed *in situ* or clinical studies are recommended in order to better understand the relative differences in performance of the various fluoride actives and formulations.

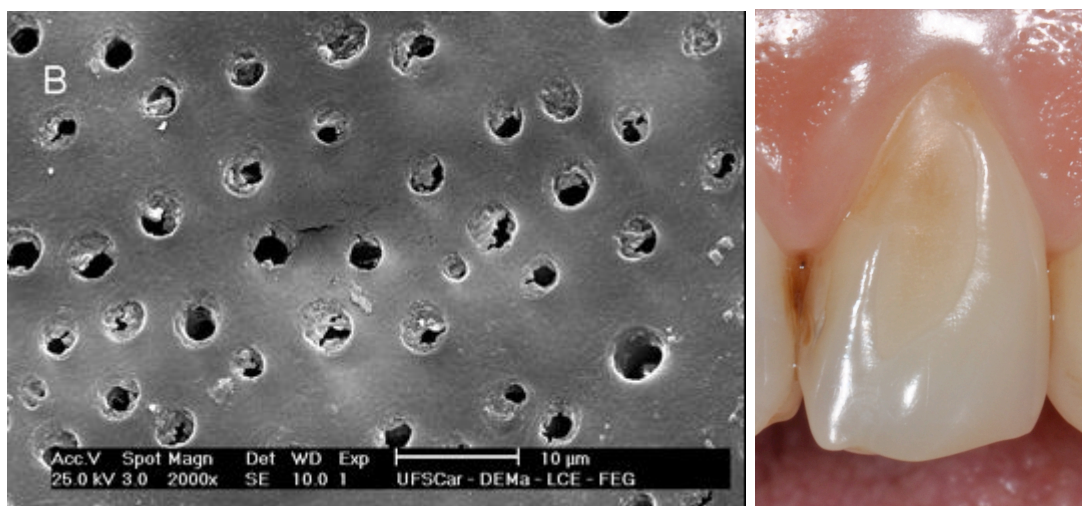
Introduction

Dental erosion is defined as substance loss by exogenous or endogenous acids without bacterial involvement. The most important sources are dietary acids [1] and those originated from the stomach, like gastric acids from regurgitation and reflux disorders [2].

In contrast to initial caries, enamel erosion is predominantly a surface phenomenon with a centripetal bulk substance loss combined with a small partly demineralised surface layer with decreased microhardness (Figures 1 and 2). In dentine, the erosive demineralisation is mostly diffusion controlled, as the increasing exposure of organic matrix hampers ion diffusion and, thus, reduces further progression of dentine erosion (Figures 3 and 4) [3,4].



Figures 1 and 2 - SEM and clinical picture of enamel erosion. The SEM is not correspondent to the clinical picture.



Figures 3 and 4 - SEM (By courtesy of Kato et al.) and clinical picture of dentine erosion. Figure 3 shows opened dentinal tubules; however, the tubules also can be partially or totally closed in the clinical situation. The SEM is not correspondent to the clinical picture.

There is evidence that the prevalence of erosion is steadily increasing [5]. Preventive strategies in the management of dental erosion consider dietary counselling, stimulation of salivary flow, modification of erosive beverages, adequate oral hygiene measures and fluoride treatment as most relevant [6].

This chapter will give an overview about the current knowledge on the use of fluorides, including conventional and metal fluorides, for the prevention of erosive and combined erosive-abrasive dental loss. Due to the fact that the histology of enamel and dentine erosion is considerably different, this chapter will be divided in: 1) fluoride and enamel erosion; 2) fluoride and dentine erosion.

Fluorides and enamel erosion

Extrinsic and/or intrinsic acids with low pH (pH 1.0-3.5) cause initially either the dissolution of the prism cores or of interprismatic areas, showing a honeycomb structure in prismatic enamel. In aprismatic enamel, the demineralisation is irregular, without a clear structural pattern. If the erosive challenge is ongoing, the dissolution process results in surface loss accompanied by a progressive softening of the surface. As the demineralised layer of eroded enamel is considerably small compared to the enamel loss, fluoride application predominately aims to prevent erosive tissue loss rather than to remineralise softened enamel.

Conventional fluorides whose beneficial effect against caries is well known [7] have been tested for prevention or control of dental erosion [8]. The potential of conventional fluorides, such as NaF and AmF, to prevent erosive demineralization is mainly related to the formation of a calcium fluoride (CaF_2)-layer [9,10] (Figure 5). This layer is assumed to behave as a physical barrier hampering the contact of the acid with the underlying enamel or to act as a mineral reservoir, which is attacked by the erosive challenge. Thereafter, calcium and fluoride released might increase the saturation level with respect to dental hard tissue in the liquid adjacent to the surface thus promoting remineralisation (Figures 6 and 7).

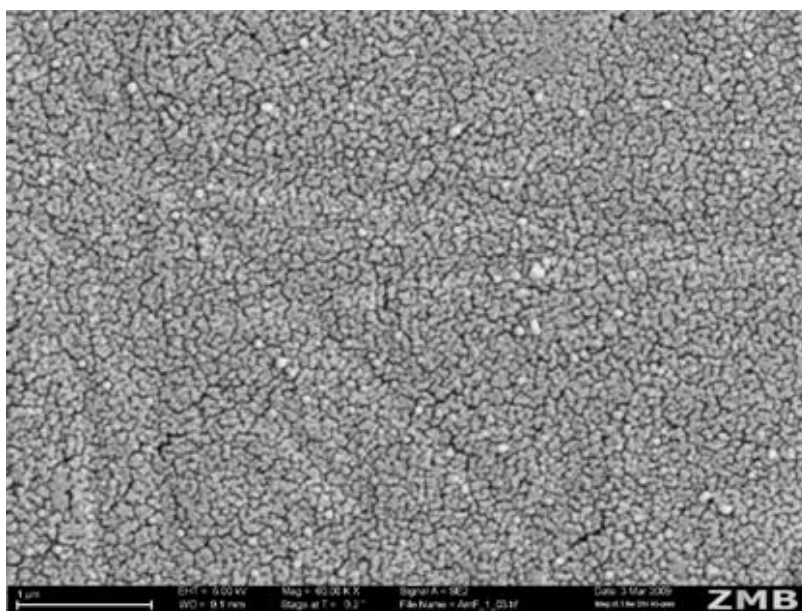


Figure 5 - SEM of enamel treated with conventional fluoride (AmF, 0.5 M F, pH 4.5, applied for 60 s).

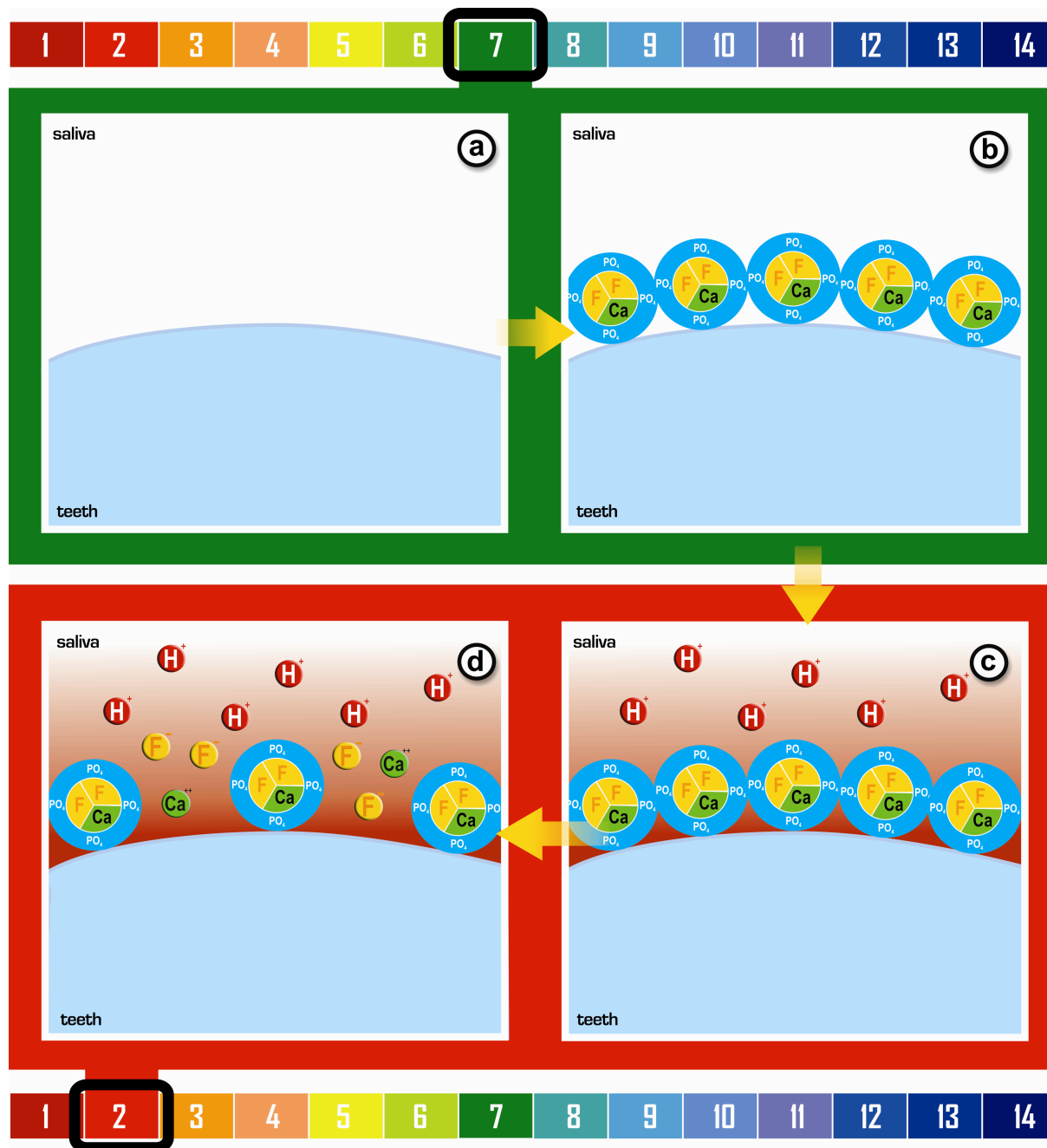


Figure 6 - Illustration of enamel treated with conventional fluoride: **a)** enamel surface, **b)** deposition of a calcium fluoride (CaF_2)-layer, **c)** CaF_2 -layer acting as a physical barrier for the erosive challenge, **d)** Progressive CaF_2 -layer dissolution.

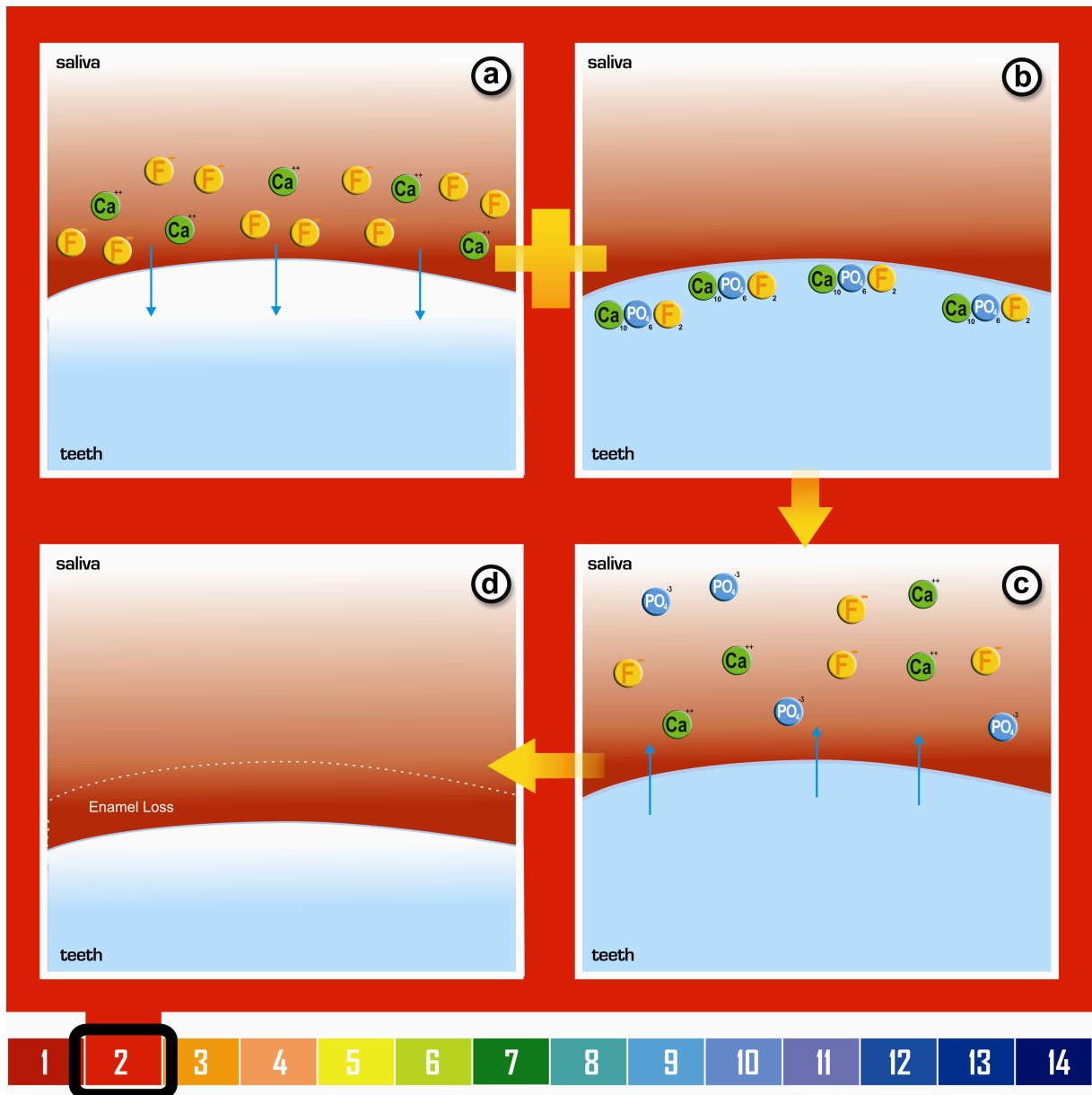


Figure 7 - Illustration of enamel treated with conventional fluoride: **a)** CaF_2 -layer final dissolution, **b)** simultaneous calcium and fluoride saturation provoking remineralisation, **c)** subsequent erosive challenge, **d)** bulk substance loss combined with a small partly demineralised surface layer.

The formation of the CaF_2 -like layer and its protective effect against demineralisation is highly dependent on the pH, the concentration of fluoride and the frequency of application. The deposition of calcium fluoride on the surface increases with increasing concentration and frequency of application and decreasing pH of the agent. Fluoride agents with a pH below 5 seem to induce a higher calcium fluoride deposition on dental surface than neutral ones [9].

Ganss *et al.* [10] evaluated the retention of calcium fluoride on human enamel under neutral and acidic conditions *in vitro* and *in situ*. Fluoride (10,000 ppm F, AmF) was applied once for 5 minutes and the enamel specimens were exposed to erosive demineralisation (3x 30s/day, 4 days *in vitro*/ 3x 2min/day, 7 days *in situ*) or neutral conditions (artificial saliva *in vitro*/ human saliva *in situ*). It was shown that more calcium fluoride was lost under erosive compared to neutral conditions *in vitro*, while the intra-oral environment was considerably protective for CaF₂-like precipitates especially on enamel.

Although toothbrushing might affect the progression of eroded dental hard tissues adversely by removing the softened layer of enamel [11,12], it was shown that the use of fluoridated (NaF) toothpastes might diminish the abrasive effect to some extent [11,12,13]. However, as the overall protective effect of toothpastes with 1,100-5,000 µg F/g is limited [14,15], the use of high-concentrated fluoride varnishes (22,600 µg F/g) was anticipated to be more effective due to their capacity to adhere to the tooth surface and create a calcium fluoride reservoir [16,17]. Indeed, the application of NaF varnish (22,600 µg F/g) was effective in reducing enamel erosion for 30 minutes of acid exposure, but the protective effect declined thereafter [18,19]. However, as placebo varnishes also showed some protection against enamel erosion and combined erosion/abrasion, it is believed that the protective effect of fluoride varnishes is mainly related to the mechanical rather than to the chemical protection [20,21].

As the anti-erosive effect of conventional fluorides requires a very intensive fluoridation regime [22], recent studies have focused on fluoride compounds which might deliver a higher level of efficacy. In this context, compounds containing polyvalent metal ions such as stannous fluoride or titanium tetrafluoride were tested. Several *in vitro* studies have shown an inhibitory effect of 0.4-10% TiF₄ solution on dental erosion [23,24,25,26,27], which is attributed not only to the effect of fluoride, but mainly to the action of titanium [23,28]. Its protective effect is related to the formation of an acid-resistant surface coating, the increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice. The glaze-like surface layer observed after the application of TiF₄ is assumed to be due to the formation of a new compound (hydrated hydrogen titanium phosphate) that might primarily act as a diffusion barrier [23,29,30,31,32] (Figures 8 and 9). The increased fluoride uptake

found after application of TiF_4 can be explained by the ability of the polyvalent metal ion to form strong fluoride complexes firmly bound to the apatite crystals [30,32].

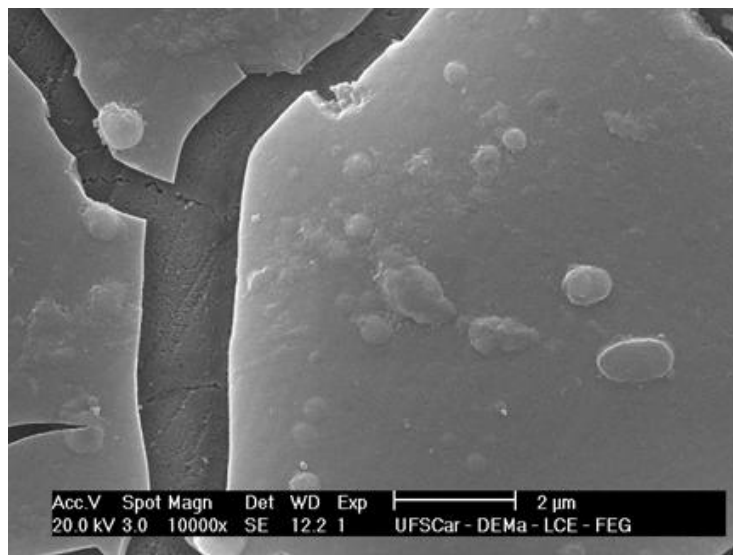


Figure 8 - SEM of enamel treated with 4% titanium tetrafluoride varnish (6h) (By courtesy of S. Karger AG, Basel. Magalhães et al. Caries Res. 2008;42:269-274).

Information regarding the efficacy of TiF_4 under clinical conditions is scarce and contradictory, as only two *in situ* studies showed 1.6% TiF_4 (0.5 M F) to be as effective as SnF_2 or AmF in the prevention of erosion or combined erosion/abrasion [33,34], while other did not show any protective effect of 4% TiF_4 [20,21,35]. The efficacy of TiF_4 is highly dependent on the pH of the agent, since it was shown that enamel erosion can be significantly reduced by TiF_4 (0.5 M F) at native pH (pH 1.2) but not at a pH buffered to 3.5 [36]. One study indicated that TiF_4 applied in the form of a varnish might be of higher efficacy than as a solution [19]. However it should be consider that the low pH of TiF_4 products does not allow self-application by the patient.

Tin-containing fluoride products have shown promising results in several studies [37,38,39 40,41]. The mode of action of tin-containing fluoride solutions is probably attributed to the formation of metal-rich surface precipitates [$\text{Ca}(\text{SnF}_3)_2$, SnOHPO_4 , $\text{Sn}_3\text{F}_3\text{PO}_4$], which were shown to be of high acid resistance [42] (Figures 9, 10 and 11). Further, tin may penetrate and become incorporated into the demineralized layer when high concentrated tin containing fluoride mouthrinses are used [38,43].

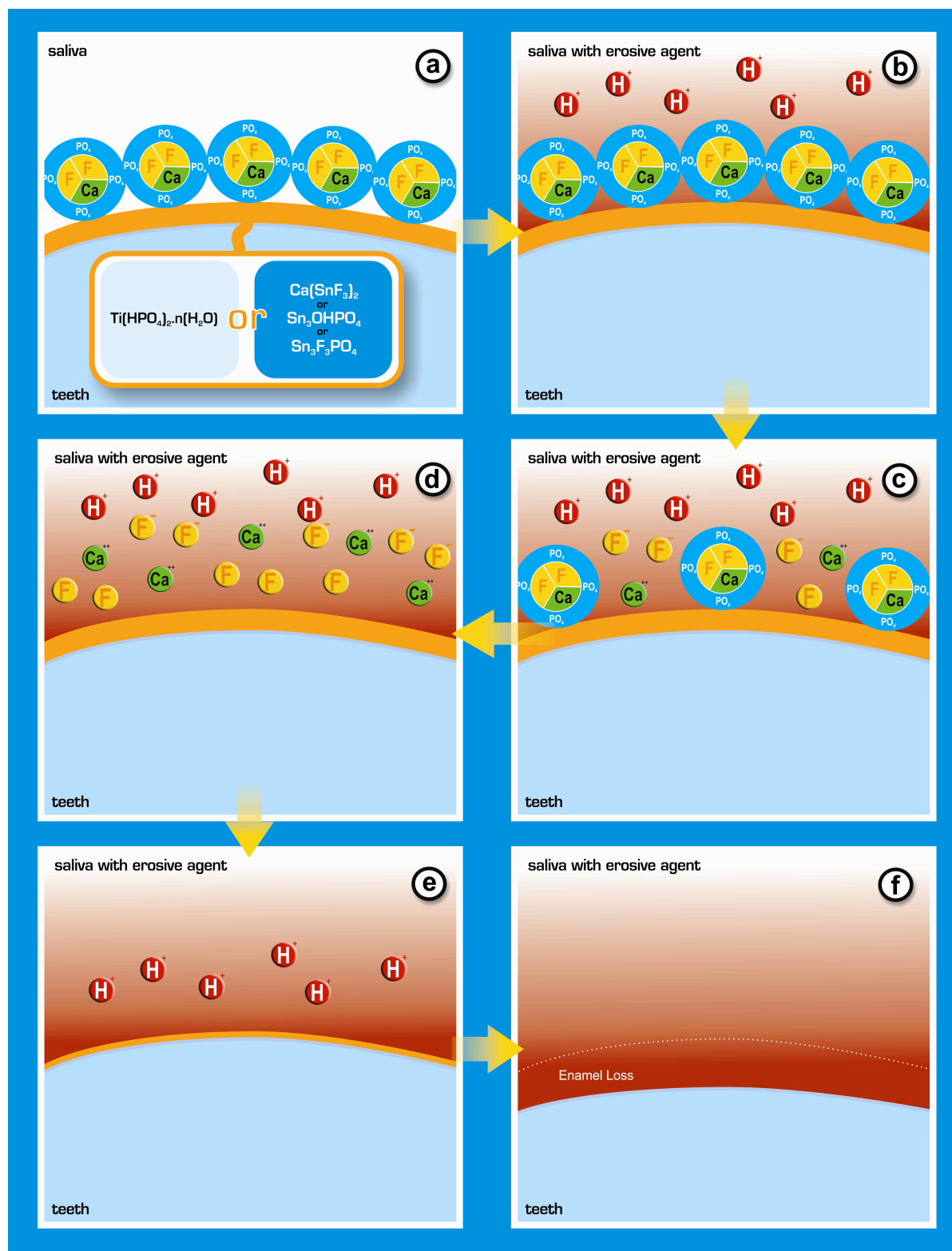


Figure 9 - Illustration of the formation of (CaF_2) -layer and an acid-resistant surface coating composed by hydrated hydrogen titanium phosphate [31] after the application of TiF_4 , or composed by metal-rich precipitates $[\text{Ca}(\text{SnF}_3)_2, \text{SnOHPO}_4, \text{Sn}_3\text{F}_3\text{PO}_4]$ after the application of tin-containing fluoride mouthrinses [40]: a) CaF_2 -layer and the metal-rich precipitates (in orange), b) erosive challenge, c) CaF_2 layer dissolution, d) CaF_2 -layer final dissolution and the preservation of the metal-rich precipitate, e) progressive erosive challenges, f) final dissolution of the metal-rich layer and a consequent enamel loss.



Figure 10 - SEM of enamel treated with SnF_2 solution (0.48 M F, pH 2.7, 3 min) before erosion (By courtesy of S. Karger AG, Basel. Yu et al. Caries Res. 2010;44:390-401).

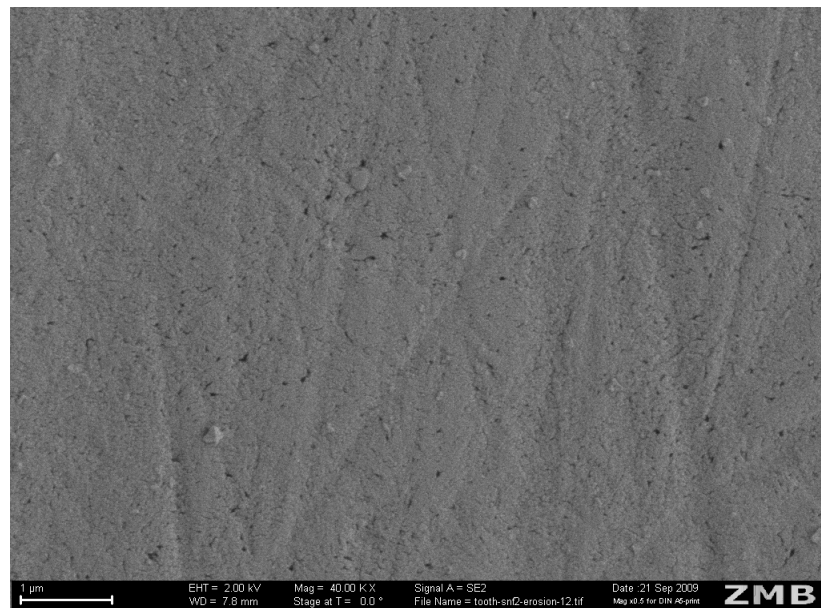


Figure 11 - SEM of enamel treated with SnF_2 solution after erosion (6x1min/day, 5 days), showing no alteration (By courtesy of S. Karger AG, Basel. Yu et al. Caries Res. 2010;44:390-401).

Ganss *et al.* [44] evaluated the relevance of cations in different fluoride compounds for their effectiveness as anti-erosive agents and showed that SnCl_2 (800 ppm Sn), NaF (250 ppm F), AmF/ SnF_2 (250 ppm F/ 390 ppm Sn) and SnF_2 (250 ppm F/ 809 ppm Sn) solutions could reduce enamel erosion. Treatment with solutions containing

SnF₂ was most effective. The combination of AmF/NaF/SnCl₂ with high (2,800 ppm Sn/ 1,500 ppm F) and low (700 ppm Sn/ 1,500 ppm F) tin concentration reduced erosion by 90% and 70%, respectively [38,39].

Some possible side effects of high concentration tin containing mouthrinses may be dull feeling on the tooth surface, astringent sensation and tooth discolouration (1,900 ppm Sn) [45]. Therefore, tin-containing solutions of lower concentration (800 ppm Sn/ 500 ppm F) were tested *in vitro* and *in situ* [46,47]. Under severe erosive conditions, the SnCl₂/NaF/AmF exhibited a high potential to reduce enamel erosion (67% reduction), and showed no adverse side effects [47]. Besides mouthrinses, tin-containing fluoride toothpastes were tested in *in vitro* protocols and shown to perform significantly better under erosive challenges when compared with NaF and MFP-containing toothpastes [41]. Further research should test specially formulated tin-containing fluoride products to minimize aesthetic negatives seen with high concentration tin-containing products, which may provide a highly effective means to help prevent dental erosion using a consumer-friendly approach.

Fluorides and dentine erosion

The preventive effect of fluorides on dentine erosion is highly dependent on the presence of the organic matrix [48]. Initial studies showed that a very intensive fluoridation combining toothpaste (0.15% F, NaF), mouthrinse (0.025% F, AmF/NaF) and gel (1.25% F, AmF/NaF) application was most effective in the prevention of dentine erosion [22,49]. However, after enzymatic removal of the organic matrix fluoride was ineffective [3,50]. It was assumed that the demineralised organic dentine matrix has a buffering capacity sufficient to prevent further dentine demineralisation especially in the presence of high amounts of fluoride [3]. Moreover, the exposed organic matrix of etched dentine involves an increased surface area and increased diffusion pathways; enhancing the amount of structurally bound and KOH-soluble fluoride compared to sound dentine [51]. However, it remains unclear to which extent the organic material is retained under clinical conditions, when the collagen layer might be affected by enzymatical and chemical degradation as well as by abrasive influences [50,52]. From the clinical appearance of dentine erosive lesions it seems likely that the collagenous layer is at least partly removed. This hypothesis might also

explain why fluorides such as NaF were less effective in dentine than in enamel under *in situ* conditions [22,10,38] but not in laboratory experiments [27,53].

The application of slightly acidic fluoride formulations such as NaF or AmF results in the formation of CaF_2 -precipitates on both enamel and dentine (Figure 12), but the precipitates are less stable on dentine than on enamel under erosive conditions [10]. Although the preventive potential of NaF and AmF solution and dentifrice on dentine erosion and combined erosion/abrasion was shown in different *in situ* studies [22,34,54], information about the ideal fluoride concentration and frequency of application is scarce. Also, the resistance of dentinal CaF_2 precipitates against abrasion was not assessed directly so far; only *in situ* study indicated that the protective potential of AmF against erosion is not affected by additional brushing treatment [34].

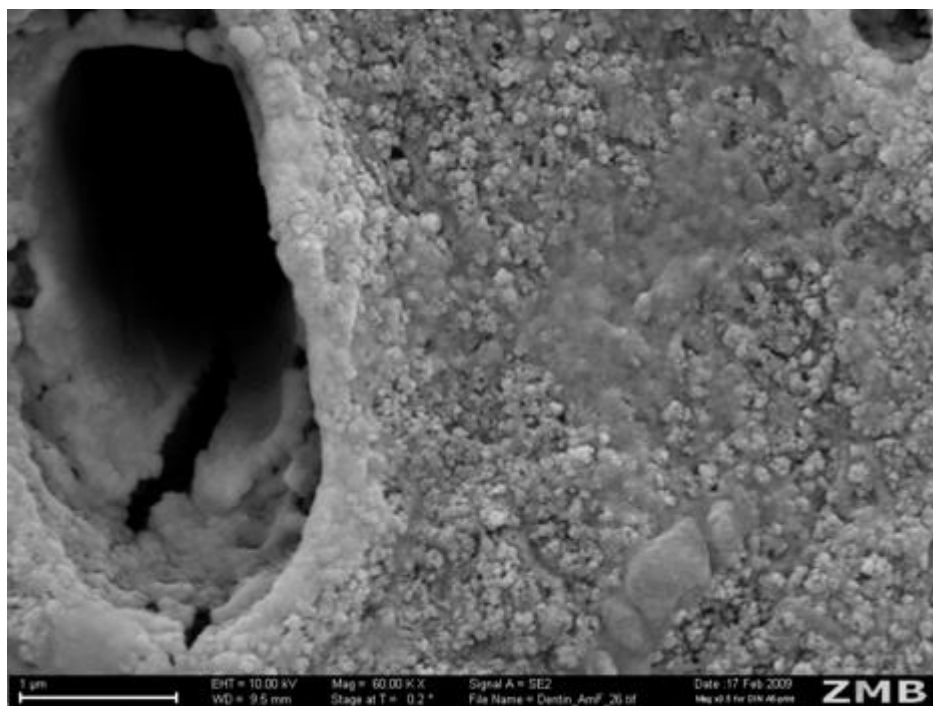


Figure 12 - SEM of dentine treated with conventional fluoride (AmF, 0.5 M F, pH 4.5, applied for 60 s).

Considering the severe and chronic acid exposure in patients suffering from dental erosion, the effect of CaF_2 -precipitates is probably limited over time [10] and fluoride compounds with a distinct potential to resist an erosive challenge are required.

Titanium tetrafluoride was shown to induce some coating on dentine surfaces, which partly covered dentinal tubules [55] (Figure 13). However, its protective potential did

not exceed the efficacy of NaF or AmF [27,34,56], and the low pH required for the efficacy of the agents do not allow for a clinical application so far [57].

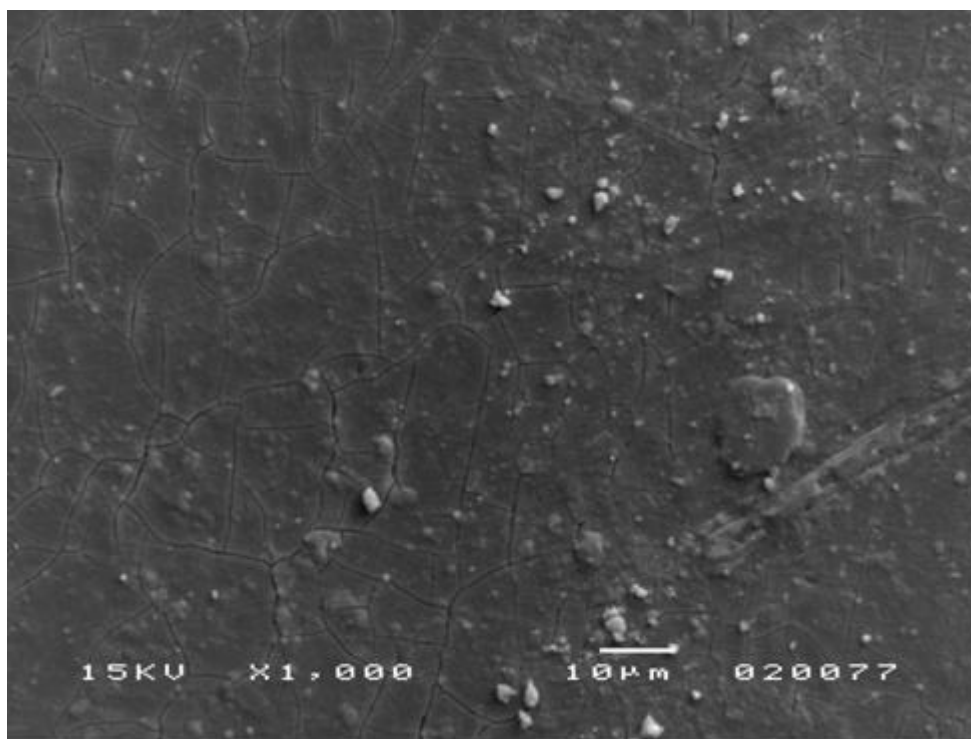


Figure 13 - SEM of dentine treated with 4% titanium tetrafluoride varnish (6h).

Tin-containing fluoride solutions have been demonstrated to exhibit promising anti-erosive effects not only on enamel but also on dentine [38,44,46]. The suggested mechanism of action is related to the incorporation of tin in mineralised dentine when the organic matrix is allowed to develop and to surface precipitation when the organic matrix is enzymatically removed [58]. In case that the organic matrix is preserved, phosphorus, phosphorylated phosphoprotein or phosphophoryn might attract the tin-ion, which is then retained in the organic matrix to some extent but accumulates also in the underlying mineralised tissue. In case that the organic matrix is removed, tin reacts with the mineral by forming different salts, e.g. $\text{Sn}(\text{OH})_2$, $\text{Sn}_2(\text{PO}_4)\text{OH}$, $\text{Ca}(\text{SnF}_3)$, $\text{Sn}_3\text{F}_3\text{PO}_4$, $\text{Sn}_2(\text{OH})\text{PO}_4$, $\text{Sn}_3\text{F}_3\text{PO}_4$ or SnHPO_4 [58]. Recent *in situ* studies demonstrated that mouthrinses containing AmF/NaF/SnCl₂ (500 ppm F, 800 ppm Sn) reduced dentine erosion by 50% and were significantly more effective than a NaF-containing mouthrinse (500 ppm F) [38,47].

Comparing the protective effect of different fluoride compounds on dentine erosion, Ganss *et al.* [48] showed that solutions containing AmF and/or SnF₂ performed only

slightly better than solutions containing NaF and/or AmF in the presence of the organic matrix. However, continuous removal of the organic matrix influenced the efficacy of the fluoride compounds distinctly and demonstrated a significantly better preventive effect of the SnF₂ and AmF/SnF₂⁻ containing solutions compared to all other solutions.

CONCLUDING REMARKS

Conventional fluorides with a known anti-cariogenic potential offer some, but limited protection against erosion as the CaF₂ precipitates formed on the surface are readily soluble in acids. Metal-containing fluoride compounds showed promising results in prevention of erosion, but might involve some adverse side effects due to the very low pH (in case of titanium tetrafluoride) and the potential to cause slight discoloration, dull feeling on the tooth surface and astringent sensation (in case of high concentrated tin containing fluoride solutions).

There is convincing evidence that fluoride, in general, can strengthen enamel against erosive acid damage, high concentration fluoride agents and/or frequent applications are considered potentially effective approaches to prevent dental erosion. However, fluorides might be more effective in enamel than in dentine, as the organic matrix influencing the efficacy of fluorides might to some extent be affected by enzymatical and chemical degradation as well as by mechanical abrasion. The use of tin-containing fluoride products might provide the best approach for effective prevention of dental erosion.

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4 Considerações finais

4 CONSIDERAÇÕES FINAIS

Com base nos resultados obtidos nestes estudos pode-se concluir que:

(1) Cárie Dentária

1.1 No estudo com ciclagem de pH simulando os processos de desmineralização e remineralização do esmalte dentário sem envolvimento de microorganismos, o verniz de TiF_4 apresentou efeito similar aos vernizes comerciais à base de NaF na redução da desmineralização, porém foi mais efetivo que os vernizes à base de NaF na remineralização de lesão artificial de cárie, especialmente nos primeiros 30 μm de profundidade. Neste estudo foi utilizada como variável de resposta a microdureza superficial e longitudinal (artigo publicado no J Dent. 2008 Feb;36(2):158-62., citado no ISI 5x, impacto 2,115).

1.2 No estudo *in situ*, com acúmulo de biofilme, simulou-se a desmineralização (com exposição à sacarose) e a remineralização do esmalte dentário. Os vernizes NaF, TiF_4 e a solução TiF_4 foram capazes de reduzir significativamente a desmineralização superficial e de subsuperfície do esmalte dentário bovino em comparação à solução NaF, verniz placebo e controle. O verniz e a solução de TiF_4 foram capazes de aumentar significativamente a remineralização superficial do esmalte dentário bovino previamente desmineralizado em comparação ao verniz e solução de NaF, verniz placebo e controle. O protocolo experimental não permitiu uma adequada remineralização de subsuperfície *in situ*. Houve uma tendência ao aumento da desmineralização das amostras previamente desmineralizadas, especialmente para aquelas que não receberam tratamento com o fluoreto, *in situ*. Portanto, o protocolo para remineralização precisa ser repetido. Neste estudo foram utilizadas como variáveis de resposta a microdureza superficial e longitudinal e os dados da microradiografia transversal (artigo em elaboração, revista a ser definida).

Portanto, mediante a desafios carigênicos, o verniz de TiF_4 tem potencial similar aos vernizes à base de NaF na prevenção da desmineralização, porém em relação à remineralização do esmalte, mais estudos são necessários.

(2) Erosão dentária

- 2.1 O verniz de TiF_4 foi mais efetivo que os vernizes comerciais à base de NaF e a solução de TiF_4 na prevenção da erosão do esmalte dentário por desafios erosivos brandos (30 minutos). A variável de resposta utilizada foi a perfilometria de contato com software desenvolvido na Universidade de Zurich (artigo publicado no *Caries Res.* 2008;42(4):269-74, citado 18x, impacto 2,926).
- 2.2 No entanto, quando os desafios erosivos se estenderam por mais de 120 minutos (60 minutos com verniz e 60 minutos sem verniz), os vernizes fluoretados foram incapazes de reduzir a erosão do esmalte. A variável de resposta foi a microdureza de superfície e a perfilometria de contato utilizando-se o Rugosímetro da Disciplina de Dentística da FOB-USP (artigo publicado no *J Dent.* 2007 Nov;35(11):858-61, citado 13x, impacto 2,115).
- 2.3 Neste estudo complementar ao trabalho 2.1, adicionaram-se controles (soluções fluoretadas) e desafios abrasivos brandos. Os vernizes à base de NaF e TiF_4 reduziram significativamente o desgaste do esmalte pelos desafios erosivos e abrasivos em comparação ao verniz placebo, porém somente o verniz de TiF_4 foi diferente do controle. As soluções fluoretadas foram ineficazes na redução do desgaste do esmalte. Neste estudo, em geral, não houve grandes diferenças entre as condições erosão e erosão associada à abrasão, o que se deve aos baixos desafios abrasivos realizados em comparação aos trabalhos anteriores, visando simular a situação clínica. A variável de resposta foi a perfilometria de contato utilizando-se o Rugosímetro da Disciplina de Dentística (artigo publicado no *Int J Paediatr Dent.* 2011 *in press* Jun 20, impacto 1,289).

- 2.4 Estes estudo foi similar ao anterior, porém realizado em dentina radicular e sem desafios abrasivos. Nenhum dos vernizes fluoretados foram eficazes na redução da erosão da dentina, sendo que o único tratamento que apresentou melhores resultados foi o verniz a base de NaF (Duraphat) no 1º dia, porém este não diferiu do placebo nos períodos estudados. A variável de resposta foi a perfilometria de contato utilizando-se o Rugosímetro da Disciplina de Dentística (artigo publicado no J Dent. 2010 Feb;38(2):153-7, citado 1x, impacto 2,115).
- 2.5 Neste trabalho, complementar ao anterior, adicionaram-se desafios abrasivos. Os vernizes fluoretados foram igualmente efetivos na redução do desgaste da dentina em comparação ao verniz placebo e controle. As soluções fluoretadas foram ineficazes na redução da erosão e abrasão da dentina. O melhor efeito do verniz fluoretado obtido neste trabalho em relação ao anterior pode ser justificado pela inclusão da abrasão, a qual poderia ter removido resíduos dos vernizes não detectados, possibilitando melhor diferenciação do efeito químico dos produtos. Ainda não se pode destacar que a diferença dos resultados pode ser devido ao tipo de perfilômetro utilizado. A variável de resposta utilizada foi a perfilometria de contato com software Marh Surf XR20 (artigo publicado no Acta Odontol Scand 2011 *in press*, impacto 1,13).
- 2.6 Na situação *in situ*, na qual amostras de esmalte e dentina radicular humanas foram submetidas a desafios erosivos e abrasivos brandos, os vernizes e as soluções fluoretadas (NaF e TiF_4) foram igualmente efetivos na redução do desgaste dentário. Neste estudo, também não houve diferença entre as condições erosão e erosão associada à abrasão, o que se deve aos baixos desafios abrasivos realizados em comparação aos trabalhos anteriores. Interessante é que neste estudo as soluções fluoretadas foram eficazes, em contraposição aos resultados dos estudos *in vitro*, o que pode ser devido ao fato de terem sido utilizados dentes humanos e principalmente à presença da película adquirida e da saliva humana, que podem ter

possibilitado melhor interação e estabilização dos compostos formados pela aplicação das soluções fluoretadas. A variável de resposta foi a perfilometria de contato utilizando-se o Rugosímetro da Disciplina de Dentística (artigo em fase de elaboração, revista não definida).

Os resultados destes trabalhos suportam que o verniz de TiF_4 tem um melhor efeito em comparação aos vernizes à base de NaF sobre o esmalte submetido aos desafios erosivos e abrasivos *in vitro*. Este efeito é perdido quando desafios erosivos são mais prolongados. Já para a dentina, os vernizes fluoretados à base de NaF e TiF_4 apresentam potencial similar, mas reduzido em relação àquele observado para o esmalte na redução do desgaste por desafios erosivos e abrasivos brandos *in vitro*. No entanto, quando o tratamento e os desafios erosivos e abrasivos brandos foram realizados *in situ*, todos os produtos fluoretados (vernizes e soluções de TiF_4 e NaF) reduziram similarmente o desgaste do esmalte e dentina. Estes dados ainda sugerem um papel importante da saliva humana e da película adquirida, tanto na ação do fluoreto quanto na progressão da erosão dentária, bem como que a ação do TiF_4 possa ser diferente em dente bovino e humano.

(3) Revisões de Literatura

- 3.1 Dentre as estratégias preventivas expostas neste artigo, enfocando os fatores químicos, biológicos e comportamentais do indivíduo, a aplicação tópica do fluoreto é a mais estudada e a que apresenta melhores resultados na redução da progressão da perda de estrutura dentária pela erosão e abrasão (artigo publicado no J Appl Oral Sci. 2009 Mar-Apr;17(2):75-86, citado 16x, impacto 0,966).
- 3.2 Neste artigo, houve um enfoque sobre o mecanismo de ação do TiF_4 e evidências científicas sobre o efeito deste sal fluoretado, o qual tem apresentado resultados superiores em comparação aos fluoretos convencionais, em função da presença do titânio que pode interagir com a apatita dentária, formando uma camada ácido-resistente, como pode ser visto nas microscopias do trabalho 2.1. Adicionalmente o baixo pH do agente pode facilitar a incorporação de fluoreto ao esmalte. Em relação à cárie dentária, há algumas

evidências clínicas do efeito positivo deste sal fluoretado, porém para a erosão dentária, os trabalhos são restritos ao laboratório, apontando para a necessidade do desenvolvimento de mais estudos (artigo publicado no Oral Health Prev Dent. 2010;8(2):159-64, sem citação).

- 3.3 Esta revisão de literatura mais recente abordou o efeito diferenciado dos sais fluoretados convencionais e dos fluoretos metálicos sobre a erosão do esmalte e dentina. Há evidência convincente que o fluoreto, em geral, pode aumentar a resistência do dente aos desafios erosivos, e que agentes com alta concentração de fluoreto bem como frequentes aplicações podem ser estratégias preventivas de alto potencial para a erosão dentária. Dentre os diferentes agentes fluoretados, as soluções fluoretadas contendo estanho (Tin) parecem ser as mais efetivas na prevenção da erosão dentária. Futuros estudos *in situ* são recomendados para um melhor entendimento do efeito dos vários agentes fluoretados e formulações (artigo publicado no Monogr Oral Sci. 2011;22:158-70).

Com base nas conclusões destes estudos, as perspectivas de pesquisas futuras são:

1. Cárie dentária:

- 1.1 Avaliar o efeito do verniz de TiF_4 , associado ou não ao dentifrício fluoretado e remoção total ou parcial do biofilme dentário, na remineralização de lesões artificiais do esmalte.
- 1.2 Avaliar eventual efeito anti-microbiano ou anti-placa do TiF_4 .
- 1.3 Avaliar a quantidade de fluoreto KOH-solúvel e fortemente aderido ao esmalte bovino e humano hígido e desmineralizado pelo tratamento com TiF_4 , tanto superficialmente como na subsuperfície do esmalte.
- 1.4 Avaliar a característica superficial do esmalte hígido e previamente desmineralizado tratado com TiF_4 por MEV-EDX.

- 1.5 Caso o efeito do TiF_4 seja superior ao NaF na remineralização *in situ*, planejar estudos clínicos longitudinais, aleatorizados e controlados para testar o efeito do produto na remineralização de manchas brancas em crianças de alto risco à cárie dentária.

2. Erosão dentária:

- 2.1 Avaliar o tipo de composto formado pela interação do TiF_4 e a apatita, utilizando hidroxiapatita sintética ou pó de esmalte e a técnica Infra-vermelho. Avaliar a característica superficial do esmalte e dentina hígidos e previamente erodidos tratados com TiF_4 por MEV-EDX.
- 2.2 Avaliar a quantidade de fluoreto KOH -solúvel e fortemente aderido ao esmalte e dentina bovinos e humanos hígidos e erodidos pelo tratamento com TiF_4 .
- 2.3 Avaliar o efeito do verniz de TiF_4 a longo prazo sobre a redução do desgaste dentário, em associação com dentifrícios fluoretados, aumentando os desafios erosivos e abrasivos *in situ*.
- 2.4 Avaliar o efeito do TiF_4 sobre amostras de esmalte previamente erodidas e sobre a dentina desmineralizada com e sem camada orgânica.
- 2.5 Potencializar o efeito do verniz de TiF_4 sobre a prevenção da erosão do esmalte e dentina, pela associação deste agente com laser de CO_2 e aplicações caseiras de fluoreto.
- 2.6 Formulação de dentifrícios e soluções de bochecho com a associação do TiF_4 e NaF , e pH entre 3,5 e 4,5.
- 2.7 Testar o potencial citotóxico do TiF_4 sobre fibroblastos.
- 2.8 Padronizar as técnicas para mensuração do perfil da superfície dentária, utilizando o Software Marh Surf XR, com adaptações que permitam sobrepor os perfis obtidos antes e após os desafios erosivos, possibilitando no futuro uma comparação mais direta entre os trabalhos, o que é especialmente crítico para a dentina.

Anexas

ANEXO A

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Processo nº 058/2009

Bauru, 16 de junho de 2009.

Senhora Professora,

O projeto de pesquisa encaminhado a este Comitê de Ética em Pesquisa em Seres Humanos, denominado **"Comparação do efeito de Tif4 e NaF, nas formas de verniz e solução, sobre a desmineralização e remineralização do esmalte dentário bovino in situ"**, de autoria de Livia Picchi Comar, que será desenvolvido sob sua orientação, foi enviado ao relator para avaliação.

Na reunião de 29 de abril de 2009 o parecer do relator, **aprovando o projeto**, foi aceito pelo Comitê, considerando que não existem infrações éticas pendentes.

Informamos que qualquer alteração efetuada no trabalho de pesquisa, o pesquisador/orientador deverá comunicar ao CEP-FOB/USP, bem como ao final do trabalho enviar um Relatório para novo parecer, o qual será utilizado para publicação científica.

Atenciosamente,

Profª Drª Maria Teresa Atta
Coordenadora

Profª Drª Ana Carolina Magalhães

Docente do Departamento de Ciências Biológicas

ANEXO B

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Processo nº 083/2008

Bauru, 28 de agosto de 2008.

Senhora Professora,

O projeto de pesquisa encaminhado a este Comitê de Ética em Pesquisa em Seres Humanos, denominado "**Avaliação do efeito de um verniz experimental de TiF4 comparado a vernizes e soluções de NaF e à solução de TiF4 sobre a erosão e abrasão do esmalte e dentina in vitro e in situ**", de autoria de Flávia Mauad Levy, que será desenvolvido sob sua orientação, foi enviado ao relator para avaliação.

Na reunião de 27 de agosto de 2008 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,

Profª Drª Maria Teresa Atta
Coordenadora

Profª Drª Marília Afonso Rabelo Buzalaf
Docente do Departamento de Ciências Biológicas