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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA
ÊNFASE EM ODONTOPEDIATRIA

EFICÁCIA DE DIFERENTES DENTIFRÍCIOS NA PREVENÇÃO DO DESGASTE DENTÁRIO EROSIVO EM DENTES PERMANENTES E DECÍDUOS

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Porto Alegre
2016

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Linha de Pesquisa

Biomateriais e Técnicas Terapêuticas em Odontologia

Tese apresentada ao Programa de Pós-Graduação em Odontologia como parte dos requisitos obrigatórios para a obtenção do título de Doutor em Odontologia, Área de concentração Clínica Odontológica/ Odontopediatria.

Orientador: Prof. Dr. Jonas de Almeida Rodrigues

Porto Alegre
2016

CIP - Catalogação na Publicação

Assunção, Cristiane Meira
Eficácia de diferentes dentifrícios na prevenção do desgaste dentário erosivo em dentes permanentes e decíduos / Cristiane Meira Assunção. -- 2016.
81 f.

Orientador: Jonas de Almeida Rodrigues.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Programa de Pós-Graduação em Odontologia, Porto Alegre, BR-RS, 2016.

1. Desgaste dentário erosivo. 2. Dentes permanentes. 3. Dentes decíduos. 4. Dentifrícios. I. Rodrigues, Jonas de Almeida, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

“Isso de querer ser exatamente aquilo que a gente é

ainda vai nos levar além.”

Paulo Leminski

DEDICATÓRIA

Dedico este trabalho à minha mãe Rose Mari (*in memoriam),*

a minha primeira orientadora, maior incentivadora e exemplo,

o meu amor maior e a minha saudade mais profunda,

AGRADECIMENTOS

“A felicidade só é real quando compartilhada”

Christopher McCandless

Ninguém conquista nada sozinho ou sem Fé. Por isso agradeço imensamente a Deus por ter colocado cada uma das pessoas as quais agradeço nesse texto em minha vida. Por ter guiado meus passos por caminhos que nunca sonhei em trilhar, por me dar forças pra continuar e a certeza que as suas bênçãos e a sua luz me acompanharão nos próximos passos.

Ao meu *dear advisor* Prof. Dr. Jonas, por sem me conhecer ter me escolhido na entrevista do Doutorado, mesmo sendo a nossa primeira conversa. Por não acreditar na primeira resposta que dei quando me falou sobre Prof Lussi e Bern. Por acreditar que eu daria conta dos 12 papers (*sorry,* ainda faltam alguns); por estar sempre *on line*; pelas sessões de terapia, até via Skype. Por ter me apresentado o ORCA e ter alargado imensamente meus horizontes. Espero que essa parceria entre um orientador ligado no 220v e uma aluna cheia de ‘TOCs’ renda muitos frutos e não se encerre com a defesa da tese.

“Stay hungry. Stay foolish.”

Steve Jobs

Agradeço aos membros da banca Profª. Drª. Marília, Prof. Dr. Maximiliano e Prof. Dr. Rodrigo, por disporem de seu tempo para participarem deste momento. Com certeza o trabalho ficará melhor após suas considerações.

Aos professores da Odontopediatria - UFRGS. Ao Prof. Dr. Fernando, por divulgar o trabalho incrível desta equipe, por me acolher tão bem, por confiar que eu seria capaz de alcançar o padrão UFRGS de qualidade. Ao Prof. Dr. Luciano por seu exemplo de comprometimento, pontualidade, por todos os ensinamentos sobre Odontologia e cultura gaúcha. A Profª. Drª. Adriela, Profª. Drª. Márcia, Prof. Jorge pela acolhida e convivência agradável em todo esse tempo na Clínica Infanto Juvenil.

À equipe do projeto plano B, que se tornou plano A’ e me preparou para a realização do projeto em Bern. Profª. Drª. Maria Carolina obrigada por toda a ajuda nessa incursão por estudos laboratoriais; Marcelo, Tatti e Nica muito obrigada por continuarem o projeto na minha ausência.

Às minhas amadas da Odontopediatria, as que ingressaram comigo e dividiram tantos momentos alegres: Ste, Fabi, Liza e a Joanna que me recebeu tão bem. As que fazem parte do time Rodrigues *et al*: Carol, Cacau, Nica, Dai. A todas as gurias (e guris) que fazem ou fizeram parte desse time incrível. Mais do que projetos, metodologias, experimentos e artigos quallis A1 o que importa e o que permanecerá será a nossa amizade, parceria, a lembrança dos momentos que vivemos juntas e os planos de sobreviver ao Doutorado e dominar o mundo.

Agradeço a toda equipe do LABIM, especialmente a Profª. Drª. Juliana que fez parte da minha prova de qualificação e a querida Luisa por toda ajuda nos inúmeros experimentos. Obrigada por me acolherem sempre tão bem!

A equipe do LAMAD que permitiu o uso dos equipamentos, especialmente a técnica Letícia pela força (literal) com as amostras.

A todas as crianças que aceitaram o pedido das ‘fadas do dente’ e doaram, ‘*tri faceiras*’, seus preciosos dentes decíduos para a realização de nossas pesquisas. É o sorriso de vocês que faz todo esse esforço valer a pena.

Às minhas *rommies* (Amanda, Daiane e Daiana), mais do que um teto em terras gaúchas vocês me deram a força e apoio pra continuar nessa saga do Doutorado.

Agradeço demais aos meus amigos *Legendaries* (Bru, Dai B, Dai C, Deisi, Renan), por todas as risadas, as festas, almoços, programas cult-hipsters, pela parceria nas viagens. Por todas as mensagens que chegavam de madrugada em Bern e me faziam rir e lembrar que tinha amigos em algum lugar distante. Vocês fazem meus dias mais alegres e leves! Que a gente nunca perca esse espírito livre e feliz que nos uniu.

Agradeço as minhas *cherries* (Imara, Lou, Fer, Wane, Vera, Rafa, Amanda), por me entenderem tão bem e compartilharem todas as minhas dúvidas existenciais, por torcerem sempre por mim, por serem a minha risada mais gostosa, o meu ombro amigo, por estarem presentes nas melhores e nas mais difíceis situações. Afinal o que o Mestrado e a Bioestatística uniram tem importância estatisticamente significativa para o resto de nossas vidas.

Aos meus mestres da Odontopediatria da minha Curitiba, Prof. Dr. Fabian, Prof. Dr. José Vitor, Prof. Dr. Renato, Profª. Drª. Estela, Profª. Drª. Fernanda. Muito obrigada por todos os ensinamentos durante a graduação, especialização e mestrado. A base sólida e o exemplo de vocês me permitiu dar passos maiores e mais seguros.

To all my friends in Bern – Switzerland - *Mercy vielmal!!*

To Prof Adrian Lussi, thank you so much for allowed me to be really part of your team, for all the support, all knowledge shared. You will be always the best example of an advisor, researcher and boss. I hope one day I became a 'boss' like you!

Mercy vielemal is not enough to say all my gratitude to Thiago who helped me with everything, literally! You helped me with my 35kg luggage from the Bern-Bahnhof to Bümpliz Nord; gave me informations, dishes and cups, all extremely necessary to start my life in Bern; shared so many knowledge, had so many patience with my anxiety.

To the most lovely and friendly lab-technician ever: Brigitte, Barbara Isabel and Samuel. If my samples were perfect, my deciduous teeth pictures and SEM images could be cover of a scientific magazine, if I didn't fall asleep during the 21.120 microhardness measurements it was because of all your help and support. All the brigadeiros and passion fruit mousses were not enough to say how happy and thankful I am for had worked with you.

To all my colleagues from the C and D floor, who kindly speak in English with me, translated the menu helping me to not eat Pferdefleisch or Kaninchen, who made all the lab reports and coffe breaks moments to share scientific knowledge, funny stories and laughs.

To Tamara and Tamara, my swiss and Spanish friends, so many good moments we shared: coffees, ice creams, city tours, hickings, laughs, plans for academic career and trips. I miss you so much, and I am waiting you here in Brazil!

To my friends from Studentenlogierhaus Fellergut, for had shared the (messy) kitchen, had tasted typical food from each homeland, had learned from different cultures. I am sure I am a better person after knowing all of you.

Aos criadores do Skype, Whatsapp, Facebook, Messenger, ao 4G suíço e a todas as tecnologias que permitiram que eu sobrevivesse à saudade imensa de todos meus amigos e familiares e também por fazê-los viajarem comigo.

Ao Programa de Pós-graduação em Odontologia da UFRGS, na pessoa de seu coordenador Prof. Dr. Cassiano, por fazer deste curso uma referência, pelas disciplinas que permitiram a formação de pensamento crítico e o desenvolvimento de habilidades essenciais a quem pretende obter o título de Doutor.

A Capes pela concessão das bolsas de Doutorado e Doutorado Sanduiche.

Por último, mas sempre em primeiro lugar, agradeço a minha família, meu pai Aldo, minha avó Annália e minhas tias Roseli e Roseni. Em Berna conheci a casa de Albert Einstein, e pude entender melhor que o tempo realmente é relativo. Esses quase quatro anos de Doutorado podem ter passado rápido pra mim, envolvida em tantas novidades (nova casa, colegas, trabalho, culturas, idioma), mas sei que para vocês o tempo se arrastou lentamente. Hoje concluo uma etapa muito desejada, e espero que a felicidade e o orgulho sentidos amenizem a falta que fiz em momentos tão importantes (felizes ou não). Mas como sempre repetia para a mãe, não há distância que separe aqueles que moram do lado de dentro da gente, no coração. Amo muito vocês!!

SUMÁRIO

RESUMO

Os dentifrícios são veículos fundamentais para a aplicação dos fluoretos. Estudos em dentes humanos e bovinos têm mostrado que os fluoretos podem apresentar efeitos diversos na prevenção e na progressão do desgaste dentário erosivo (DDE). No entanto, pouco se sabe sobre seu efeito em dentes decíduos. *Objetivo:* 1) Avaliar o efeito preventivo de dentifrícios fluoretados por meio da intensidade de reflexão da superfície especular (IRS), da microdureza de superfície (MDS) e da perda da superfície calculada (PSC) utilizando um modelo inicial de erosão/abrasão; 2) avaliar por meio de perfilometria a perda de superfície (PS) em um modelo de erosão/abrasão avançado e 3) comparar esse efeito preventivo entre os dentes permanentes (DP) e dentes decíduos (dd). *Material e Métodos:* Amostras de esmalte de dentes permanentes (n = 100) e decíduos (n = 100) foram aleatoriamente divididos em cinco grupos (n = 20) de acordo com os dentifrícios testados: G1 – dentifrício placebo (0ppm), G2 – dentifrício com NaF (controle positivo, 1500ppm, Crest®, P & G), G3 - dentifrício anti-erosão com AmF-NaF-SnCl (1400ppm, elmex Erosion Protection®, GABA - Colgate), G4 - dentifrício com SnF (1100ppm, Sensodyne Repair&Protect®, GSK), G5 - dentifrício anti-erosão com NaF para crianças (1450ppm, Sensodyne ProNamel Junior®, GSK). Metade de cada superfície de esmalte foi coberta com resina à base de metacrilato para criar uma área hígida de referência. As amostras foram então submetidas a ciclos de erosão/abrasão, cinco no protocolo inicial e mais 25 para o protocolo avançado, totalizando 30 ciclos para este último protocolo. Em cada ciclo, as amostras foram incubadas em saliva artificial por 1h hora, submetidas ao desafio erosivo (3min; ácido cítrico 1%; pH 3.6; a 25 ° C) e à abrasão (2min de imersão no *slurry*; 50 movimentos de escovação; 200 g). Os efeitos das duas co-variáveis "dente" e "dentifrício" foram analisados através do teste ANOVA e as comparações entre os dentifrícios foram realizadas por meio do teste de Kruskal-Wallis e entre os tipos de dente (DP e dd) utilizando o teste de Wilcoxon, com nível de significância de 5%. *Resultados:* No protocolo inicial, considerando os resultados de MDS, as amostras do grupo do dentifrício placebo apresentaram valores significativamente menores para os ‘DP’ do que os outros grupos ($p<0.05$), no entanto sem diferença entre os dentifrícios fluoretados. Para os ‘dd’, o dentifrício placebo (G1) e o dentifrício com SnF (G4) também mostraram valores significativamente menores do que os outros grupos ($p<0.05$). Nos resultados de IRS, o dentifrício com SnF (G4) apresentou valores menores em ambos os tipos de dente. Além disso, os dentes decíduos apresentaram significativamente maiores valores de IRS que os dentes permanentes, exceto no grupo do dentifrício anti-erosão com AmF-NaF-SnCl (G3). Os dentes decíduos apresentaram maior PSC do que dentes permanentes, exceto no G3. No protocolo avançado de erosão/abrasão os dentes decíduos (dd) mostraram PS significativamente maior do que os dentes permanentes (DP) em todos os grupos ($p < 0.001$). Os valores médios de PS de cada grupo foram: G1 DP 18.18µm (± 3.98), dd 25.65µm (± 9.21); G2 DP 14.76µm (± 2.82), dd 18.11µm (± 3.92); G3 DP 12.62µm (± 5.29), dd 15.61µm (± 6.70); G4 DP 17.12µm (± 2.24), dd 23.41µm (± 7.9); G5 DP 13.24µm (± 1.29), dd 18.28µm (± 8.96). *Conclusões:* No protocolo de erosão/abrasão inicial dentes decíduos apresentaram valores mais baixos MDS, valores de IRS mais elevados e maior PSC do que os dentes permanentes durante o experimento. O dentifrício anti-erosão com NaF para crianças apresentou os menores valores de PSC em ambos os dentes permanentes e decíduos, com um melhor efeito preventivo. No protocolo avançado o dentifrício anti-erosão com AmF-NaF-SnCl apresentou o melhor efeito preventivo contra desgaste erosivo nos dentes permanentes. Em dentes decíduos os dentifrícios com NaF (G2), anti-erosão com AmF-NaF-SnCl (G3) e anti-erosão com NaF para crianças (G5) mostraram efeito semelhante.

Palavras-chave: Desgaste Erosivo, Dentes Permanentes, Dentes Decíduos, Dentifrício.

ABSTRACT

Toothpastes are key vehicles for fluorides application. Studies have shown that various fluorides have different preventive effect on erosive tooth wear (ETW) progression. Little is known about their effect on deciduous teeth. *Aim:* 1) To evaluate the preventive effect of the toothpastes through surface specular reflection intensity (SRI), surface microhardness (SMH) and calculated surface loss (CSL) in an initial erosion/abrasion model; 2) to evaluate through profilometry the surface loss (SL) in a severe erosion/abrasion model and 3) to compare this preventive effect between permanent teeth (PT) and deciduous teeth (dt). *Material and Methods:* Enamel samples of permanent (n=100) and deciduous teeth (n=100) were randomly divided into five groups according to toothpastes tested (n=20). G1 – placebo toothpaste (0ppm), G2 – NaF toothpaste (positive control, 1500ppm, Crest®, P&G), G3 – AmF-NaF-SnCl antierosion toothpaste (1400ppm, elmex Erosion Protection®, GABA – Colgate), G4 – SnF toothpaste (1100ppm, Sensodyne Repair®, GSK), G5 – NaF antierosion toothpaste for children (1450ppm, Sensodyne ProNamel Junior®, GSK). Half of enamel sample surfaces were covered with methacrylate-based resin to create a sound reference area. The samples were submitted to erosion-abrasion cycles, 5 in initial protocol and more 25 in severe protocol, totalizing 30 cycles at the end. In each cycle samples were incubated in artificial saliva (1h), submitted to erosive challenge (3min; 1% citric acid; pH3.6; at 25°C) and to toothbrush abrasion (2min immersion in slurry; 50 strokes; 200g). The effects of the two covariables "tooth" and "toothpaste" were analyzed by ANOVA Comparisons among toothpastes were evaluated using Kruskal-Wallis-tests and between PT and dt using Wilcoxon's rank sum test. *Results:* In initial protocol, considering the SMH results, placebo toothpaste showed significantly lower SMH values in PT than the other toothpastes ($p<0.05$), with no differences between the toothpastes. In dt, placebo and G4 also showed significantly different values than the other groups ($p<0.05$). In SRI results, SnF toothpaste (G4) showed lower *erf* values in both PT and dt. Deciduous teeth presented significantly higher SRI than permanent ($p<0.05$), except on AmF-NaF-SnCl antierosion group (G3). Deciduous teeth presented generally higher CSL than PT, except for G3. In the severe protocol deciduous teeth (dt) showed significant higher SL than permanent teeth (PT) in all groups ($p<0.001$). The mean values of SL of each group were: G1 PT 18.18(±3.98), dt 25.65(±9.21); G2 PT 14.76(±2.82), dt 18.11(±3.92); G3 PT 12.62(±5.29), dt 15.61(±6.70); G4 PT 17.12(±2.24), dt 23.41(±7.9); G5 PT 13.24(±1.29), dt 18.28(±8.96). Conclusions: In initial protocol deciduous teeth presented lower SMH values, higher SRI values and higher surface loss than permanent teeth during the experiment. The NaF antierosion toothpaste for children presented the lowest values of SL in both permanent and deciduous teeth, with a better preventive effect. In severe protocol, AmF-NaF-SnCl antierosion toothpaste showed the best preventive effect against erosion-abrasion cycles in permanent teeth. In deciduous teeth NaF toothpaste, AmF-NaF-SnCl antierosion toothpaste and NaF antierosion toothpaste for children showed similar effect.

Key-words: Erosive Tooth Wear, Permanent Teeth, Deciduous Teeth, Toothpaste.

ANTECEDENTES E JUSTIFICATIVAS

DEFINIÇÃO

Desgaste dentário erosivo é definido como um processo químico mecânico que resulta em uma perda cumulativa de tecido dentário duro, o qual não é causado por bactérias (Carvalho *et al,* 2015). Este termo foi definido pela Federação Europeia de Odontologia Conservadora (European Federation of Conservative Dentistry - EFCD), e teve como objetivo orientar os cirurgiões-dentistas a realizar um diagnóstico preciso e tomar as decisões de tratamento frente a esta condição, cuja prevalência tem aumentado nos últimos anos.

Os termos “erosão”, “erosão dentária” e “desgaste dentário erosivo” têm sido usados indistintamente quando se referem aos efeitos de ácidos, não provenientes do metabolismo bacteriano, na superfície dentária. Quando a superfície dentária é primeiramente atingida por ácidos, estes causam uma perda da integridade estrutural que deixa uma camada mais amolecida na superfície, tornando-a mais suscetível às forças abrasivas. Estas forças abrasivas removem esta camada mais amolecida do esmalte, causando perda substancial de tecido duro. A perda de tecido dentário duro teoricamente também pode ocorrer sem a ação significativa de abrasão, e alguns casos de desafio erosivo prolongado e repetitivo (ex. vômitos) podem resultar em erosão dentária. No entanto, as evidências científicas disponíveis sugerem que os desafios erosivos *in vivo* são muito breves, consequentemente erosão por si só talvez não seja a causa mais direta de perda de superfície dental (Lussi e Carvalho, 2014). Por essa razão Shellis *et al* (2011) sugerem que o termo “erosão dentária” inclua apenas os casos de desmineralização parcial e inicial (amolecimento) e qualquer perda de superfície causada somente pela exposição excessiva a ácidos, enquanto que o termo “desgaste dentário erosivo” deve incluir os efeitos de qualquer força abrasiva mecânica após o amolecimento do tecido dentário (esmalte ou dentina). Portanto, os termos “erosão dentária” e “desgaste dentário erosivo” devem ser usados para se referir aos processos químico e químico-mecânico respectivamente (Lussi e Carvalho, 2014).

PREVALÊNCIA

Estudos epidemiológicos sugerem que ou a prevalência do desgaste dentário erosivo está aumentando ou há um aumento do diagnóstico inicial desta condição, principalmente, em adultos jovens e adolescentes (Holbrook *et al*, 2009). Atualmente, o desgaste dentário erosivo tem sido considerado um tópico de crescente interesse e preocupação na prática clínica diária, e a sua prevalência tem variado de 4 a 82% em adultos e de 10 a 80% em crianças (Jaeggi e Lussi, 2006; Taji e Seow, 2010).

A grande variação nos resultados dos estudos em diferentes países parece dever-se, principalmente, a diferenças nas populações estudadas, assim como a variações nos níveis de consumo de bebidas ácidas e da diversidade de índices utilizados para o diagnóstico (Taji e Seow, 2010). A idade também parece ser um fator importante, uma vez que, quanto mais tempo o dente estiver exposto ao meio bucal, maiores os níveis de desgaste erosivo e mais facilmente esta alteração pode ser detectada (Taji e Seow, 2010). Além disso, a maioria dos estudos analisa o desgaste erosivo em dentes específicos e não fornecem informações sobre a distribuição e severidade de lesões erosivas na dentição como um todo (Wienegan *et al*, 2006).

Estudos epidemiológicos que investigaram o desgaste erosivo na dentição decídua são raros. Uma prevalência de 0,6% em crianças de até seis anos foi encontrada por Moimaz *et al* (2013) e de 13% em crianças entre 2 a 4 anos por Habib *et al* (2013). No Brasil alguns estudos da base populacional foram realizados, especialmente em adolescentes. Na tabela 1 estão listados os estudos brasileiros publicados, índices utilizados e prevalência encontrada em adolescentes. No entanto dados sobre a prevalência em dentes decíduos ou em adultos ainda é escassa. A avaliação de desgaste dentário erosivo não fez parte dos últimos levantamentos de saúde bucal realizados pelo Sistema Único de Saúde (SB-Brasil – SUS).

Autores	**Local**	**N**	**Idade (anos)**	**Prevalência (%)**	**Índice**
Alves *et al,* 2015	RS	1.528	12	15%	BEWE
Aguiar *et al*, 2014	PB	675	15-19	21%	O’Sullivan
Vargas-Ferreira *et al*, 2011	RS	944	11-14	7,2%	O’Sullivan
Gurgel *et al*, 2011	SP	414	12 e 16	20%	TWI modificado
Mangueira *et al*, 2009	PB	983	12	38,2%	O’Sullivan
Correr *et al*, 2009	SP	389	12	26%	O’Sullivan
Auad *et al*, 2007	MG	458	13-14	34%	O’Brien
Peres *et al*, 2005	SC	391	12	13%	O’Sullivan

Tabela 1. Prevalência de desgaste dentário erosivo em adolescentes em estudos realizados no Brasil.

DIAGNÓSTICO CLÍNICO E CLASSIFICAÇÃO

Com a diminuição da prevalência da doença cárie, e consequentemente a diminuição de perdas dentárias, um maior número de pessoas tem conservado seus dentes íntegros por um período maior. Estas superfícies dentárias acabam sendo expostas a diferentes agentes agressores, químicos e físicos, que podem contribuem para o desgaste dentário. Devido à característica multifatorial do desgaste dentário erosivo, o seu diagnóstico diferencial pressupõe que além do exame clínico, a anamnese e os hábitos dietéticos também sejam levados em conta (Carvalho *et al,* 2015).

Em geral, devido ao desgaste erosivo nos estágios iniciais, uma alteração pode ser observada nas propriedades ópticas do esmalte, com alteração no brilho. O esmalte se apresenta liso, polido, com aumento da translucidez incisal. Os sinais típicos em superfícies oclusais são escavação das cúspides, com aspecto de vulcão e achatamento. Num estágio avançado, toda a anatomia da superfície oclusal, seus sulcos, fissuras e fóssulas, podem ser perdidas, dando lugar a uma superfície escavada, lisa e brilhante. Nas superfícies lisas, ocorre a perda das periquimáceas e da rugosidade natural dos dentes anteriores principalmente, tornando a superfície lisa e brilhante. Um sinal característico é o contorno da margem gengival intacto, devido à proteção do fluido crevicular gengival contra a ação dos ácidos (Lussi, Jaeggi, 2008; Carvalho *et al,* 2015). Essas lesões podem ocorrer em dentes permanentes e decíduos, podendo também atingir o tecido dentinário. As lesões podem ser localizadas (em um único grupo de dentes, na superfície lingual ou vestibular), generalizadas ou assimétricas, dependendo da origem do ácido (extrínseco ou intrínseco).

Com o objetivo de oferecer um sistema de escores simples, que pudesse compilar os critérios de diagnósticos já existentes, foi elaborado o BEWE (Basic Erosive Wear Examination, Barlett *et al*, 2008). O BEWE é um sistema de escores simples, reprodutível e transferível, utilizado para registrar exames clínicos de rotina bem como auxiliar na decisão de tratamento e acompanhamento das lesões de desgaste dental erosivo. A superfície mais afetada de cada um dos sextantes é registrada através de um escore com quatro níveis e a soma dos escores classifica e indica o grau de severidade, guiando assim o tratamento e acompanhamento desta condição.

Escore	**Descrição**
0	Superfície hígida, sem desgaste dentário.
1	Perda inicial da textura de superfície.
2*	Defeito distinto, perda de estrutura dentária <50% da área da superfície.
3*	Perda de tecido mineralizado em mais de 50% da área da superfície.

* Nos escores 2 e 3 a dentina está frequentemente envolvida.

Tabela 2. Descrição dos escores do BEWE.

ETIOLOGIA

O primeiro passo para o desenvolvimento do desgaste dentário erosivo é a desmineralização ou amolecimento da superfície dentária pela ação de substancias erosivas, ácidos de origem intrínseca ou extrínseca. Dentre os agentes extrínsecos podem-se citar alguns componentes dietéticos como bebidas industrializadas (refrigerantes, sucos, isotônicos e energéticos) e medicamentos com baixo pH (tabletes de vitamina C, formulações contendo ácido acetilsalicílico). O agente erosivo intrínseco principal é o suco gástrico, que pode atingir a cavidade oral devido à doença do refluxo gastroesofágico ou através de vômitos frequentes relacionados a problemas neurológicos ou distúrbios alimentares como a bulimia (Carvalho *et al,* 2015, Lussi e Carvalho, 2014).

Deve-se ressaltar que, ao contrário da doença cárie, não há um pH específico para o início do processo de erosão dentária ou desgaste dentário erosivo. Para determinar o potencial erosivo de uma bebida deve-se levar em conta a sua capacidade de dissolução, adesividade e conteúdo de Cálcio e Fosfato além do modo, frequência e momento de ingestão (Taji e Seow, 2010; Wang e Lussi, 2010).

A saliva também tem um papel protetor crucial no desenvolvimento do desgaste dentário erosivo. Além de diluir e remover as substâncias ácidas da cavidade oral, através da capacidade tampão neutraliza os ácidos, diminuindo a intensidade e duração do desafio erosivo *in vivo*. As proteínas salivares também são parte essencial na formação da película adquirida, que forma uma barreira semipermeável protetora para os ataques ácidos à superfície dentária. Qualquer alteração no fluxo salivar ou capacidade tampão, devido a doenças como Síndrome de Sjögren ou uso contínuo de medicamentos (antidepressivos, anti-hipertensivos) pode suprimir essa capacidade protetora da saliva e aumentar o risco do paciente apresentar lesões de desgaste erosivo (Carvalho *et al,* 2015, Lussi e Carvalho, 2014).

No diagrama abaixo estão representados os fatores etiológicos e associados ao desgaste dentário erosivo, ilustrando o aspecto multifatorial desta condição.

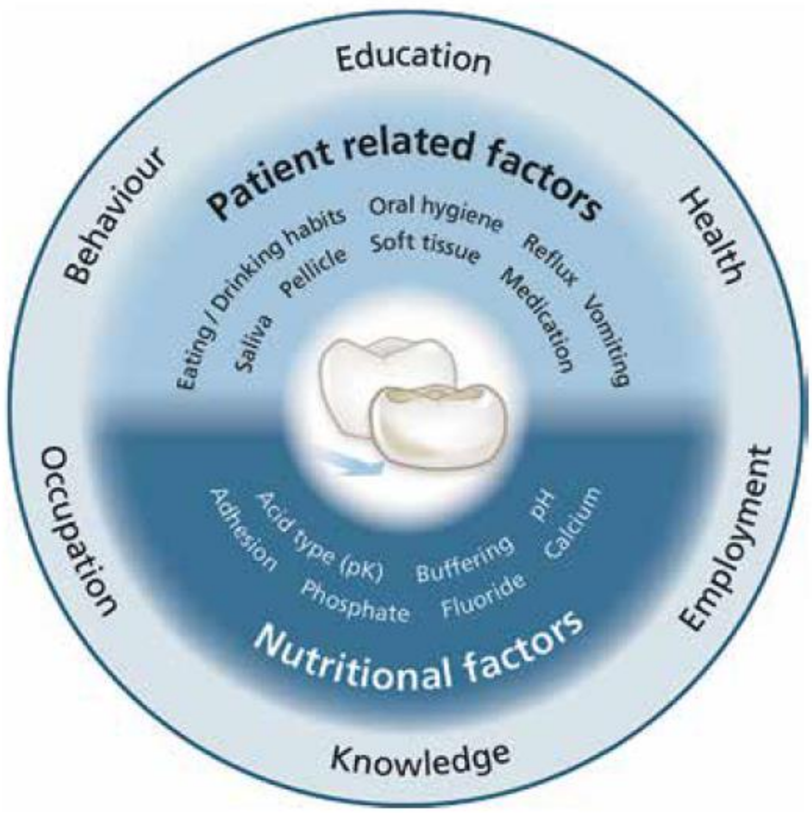

Figura 1. Diagrama representativo dos fatores etiológicos e associados ao desenvolvimento de desgaste dentário erosivo. (Lussi e Carvalho, 2014).

PREVENÇÃO

Uma vez avaliado o risco do paciente e diagnosticada a perda inicial de tecido dentário duro, medidas de prevenção da continuidade do processo devem ser estabelecidas. Métodos convencionais de prevenção incluem avaliação da dieta e restrição do contato com comidas e bebidas ácidas, educação das crianças e dos pais, e otimização do mecanismo protetor da saliva. Entretanto, o sucesso desses métodos depende da aceitabilidade e do comprometimento do paciente e dos seus responsáveis. Assim, métodos de prevenção que atuem através de produtos aplicados diretamente à estrutura dentária estão sendo estudados (Taji e Seow, 2010). Diferentes formulações de fluoretos (mono ou polivalentes), e diferentes veículos como dentifrícios, colutórios para uso diário pelo paciente; soluções, vernizes e géis para aplicação profissional têm sido estudados em busca de uma melhor e mais efetiva ação preventiva contra a erosão e o desgaste dentário erosivo.

A escovação dentária adequada não é prejudicial à superfície dentária sadia, porém após exposição a ácidos, a superfície dentária amolecida fica mais vulnerável as forças abrasivas da escovação. Porém, uma vez que o esmalte não é remineralizado pela saliva num curto período de tempo, ele será desgastado mesmo na ausência de escovação, pela ação dos tecidos moles (língua, bochecha). Deste modo, adiar a escovação após ingerir produtos ácidos não é uma medida preventiva efetiva, especialmente porque a escovação serve como veículo para os fluoretos agirem na prevenção tanto da cárie quanto no desgaste erosivo (Lussi e Carvalho, 2014; Carvalho *et al*, 2015).

DENTIFRÍCIOS E DESGASTE DENTÁRIO EROSIVO

O principal objetivo dos ingredientes ativos dos dentifrícios é modificar positivamente a película adquirida e aumentar a resistência da estrutura dentária frente ao desafio erosivo. O principal problema no uso dos dentifrícios está na sua ação abrasiva, desejável para adequada remoção de biofilme e limpeza da superfície dentária, mas que pode se sobrepor à ação preventiva dos ingredientes ativos. Os efeitos preventivos idealmente devem ocorrer sem nenhum efeito colateral, e o dentifrício deve ser de uso fácil, sendo um requisito fundamental para a adesão ao tratamento preventivo diário (Huysmans *et al,* 2014). Dentifrícios fluoretados têm demonstrado certa ação preventiva, mas buscando uma ação mais efetiva, outros ingredientes ativos têm sido estudados. Cátions de metais polivalentes, sais de Ca/P em nanopartículas, fosfatos, proteínas e vários biopolímeros como quitosana são substâncias que vem sido testadas (Ganss *et al,* 2014).

Os dentifrícios convencionais a base de fluoreto de sódio apresentaram desde nenhum efeito preventivo até 37% mais proteção em esmalte e de nenhum a 32% mais proteção em dentina, quando comparado ao dentifrício placebo sem adição de fluoretos (Carvalho *et al*, 2014). Resultados controversos foram encontrados quando formulações com alta concentração de flúor foram testadas. Um estudo *in vitro* comparou dentifrícios com 1.100ppm e 5.000ppm de NaF e demonstrou ação preventiva de 26% e 53% respectivamente quando comparados ao dentifrício placebo, utilizados durante a escovação dentária. Quando aplicados na forma de *slurry* (dentifrício diluído em água destilada ou saliva artificial) a ação preventiva foi semelhante: 27% e 57% (Moretto *et al,* 2010). Num estudo *in situ* o dentifrício de 5.000ppm NaF apresentou 55% mais efeito protetor que um com 1.450ppm, ao ser aplicado na forma de *slurry,* sem desafio abrasivo associado (Ren *et al,* 2011). Em outros estudos *in situ*, nenhum benefício significativo foi encontrado com a aplicação de dentifrício de alta concentração (5.000ppm) em esmalte ou dentina, submetidos a desafio erosivo e/ou abrasivo (Rios *et al,* 2008; Magalhães *et al,* 2008).

Alguns íons metálicos polivalentes como estanho e fluoreto de amina foram capazes de produzir precipitados mais resistentes aos desafios ácidos na superfície dentária. Dentifrícios contendo fluoreto de estanho, quando aplicados em esmalte na forma de *slurry*, mostraram uma redução na perda de superfície variando de 55% a 95% quando comparados a dentifrícios não fluoretados, embora este efeito diminua quando desafios abrasivos através da escovação foram associados. Esta redução no efeito protetor pode ser explicada pela a ação física das partículas abrasivas dos dentifrícios, que interferem com a precipitação dos sais de estanho (Ganss *et al,* 2014; Carvalho *et al*, 2014). Alguns pacientes se queixam do gosto metálico de dentifrícios com estanho, sendo um fator que limita o uso contínuo, principalmente em crianças e adolescentes, mais acostumados com dentifrícios de sabores agradáveis que estimulam o seu uso.

O efeito da abrasividade dos dentifrícios também tem sido bastante investigado. Em substrato hígido, a abrasão causada pelos dentifrícios é geralmente bem baixa, já em superfícies submetidas a desafios erosivos, a escovação pode aumentar a perda de superfície significativamente. Histologicamente, sabe-se que o esmalte submetido a desafio erosivo tem os cristais da superfície mais facilmente removidos até mesmo pela fricção dos tecidos moles subjacentes, e ainda mais pela ação da escovação dentária e dos abrasivos contidos nos dentifrícios. Porém estudos sobre a abrasividade em esmalte e/ou dentina submetidos a desafios erosivos e abrasivos ainda são bastante escassos. A interação entre fluoretos e abrasivos também tem sido pouco investigada (Ganss *et al,* 2014). Os dados fornecidos pelos fabricantes dos dentifrícios geralmente se referem ao valor de abrasividade relativa em dentina (RDA), porém alguns estudos demonstraram que esse valor não pode ser transposto para o esmalte (Ganss *et al*, 2013; Philpotts et al; 2005; Pickles *et al,* 2005). Outras propriedades como viscosidade e molhabilidade e outros ingredientes além dos fluoretos, também podem interferir na abrasividade dos dentifrícios e necessitam ser mais profundamente investigados (Ganss *et al,* 2014).

A complexa interação entre os ingredientes ativos e abrasivos na superfície dentária, e a ação de todos os excipientes dos dentifrícios ainda não foram totalmente elucidados. A evidência científica disponível até o momento é proveniente de estudos laboratoriais e pouca evidência de estudos clínicos com pacientes foi produzida. No entanto, os resultados desses estudos *in vitro e in situ* apontam que há campo para desenvolvimento de dentifrícios mais efetivos na prevenção do desgaste dentário erosivo (Ganss *et al,* 2014).

DIFERENÇAS ENTRE DENTES PERMANENTES E DECÍDUOS

Além das diferenças anatômicas entre dentes permanentes e decíduos, os quais são menores e com uma camada significativamente mais fina de esmalte, diferenças histológicas podem influenciar a susceptibilidade a dissolução por ácidos e consequentemente levar a diferentes padrões de desgaste dentário erosivo (Carvalho *et al,* 2014).

O esmalte dos dentes decíduos apresenta prismas menores, com limites mais completos e espalhados quando comparados aos prismas de dentes permanentes. Esta característica sugere que o esmalte decíduo seja mais poroso que o permanente. O conteúdo orgânico também e diferente entre os dois tipos de dente, podendo variar de 0.7 a

12% em dentes decíduos e de 0.4 a 0,8% nos permanentes. Quanto ao conteúdo mineral, o esmalte decíduo é significativamente menos mineralizado que o permanente, sendo também mais solúvel devido ao tipo de carbonato predominante em sua composição. Essas diferenças histológicas podem ser responsáveis pelo fato de que o esmalte decíduo apresenta menores valores de microdureza superficial e elasticidade que os dentes permanentes, sendo desse modo mais suscetível a dissolução, abrasão e atrição (Carvalho *et al,* 2014).

No entanto, resultados de diversos estudos laboratoriais não conseguiram confirmar categoricamente essa diferença. Amaechi *et al* (1999) avaliou a perda de superfície dentária nos dois tipos de dente após imersão em suco de laranja num período de tempo que variou de 6 a 12 horas. Neste estudo, os dentes decíduos apresentaram maior perda mineral e uma progressão mais rápida de lesões erosivas que os dentes permanentes. Resultados parecidos foram encontrados em um estudo *in situ*, onde dentes decíduos apresentaram maior perda de tecido dentário duro que os permanentes, sendo essa perda progressiva ao longo dos ciclos (Hunter *et al*, 2000a). Porém o mesmo protocolo de desafio erosivo, quando reproduzido *in vitro* pelo mesmo grupo não encontrou diferenças estatisticamente significativas entre os dois tipos de dente (Hunter *et al*, 2000b). Essa diferença foi atribuída pelos autores à ação protetora da película adquirida, embora outros estudos não tenham conseguido confirmar esse efeito.

A composição da película adquirida varia conforme a idade, devido as variação na composição de proteínas e da saliva. Em um estudo *in vitro*, amostras de saliva de adultos e crianças foram utilizadas para a formação de película adquirida em dentes decíduos e permanentes, previamente ao desafio erosivo. Os resultados de microdureza superficial demonstraram que película de adultos e crianças promoveram diferentes efeitos preventivos contra erosão dentária. A película adquirida formada por saliva de adultos proporcionou melhor proteção em dentes permanentes e da mesma forma a de crianças em dentes decíduos (Carvalho *et al*, 2016). Nenhum dos estudos mencionados acima incluiu desafios abrasivos na avaliação entre dentes permanentes e decíduos.

Estudos epidemiológicos, no entanto, demonstraram que os dentes decíduos possuem menor resistência ao desgaste que os permanentes (Kreulen *et al*, 2010). Esse fato sugere que diversos fatores contribuem para o desgaste erosivo *in vivo* e não foram completamente reproduzidos em estudos laboratoriais (Carvalho *et al,* 2014).

MÉTODOS DE AVALIAÇÃO EM LABORATÓRIO

Diversos métodos têm sido utilizados para avaliar a perda de tecido dentário e a camada amolecida em esmalte, formada pela ação de desafios erosivos. Em uma revisão de literatura, Schlueter *et al* (2011) avaliaram os métodos mais utilizados na análise de desgaste dentário erosivo. A perfilometria foi o método quantitativo mais comumente empregado para avaliar esmalte e/ou dentina em estudos *in vitro, in situ* e também em alguns estudos clínicos (usando modelos de gesso de arcadas de pacientes) seguido pela avaliação quantitativa de dureza superficial (esmalte) e microrradiografia (dentina). A microscopia eletrônica de varredura foi o método qualitativo mais utilizado para avaliação tanto do esmalte quanto da dentina. Outras técnicas também avaliaram o conteúdo mineral perdido nos desafios erosivos através de análises químicas (espectroscopia de energia dispersiva, análise de cálcio e flúor entre outros elementos). A caracterização da superfície submetida à erosão ou a desgaste dentário erosivo também pode ser feita através da rugosidade superficial, microscopia de força atômica, intensidade de reflexão da superfície especular, fluorescência quantitativa induzida por luz, dentre outros métodos (Attin e Wegehaupt, 2014). A seguir, apenas os métodos utilizados nos artigos subsequentes serão descritos em detalhe.

Microdureza superficial e perda de superfície calculada:

Esse é um método relativamente barato e eficaz para obter informações precisas sobre as mudanças na estrutura dental em desafios erosivos iniciais (Schlueter *et al*, 2011) Através da mensuração de microdureza superficial, estágios iniciais de dissolução do esmalte e dentina, os quais estão associados a um enfraquecimento da superfície, podem ser detectados. O método básico para essa mensuração consiste em uma endentação de uma ponta diamantada com dimensões geométricas conhecidas por um tempo e carga determinados. Para essa análise, na maioria das vezes são utilizadas pontas diamantadas de Vickers ou Knoop, em superfícies previamente polidas e paralelas, para obtenção de uma melhor precisão das mensurações. O comprimento das endentações é medido e os valores de dureza calculados através de fórmulas específicas. Em esmalte o comprimento das endentações não depende do tempo ou humidade, em dentina devido à elasticidade do conteúdo orgânico não é recomendável que as medições sejam feitas logo após a endentação (Attin e Wegehaupt, 2014).

Nas superfícies submetidas a desafios erosivos contorno das endentações nem sempre é bem demarcado, podendo dificultar a técnica. A área adjacente às endentações também é alterada, em até 10 vezes o comprimento da endentação. Para minimizar esse efeito, micro endentações para determinar o efeito erosivo nas superfícies podem ser realizadas com menos pressão, e uma carga de 50g apenas (Attin e Wegehaupt, 2014).

A preferência pelo método de Knoop deve-se ao fato que este determina melhor as alterações na camada mais superficial, com uma menor penetração e um maior comprimento de endentação, facilitando a mensuração. Com isso é possível diferenciar o potencial erosivo de diversas substâncias, mesmo com um curto período de exposição aos agentes ácidos (Attin e Wegehaupt, 2014).

A avaliação da abrasão nas superfícies de esmalte também pode ser realizada através da profundidade e comprimento das endentações. A diferença entre a profundidade antes e depois da abrasão fornece uma mensuração direta da quantidade de substância foi perdida pelas forças abrasivas neste local. O principio deste método esta no fato que a parte mais profunda da endentação permanece inalterada e não é totalmente removida com a abrasão. Apenas o tecido amolecido ao redor é removido, então o contorno piramidal da parte externa da endentação (e consequentemente seu comprimento) é reduzido. O comprimento da endentação é medido logo após o desafio erosivo e medido novamente após o desafio abrasivo, na mesma endentação. Utilizando o valor do comprimento da cada endentação (*cE*), o valor de profundidade de cada endentação (*pE*) é calculado através da seguinte fórmula:

$$pE= cE / 2.\tan \alpha$$

Nessa fórmula α = 3.75° é uma constante relacionada ao tamanho da ponta diamantada Knoop. A quantidade de superfície perdida através da abrasão é então calculada pela diferença entre as profundidades da mesma endentação antes e após a abrasão, e geralmente expressa em *µm* (Carvalho e Lussi, 2014). Esse método é indicado para avaliação de pequenas perdas de superfície, em experimentos com diversos ciclos, possibilitando diversas mensurações e a integridade das amostras.

Intensidade de reflexão da superfície especular (IRS)

Através da ação de ácidos na superfície dentária, ocorre uma perda inicial de conteúdo mineral e também se observa uma mudança nas propriedades ópticas do esmalte, devido ao aumento da sua porosidade. Após o desafio abrasivo, essa camada amolecida mais superficial é parcialmente removida e a superfície passa por um polimento com a ação da escovação, voltando a apresentar aspectos mais próximos da superfície hígida, aumentando assim seu brilho. Essa mudança pode ser mensurada através de um dispositivo óptico (Rakhmatullina *et al*, 2013).

Esse dispositivo é equipado com laser de diodo (oeMarket, Cherrybrook, Austrália), que emite um feixe de laser (635 nm) sobre a superfície dentária. A luz refletida é captada e, em seguida, medida com um fotodiodo (FDS100, Thorlabs, Dachau, Alemanha). A ponta do dispositivo deve ser colocada diretamente sobre a superfície da amostra, e em seguida, inclinada manualmente em ângulos diferentes até que o ponto de maior intensidade de reflexão seja atingido (Rakhmatullina *et al*, 2013). O dispositivo é conectado a um computador, onde o ponto de maior intensidade de reflexão pode ser capturado e expresso em um valor numérico que corresponde ao fator de reflexão de esmalte (*fer*).

Em estudos *in vitro*, as amostras inicialmente apresentaram-se lisas e extremamente polidas. O valor inicial de refletividade do esmalte é o mais alto, e mesmo após a ação do desafio erosivo, este diminuiu drasticamente. Um aumento nesse valor pode ser observado após os desafios abrasivos, porém não se aproxima dos valores iniciais. Esse dispositivo apresentou bons resultados e tem chances de ser usado clinicamente, como auxiliar no diagnóstico e proservação de casos de desgaste dentário erosivo (Rakhmatullina *et al*, 2013).

Perfilometria de superfície

A perfilometria de superfície quantifica a perda de superfície da estrutura dental, em relação a uma área de referência não submetida a desafios erosivos e/ou abrasivos. Também é capaz de avaliar a rugosidade superficial, com maior precisão nos estágios iniciais de desgaste dentário erosivo (Schlueter *et al*, 2011). Através desse método a superfície da amostra é escaneada, dando origem a um perfil em duas ou três dimensões, usando um perfilômetro de contato (agulha ou ponta diamantada) ou através de um feixe de luz (laser de luz azul ou branca).

O perfilômetro com feixe de laser apresenta uma maior resolução que o de contato. Além disso, a superfície dentária mais externa, após ser submetida a desafios erosivos, apresenta-se mais suscetível a forças mecânicas, então a precisão da medida através do perfilômetro de contato pode ser prejudicada pela possibilidade de penetração da agulha ou ponta diamantada nessa camada mais desmineralizada, superestimando assim a perda de superfície (Attin e Wegehaupt, 2014).

Um perfilômetro com laser de luz branca é capaz de medir uma diferença vertical entre 300µm e 10mm, dependendo do sensor utilizado, o que permite mensurar pontos bastante profundos de superfícies dentárias naturais com desgaste erosivo. Porém, para uma maior acurácia, amostras lisas e bem polidas são desejáveis, sendo possível detectar com precisão perdas a partir de 0.5 µm (Schlueter *et al*, 2011).

Este método também pode ser utilizado em superfícies dentárias naturais e em mensurações indiretas intra-orais através de réplicas dos dentes afetados (Attin e Wegehaupt, 2014).

Microscopia Eletrônica de Varredura (MEV)

Este é um método qualitativo, essencial para avaliação de mudanças ultraestruturais, associadas a desafios erosivos e abrasivos, tanto em esmalte quanto em dentina. Para a avaliação através da MEV convencional usa-se um sistema de vácuo e as amostras são recobertas por uma fina camada de metal (ouro) ou carbono, sendo desse modo uma metodologia destrutiva, impossibilitando o uso posterior da mesma amostra. Mais recentemente a avaliação de superfícies naturais através da MEV, com uma menor aplicação de vácuo e sem recobrimento da amostra tem sido utilizada, porém as imagens apresentam uma menor resolução que a convencional (Schlueter *et al*, 2011).

As imagens de MEV possibilitam avaliar a eficácia da película adquirida em proteger a superfície subjacente esmalte da dissolução pelos ácidos do desafio erosivo. Também pode mostrar as partículas resultantes da dissolução dos minerais por diferentes ácidos, depositadas superficialmente na estrutura dentária (Attin e Wegehaupt, 2014).

Considerando o que foi exposto, deve-se reconhecer que ainda há muitas lacunas no entendimento do processo do desgaste dentário erosivo. O mecanismo de ação dos dentifrícios na prevenção desta condição ainda não foi propriamente estabelecido, assim como a melhor combinação entre as diferentes combinações de fluoretos e a medida da abrasividade.

A diferença na susceptibilidade entre dentes permanentes e decíduos também ainda não foi detalhadamente explorada, muito pelo fato de desenhos de estudo não incluírem desafios abrasivos e pelo reduzido número de estudos *in vitro* ou *in situ* com dentes decíduos.

O melhor entendimento dessa condição, inicialmente através de estudos laboratoriais, pode refletir em medidas de prevenção mais efetivas e em dados de prevalência mais baixo no futuro.

OBJETIVOS

3.1. Objetivo Geral:

O objetivo geral deste estudo foi investigar o efeito protetor de diferentes dentifrícios fluoretados frente ao desgaste erosivo inicial e avançado de dentes permanentes e decíduos.

3.2. Objetivos específicos:

Artigo 1:

- Investigar efeito preventivo de diferentes dentifrícios fluoretados no desgaste erosivo inicial em dentes decíduos e permanentes humanos.
- Avaliar a microdureza superficial, a refletividade da superfície do esmalte e a perda de substância calculada em dentes decíduos e permanentes frente a um modelo de desgaste erosivo inicial.

Artigo 2:

- Investigar efeito preventivo de diferentes dentifrícios fluoretados no desgaste erosivo avançado em dentes decíduos e permanentes humanos.
- Mensurar a perda de superfície através da perfilometria em dentes decíduos e permanentes frente a um modelo de desgaste erosivo avançado.

ARTIGOS CIENTÍFICOS

ARTIGO 1: Efficacy of toothpastes in prevention of initial erosive tooth wear in permanent and deciduous teeth

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Efficacy of toothpastes in the prevention of initial erosive tooth wear in deciduous and permanent teeth.

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Abstract

Objective: to evaluate the preventive effect of different toothpastes in an initial erosion-abrasion model in permanent (PT) and deciduous teeth (dt). *Material and Methods:* Enamel samples of permanent (n=100) and deciduous teeth (n=100) were randomly divided into five groups (n=20) according to the toothpastes tested: G1 – Placebo toothpaste (negative control, 0ppm), G2 – NaF toothpaste (control, 1500ppm, Crest®, P&G), G3 – AmF-NaF-SnCl anti-erosion toothpaste (1400ppm, elmex Erosion Protection®, GABA – Colgate), G4 – SnF toothpaste (1100ppm, Sensodyne Repair&Protect®, GSK), G5 – NaF anti-erosion toothpaste for children (1450ppm, Sensodyne ProNamel Junior®, GSK). The samples were submitted to five erosion-abrasion cycles. In each cycle, the samples were incubated in artificial saliva (1h), submitted to erosive challenge (3min; 1% citric acid; pH3.6; at 25°C) and to toothbrush abrasion (2min immersion in slurry; 50 strokes; 200g). Surface microhardness (SMH), surface specular reflection intensity (SRI), and cumulative surface loss (CSL) were measured. Testing time effect as a factor in the setting of repeated measures, a non-parametrical ANOVA for longitudinal data was performed. Comparisons among toothpastes were evaluated using Kruskal-Wallis-tests and between PT and dt using Wilcoxon's rank sum test. *Results:* Considering the SMH results, placebo toothpaste showed significantly lower SMH values in PT than the other toothpastes (p<0.05), with no differences between them. In dt, placebo and G4 also showed significantly different values than the other groups (p<0.05). In SRI results, SnF toothpaste (G4) showed lower *erf* values in both PT and dt. Deciduous teeth presented significantly higher SRI than permanent (p<0.05), except on AmF-NaF-SnCl anti-erosion group (G3). Deciduous teeth presented generally higher CSL than PT, except for G3. *Conclusions:* Deciduous teeth were more prone to mineral loss than permanent teeth. NaF anti-erosion toothpaste for children presented better efficacy for both permanent and deciduous teeth, while AmF-NaF-SnCl anti-erosion toothpaste showed a better preventive effect only for deciduous teeth.

Key-words: Erosive Tooth Wear, Permanent Teeth, Deciduous Teeth, Toothpaste.

1. Introduction

Erosive tooth wear (ETW) is defined as the loss of tooth substance by mechanical and chemical processes not involving bacteria (LUSSI and CARVALHO, 2014). The most important sources are dietary acids (acidic foods and drinks) and gastric acids (regurgitation and reflux disorders) (CARVALHO *et al,* 2015; LUSSI and CARVALHO, 2015; LUSSI *et al*, 2004; BARTLETT, 2006). ETW is increasingly recognized as a common condition in pediatric dentistry with complications such as tooth sensitivity, altered aesthetics and loss of occlusal vertical dimension. The prevalence of ETW in children has been reported to range from 10% to over 80%, most of initial stages (TAJI, SEOW, 2010). Regarding deciduous teeth in children up to 7 years old, some results from a systematic review indicate that the prevalence of ETW into the dentin increases significantly with age (KREULEN *et al*, 2010).

Besides these facts, erosive tooth wear in deciduous teeth should not be considered as a short-term physiological process, but a predicting factor of wear in permanent dentition (CARVALHO *et al*, 2014; GANSS *et al,* 2001). The management of ETW in children should include an early diagnosis, the evaluation of different etiological factors, risk identification and the proposal of preventive measures to delay the advance of the condition (CARVALHO *et al*, 2014). Because surface loss is an irreversible condition, prompt preventive measures, during the early stages of ETW, are essential to minimize this condition (CARVALHO, LUSSI, 2014).

Most information about the preventive effect of toothpastes in ETW comes from *in vitro* and *in situ* studies. The benefits of toothpaste compounds associate to fluoride are higher than the adverse effects, such as abrasivity. Regarding to ETW prevention, various active ingredients were tested, and the ability of forming acid-resistance precipitates on dental surface were evaluated (MAGALHÃES *et al,* 2014). Several *in vitro* and *in situ* studies produced enough evidence that, when comparing to non-fluoride toothpastes, fluoride-containing toothpastes have a certain preventive effect against ETW, increasing the acid resistance of tooth surface or pellicles (GANSS *et al*, 2014). However, for sodium fluoride toothpastes to promote more acid-resistance precipitates other formulations have been tested using amine or stannous fluoride compounds, as well as phosphates or biopolymer additives (GANSS *et al*, 2014).

Ganss *et al* (2011) evaluated the effect of commercial toothpastes with anti-erosion claim in comparison to conventional fluoride toothpastes in terms of slurry and additional abrasion effects. Conventional NaF toothpastes were able to reduce erosive tissue loss even in severe erosive conditions, but had limited efficacy regarding brushing abrasion. The formulations with anti-erosive claims were not superior, although tin-containing toothpaste had promising anti-erosion potential, which was, unfortunately, counteracted by the effects of abrasion (GANSS *et al*, 2011).

The preventive effect of different formulations of toothpastes, mouth rinses and varnishes in ETW had been tested by several studies, most of them in permanent teeth (GANSS, *et al,*2011; SCHLUETER *et al,* 2009; LUSSI *et al,* 2004; MAGALHÃES *et al,*2008). But some considerable differences between permanent and deciduous teeth should be addressed. The enamel of deciduous teeth is less mineralized, and its surface hardness and elasticity is lower, so could be assumed that deciduous teeth are more susceptible to acidic dissolution than permanent teeth. However laboratory studies showed conflicting results, epidemiological studies imply that deciduous teeth are less wear resistant (CARVALHO *et al*, 2014).

The outermost layer of aprismatic enamel and the absence of perikymata in deciduous teeth turns the erosive tooth wear diagnosis in its early stages a difficult tool in daily practice (CARVALHO *et al*, 2014). Optical assessment of ETW has been tested to improve the diagnosis. The mode of action of these devices is based on the fact that abrasion of the eroded teeth resulted in the partial removal of the softened enamel tissue and an increase in the specular reflection intensity due to smoothing of the etched enamel surfaces. Nevertheless, an exponential change in the reflection signal with erosion progression could be measured on the eroded and abraded samples, indicating that the optical method can be further applied for ETW assessment of the abraded flat surfaces *in situ* or even *in vivo* (LUSSI *et al*, 2012).

In laboratory studies, the use of different methods could be applied to evaluate ETW. Early stages of enamel and dentin dissolution, which are associated with surface weakening, can be determined through surface hardness measurements. Detection of enamel abrasion is also possible by calculating the depth of the indentations, once the ground of the indentation is not changed and is not removed by abrasion. Only the surrounding tissue on the surface is removed, and the surface loss could be calculated from the change in indentation length (ATTIN, 2014).

Considering the presumed difference between deciduous and permanent enamel, and the lack of information about the preventive effect of some commercial toothpastes in ETW on deciduous teeth, the aim of this present study is to evaluate the preventive effect of different toothpastes in an initial erosion-abrasion model and compare this effect in permanent and deciduous teeth. The null hypothesis tested was that permanent and deciduous teeth were similar regarding surface microhardness (SMH) decrease, specular reflection intensity (SRI) variation or calculated surface loss (CSL).

2. Materials and Methods

Experimental design and groups

The following parameters were included in the present study: surface specular reflection intensity (SRI), surface microhardness (SMH) and calculated surface loss. The sample size calculation was based on the difference of surface loss in different toothpastes treatments of a previous study and considered the balanced one-way analysis of variance power calculation, with level of significance set to 0.05, and power at 80%, and indicated a minimum number of 16 in each group (CARVALHO e LUSSI, 2014). In order to have extra samples for SEM images and counting with possible losses during the experiment 20 samples were randomly selected for each group. The cyclic design of the experiment is presented in Figure 1.

Sample preparation

Sound premolars (n=100) and deciduous molars (n=100) were randomly selected from a pool of extracted teeth. The present experiment was carried out in accordance with the approved guidelines and regulations of the local ethical committee (Kantonale Ethikkommission: KEK). The teeth were extracted by dental practitioners in Switzerland, pooled into two groups of deciduous and permanent teeth, and stored in chloramine until the time of the experiment. Before the donation, the patients (and parents, in case of deciduous teeth) were informed about the use of their teeth for research purposes. Oral consent was obtained by all patients (and parents) for the use of the teeth in research..

The crowns were separated from the roots using Isomet® Low Speed Saw (Buehler, Düsseldorf, Germany); cross-sectioned into lingual and buccal sides and painted with a layer of nail varnish before embedding into resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany). To obtain a flat, parallel, highly smooth enamel surface the samples were ground and polished. Under constant tap water cooling, the samples were abraded using a Knuth Rotor machine (LabPol 21, Struers, Copenhagen, Denmark) with silicon carbide paper discs (grain size 1000, 2400, 4000) for 30 seconds each grain. Later, samples were polished for 60 seconds with 3 µm diamond abrasive on Struers polishing cloth under constant cooling (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark). Between each abrading and polishing step, as well as after the final polishing, all samples were sonicated for 1 minute in distilled water.

To select samples with the same mineral content, initial microhardness measurements were performed (50g/10s indentations - UHL VMHT Microhardness Tester, UHL technische Mikroskopie GmbH & Co. KG, Aßlar, Germany), samples with KHN values between 315-420KHN were select, totalizing 100 permanent and 100 deciduous teeth samples.

All samples were stored in a mineral solution (1.5 mmol/l CaCl2, 1.0 mmol/l KH2PO4, 50 mmol/l NaCl, pH = 7.0)(ZERO *et al*, 1990) and underwent a further polishing with 1 µm diamond abrasive paste (60 seconds, LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark) immediately prior to the beginning of experimental phase. The enamel samples were randomly divided into five groups (n= 20 for both permanent and deciduous teeth). Five different toothpastes were tested in this study (Table 1).

Erosion-abrasion cycles

The samples were submitted to five erosion-abrasion cycles, one cycle per day. Prior to erosive challenge the samples were incubated in artificial saliva ($CaCl_2 2H_2O$; KH_2PO_4; NaCl; KCl; Mucin – pH7.4) (NEWBY *et al*, 2006) at 37°C, for a period of 1 hour, dipped in deionized water three times to remove the solution excess from their surfaces, and gently dried with oil free air. Initial SRI and SMH measurements were performed.

The erosive challenge consisted in sample immersion for 3 minutes on 30mL of 1% citric acid (pH 3.6) at 25°C, under constant agitation (70 rpm). The samples were washed, dried, and SRI and SMH measurements were repeated.

Afterwards, the samples were submitted to abrasion, by immersing each sample in toothpaste slurry for 2 min, and within this time the sample was brushed in the brushing machine (50 strokes; 200g force). Toothpaste slurries were prepared daily by mixing 20g of toothpaste with 40g of artificial saliva ($CaCl_2 2H_2O$; KH_2PO_4; NaCl; KCl; Mucin – pH7.4) (NEWBY, 2006). After each abrasion challenge, surface loss was calculated and final SRI and SMH measurements were performed. The samples were stored in relative humidity at 4ºC overnight for the following cycles.

Surface microhardness (SMH) measurements

SMH measurements were performed using a Knoop diamond under a load of 50 ponds and a dwell time of 10 s (UHL VMHT Microhardness Tester, UHL Technischer Mikroskopie, Aßlar, Germany). Six indentations were made on a defect-free surface area of the enamel. The length of each indentation was measured and the hardness value calculated. The hardness value for each experimental step was considered the average from the six indentations. SMH was made initially (at baseline), and after each erosive and abrasive challenge.

The percentage difference in SMH (ΔSMH) was calculated using the formula $\Delta SMH_i = SMH_i / SMH_0 \times 100$, where SMH_0 is the initial SMH and SMH_i is the value after the *i*th SMH measurement (*i* = 1, 2, 3…) taken after the *i*th erosion-abrasion cycles.

Enamel specular reflection intensity (SRI) measurements

Enamel SRI measurements were carried out using an optical pen-size reflectometer (RAKHMATULLINA *et al*, 2013). The reflectometer is fitted with laser diode (oeMarket, Cherrybrook, Australia), which emits a laser beam (635 nm) onto the surface of the sample. The reflected light is then captured and measured with a photodiode (FDS100, Thorlabs, Dachau, Germany). The tip of the reflectometer was placed directly onto the enamel sample surface, and was then manually inclined in different angles until the point of highest reflection intensity was registered (RAKHMATULLINA *et al*, 2013). The reflectometer was attached to a computer, where the point of highest reflection intensity was captured and expressed as enamel reflection factor (*erf)* value. The percentage difference in SRI (ΔSRI*i*) was calculated using the formula $\Delta SRIi = SRIi / SRI_0 \times 100$, where SRI_0 is the initial SRI (measured at baseline) and SRI*i* is the value after the *i*th SRI measurement (*i* = 1, 2, 3…) taken after the erosion or abrasion cycles.

Calculated enamel surface loss

A set of six Knoop indentations was made on the enamel surface (50g/10s - UHL VMHT Microhardness Tester, UHL Technischer Mikroskopie, Aßlar, Germany). The lengths of these indentations were measured after the erosive cycles and remeasured immediately after the abrasive cycles (JAEGGI and LUSSI, 1999). Using these length values (L), each indentation depth (D) was calculated according to the equation $D = L/2 \cdot \tan \alpha$, where $\alpha = 3.75°$, a constant parameter of the diamond indenter. Prior to the abrasive challenge, the indentations were longer (and therefore deeper) than after the abrasion. The difference in indentation depth (µm) before and after the toothpaste abrasion treatment was calculated for each indentation (calculated enamel surface loss), and this value represent the amount of enamel abraded away from the indentation surroundings (CARVALHO and LUSSI, 2014). The average value of the six indentations was considered as the calculated enamel surface loss after each abrasive challenge. The cumulative surface loss corresponded to the sum of each calculated enamel surface loss value during the 5 erosion-abrasion cycles.

Scanning electron microscopy (SEM) analyses

Two samples from each group were randomly selected and sputtered with gold using a Baltec MED 020 sputtering instrument (20 mA, 0.015 mbar, in argon atmosphere). The samples were taken to the SEM chamber (JSM-6010 PLUS/LV SEM, JEOL, Tokyo, Japan) for analyses. SEM images of the enamel surfaces were taken at 1000× and 4000× magnifications, with acceleration voltage of 5 kV.

Statistical analyses

Testing time effect as a factor in the setting of repeated measures, a non-parametrical ANOVA for longitudinal data was performed. Comparisons among toothpastes were evaluated using Kruskal-Wallis-tests and between permanent and deciduous teeth using Wilcoxon's rank sum test. No correction for multiple testing was applied due to the explorative nature of the study. Level of significance is set to 0.05. All statistical results were calculated with R (version 3.1.0).

3. Results

3.1 Surface microhardness (SMH) measurements

A signifficant decrease in SMH values throughout the experiment was observed in both permanent and deciduous teeth for all toothpaste groups ($p<0.001$). The higher reduction in SMH values occurred after the first erosion challenge, and after the abrasion challenges, the SMH values increased. In permanent teeth, the negative control toothpaste caused significantly greater enamel softening at the end point, after the 5th cycle (Figure 2). All fluoride toothpastes caused significantly less enamel softening than the control group ($p<0.001$), but they were not different from each other. In deciduous teeth, the negative control group also caused greater SMH change (Figure 3). Throughout the experiment, all fluoride toothpastes were significantly different from the negative control group ($p<0.001$), but at the end of the experiment, group 5 (NaF anti-erosion toothpaste for children) presented a decrease in SMH values, and was not significantly different to the placebo toothpaste ($p= 0.116$).

To compare permanent and deciduous teeth, ∆SMH were calculated, once deciduous teeth presented lower SMH values at baseline (KHN 358±1.35 and 390±7.55 respectively, $p<0.05$). The total amount of ∆SMH change was similar in all fluoride toothpastes at the end of experiment (Figure 4). Deciduous teeth treated presented a higher decrease in ∆SMH, which was significantly different from permanent teeth in all toothpastes tested ($p < 0.05$).

3.2 Enamel specular reflection intensity (SRI) measurements

Similar as the SMH results, the SRI values decreased and presented the zig-zag pattern with a small increase after the abrasion challenges and small decrease after erosion challenges (Figures 5 and 6). In both permanent and deciduous teeth, the five toothpastes were significantly different from each other at the last measurement. Deciduous teeth had higher SRI values than permanent teeth, with significant difference in placebo (Group 1; p=0.017) and AmF-NaF-SnCl anti-erosion (Group 3; p=0.041) toothpastes.

Comparing the ∆SRI in permanent teeth, only the AmF-NaF-SnCl anti-erosion toothpaste (group 3) presented lower and significant different values (p<0.001). In deciduous teeth, both SnF toothpaste and NaF anti-erosion toothpaste for children showed significantly different values to all other toothpastes (p<0.001). Comparing the ∆SRI between permanent and deciduous teeth, no significant difference were noticed in all toothpastes tested, p > 0.05 (Figure 7).

3.3 Calculated enamel surface loss

Different toothpastes caused significantly different values of surface loss throughout the experiment (p<0.001). After each cycle, there was a progressive increase of surface loss. In permanent teeth, the NaF toothpaste (G2) caused more surface loss. The placebo (G1) and NaF anti-erosion for children (G5) toothpastes showed significantly less surface loss than the others groups (p<0.001) (Figure 8). In deciduous teeth, the NaF and SnF toothpastes (G2 and G4) showed the highest surface loss values, significantly different from the others toothpastes (p<0.001) (Figure 9).

Deciduous teeth presented significantly more surface loss than permanent teeth during the whole experiment in four groups (p<0.001). The only exception was for AmF-NaF-SnCl anti-erosion toothpaste (G3). This toothpaste caused similar surface loss in both deciduous and permanent teeth (p> 0.05).

3.4 SEM images

On SEM images (Figure 10), the effect of erosion/abrasion cycles on enamel surface of permanent and deciduous teeth could be observed. In both types of teeth, AmF-NaF-SnCl anti-erosion (G3), SnF (G4) and NaF anti-erosion for children (G5) toothpastes were able to preserve the prisms structure in a better way than placebo (G1) and NaF toothpastes (G2). On AmF-NaF-SnCl anti-erosion toothpaste less signs of erosive demineralization on the enamel surface could be observed. No distinguished aspects could be observed between permanent and deciduous teeth on either lower (400x) or higher magnification (1000x).

4. Discussion

The prevalence of erosive tooth wear (ETW) has been increasing during the last years, especially in young people (TAJI and SEOW, 2010; KREULEN *et al,* 2010). There are a variety of products, especially toothpastes, that claim to provide ETW protection, but most of them were tested on permanent teeth. To our knowledge, this is the first study to investigate the anti-erosion effect of toothpastes on deciduous teeth, and its effect was compared using permanent teeth in an initial erosion-abrasion model.

Studies have shown controversial results on whether deciduous teeth are more susceptible to ETW than permanent teeth (CARVALHO *et al*, 2014). In the present study, deciduous teeth presented lower initial surface hardness than permanent teeth (KHN 358±1.35 and 390±7.55 respectively). Moreover, when treated with a non-fluoride containing placebo toothpaste, deciduous teeth had significantly greater softening than permanent teeth ($p<0.05$). On the other hand, no signifficant differences were observed between the two types of teeth when fluoride toothpastes were used Therefore, the preventive effect provided by the fluoride toothpastes was similar for both types of teeth.

The variation on SMH during the erosion-abrasion cycles in both permanent and deciduous teeth was similar to a previous study (CARVALHO and LUSSI, 2014). The greater SMH decrease occurred at first erosion challenge, and after each abrasion challenge a small increase in SMH was observed. This is due to the fact that the enamel surface was softened after the erosive challenge, and this outermost softened layer was removed by tooth brushing during the abrasion challenge. The treatment with different toothpastes was also responsible for the different decrease on SMH; as expected the placebo toothpaste group presented a higher decrease compared to the fluoride toothpaste groups (CARVALHO and LUSSI, 2014). When treated with the placebo toothpaste, the decrease in SMH after the erosive challenge was greater than in other groups. For groups treated with the fluoride toothpastes (groups 2, 3, 4, and 5), SMH did not decrease to such low values, as those observed on the placebo toothpaste group. Therefore, it could be observed that fluoride toothpastes significantly protected both permanent and deciduous teeth, when compared to placebo toothpaste. However, on the last cycle, only the NaF anti-erosion toothpaste for children caused a decrease in SMH values in deciduous teeth, leading to a non-statistically significant difference compared to the placebo toothpaste group.

Not only does the acid cause a decrease in surface hardness after an exposure, but also lead the enamel to a rougher surface. This roughened surface causes a decrease in surface reflection (RAKHMATULLINA *et al*, 2011), as observed in the SRI results. The subsequent abrasion challenge then partially removes this softened layer, leaving a slightly smoother enamel surface (LUSSI *et al*, 2012). This was also observed in the SRI results, where an increase-decrease pattern was observed. This zig-zag pattern is in accordance to previous *in vitro* studies (JAEGGI and LUSSI, 1999; BREVIK *et al,* 2013; RAKHMATULLINA *et al*, 2011). Considering the *SRI* values, both permanent and deciduous teeth treated with the SnF toothpaste (G4) showed significantly lower *SRI* values than others groups (p<0.001, Figures 5 and 6). The SnF toothpaste (Sensodyne Repair&Protect@, GSK) has been originally indicated by the manufacturer for dentine sensitivity treatment. This toothpaste caused a different wear pattern on the enamel surface, thus possibly leading to the lower *SRI* values found.

Enamel surface loss was observed in all samples, and none of the tested toothpastes were able to fully prevent ETW. The NaF and SnF toothpastes groups (G2 and G4) showed greater surface loss results in both permanent and deciduous teeth (Figures 8 and 9). In deciduous teeth, the anti-erosion toothpastes (AmF-NaF-SnCl anti-erosion - G3 and NaF anti-erosion toothpaste for children - G4) caused the least enamel surface loss, statistically different from the others groups. In permanent teeth, the NaF anti-erosion toothpaste for children (G5) showed lower and statistically different surface loss, with exception to the placebo toothpaste. Since the placebo toothpaste had no active ingredients (F- or Sn^{2+}), it was expected that this toothpaste would cause greater SMH decreased and, hence, greater surface loss. Surprisingly, despite the greater surface softening observed on samples treated with the placebo toothpaste, this toothpaste presented remarkably low enamel surface loss values, for permanent teeth (2.16 µm) and for deciduous teeth (3.31 µm). This fact can be explained, not only by the fluoride (and stannous) content in toothpastes, but most importantly by the complex composition of the toothpastes.

The daily use of toothpastes is a settled part of oral hygiene practices and the main vehicle for active ingredients, mainly fluorides and stannous compounds. These active ingredients should increase the acid resistance of tooth surface and pellicles (CARVALHO *et al*, 2014), leading to less tooth wear. Nevertheless, toothpastes also contain abrasives, which are an important part of tooth cleaning, and they could also counteract the positive effects from the active ingredients (GANSS *et al*, 2013). Toothpaste abrasivity is usually measured by RDA values (Relative Dentine

Abrasivity), but RDA values are mostly a measure of dentine abrasivity and they do not quite reflect enamel abrasivity. Moreover, even REA values (Relative Enamel Abrasivity) do not represent the rate of enamel wear in an erosion-abrasion experiment. In the study by Philpotts *et al.* (2005), the authors tested seven different toothpastes that presented different RDA and REA values. Comparing two of these toothpastes, toothpaste D had an RDA value of 90 and an REA value of 4.1, whereas toothpaste F had an RDA value more than twice as high (RDA 204) and an REA value twice as low (REA 2.1) (PHILPOTTS *et al*, 2005). These values go to show that RDA and REA values do not always correlate. Furthermore, Ganss *et al*. (2011) have already raised the issue that RDA values provided by companies do not necessarily represent RDA values observed in the laboratory. In this case, even though RDA values of the toothpastes used in this study were obtained from the manufacturers (Table 1), the RDA values may not necessarily indicate the rate of enamel wear. Additionally, in regards to the enamel wear caused by the toothpastes in the study by Philpotts *et al*. (2005), it could be observed that, despite the discrepant RDA and REA values for toothpaste D and F, both these toothpastes caused similar amounts of abrasion. The fact that RDA and REA values may not represent enamel wear rates was also observed in the study by Pickles *et al*. (2005). To sum up, in this study, the RDA values for the placebo toothpaste may not necessarily represent the true abrasivity of the toothpaste in regards to enamel erosive wear, once these values refers to sound dentin abrasivity.

Still, other studies however, have shown that toothpaste abrasivity can play a major role on the wear rate of acid-softened enamel. In the study by Wiegand *et al* (2008), the authors showed that abrasive-free toothpaste caused significantly less tissue loss than toothpastes with medium and high abrasivity, but the amount of enamel wear was generally similar for the two toothpastes with medium and high REA values. Hara *et al*. (2009) also presented similar results, and, additionally, the authors also showed that fluoride-containing toothpastes promoted significantly less abrasion than toothpastes without fluoride. Strikingly, in the present study, we observed that the placebo toothpaste (which, according to the manufacturer, is without fluoride and has RDA value of 80-100) caused significantly less enamel wear than the other toothpastes, especially in permanent teeth.

The difference between the present study and the abovementioned studies (WIEGAND *et al,* 2008; HARA *et al*, 2009) is that the two other studies used far more erosion and abrasion in their experimental setups. This difference between the severity of erosive and (most importantly) abrasive challenges could account for the differences in results. In the study by Hara *et al*. the number of toothbrush strokes per cycle (500 strokes) was probably enough to remove the whole softened enamel layer In this case, we are in agreement with Carvalho and Lussi (2014), that the mode of action of the fluoride toothpastes is rather related to a partial inhibition of enamel softening during the subsequent erosive challenge, and the fluoride itself is not able to prevent substance loss when the softened enamel is brushed away (CARVALHO and LUSSI, 2014).

The scenario is, however, different, when less toothbrush strokes are used (like in the case of the present study: initial erosion-abrasion). If the samples are brushed for less time or with fewer strokes, not all the softened layer would be removed. In this case, we suggest that the active ingredients (fluoride and stannous) do not play such an important role on prevention in this initial erosion-abrasion model. The active ingredients will only play a more significant role with the increase in the number of toothpaste application (more experimental cycles). Furthermore, since not all the softened layer was removed in this experiment, because we had only 50 toothbrush strokes per experimental cycle, it can be suggested that the amount of enamel loss was more dependent on the abrasivity of the toothpaste. In other words, the active ingredients of the toothpastes (fluoride or stannous ions) would probably have a greater effect with more cycles (more toothpaste applications), when they would be able to form a protective layer on enamel and, thus, hinder further acid attacks.

The SEM images helped to illustrate the different patterns of erosive tooth wear among the five groups (Figure 10). The placebo toothpaste showed a more disorganized surface, which represents the more severe effect of the acid demineralization. The AmF-NaF-SnCl anti-erosion and NaF anti-erosion for children toothpastes showed tooth structure more intact, with the enamel prisms with a more defined contour.

The toothpaste compounds associate to fluoride, or its presence, in toothpastes tested seemed not to be the key factor involved on the different results showed among the groups. Others toothpaste properties such as RDA, REA, wettability and viscosity could have an important action on initial erosive tooth wear in both permanent and deciduous teeth.

5. Conclusions

Deciduous teeth were more prone to surface loss than permanent teeth. NaF anti-erosion toothpaste for children presented better efficacy for both permanent and deciduous teeth, while AmF-NaF-SnCl anti-erosion toothpaste showed a better preventive effect only for deciduous teeth, regarding to the main variable evaluated in this study, the calculated surface loss.

6. Acknowledgments

The authors appreciate the support of CAPES Foundation (Ministry of Education of Brazil) for the PhD student scholarship. We are grateful to G Fischer and Prof. J. Hüsler from the Institute of Mathematical Statistics and Actuarial Science, University of Bern, for all statistical analysis. The toothpastes used in the present study were provided by GABA-Colgate and GlaxoSmithKline. We declare that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors were involved on designing the experiments, critical analysis of the results and writing the paper. Data collection was performed by CMA. Furthermore, the authors are very grateful to Barbara Beyeler, Brigitte Megert, Isabel Hug, and Samuel Furrer, members of the Department of Preventive, Restorative and Pediatric Dentistry, University of Bern.

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Table 1. Description of groups and toothpastes ingredients used in the study.

Group	Active ingredient	RDA values	Brand / Manufacturer
G1 – Negative control	None	80-100	Placebo toothpaste (GABA – Colgate)
G2 – NaF toothpaste Positive control	NaF (1500ppm)	120	Crest® (P&G)
G3 – AmF-NaF-SnCl anti-erosion toothpaste	NaF/AmF/SnCl (1400ppm)	40-60	elmex Erosion Protection® (GABA – Colgate)
G4 – SnF toothpaste	SnF_2 anhydrous (1100ppm)	119	Sensodyne Repair&Protect® (GSK)
G5 – NaF anti-erosion toothpaste for children	NaF (1450ppm)	30-40	Sensodyne ProNamel for children® (GSK)

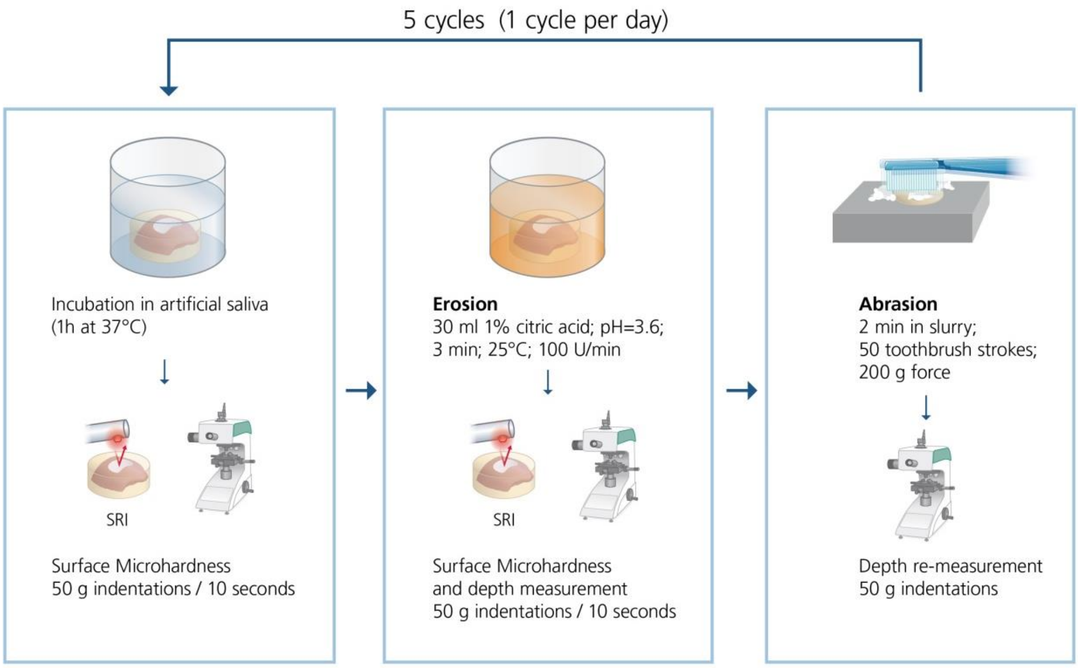

Figure 1. Study design

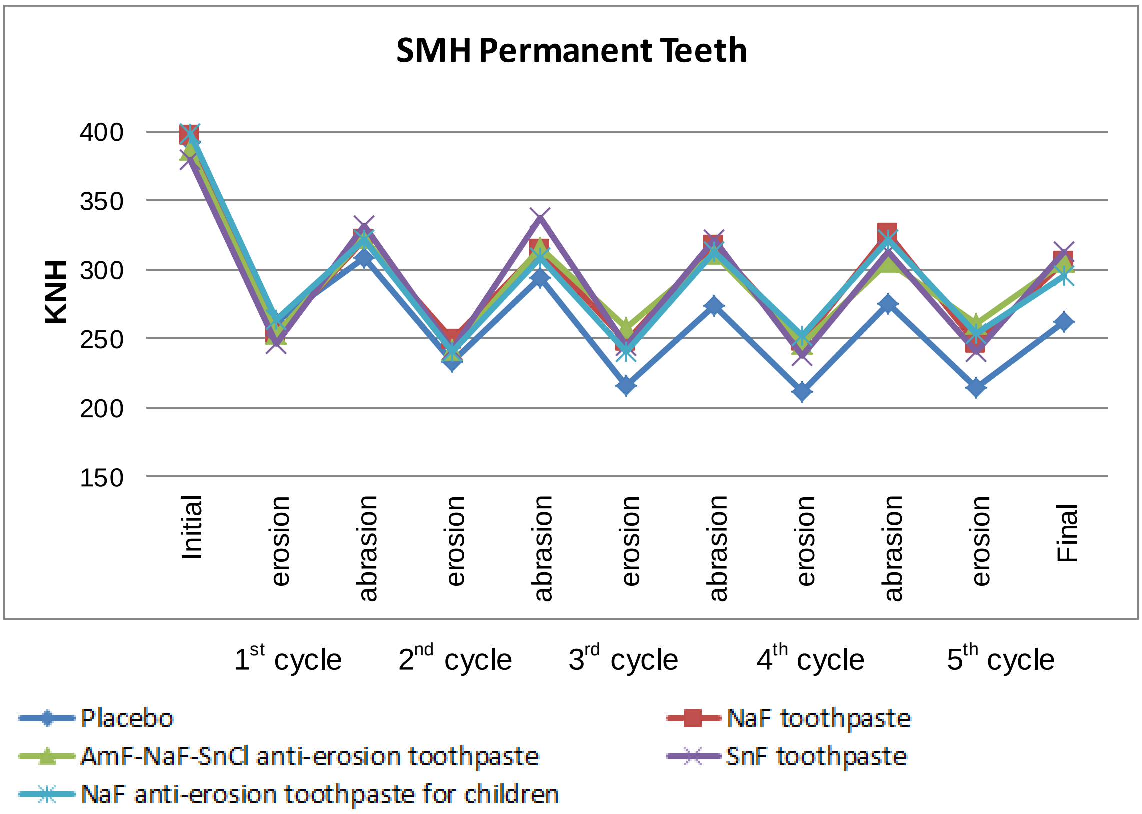

Figure 2. Mean surface microhardness after each erosion-abrasion cycles in permanent teeth, according to the different groups. (n=20)

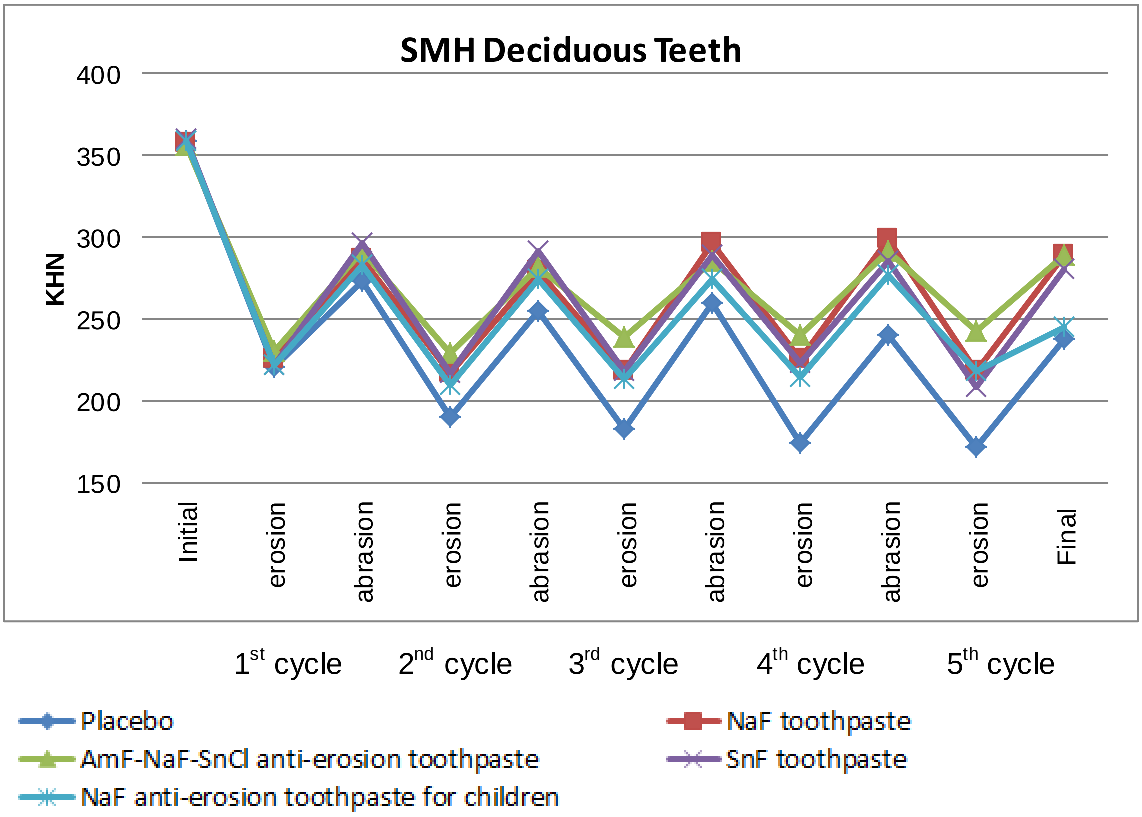

Figure 3. Mean surface microhardness after each erosion-abrasion cycles in deciduous teeth, according to the different groups. (n=20)

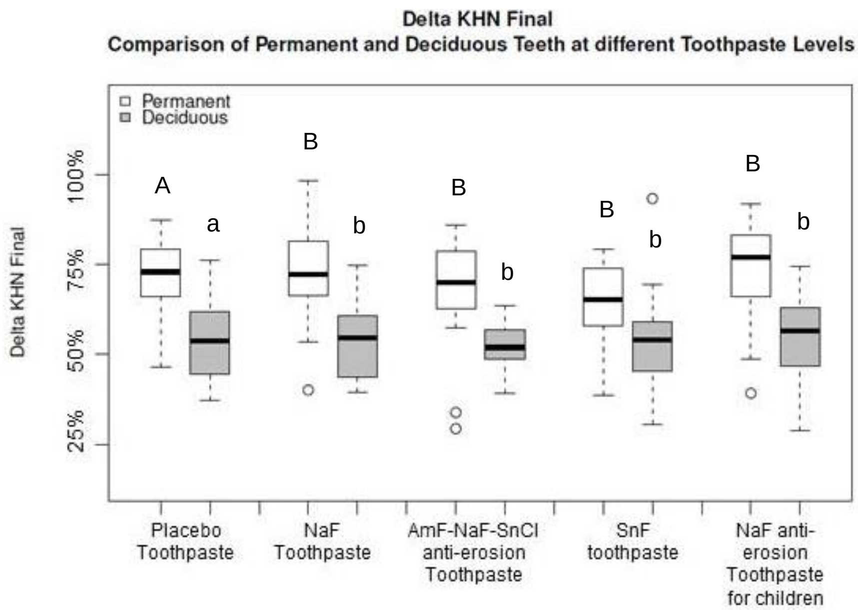

Figure 4. Percentage difference in SMH (∆SMH) after erosion-abrasion challenges in permanent and deciduous teeth, according to the different groups. (n=20) Statistically significant differences are shown with different letters: lower case letters represent deciduous teeth; upper case letters represent permanent teeth.

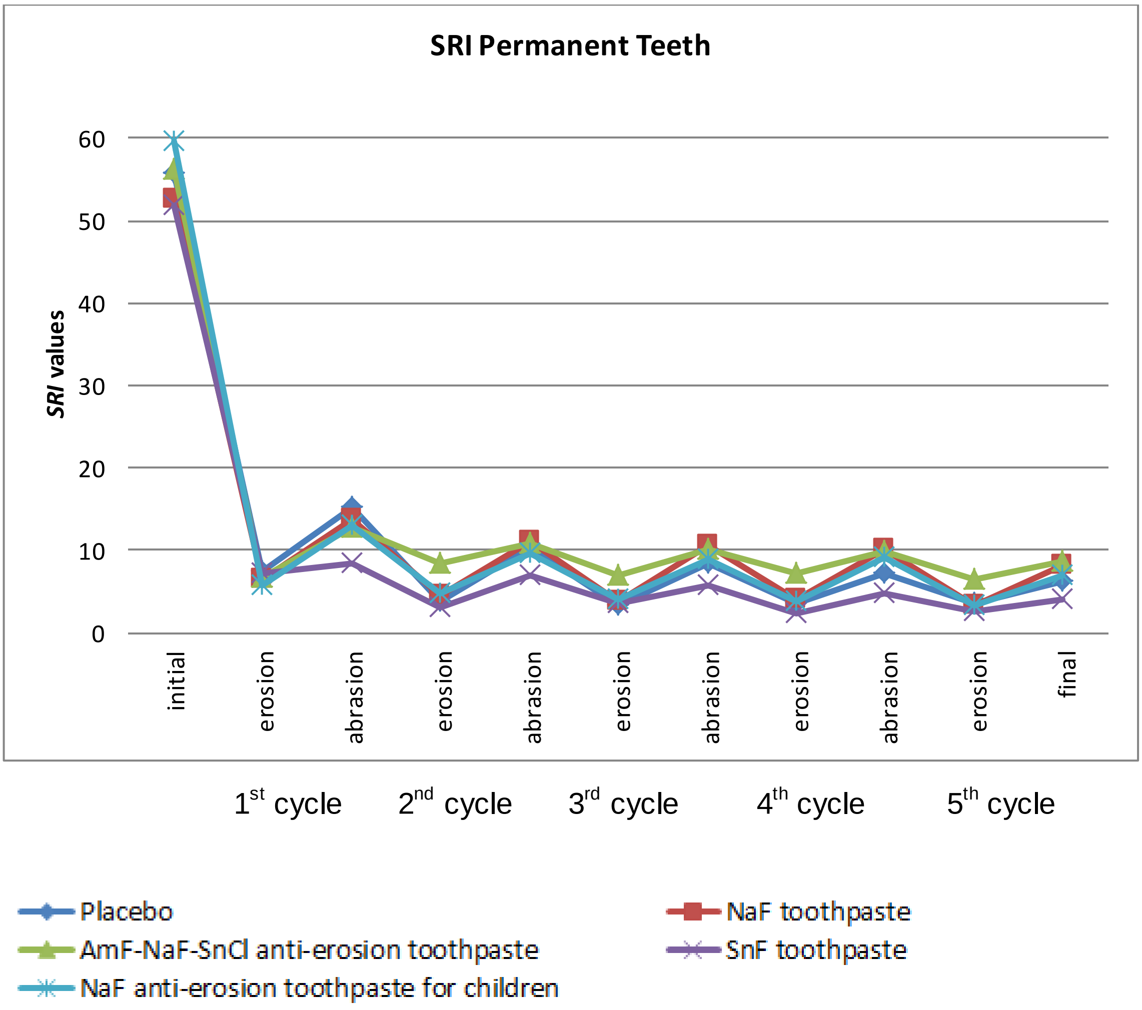

Figure 5. Mean enamel specular reflection intensity (SRI) during the erosion-abrasion cycles in permanent, according to the different groups. (n=20)

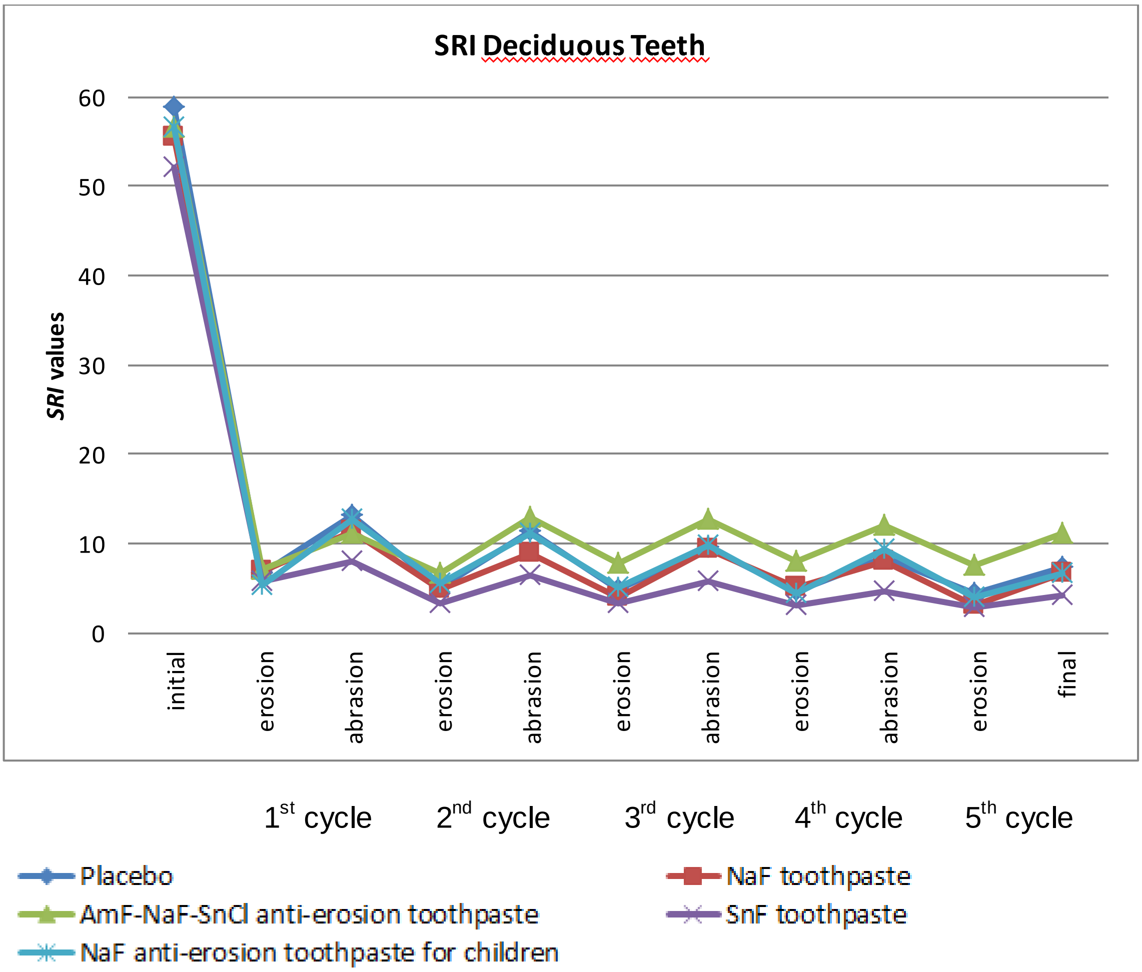

Figure 6. Mean enamel specular reflection intensity (SRI) during the erosion-abrasion cycles in deciduous teeth, according to the different groups. (n=20)

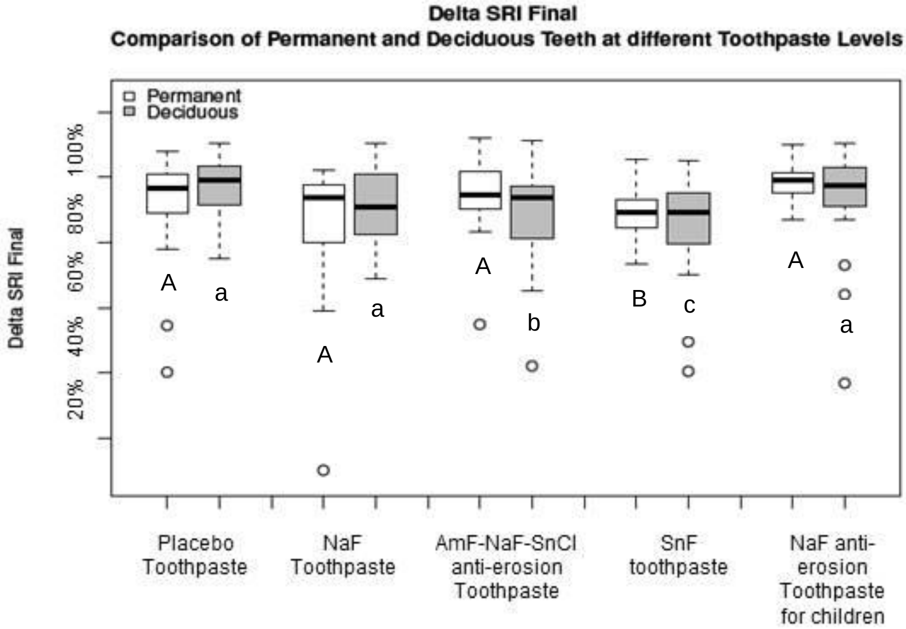

Figure 7. Percentage difference in SRI (ΔSRI*)* after erosion-abrasion challenges in permanent and deciduous teeth, according to the different groups. (n=20). Statistically significant differences are shown with different letters: lower case letters represent deciduous teeth; upper case letters represent permanent teeth.

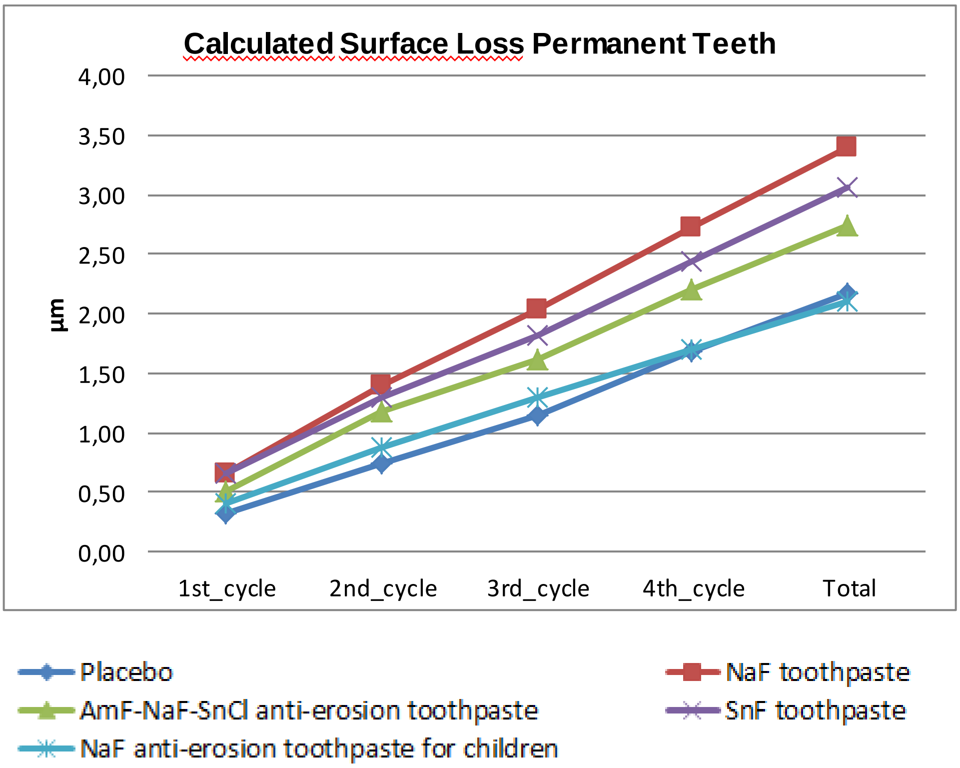

Figure 8. Calculated surface loss during the erosion-abrasion cycles in permanent and deciduous teeth, according to the different groups. (n=20)

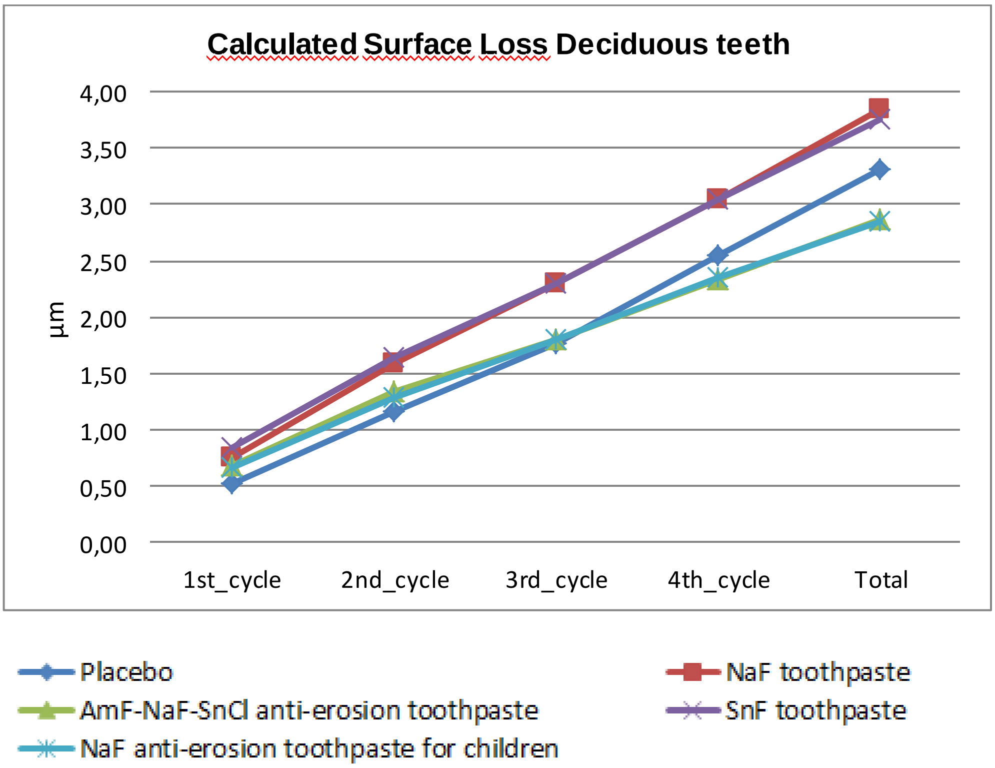

Figure 9. Calculated surface loss during the erosion-abrasion cycles in permanent and deciduous teeth, according to the different groups. (n=20)

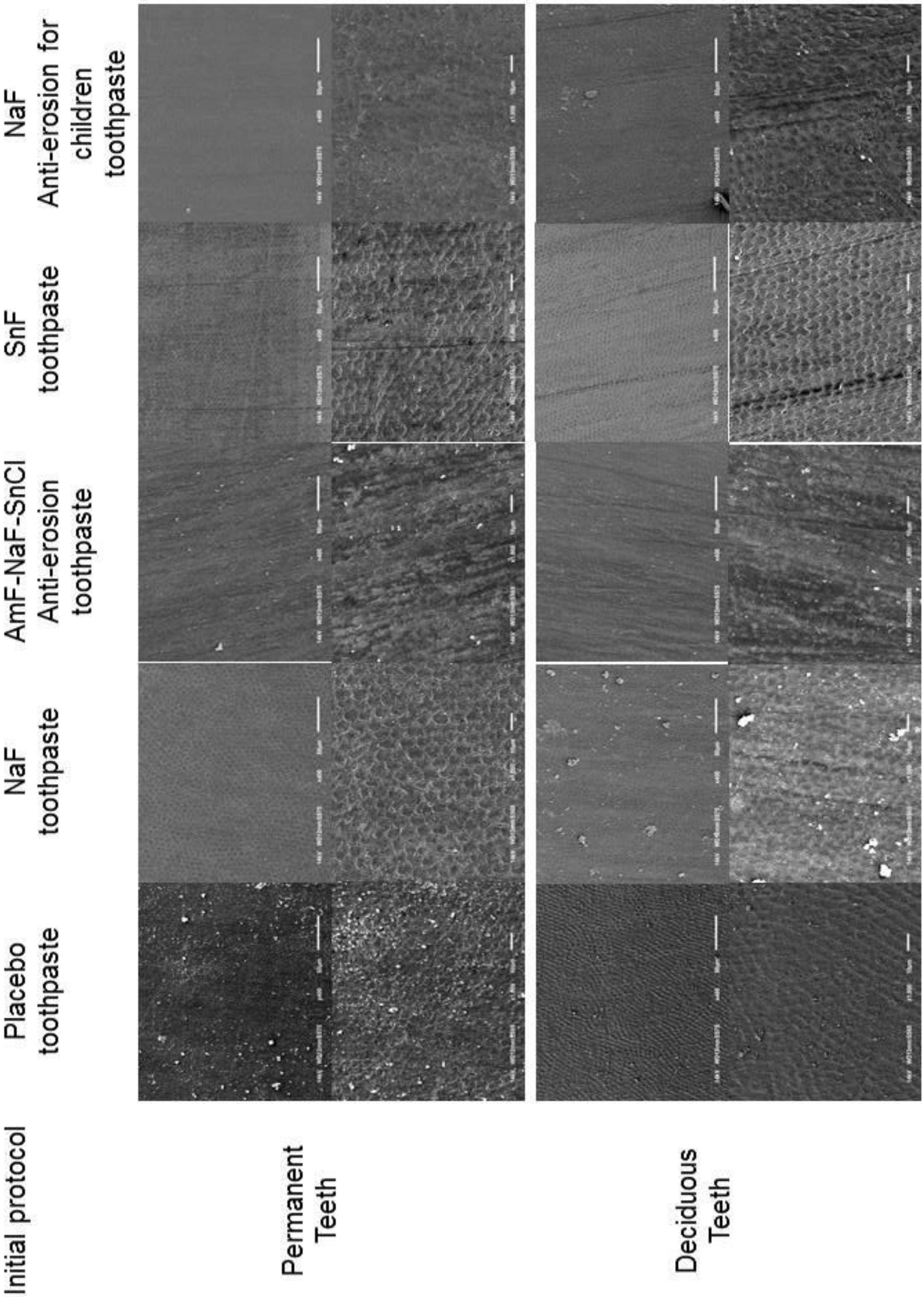
Initial protocol
Placebo toothpaste
NaF toothpaste
AmF-NaF-SnCl Anti-erosion toothpaste
SnF toothpaste
NaF Anti-erosion for children toothpaste
Permanent Teeth
Deciduous Teeth

Figure 10. SEM images of permanent and deciduous teeth after 5 erosion/abrasion cycles, according to toothpaste groups in lower (400x) and higher (1000x) magnification.

ARTIGO 2: Do different fluoride toothpastes have similar preventive effect in permanent and deciduous teeth against erosive tooth wear?

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Do different fluoride toothpastes have similar preventive effect in permanent and deciduous teeth against erosive tooth wear?

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ABSTRACT

Toothpastes are key vehicles for fluoride application. Studies have shown that different fluoride compounds have some preventive effect on erosion/abrasion progression. Little is known about their effect on deciduous teeth. *Aim*: To evaluate the preventive effect of different toothpastes on surface loss (SL) after an erosion/abrasion model and to compare this effect between permanent and deciduous teeth. *Material and Methods:* Enamel samples of permanent (n=85) and deciduous teeth (n=85) were randomly divided into five groups according to toothpastes tested (n=17). G1 – Placebo toothpaste (0ppm), G2 – NaF toothpaste (positive control, 1500ppm, Crest®, P&G), G3 – AmF-NaF-SnCl anti-erosion toothpaste (1400ppm, elmex Erosion Protection®, GABA – Colgate), G4 – SnF toothpaste (1100ppm, Sensodyne Repair&Protect®, GSK), G5 – NaF anti-erosion toothpaste for children (1450ppm, Sensodyne ProNamel Junior®, GSK). Half of enamel samples were covered with methacrylate-based resin to create a reference area. Samples were submitted to 30 erosion-abrasion cycles. In each cycle, samples were incubated in artificial saliva (1h), submitted to an erosive challenge (3min; 1% citric acid; pH3.6; at 25°C) and to toothbrush abrasion (2min immersion in slurry; 50 strokes; 200g). At the end of the experiment, surface loss (µm; mean ± SD) was quantified profilometrically with an optical, contactless measuring device. The effects of the two covariables "tooth" and "toothpaste" were analyzed by non-parametric ANOVA and variables with significant effects were tested by Wilcoxon tests. *Results:* Deciduous teeth (dt) showed significantly higher SL than permanent teeth (PT) in all groups ($p<0.001$). The mean values of SL of each group were: G1 PT 18.18 (±3.98), dt 25.65 (±9.21); G2 PT 14.76 (±2.82), dt 18.11 (±3.92); G3 PT 12.62 (±5.29), dt 15.61 (±6.70); G4 PT 17.12 (±2.24), dt 23.41 (±7.9); G5 PT 13.24 (±1.29), dt 18.28 (±8.96). *Conclusions:* In permanent teeth AmF-NaF-SnCl anti-erosion toothpaste showed the best preventive effect against erosion-abrasion cycles. In deciduous teeth NaF toothpaste, AmF-NaF-SnCl anti-erosion toothpaste and NaF anti-erosion toothpaste for children showed similar effect.

Key-words: Erosive Tooth Wear, Permanent Teeth, Deciduous Teeth, Toothpaste.

INTRODUCTION

Erosive tooth wear (ETW) is a condition with growing concern in research and daily practice (LUSSI and CARVALHO, 2014). Surface loss, one of the major consequences of ETW, is an irreversible condition that can cause complications such as tooth sensitivity, altered aesthetics and loss of occlusal vertical dimension (TAJI, SEOW, 2010).

Toothpastes are an important part of oral hygiene practices. They are the main vehicle for fluoride compounds, and they have an established preventive effect on both dental caries and erosive tooth wear. For erosive tooth wear prevention, the active ingredients from toothpastes are supposed to increase the acid resistance of tooth surface and pellicles (MAGALHÃES *et al,* 2014; CARVALHO *et al*, 2014). Apart from conventional sodium fluoride toothpastes, which presented a range between 0 to 37% more preventive effects than placebo toothpaste, other compounds have been investigated. Some polyvalent metal ions, such as stannous or titanium, in combination to sodium or amine fluorides have shown to produce more acid-resistant precipitates. Stannous-containing toothpastes, when applied as slurries, showed protection effect from 55 to 95%, although this effect decreased with toothbrush abrasion (CARVALHO *et al*, 2014). This reduction was explained by the physical action of the abrasive particles, which interfere on stannous precipitation.

It is important to diagnose the first erosive lesions still during childhood, in the primary dentition, once ETW in the primary dentition is predictive for ETW in permanent dentition (GANSS *et al*, 2001; CARVALHO *et al*, 2014). The enamel of deciduous teeth is significantly softer than permanent enamel, so it is expected to be more susceptible to erosion and abrasion challenges. Although some laboratory studies found contradictory results, especially studies that are not associated to abrasion cycles, epidemiological surveys demonstrated that deciduous teeth are less wear resistant than permanent teeth (KREULEN *et al,* 2010; CARVALHO *et al*, 2014).

Some studies have already evaluated the association of erosive and abrasive challenges in permanent and deciduous teeth. Correr *et al* (2007) tested neutral and acidic toothpastes slurries in an oral wear simulator, and they observed that deciduous enamel presented more wear than permanent teeth. Attin *et al* (2007) found only negligible differences between the two types of teeth when erosion or erosion and abrasion challenges were performed. In a previous study of our research group, deciduous teeth presented higher surface loss after initial erosion-abrasion cycles than permanent teeth, in four out of five different toothpastes tested. Only with an AmF-NaF-

SnCl anti-erosion toothpaste (elmex - GABA®) deciduous and permanent teeth did not present significantly different surface loss values (ASSUNÇÃO *et al*, unpublished data).

These contradictory results found in laboratory studies could be explained by different protocols (acid exposures, brushing times, type of teeth) and different surface loss measuring methods used. One of the most used methods for surface loss assessment in laboratory studies is surface profilometry. This method quantifies the loss of dental tissue in relation to a non-treated reference area. The non-contact profilometry uses a probe with laser light (white or blue), which has a high precision, detecting surface loss which exceeds about 0.4µm (SCHLUETER, *et al,* 2011; ATTIN and WEGEHAUPT, 2014).

There are several products available in the market, especially toothpastes that claim to provide erosive tooth wear protection, and some of them are indicated for children. However, regarding the contradictory results previously mentioned, the effect of these different toothpastes on deciduous teeth are not yet fully understood. Therefore, the aim of the present study was to evaluate the preventive effect of different toothpastes on surface loss (SL) in an erosion/abrasion model and to compare this effect between permanent and deciduous teeth.

MATERIALS AND METHODS

Experimental design and groups

The experimental set up is shown in Figure 1. We tested five different toothpastes, and their composition is described in Table 1.

We used a total of 30 cycles in the present study. Data on the first five cycles are presented in Assunção *et al,* unpublished data. The samples used in this study were the same as those from this previous research, but the experiment was furthered until 30 cycles. The sample size calculation considered the balanced one-way analysis of variance power calculation, with level of significance set to 0.05, and power at 80%, and indicated a minimum number of 16 in each group. On our first study, 20 samples of each group were submitted to five erosion/abrasion cycles, three samples were then used for SEM evaluation, and the remained samples (n=17) continued the erosion/abrasion cycles for this study.

Sample preparation

Sound human deciduous molars and (permanent) premolars were randomly selected from a pool of extracted teeth. The present experiment was carried out in accordance with the approved guidelines and regulations of the local ethical committee

(Kantonale Ethikkommission: KEK). The teeth were extracted by dental practitioners in Switzerland, pooled into two groups of deciduous and permanent teeth, and stored in chloramine until the time of the experiment. Before the donation, the patients (and parents, in case of deciduous teeth) were informed about the use of their teeth for research purposes. Oral consent was obtained by all patients (and parents) for the use of the teeth in research.

The crowns were separated from the roots using Isomet® Low Speed Saw (Buehler, Düsseldorf, Germany), cross- sectioned into lingual and buccal sides and embedded into resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany). To obtain a flat, parallel, highly smooth enamel surface the samples were grinded and polished. Under constant tap water cooling, the samples were abraded using a Knuth Rotor machine (LabPol 21, Struers, Copenhagen, Denmark) with silicon carbide paper discs (grain size 1000, 2400, 4000) for 30 seconds each grain. Later, samples were polished for 60 seconds with 3 µm diamond abrasive on Struers polishing cloth under constant cooling (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers, Copenhagen, Denmark). Between each abrading and polishing step, as well as after the final polishing, all samples were sonicated for 1 minute in distilled water. This procedure removed a 200 µm layer of the surface enamel. All samples were stored in a mineral solution until the start of the experiment (1.5 mmol/l CaCl2, 1.0 mmol/l KH2PO4, 50 mmol/l NaCl, pH = 7.0) (ZERO et al, 1990).

Immediately prior to the experiment, the samples were further polished for 60 seconds with 1 µm diamond abrasive on Struers polishing cloth under constant cooling (LaboPol-6, DP-Mol Polishing). Then, to create a reference area of sound enamel (not submitted to erosion/abrasion cycles), half of the enamel surface was protected with a viscous methacrylate-based utility resin (LC Block-Out Resin - Ultradent®, Utah - USA). This resin was applied carefully with a probe and light-cured for 30 seconds (470 mW/cm^2, Ortholux LED Curing Light, 3M Unitek, CA, EUA). No acid etching or adhesive was used, to allow easy removal of the resin once the experiment ended.

Erosion/abrasion cycles

The samples were submitted to thirty erosion/abrasion cycles. Throughout the experiment (between experimental cycles), the samples were kept in a 100% humidity chamber. Each cycle consisted of incubating the samples in artificial saliva (CaCl22H2O; KH2PO4; NaCl; KCl; Mucin – pH7.4; NEWBY, 2006) at 37°C (NEWBY, 2006), for a period of 1 hour, then washing them with deionized water to remove the excess solution from their surfaces, and gently drying them with oil free air. The erosive challenge consisted in individually immersing the samples for 3 minutes in 30 mL of 1%

citric acid (pH 3.6) at 25°C, under constant agitation (70 rpm). Afterwards, the samples were submitted to abrasion, by individually immersing each sample in toothpaste slurry for 2 min, and, within this time, the samples were brushed in a brushing machine (50 strokes; 200g force). The toothbrushes used were the American Dental Association reference flat trim brush. Toothpaste slurries were prepared daily by mixing 25g of toothpaste with 50g of artificial saliva.

Enamel surface loss measured by Laser Profilometry

After the erosion-abrasion cycles, we carefully removed the resin covering half of the enamel surface with a scalpel, thus exposing the sound enamel layer (reference area). This reference area was checked in an optic microscope (40x magnification) with regard to defects or resin remnants. Profilometric readings, measuring the step height from the sound layer to the eroded-abraded layer were taken. The measurement was performed by a blind-examiner, with an optical, contactless measuring device (MicroProf; Fries Research & Technology GmbH, Bergisch-Gladbach, Germany; sensor H0). On each specimen three traces were made with a total length of 2 mm (200 pixels, 1 mm on reference and 1 mm on experimental area) at 200 µm intervals. Traces were interpreted with special software (Mark III, Fries Research & Technology GmbH). Regression lines were then constructed at both the reference and the experimental areas, with a length of 0.5 mm each. The vertical distance between the regression lines was defined as the amount of surface loss (µm). Sample's surface loss was expressed as the mean of three traces.

Statistical analyses

Non-parametrical methods were applied due to non-normally distributed data. The absence of normality was checked computing QQ plots as well as by the *p* value according to Shapiro Wilk's test ($p<0.0001$). The effects of the two covariables "tooth" and "toothpaste" on the surface loss was analyzed with a non-parametric ANOVA. Variables with significant effects in the non-parametric ANOVA were tested afterwards with exact Wilcoxon rank sum tests. Level of significance was set to 0.05. All statistical results were calculated with R (Versions 3.1.0 and 3.2.2).

RESULTS

Both variables "tooth" and "toothpaste" were highly significant on the global analysis with ANOVA (<0.0001). Comparing the surface loss between permanent and deciduous teeth, there was statistical differences in all groups, and deciduous teeth presented significantly greater surface loss (Table 2).

Figure 2 shows the distribution of surface loss on both permanent and deciduous teeth. Permanent teeth showed generally lower surface loss values, and also smaller standard deviation.

In permanent teeth, placebo (G1) and SnF (G4) toothpastes presented the higher SL values, and they were not statistically different from each other. Other groups presented significantly lower SL, and the lowest SL value was observed in the AmF-NaF-SnCl anti-erosion toothpaste group (G3) (Figure 2, white boxes).

Similarly in deciduous teeth, placebo (G1) and SnF (G4) toothpastes presented the greatest SL values, and they were not significant different from each other. The other toothpaste groups presented significantly lower SL, and they were not statistically different from each other (Figure 2, gray boxes).

On SEM images (Figure 3), the effect of 30 erosion/abrasion cycles on enamel surface of permanent and deciduous teeth according to different toothpastes could be observed. In both permanent and deciduous teeth, the placebo toothpaste was not able to maintain the enamel prisms structure. On the other hand, AmF-NaF-SnCl anti-erosion (G3), SnF (G4) and NaF anti-erosion for children (G5) toothpastes showed a better preventive action on ETW. No distinguished aspects could be observed between permanent and deciduous teeth on lower (400x) or higher magnification (1000x).

On figure 4 one example of the step produced between the sound reference and the experimental areas could be observed, on a deciduous tooth sample, submitted to 30 erosion/abrasion cycles and treated with SnF toothpaste.

DISCUSSION

The prevalence of erosive tooth wear has increased in the past years, as a reflection of a generalized growth in acid food and beverages consumption, and has changed the perception of this condition in research and daily dental care practice (LUSSI and CARVALHO, 2014). It is important to maintain an increased awareness of the management of ETW in long-term health of both deciduous and permanent dentition, once ETW is a progressive and chronical condition. The amount of surface loss that is considered acceptable depends on teeth lifespan, and could vary from deciduous to permanent teeth (TAJI, SEOW, 2010).

Until now just three other studies have evaluated if permanent and deciduous teeth have different susceptibility to erosion-abrasion (CORRER *et al,* 2007; ATTIN *et al,* 2007, ASSUNÇÃO *et al, unpublished data*). Attin *et al* (2007) observed no differences between deciduous and permanent teeth, when erosion and abrasion challenges were associated. In contradiction, when Correr *et al* (2007) compared erosive tooth wear in both types of teeth using neutral and acidic slurries in an oral wear simulator, the authors observed that deciduous teeth presented more wear than permanent enamel. Additionally, in a previous study (Assunção *et al*, unpublished data), the same erosion-abrasion protocol was used were reported. It was observed that deciduous teeth generally presented greater surface loss than permanent teeth after initial erosion-abrasion challenges. In the present study, it was showed that this pattern continued after severe erosion-abrasion challenges, and deciduous teeth presented significantly more surface loss than permanent teeth for all toothpaste groups (Table 2, Figure 2). These differences may be explained in regards to the different times of acid exposure, slurry composition and brushing cycles.

In the study by Attin et al. (2007), the authors used 1 min erosive challenge followed by an abrasive challenge of 100 toothbrush strokes, whereas in the present study 3 min erosive challenge and only 50 toothbrush strokes were used. Some studies (LIPPERT et al. 2004; LUSSI et al. 2000) with relatively short demineralizing periods (up to 5 min) have shown no differences in the demineralization between deciduous and permanent teeth. It is, therefore, possible that the erosive challenges used in the above mentioned studies (up to 3 min) caused similar changes in surface hardness (similar amounts of surface softening) between deciduous and permanent teeth. However, deciduous teeth are generally softer than permanent teeth at baseline, so they will rather be more vulnerable to the abrasive actions of the toothbrush. Therefore, after a 3 min erosive challenge used in the present study, the 50 toothbrush strokes was able to remove different amounts of enamel from deciduous and permanent teeth. Consequently, it was possible to observe differences between deciduous and permanent teeth. This is also in accordance with the suggestions previously made by Hunter et al. (2000), that erosive wear in deciduous teeth appears rather over time and/or with increasing presence of the acid. In addition, we further propose that the ratio of acid exposure: toothbrushing time is also an important factor when assessing different enamel substrates or toothpastes.

In this context, the association of abrasion challenges is a key factor when differences in toothpastes treatment are evaluated. Magalhães *et al* (2007) evaluated the influence of fluoride dentifrice on brushing abrasion of eroded enamel. The authors showed a protective effect the fluoride dentifrice, when compared to a non-fluoride dentifrice. The mode of action of these toothpastes is due to the precipitation of a CaF_2 – like layer onto the enamel surface. This is in accordance with our results, where the NaF toothpaste presented better results than the placebo toothpaste. This CaF_2 – like layer, however, is not stable, and it is readily soluble in acidic challenges, so better results are observed when fluoride is associate with polyvalent metal ions, especially titanium and stannous. In our study, the AmF-NaF-SnCl anti-erosion toothpaste (G3) had the lowest surface loss values in permanent and deciduous teeth. This is in accordance to previous studies (HOVE *et al,* 2014; GANSS *et al*, 2012; GANSS *et al,* 2011; SCHLUETER *et al,* 2013; CARVALHO and LUSSI, 2014). The Sn-containing salts form a more resistant layer on the enamel surface (SCHLUETER *et al*, 2009), and it can interact with the salivary pellicle (HARA *et al*, 2013), or be incorporated into the demineralized enamel surface (SCHLUETER *et al,* 2009). Remarkably, this toothpaste showed similar surface loss in deciduous teeth as the NaF toothpastes (G2 and G5). Although the mode of action of the Sn-containing toothpaste was meticulously outlined by Schlueter et al (2009), the authors have mostly focused on permanent teeth. Regarding deciduous teeth, our results suggest a slightly different mode of action for Sn-containing products, where they provide slightly less protection for deciduous teeth in comparison to permanent teeth. However, further studies are still necessary to fully elucidate the interaction of Sn with deciduous enamel.

Moreover, our results also suggest that Sn might not the most important factor in the protection against erosion and abrasion. SnF toothpaste (G4) did not provide a significantly better protection in both deciduous and permanent enamel, presenting similar results as the placebo.

Although Sensodyne Repair&Protect (SnF toothpaste, G4) has SnF as active ingredient, our study showed that it caused considerable loss of enamel surface. In this regard, Sn2+ was not capable of promoting an effective protection for enamel against erosive tooth wear. This fact could be partially explained by the higher RDA value (119), compared to AmF-NaF-SnCl anti-erosion toothpaste (40-60). In other words, the SnF toothpaste probably did not form a protective layer on the enamel surface, but rather removed more of the softened enamel layer. On the other hand, this SnF toothpaste was conceived for use on dentine, and other studies have shown that it is capable of forming a protective layer over the dentine surface, and of occluding the dentinal tubules (AMAECHI *et al,* 20015; LOPES *et al,* 2015). Hence, this toothpaste will probably play a more important role in dentine hypersensitivity.

The better protection observed in the AmF-NaF-SnCl anti-erosion toothpaste could then be related to the chitosan bio-polymer (CARVALHO and LUSSI 2014), which interacts with the Sn, improving the anti-erosion effect of the toothpaste (SCHLUETER *et al,* 2013; SCHLUETER *et al,* 2014; GANSS *et al,* 2012; GANSS *et al,* 2011).

CONCLUSIONS

There are several products available in the market that claim better erosive tooth wear protection, some of them are even indicated for children. Despite this fact, little is know on how these toothpastes behave on deciduous teeth, and this is one of the studies that investigated the preventive effect of some commercial anti-erosion toothpastes on deciduous teeth. We showed that deciduous teeth presented significantly higher surface loss than permanent teeth, with all toothpastes tested. Furthermore, these toothpastes showed different preventive effects between permanent and deciduous teeth. The AmF-NaF-SnCl anti-erosion toothpaste (G3) showed the best preventive effect against erosion-abrasion cycles in permanent teeth. In deciduous teeth, the NaF toothpaste (G2), AmF-NaF-SnCl anti-erosion toothpaste (G3) and NaF anti-erosion toothpaste for children (G5) showed better protection in relation to placebo, but similar effects between themselves.

ACKNOWLEDGMENTS

The authors appreciate the support of CAPES Foundation (Ministry of Education of Brazil) for the PhD student scholarship, and the support from the Department of Preventive, Restorative and Pediatric dentistry, University of Bern. We are grateful to Gabriel Fischer and Prof. J. Hüsler from the Institute of Mathematical Statistics and Actuarial Science, University of Bern, for all statistical analysis. The toothpastes used in the present study were provided by GABA-Colgate and GlaxoSmithKline. We declare that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors were involved on designing the experiments, critical analysis of the results and writing the paper. Data collection was performed by CMA and NS. Furthermore, the authors are very grateful to Barbara Beyeler, Brigitte Megert, Isabel Hug, and Samuel Furrer, members of the Department of Preventive, Restorative and Pediatric Dentistry, University of Bern.

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Figures and Tables

Table 1. Description of groups and toothpastes ingredients used in the study.

Group	Active ingredient	RDA values	Brand / Manufacturer
G1 – Negative control	None	80-100	Placebo toothpaste (GABA – Colgate)
G2 – NaF toothpaste Positive control	NaF (1500ppm)	120	Crest® (P&G)
G3 – AmF-NaF-SnCl anti-erosion toothpaste	AmF-NaF-SnCl (1400ppm)	40-60	elmex Erosion Protection® (GABA – Colgate)
G4 – SnF toothpaste	SnF_2 anhydrous (1100ppm)	119	Sensodyne Repair&Protect® (GSK)
G5 – NaF anti-erosion toothpaste for children	NaF (1450ppm)	30-40	Sensodyne ProNamel for children® (GSK)

Table 2. Mean values of surface loss in permanent and deciduous teeth, according to different groups. Exact Wilcoxon rank sum tests (n=17)

Group	**Surface Loss (µm)**		
	Permanent Teeth	**Deciduous Teeth**	
	Mean (SD)	Mean (SD)	*p* value
G1 – Negative control	18.18 (±3.98)	25.65 (±9.21)	0.003
G2 – NaF toothpaste Positive control	14.76 (±2.82)	18.11 (±3.92)	0.018
G3 – AmF-NaF-SnCl anti-erosion toothpaste	12.62 (±5.29)	15.61 (±6.70)	0.020
G4 – SnF toothpaste	17.12 (±2.24)	23.41 (±7.49)	<0.000
G5 – NaF anti-erosion toothpaste for children	13.24 (±1.29)	18.28 (±8.96)	0.020

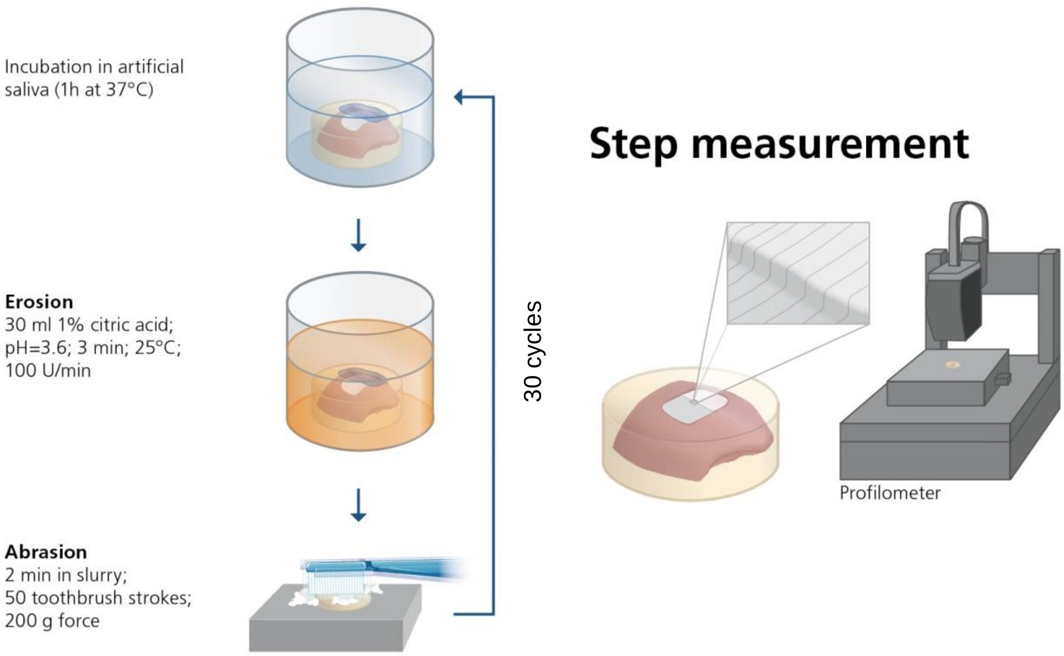

Figure 1. Study design.

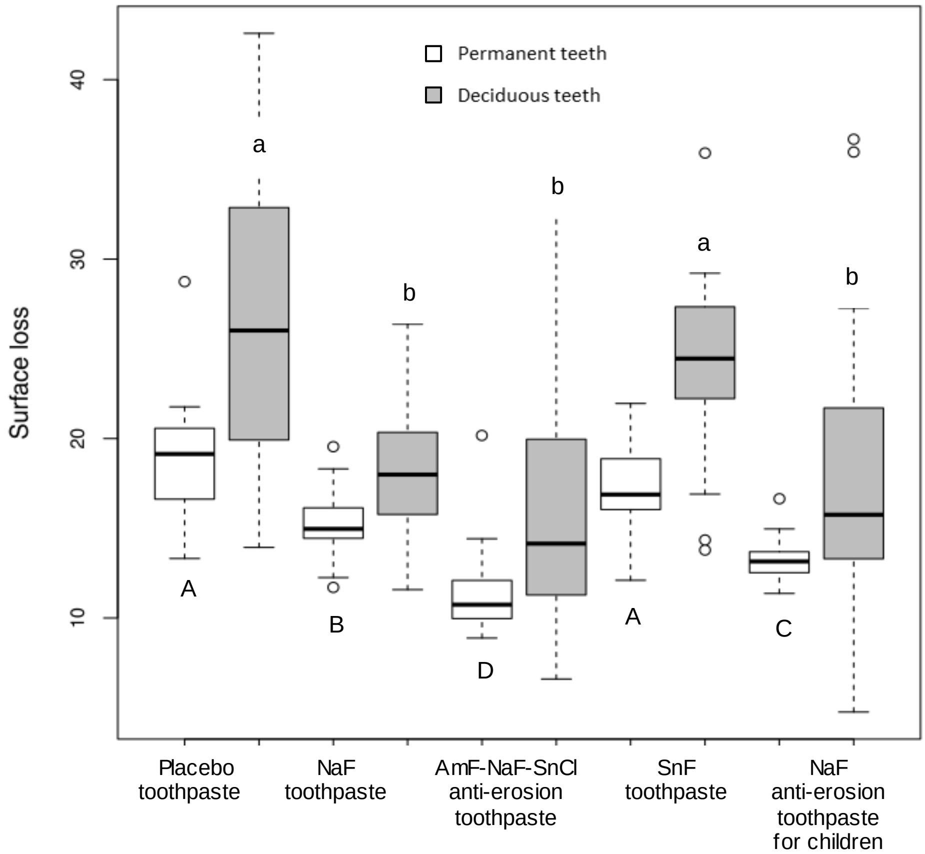

Figure 2. Mean values of surface loss in permanent and deciduous teeth according to different toothpastes. (n=17) Statistically significant differences are shown with different letters: lower case letters represent deciduous teeth; upper case letters represent permanent teeth.

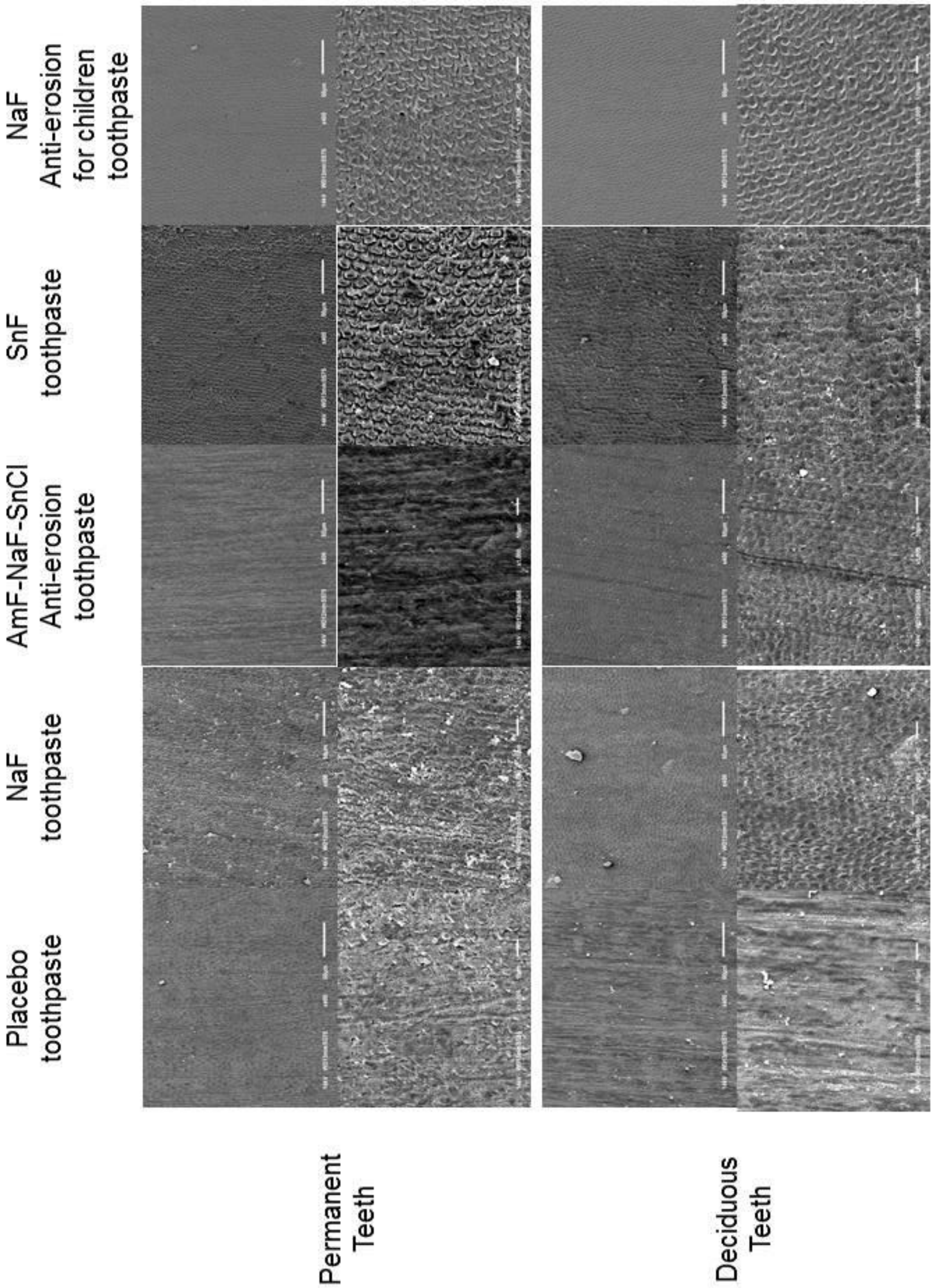

Figure 3. SEM images of permanent and deciduous teeth after 30 erosion/abrasion cycles, according to toothpaste groups in lower (400x) and higher (1000x) magnification.

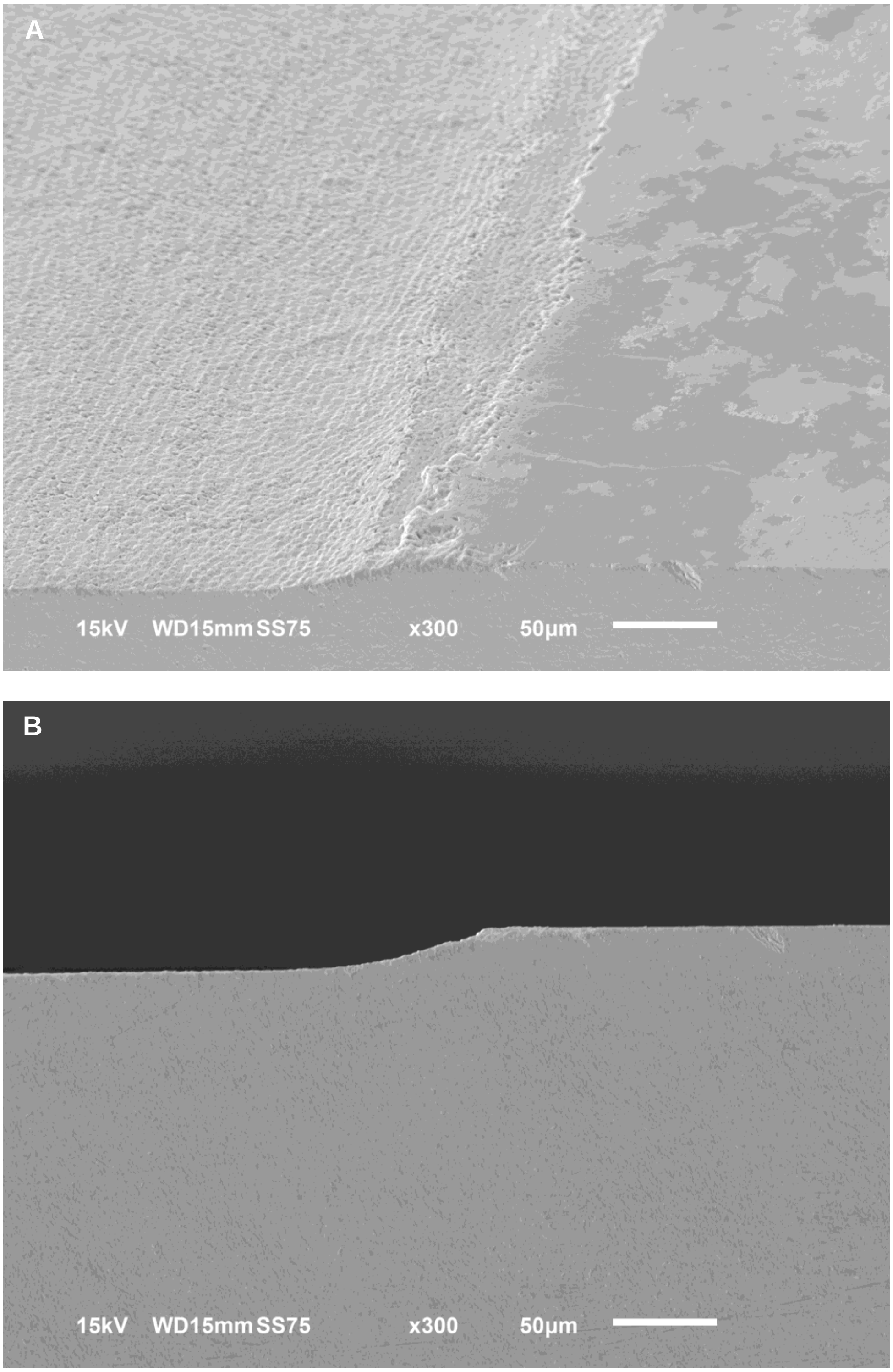

Figure 4. SEM images of the step between the reference sound enamel area and the experimental area, submitted to erosion/abrasion cycles. Deciduous teeth, treated with SnF toothpaste (Sensodyne Repair&Protect, GSK). (A) Image at 300x magnification, 30º. (B) Image at 300x magnification, cross-sectional view.

CONSIDERAÇÕES FINAIS

Este é um dos primeiros estudos que buscou investigar o efeito de dentifrícios disponíveis no mercado na prevenção de desgaste dentário erosivo em dentes decíduos e que comparou esse efeito aos dentes permanentes.

Considerando os resultados do protocolo inicial, dentes decíduos apresentaram menor dureza superficial, maior intensidade superfície de reflexão e maior perda de superfície calculada do que os dentes permanentes durante o experimento. Nos resultados de perda de superfície calculada, pode-se observar que essa perda foi progressiva ao longo dos cinco ciclos. Em dentes permanentes a maior perda de superfície calculada foi encontrada no grupo do dentifrício de NaF. Os dentifrícios placebo e anti-erosão contendo AmF-NaF-SnCl apresentaram o melhor efeito preventivo, com menor perda de superfície calculada ao final do experimento. Em dentes decíduos os valores significativamente maiores de perda de superfície foram apresentados pelos grupos dos dentifrícios contendo NaF e SnF. O melhor efeito preventivo foi representado pelos dentifrícios placebo, anti-erosão com AmF-NaF-SnCl e anti-erosão com NaF para crianças. Considerando todas variáveis estudadas, pode-se concluir que o dentifrício anti-erosão contendo NaF para crianças apresentou o melhor efeito preventivo.

Os resultados surpreendentes na perda de superfície apresentados pelo dentifrício placebo podem ser explicados por algumas propriedades como a abrasividade relativa em dentina (RDA). Mesmo apresentando menores valores de microdureza superficial, a camada amolecida não foi totalmente removida pela ação abrasiva do dentifrício placebo, que apresentou valor menor de RDA quando comparado aos dentifrícios com NaF e SnF. Esse dado aponta para outros aspectos em relação aos dentifrícios que podem ser mais profundamente explorados em futuros estudos. Os valores de RDA foram fornecidos pelos fabricantes, porém se referem a abrasividade testada em dentina, não em esmalte. A ação desses abrasivos pode ser diferente em esmalte e também entre dentes permanentes e decíduos.

Nos resultados do protocolo avançado, os dentes decíduos apresentaram perda de superfície significativamente maior do que os dentes permanentes em todos os dentifrícios testados. Esses dentifrícios mostraram efeito preventivo diferente em dentes permanentes e decíduos. O dentifrício anti-erosão com AmF-NaF-SnCl apresentou o melhor efeito preventivo contra ciclos de erosão-abrasão nos dentes permanentes. Em dentes decíduos o dentifrício com NaF, anti-erosão contendo AmF-NaF-SnCl e anti-erosão contendo NaF para crianças mostraram efeito preventivo semelhante.

Outros ingredientes, ativos ou não, dos dentifrícios devem ser mais detalhadamente estudados, em busca da elucidação dos mecanismos de prevenção ao desgaste dentário erosivo. O delineamento de estudos que incluam a abrasão deve ser priorizado, uma vez que a ação mecânica da escovação durante o uso do dentifrício faz parte da rotina de higiene bucal dos pacientes.

Neste estudo, dentes decíduos e permanentes responderam de forma diferente aos mesmos ciclos de erosão/abrasão, desta forma, um dentifrício que tem boa ação preventiva contra o desgaste dentário erosivo em dentes permanentes não necessariamente pode ser indicado para uso em pacientes com dentição decídua.

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APÊNDICE

Declaração da Universidade de Berna a respeito dos dentes utilizados nesta tese:

b
UNIVERSITÄT
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Klinik für Zahnerhaltung, Präventiv-
Und Kinderzahnmedizin

Bern, 25th February 2016

To whom it may concern

I herewith confirm that the teeth used in the study carried out by Cristiane Meira Assunção were donated to the University of Bern with the consent of the patients. These teeth are from a pooled bio-bank, and they are considered irreversibly anonymized. Studies using this kind of specimens do not need prior permission from an Ethical Committee in Switzerland.

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