Faculdade de Odontologia de Araçatuba

Universidade Estadual Paulista “Julio de Mesquita Filho”

Efeito de fluoretos presentes em dentifrícios na prevenção da erosão de restaurações de resina composta e cimento de ionômero de vidro de esmalte e dentina erodidos

ARAÇATUBA - SP

2019

Mariana Dias Moda

Efeito de fluoretos presentes em dentifrícios na prevenção da erosão de restaurações de resina composta e cimento de ionômero de vidro de esmalte e dentina erodidos

Tese apresentada à Faculdade de Odontologia, Campus de Araçatuba, da Universidade Estadual Paulista "Júlio de Mesquita Filho", como parte integrante dos requisitos para obtenção do título de DOUTOR, pelo Programa de Pós-Graduação em Odontologia, área de Concentração em Dentística.

Orientadora: Profa. Ass. Dra. Ticiane Cestari Fagundes Tozzi

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Moda, Mariana Dias.

M689e Efeito de fluoretos presentes em dentifrícios na prevenção da erosão de restaurações de resina composta e cimento de ionômero de vidro de esmalte e dentina erodidos / Mariana Dias Moda. – Araçatuba, 2019

107 f. : il. ; tab.

Tese (Doutorado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba

Orientadora: Profa. Ticiane Cestari Fagundes Tozzi

1. Abrasão dentária 2. Cimentos de ionômeros de vidro 3. Fluoretos de estanho 4. Erosão dentária 5. Resinas compostas I. T.

Black D2

CDD 617.6

Claudio Hideo Matsumoto – CRB-8/5550

Dedicatória

Dedicatória

A Deus

Pela presença onipotente em todos os momentos da minha vida. A essa força tão divina e presente, que acalenta e impulsiona nos momentos mais difíceis. Obrigada meu Deus, por tudo!!!

Aos meus pais,

Marcos e Heloísa,

Pela força, dedicação, paciência e pela imensa contribuição para que eu pudesse realizar muitos sonhos e alcançar objetivos. Sou extremamente grata por tudo que fizeram em minha vida. Obrigada pelo maior presente que vocês puderam me dar: a vida! Amo vocês!!!

À minha irmã Lígia,

Não tenho palavras para descrever o quanto a admiro. Muitas lembranças de infância que estarão sempre vivas. Estarei sempre com você, conte sempre comigo! Te amo!

Aos meus avós,

Antonio, Neide, Antenor e Elza,

Alguns infelizmente não estão mais presentes... eternas e doces lembranças que guardarei sempre comigo. Saudade eterna!

Agradecimentos Especiais

Agradecimentos Especiais

À minha orientadora,

Profa. Ticiane Cestari Fagundes Tozzi

Já convivemos há 6 anos, desde quando me co-orientou no mestrado, e desde então fizemos muitas parcerias, trabalhos e projetos. Sou muito grata a você pela oportunidade de trabalhar com vários de seus alunos de iniciação científica, isso certamente me fez aprender muito. Além de toda a sua competência e responsabilidade com a pesquisa. Te admiro muito. Finalizo meu doutorado com o coração cheio de gratidão.

Muito Obrigada!!!

Ao Prof. Paulo Henrique dos Santos

Foi meu orientador no mestrado e tenho muito orgulho de ter convivido com um professor tão competente e humilde. Quero agradecê-lo por todos os ensinamentos, de prezar sempre pela ética em pesquisa, pela paciência e sobretudo pelos belos trabalhos que realizamos juntos! Uma honra ter sido sua orientada.

Muito Obrigada!!!

Ana Carolina Souza Barboza Haber

Ana Carol, o que dizer de você! Te conheci quando ainda era aluna de iniciação científica e, desde então, sempre convivemos juntas. Gosto muito de você, pelo coração que você tem e sempre tem algo de bom a dizer! Conte comigo sempre que precisar, pois você ganhou uma amiga para a vida toda. Torço muito pelo seu sucesso!! Adoro você!

Lívia Maria Alves Valentim da Silva

Lívia! Fico sem palavras para descrever o quanto gosto e torço por você. Que é uma pessoa tão doce, competente e sobretudo tão humilde, pelo grande conhecimento que já possui. Obrigada pela convivência, eu adoro você. Sentirei muitas saudades!!

Marjorie de Oliveira Gallinari

Marjô, querida, sempre te admirei e a tive como exemplo aqui na pós. Lembro de quando iniciamos a nossa caminhada juntas no mestrado... Você tem competência de sobra e principalmente humildade! Muiiito sucesso a você nessa nova etapa. Te adoro!!!

Janaína Cardoso Moreira

Jana quantas histórias hein! Estamos juntas desde 2014, uma pena ter nos deixado no doutorado, mas foi por uma boa causa e está numa cidade que eu guardo no coração... ê São José do Rio Preto! Saiba que torço muito por você e pelo seu sucesso como docente, que já está tendo!! Adoro você!

Morganna Borges de Almeida Souza

Morgs querida! Te conheci também quando era aluna de iniciação científica, sempre muito educada e gentil! E agora será mamãe!! Fiquei muito feliz por você, querida! Sucesso em tudo na sua vida! Adoro você!!

Renata Parpinelli de Oliveira

Rê querida! Trabalhamos juntas no seu projeto de iniciação científica e na minha tese. Quero te dizer que foi muito bom conviver com você, que é uma menina tão bem humorada e doce! Te desejo todo sucesso desse mundo! Conte comigo sempre que precisar! Adoro você!

Jorge Orlando Cuellar Mansilla e Fábio Ricardo Macedo

Queridos, muito obrigada pela amizade, pelos conselhos, pela troca de experiências, risadas, enfim por tudo! Vocês foram essenciais para que eu pudesse evoluir nesse último ano. Gracias!!

As queridas alunas de iniciação científica: *Érika Mayumi Omoto, Laryssa de Castro Oliveira, Luana Ribeiro do Vale, Morgana Fernandes de Freitas Farina, Renata Parpinelli de Oliveira, Taísa Zago de Freitas.* Saibam que foi muito bom conviver com vocês, contem sempre comigo!

Aos colegas e amigos de pós-graduação,

Ana Carolina Souza Barboza Haber, Ana Teresa Maluly Proni, Bruna de Oliveira Reis, Caio César Pavani, Diego Felipe Mardegan Gonçalves, Fábio Martins Salomão, Fabrício

Daniel Finotti Guarnieri, Fernanda de Souza e Silva Ramos, Glívia Queiroz Lima, Henrico Badaoui Strazzi Sahyon, Ísis Almela Endo Hoshino, Janaína Cardoso Moreira, Lara Maria Bueno Esteves, Laura Molinar Franco, Lívia Maria Alves Valentim da Silva, Lucas Silveira Machado, Marjorie de Oliveira Gallinari, Morganna Borges de Almeida Souza, Sibele de Alcântara, Thaís Yumi Umeda Suzuki, Vanessa Rahal.

Agradecimentos

Agradecimentos

À Faculdade de Odontologia do Campus de Araçatuba - UNESP, ao **diretor Prof. Dr. Glauco Issamu Miyahara e ao vice-diretor Prof. Dr. Alberto Carlos Botazzo Delbem** pela gentileza e atenção com que nos recebem.

Ao coordenador do curso de Pós-Graduação em Odontologia, Prof. Dr. André Luiz Fraga Briso, pelo seu empenho para que possamos ter uma formação de pós-graduação de excelência. Tenho grande admiração pelo senhor! Muito obrigada!

O presente trabalho foi realizado com apoio da **Cordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001,** através da concessão da bolsa de doutorado, de suma importância para que esse trabalho fosse realizado. Muito Obrigada!

Aos professores da banca de qualificação, representada pelo **Prof. Dr. Paulo Henrique Dos Santos e Prof. Dr. Wirley Gonçalves Assunção**. Muito obrigada por terem aceito nosso convite e contribuído sobremaneira para o meu crescimento.

Aos professores da banca examinadora, representada pelo **Prof. Dr. André Luiz Fraga Briso, Profa. Dra. Linda Wang, Prof. Dr. Juliano Pelim Pessan e Profa. Dra. Núbia Inocencya Pavesi Pini**, muitíssimo obrigada por terem aceito meu convite em participar deste momento tão especial e importante. Será uma honra tê-los em minha defesa. Muito obrigada!

Às funcionárias da Seção de Pós-Graduação, Cristiane Lui, Lilian Mada e Valéria Zagato, por todo o suporte, paciência e atenção com que nos tratam.

Aos bibliotecários, Ana, Cláudia, Cláudio, Denise por estarem sempre dispostos a nos ajudar. Obrigada por todo o valioso apoio prestado.

A todos professores do curso de Pós-Graduação, pela oportunidade de aprendizado constantes.

Aos alunos da turma de graduação e pós-graduação pelas experiências compartilhadas ao longo de todo o curso.

Aos professores da disciplina de Dentísitca,
Prof. Dr. André Luiz Fraga Briso, Prof. Dr. Renato Herman Sundfeld, Prof. Dr. Ricardo Coelho Okida, Prof. Dr. Sílvio José Mauro, Profa. Dra. Ticiane Cestari Fagundes. Muito obrigada por ter o privilégio de conviver e aprender com cada um de vocês. Em especial ao Prof. Dr. Ricardo Coelho Okida e, à minha orientadora, Profa. Dra. Ticiane Cestari Fagundes, pelo aprendizado que tive com vocês no acompanhamento às clínicas de graduação.

Aos funcionários do Departamento de Odontologia Restauradora, Carlos Suetake, Jorge Trevelim, e aqueles que não estão mais no convívio diário, mas que estiveram conosco durante muitos anos sempre nos ajudando muito. Elaine Cristina Francischini Ferreira, Nelci Vieira, Peterson Moura, por sempre estarem dispostos a nos ajudar, por fazer nossos dias mais agradáveis. Muito obrigada!

Ao Departamento de Odontologia Restauradora, a todos que fazem parte desse departamento, tornando um lugar agradável de se trabalhar e conviver. Sentirei muitas saudades!

Aos professores da disciplina de Materiais Dentários,

Profa. Dra. Aimeé Maria Guiotti, Profa. Dra. Maria Cristina Rosifini Alves Rezende e, em especial, ao querido Prof. Dr. Paulo Henrique dos Santos, pela oportunidade de ter acompanhado os alunos de graduação nas aulas teóricas e práticas ministradas. E pela valiosa oportunidade de ter podido ministrar aulas na sua disciplina, muito obrigada Professor Paulo Henrique dos Santos!

Aos Prof. Dr. Alberto Carlos Botazzo Delbem do programa de Pós-graduação em Ciência Odontológica, pela autorização para que eu pudesse utilizar o laboratório de Odontopediatria e por toda a ajuda em etapas laboratoriais indispensáveis para a realização deste trabalho. Muito obrigada!

Ao Laboratório de Microscopia Eletrônica, da Faculdade de Ciência e Tecnologia de Presidente Prudente – UNESP, pela disponibilidade na realização da microscopia eletrônica de varredura e análise de EDS, indispensáveis para este trabalho.

Ao Dr. André Assmann, pelo suporte e por todo o empenho para que pudéssemos realizar as análises de nanonudreza. Agradeço demais a você e ao Departamento de Física da **Universidade Estadual de Ponta Grossa (UEPG)** pela gentileza e disponibilidade!

Ao Dr. Leonardo Negri Furini pela realização das análises de microscopia Raman. Léo, muito obrigada a você e ao Departamento de Física da **Faculdade de Ciência e Tecnologia de Presidente Prudente – UNESP**, pela disponibilidade e toda a ajuda. Sucesso a você nessa nova fase! Você merece!

Ao CNPEM (Centro Nacional de Pesquisas em Energia de Materiais), em especial ao Llnano, sob a responsabilidade de Carlos Alberto Rodrigues Costa. Agradeço pela disponibilidade e gentileza para que pudéssemos realizar as análises de AFM. Muitíssimo Obrigada!

À minha querida **Universidade Federal do Paraná (UFPR)** da qual tenho muito orgulho de ter sido aluna egressa. Saudades imensas!

Epigrafe

Epígrafe

"O êxito da vida não se mede pelo caminho que você conquistou, mas sim pelas dificuldades que superou no caminho"

Abraham Lincoln

Resumo geral

MODA, M.D. **Efeito de fluoretos presentes em dentifrícios na prevenção da erosão de restaurações de resina composta e cimento de ionômero de vidro de esmalte e dentina erodidos** [Tese]. Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista “Júlio de Mesquita Filho”; Araçatuba, 2019.

Resumo Geral

O objetivo desse estudo foi avaliar o desgaste, as propriedades mecânicas, topografia e composição química dos substratos dentários e materiais restauradores após ciclo erosivo/abrasivo, utilizando diferentes dentifrícios. Foram utilizados 244 blocos, sendo 122 de esmalte e 122 blocos de dentina, medindo 4 x 4 mm, obtidos a partir de incisivos bovinos que foram cortados e polidos. Cada amostra continha um bloco de esmalte e um de dentina, entre os blocos foram confeccionadas restaurações com cimento de ionômero de vidro modificado por resina (CIVMR) e resina composta (RC). Após as restaurações, a hemiface de cada amostra foi recoberta com verniz ácido-resistente, afim de produzir o lado controle. Esses dois grupos foram subdivididos em três grupos, de acordo com o dentifrício utilizado no processo de abrasão: SF - sem flúor (controle negativo), NaF - com fluoreto de sódio 1450 ppm de F (controle positivo) e SnF_2 - com fluoreto de estanho 1100 ppm de F. O ácido cítrico a 0,05 M, pH= 3.2, foi utilizado nos ciclos de erosão, sendo realizados 4x/dia, 2 minutos cada, com intervalos de 1 hora entre cada ciclo. Os espécimes foram submetidos à abrasão (2x/dia, ao final do primeiro e último ciclo erosivo/dia), aplicando o *slurry* (1:3) sobre as amostras por 2 minutos, seguidos de 15 segundos de escovação por espécime (200 g por 15 s), ao longo de 5 dias. Na sequência, o verniz ácido resistente foi removido da hemiface de cada amostra e estas foram analisadas quanto ao desgaste das superfícies através de perfilometria (n=12), microdureza, apenas dos materiais restauradores (n =12), topografia por microscopia de força atômica (AFM) (n=2), nanodureza (H) e módulo de elasticidade (Er) (n=5), composição química através de energia dispersiva de raios-X (EDS) (n=3), microscopia Raman (n= 5). Os dados de perfilometria, microdureza dos materiais restauradores, (H / Er) e EDS e Raman foram submetidos a ANOVA dois fatores medidas repetidas e teste de Tukey ($p < 0,05$). Em relação às imagens de AFM foram analisadas apenas qualitativamente. O dentifrício NaF promoveu o maior desgaste nas superfícies dentinárias adjacentes ao CIVMR e RC. Apenas as interfaces adjacentes ao esmalte sofreram influência do dentifrício. Os mais baixos valores de microdureza foram observados para CIVMR quando se utilizou o dentifrício SnF_2 ($p < 0,05$). Em relação aos valores de H e Er, pode-se notar que não houve diferenças entre os dentifrícios ($p > 0,05$), apenas entre as superfícies dentro de cada dentifrício ($p < 0,05$). Em relação às superfícies controle e erodida, apenas RC manteve seus valores constantes após erosão (p> 0,05). Em relação à composição química, os substratos dentários erodidos mostraram menores concentrações de cálcio e fosfato, enquanto para a superfície do material ionomérico houve uma diminuição de flúor e aumento de cálcio para as superfícies erodidas; as superfícies de resina composta mostraram-se inalteradas em sua composição química após os desafios erosivos (p > 0.05). Os dentifrícios não foram capazes de promover diferença nas propriedades mecânicas das superfícies após ciclo erosivo-abrasivo. Entretanto, promoveram diferenças quanto ao desgaste, composição química e topografia das superfícies, à exceção das superfícies de resina composta.

Palavras – chave: Abrasão dentária. Cimentos de ionômeros de vidro. Fluoretos de estanho. Erosão dentária. Resinas compostas.

Listas &
Sumário

Listas de Figuras

Capítulo 1

Capítulo 2

Listas de Tabelas

Capítulo 1

Capítulo 2

Listas de abreviaturas, símbolos e siglas

α = nível de significância / significance level
ºC = graus Celsius / degree Celsius
% = porcentagem / percentage
> = maior / higher
< = menor / lower
= igual / equal
µm = micrometro / micrometer
AFM = Microscopia de força atômica / Amomic Force Microscopy
ANOVA = Análise da variância
CEP = Comitê de Ética e Pesquisa
CR = Composite resin
DOM = Desmineralised organic matrix
Dr = Doutor
Dra = Doutora
EDS = Espectroscopia de energia dispersiva de raios-X / dispersive energy x-ray spectroscopy
ETW = Erosive tooth wear
g = grama / grams
MEV = Microscopia Eletrônica de Varredura
mg = miligrama
mm = milímetro/ milimeter (unidade de medida equivalente a 10^{-3m})
min = minuto / minute
NaF = fluoreto de sódio/ sodium fluoride
RDA= abrasividade relativa da dentina/ relative dentin abrasivity
RMGIC = Resin-modified glass ionomer cement
SEM = Scanning electron microscopy
SF = sem flúor
SnF_2 = fluoreto de estanho/stannous fluoride
UNESP = Universidade Estadual Paulista “Julio de Mesquita Filho”
WF = without fluoride

Sumário

General Introduction

General Introduction

Erosion tooth wear (ETW) has become an important topic in dental research over the past decade (SCHLUETER et al., 2019), their nomenclature was better standardized by these authors being that the definition of tooth wear includes all noncarious lesions, e.g. attrition, abrasion and abfraction. In contrast, erosive tooth wear is defined when the erosive process is the primary cause of injury (SCHLUETER et al., 2019). Particularly, ETW is attributed to the damage of chemical-mechanical process through of cumulative tooth surface loss caused by the intrinsic or extrinsic acids (SCHLUETER et al., 2019; MARRO et al., 2018; CARVALHO et al., 2015), such as gastroesophageal reflux disorder (e.g., bulimia nervosa) and high consumption of acidic food and drink. The made of consumption of soft drinks influences ETW because the erosive potential of acidic drinks is lower when swallowed in larger gulps and a shorter period than when sipped over an extended period (CARVALHO et al., 2015). Other factors may be associated with susceptibility to ETW, such as: saliva to dilute acidic substances, erosive episode intervals, and saliva proteins, which can produce a protective film that minimizes the erosive process (CARVALHO et al., 2015).

Under clinical situations, the occurrence of erosion is associated with abrasion, so that, *in vitro* and *in situ* protocols should reproduce the clinical condition (WIEGAND et al., 2014; ALGHILAN et al., 2015; SCHLUETER et al., 2016). The abrasion process from brushing teeth can accentuate tissue loss when the it was previously softened by erosion (GANSS et al., 2009; GANSS et al., 2014; SCHLUETER et al., 2016).

Teeth with ETW have a loss of morphology and contour such as flattening of the occlusal structures and cupping of the cusps with the presence of concavities that are generally more wide than deep (CARVALHO et al., 2015). The correct diagnosis of ETW’s initial stages can be difficult to perform, recent studies developed tools for the diagnosis of these lesions in the early-stages using 3D images, photographs, and tomography (MARRO et al., 2018; KUMAR et al., 2018, SAHYOUN et al., 2019).

When lesions have already formed cavities, patients usually visit a dental professional due to dentin sensitivity, thus, restorative treatment is necessary (CARVALHO et al., 2015). The objective, in these cases, is minimizing or stopping the evolution of the erosive process, reducing symptoms of tooth sensitivity, and reestablishing the shape and aesthetics (CARVALHO et al., 2015). Restoration of teeth with ETW is challenging because restorative materials adjacent to the dental surface may protrude as a result of a loss of natural tissue over time, but not the restorative material (CARVALHO et al., 2015).

After restorations, ETW may continue to propagate and erode both dental tissues and restorative materials. Thus, the use of toothpaste is important not only for dental tissues but also for the maintenance of the restorative materials properties. Some studies evaluated the protective effects of toothpaste on the enamel or dentin using different active compounds, such as sodium fluoride, stannous fluoride, and chitosan (GANSS et al., 2016; PINI et al., 2018, GANSS et al., 2017; YOUNG et al. 2006). Furthermore, the concentration or even solution viscosity and frequency can promote better tissue protection (SAKAE et al., 2018).

However, few studies evaluated eroded tissues in association with restorative materials. Thus, the present study investigated the effect of toothpaste with different active compounds on restorative materials by analyzing wear, mechanical properties and topography images (Chapter 1) and chemical composition and nanomechanical properties (Chapter 2).

Capítulo 1

Effects of different toothpastes on erosion protection of composite resin and glass ionomer cement restorations in enamel and dentin

Mariana Dias Moda[a], André Luiz Fraga Briso[a], Renata Parpinelli de Oliveira[a], Núbia Inocencya Pavesi Pini[b], Diego Felipe Mardegan Gonçalves[a], Paulo Henrique dos Santos[c], Ticiane Cestari Fagundes[a]

[a]Department of Restorative Dentistry, Araçatuba School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil. Rua José Bonifácio 1193 Araçatuba, SP, Postal code 16015-050, Brazil.

[b]Department of Restorative Dentistry and Prosthodontics, Ingá University Center UNINGÁ, Maringá, Paraná. Rod. PR 317, 6114 Parque Industrial 200, 87035-510, Brazil.

[c]Department of Dental Materials and Prosthodontics, Araçatuba School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil. Rua José Bonifácio 1193 Araçatuba, SP, Postal code 16015-050, Brazil.

***Corresponding author**

Ticiane Cestari Fagundes

Department of Restorative Dentistry

Araçatuba School of Dentistry, São Paulo State University (UNESP)

Street Address: José Bonifácio, 1193

Araçatuba, SP, Brazil

Postal code: 16015-050

Phone number: (+55)183636-3347

Email: ticiane.fagundes@unesp.br

MODA, M.D. **Effects of different toothpastes on erosion protection of composite resin and glass ionomer cement restorations in enamel and dentin.** [Tese]. Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista “Júlio de Mesquita Filho”; Araçatuba, 2019.

2.1 Abstract

Objectives: This study aimed to evaluate the effects of toothpastes on the enamel, dentin, composite resin, and resin-modified glass ionomer cement through analisys of wear and mechanical properties after cycles of erosion and abrasion. *Materal and Methods:* One hundred fourty four bovine incisors were obtained, being 72 blocks of enamel and 72 blocks of dentin (4×4-mm). Half of the samples were restored with composite resin (CR - Filtek Z350 XT) and the other half with resin-modified glass ionomer cement (RMGIC - Fuji II LC). After restoration, the samples were submitted to a demineralization cycle (5 days, 4 × 2 min/day, 1% citric acid, pH 3.2) and exposed to abrasion using different toothpastes (2 × 15 s/day), with n=12 for each toothpaste: without fluoride (WF), sodium fluoride (NaF – 1450 ppm F), and stannous fluoride (SnF_2 – 1100 ppm F). Surface wear was investigated by profilometry (dental substrates and restorative materials), as well as the wear of the restorative interfaces (dental substrates/restorative materials). The surface microhardness of the restorative materials was also analyzed. Atomic force microscopy (AFM) analysis was performed on all representative surfaces. The data were analyzed by two-way ANOVA and Tukey tests ($\alpha = 0.05$). *Results:* The NaF toothpaste caused the most wear on the dentin surfaces ($p < 0.05$); restorative interfaces with enamel were affected by the toothpastes, and the microhardness of the RMGIC was most affected by the SnF_2 toothpaste ($p < 0.05$). *Conclusions:* The WF and SnF_2 toothpastes produced similar results on dental surfaces. However, the SnF_2 toothpaste decreased the microhardness of RMGIC, and had the most effect on the enamel wear of the restorative interface.

Clinical relevance: Restorative materials, especially ionomeric ones, undergo changes when erosion is combined with Sn-based toothpaste.

Keywords: abrasion; composite resin; erosion; glass ionomer cement; stannous ions.

2.2 Introduction[1]

The number of patients with erosive tooth wear (ETW) has increased, causing growing clinical concern [1]. ETW is the loss of dental substrate combined by physical forces, such as toothbrushing, and acids present in the oral cavity [1,2]. These acids may come from external sources, such as fruit juices and soft drinks that are rich in citric acid, or from internal sources such as the acids from gastroesophageal disorders which can potentially damage dental substrates over time [2-4]. The treatment of ETW relies on strategies to strengthen the resistance of dental tissues against erosion and the use of restorative treatments when necessary [5,6]. Composite resins (CR) and resin-modified glass ionomer cements (RMGIC) are commonly used for restoration [7], but they can be affected by erosive acids which could potentially decrease their clinical effectiveness and longevity [8], through the breakdown of its organic matrix, for example [7].

The erosive process on enamel and dentin is different, being that on enamel, erosion occurs by the dissolution of hydroxyapatite [9]. In contrast, dentin erosion begins in peritubular dentin, exposing the organic matrix that is rich in collagen fibers and water [9]. In the case of severe ETW, the exposure of the organic demineralized matrix of dentin results in hypersensitivity in many patients, as well as tissue loss [9,10]. Clinically, the tooth may develop shallow defects and even loss occlusal [11]. Several factors can affect the interaction between acids and dental tissues and the development of ETW such as: the composition of saliva and its protective capacity, the force applied during brushing, and the toothpaste type and abrasiveness [2].

Many studies have investigated various toothpastes with anti-erosive compounds and their action on eroded enamel and dentin [12-16]. Numerous toothpastes contain active compounds other than sodium fluoride (NaF), which has limited efficacy against

---

[1] Normalização segundo a revista Clinical Oral Investigations (ANEXO E).

ETW, [17], such as hydroxyapatite nanoparticles, zinc-carbonate-hydroxyapatite nanoparticles, potassium nitrate, chitosan, and stannous salts, due to protective action on the eroded substrate [12,16]. These anti-erosive toothpastes, especially those containing stannous ions, on the enamel surface would act forming a precipitate erosion resistant and may reduce dentin hypersensitivity through forming a compound that potentially occludes the dentin tubules, thereby reducing fluid movement within tubules and the effect of external stimuli [18]. However, some toothpastes with anti-erosive claims may present high relative dentin abrasivity (RDA) [19]. Thus, there is no consensus in the literature about which toothpastes are the most recommended, especially when restored erosive lesions are present [2].

The aim of this study was to evaluate the effects of toothpastes on the surface wear of enamel, dentin, composite resin, and resin-modified glass ionomer cement as well as the mechanical properties of restorative materials after erosive-abrasive cycles and topography analysis of the surfaces through representative images of Atomic force microscopy (AFM). Therefore, this study hypothesized that: (1) there would be no difference in the loss of dental tissues, restorative material surfaces, or restorative interfaces after erosive-abrasive cycles among the toothpastes, (2) there would be no differences in wear among the analyzed surfaces when a single type of toothpaste is evaluated after the erosive-abrasive cycles, and (3) there would be no difference in microhardness for the analyzed surfaces of the restorative materials among the toothpastes.

2.3 Materials and Methods

This study was previously approved by the local animal ethical committee (process # 00452-2017). Sample size was determined by the results of the pilot test (n =

12) with test power at 80%, utilizing the minimal difference among the averages and the standard deviation average. Bovine incisors were stored in a 0.1% aqueous solution of thymol for 30 days. The study flowchart is presented in Figure 1.

Two specimens (one of dentin and other of enamel) were embedded in acrylic resin using a metal matrix with a 1-mm distance for restoration, as previously performed by Alghilan and others [6]. A cavity was created in the mesial side of each block using a diamond tip (#1090, KG Sorensen, Barueri, SP, Brazil) at high-rotation, and at the end of the preparation, the box-shaped cavity was 2 mm wide. The samples restored with composite resin were previously conditioned with phosphoric acid to 37% por 20s. Both the cavities were filled with respective restorative material according to the manufacturer's instructions and covered with a polyester strip. A glass slide was placed over the strip and a static load of 0.53 kg was applied using a heavy glass slab to allow excess material to spill over the top of the cavity margins and to ensure that the material was flush with the surface of enamel and dentin [6]. Subsequently, the glass slab was removed and materials were cured through the polyester strip and glass slide using a light curing unit with irradiance of 1000 mW/cm^2 (Kavo, Joinville, SC, Brazil). Seventy-two samples were restored using CR (Filtek Z350 XT, 3M ESPE, St. Paul, MN, USA) and photocured for 20 s (Kavo, Joinville, SC, Brazil). Seventy-two other samples were restored using RMGIC (Fuji II LC, GC Corporation, Tokyo, Japan) and photocured for 40 s, coated with petroleum jelly, and kept under humid conditions at 37 °C for 7 days. After storage, the samples were polished as previously described for excess removal. A hemiface of each specimen was protected using an acid-resistant varnish (Colorama, São Paulo, SP, Brazil) to create a control surface.

The specimens were randomly assigned to the 3 experimental groups: without fluoride (WF: Curaprox Enzycal Zero, Trybol AG, Neuhausen AM Rheinfall, Swiss), sodium fluoride (NaF: Colgate total 12, Palmolive, Sao Bernardo do Campo, SP, Brazil.), and stannous fluoride (SnF_2: Crest Pro-Health, P&G, Cincinnati, USA). The specifications of toothpastes and restorative materials are described in Table 1.

Erosive/Abrasive Cycling

Specimens were subjected to 5 days of erosive and abrasive cycles. Erosive cycles were performed four times per day and the specimen abrasion simulations were done after the first and last erosive cycles. The samples were eroded by immersion in 250 mL of citric acid (PA; Merck, Darmstadt, Germany, pH = 3.2) shaken for 2 min in an orbital shaker (Tecnal TE – 420, Piracicaba, SP, Brazil) and stirred (70/min) [13]. After the first and last erosive cycles, 2 mL of the toothpaste slurry solution, made with distilled water at a ratio of 1:3, was pipetted onto the samples for the dental abrasion simulations and an electric brush was applied, moving in a circular motion (Oral-B Plak Control Ultra; Braun, Frankfurt, Germany) under a weight of 200 g for 15 s. and immersion in the slurry until 2 min [21]. Each erosion cycle was performed at 1h intervals and in the interim the samples were stored at 37 ºC in artificial saliva (1,5 mmol l-1 Ca(NO3)2.4H20; 0.9 mmL l-1 NaH2PO4.2H2O; 150 mmol l-1 KCl, 0.1 mol l-1 Tampão Tris; 0.03 ppm F; pH 7.0) [13]. At the end of the 5-day experimental period, the acid-resistant layer was removed, and the samples were stored at 100% humidity.

Surface Wear and Microhardness Analyses

The surface wear was determined by a mechanical contact profilometer (Surftest SJ 400, Mitutoyo American Corporation, Aurora, IL, USA). In the center of each specimen 3 traces were made at intervals of 0.5 mm, each one 2 mm in length (1 mm for the control and 1 mm for the experimental area). Measurements were made on the dental surfaces (E and D), restorative materials (RMGIC and CR) and restorative interfaces (enamel/RMGIC, enamel/CR, RMGIC/dentin, CR/dentin) also at intervals of 0.5 mm.

The scans were interpreted with specific software (Surftest – SV 2100, Mitutoyo American Corporation, Aurora, IL, USA) along with the profilometer evaluation of the regression lines between the control and experimental sides. The wear was measured in micrometers and defined as the vertical distance between the regression lines on the control surface (previously protected by the acid resistant varnish) and the area subjected to erosive/abrasive cycling.

Surface microhardness of restorative materials (control and eroded CR, control RMGIC and eroded RMGIC) were evaluated using five indentations (Micromet 5114 e OminiMet Software – Buehler, Lake Bluff, IL, EUA) in the center of the control and eroded surfaces with a Knoop (KHN) diamond indenter loaded with 50 g and with a dwell time of 15 seconds [22].

Atomic Force Microscopy (AFM)

Two representative samples from each group were observed under AFM to visualize the topography aspect (Park NX10, Park Systems Corp. Suwon, South Korea). Samples were scanned with a high frequency silicon probe tip. The scan rate was 0.30 Hz (9 µm / s) with a resolution of 256 x 256 pixels. A three-dimensional image of 30 µm x 30 µm was obtained of 6 regions mentioned above (software - Gwyddion 2.5, Prague, Czech Republic).

Statistical analyses

The statistical analyses were performed using Sigma Plot 12.5 software (Systat Software, San Jose, CA, USA). The data were submitted to Shapiro-Wilk test for normality, since they were within normality, the data were submitted to ANOVA. The profilometry data were submitted to two-way ANOVA and microhardness data to two-way ANOVA repeated measurements. The Tukey post hoc test was used and the level of significance was $\alpha = 0.05$.

2.4 Results

The results for the surface wear on dental substrates and restorative materials are described in Table 2. For all toothpastes, the enamel surfaces (ECR and ERMGIC) showed lower wear than the dentin surfaces (DCR and DRMGIC). The NaF toothpaste caused more wear on the ECR, DCR and DRMGIC than the WF and SnF_2 toothpastes ($p < 0.05$). In contrast, no differences were found among the toothpastes when CR, RMGIC, and ERMGIC surfaces were analyzed ($p > 0.05$). Both restorative materials had less wear than the enamel and dentin surfaces. However, ERMGIC presented similarity to RMGIC only for the SnF_2 toothpaste ($p > 0.05$).

The negative values of surface wear on the restorative surfaces (Table 3), show that tissue loss (enamel and dentin) was higher than wear on the restorative material surfaces, with the exception of the analysis between Enamel/RMGIC interfaces. There was higher wear on the NaF toothpaste than the SnF_2 for the Enamel/CR ($p < 0.05$) and the SnF_2 toothpaste caused the most wear in the Enamel/RMGIC, with statistical difference for the other groups ($p < 0.05$). There were no differences in the level of wear on dentin interfaces($p > 0.05$). In comparing the interfaces with different materials within the same dental substrate, it is apparent that the RMGIC had more surface loss than the enamel. The Dentin/RMGIC interfaces showed lower values than Dentin/CR ($p < 0.05$).

The surface microhardness results of the restorative materials are described in Table 4. Only for ionomeric surfaces did the type of toothpaste influence the results. The lowest microhardness values were found with the SnF_2 toothpaste ($p < 0.05$). Comparing the surfaces, WF and SnF_2 presented the following sequence: control CR = eroded CR > eroded RMGIC > control RMGIC. However, for the NaF toothpaste, the eroded CR was similar to the control RMGIC.

Representative AFM images can be seen in Figure 2. Since all eroded surfaces showed some differences from the control ones, only images of eroded surfaces were presented and the main aim is to demonstrate the differences among the toothpastes on the topography surfaces. Few alterations of enamel and CR surfaces was found when comparing the toothpastes after erosive-abrasive cycles (Figures 2 a,b,c,j,k,l). The free-fluoride toothpaste presented large dentinal tubules with collagen fibers exposed. In contrast, both NaF and SnF_2 toothpastes showed partially obliterated dentinal tubules, probably due to mineral precipitation (Figure 2 d,e,f). Greater alterations were found in erosive surfaces for RMGIC, indepently of toothpaste (Figures 2 g,h,i).

2.5 Discussion

The aim of this study was to evaluate the effects of toothpastes on the surface wear of enamel, dentin, composite resin, and resin-modified glass ionomer cement as well as the mechanical properties of restorative materials after erosive-abrasive cycles and topography analysis of the surfaces through representative images of Atomic force microscopy (AFM).

The specimen preparation design in this study was based on a previous study by Alghilan and others [6]. The use of different restoration materials optimized the number of samples and allowed for a correct analysis of surface wear at the same time. Citric acid was chosen for the erosive cycles since it is the most common type of acid found in acidic beverages consumed and is most-commonly used in studies about erosive challenges [23,24]. The present study aimed to evaluate the behavior of toothpastes that contain different abrasives according to the RDA provided by the manufacturers. Although they are toothpastes manufactured in different countries, these were chosen because they contain active ingredients from previous studies cited.

Profilometry is a quantitative method used to evaluate the loss of dental tissue in relation to a non-treated control area, which is considered the standard method to analyze tissue loss *in vitro*, and *in situ* for erosion or erosion-abrasion simulations [25,26]. Schlueter et al. [26] investigated the different methods of profilometry (non-contact or contact), differences in the dentin tissue (wet or dry), and presence or absence of the desmineralised organic matrix (DOM). The authors concluded that the best method to evaluate dentin was non-contact profilometry without DOM. Although the contact device may overestimate the results of tissue loss, this method allows samples to be measured in a wet environment unlike the non-contact method which uses a light probe [25].

A microhardness analysis was done to evaluate the hardness of material through of indentation noted on the applied load [25]. Its use was limited only to the restorative materials since the analysis of eroded dental substrates can be unviable if it is too eroded [25]. In these cases, other types of analysis of hardness may be employed, [25] such as ultra-microhardness utilized for the studies related to eroded dentin [24,27].

Regarding AFM, this technique uses microscopy through a probe that can reach resolutions of molecular and atomic levels and provides topographic aspects, with possible effects of differences between demineralized and remineralized surfaces [25,28], as well as the influence of acids, varnishes or toothpastes [29]. This analysis allows the measurements under ambient conditions (air or liquid), minimizing possible artefacts; however, it takes a long time to scan a single region, an example of 0.5 x 0.5 mm consumes a time of 60 minutes [25].

The first null hypothesis was rejected since there were differences in wear among the toothpastes for dental substrates and restorative interfaces involving enamel after erosive-abrasive cycles. The NaF toothpaste presented higher levels of wear than the WF and the SnF_2 toothpastes in the ECR, DCR, and DRMGIC surfaces. Corroboring with

Ganss and others [14]. Commonly, under demineralization conditions, NaF toothpastes can form prepicited CaF_2 molecules on the enamel surface [5]. However, in the case of extreme acidic conditions, such as in the erosion cycles, this molecule is unstable, easily soluble, and its protective effect could be insufficient [5]. Additionally, the beneficial effects of fluoride on ETW is strongly dependent on the other compounds it is combined with [17]. Others studies have shown that fluoride and polyvalent metal ions such as stannous have promoted better results [15,16]. Furthermore, the concentration of the silica abrasive particles may affect the loss of those dental substrates [15,19], especially with toothbrushing [5,30].

The WF and SnF_2 toothpastes had similar wear on ECR, DCR, and DRMGIC surfaces. WF is a free-fluoride toothpaste, while SnF_2 is considered an anti-erosive toothpaste. Although the SnF_2 toothpastes have anti-erosive properties, silica abrasive particles in toothpaste may decrease their effectiveness because they bind to stannous ions, thereby decreasing their anti-erosive actions [15]. Additionally, these particles can hinder the development of an Sn-rich zone due to the removal of the most superficial enamel structure [15] which may have caused the SnF_2 toothpastes to behave similar to a fluoride-free toothpaste. Ganss and others [14] compared several toothpastes (those without fluoride, and those containing Sn, NaF and hydroxyapatite) and showed the lowest enamel loss in stannous-containing toothpastes (5.4 μm), very similar to our results (4.95 to 5.03 μm). They also found that the concentration of abrasive components in stannous toothpastes has to be greater than 10%, or approximately 20% by weight, to achieve any beneficial effects [15]. Theorically, stannous fluoride is more resistant to erosion since this compound forms a layer on the demineralized enamel and occludes the dentinal tubules after an erosive process [5]. Stannous and sodium fluoride-based toothpastes appear to have acted on dentinal tubule occlusion (Figure 2d-f). For the restorative materials, the toothpastes did not significantly affect their wear level and topography (Figure 2 g-l).

Regarding the restorative interfaces, SnF_2 promoted higher wear compared to WF and NaF for Enamel/RMGIC, and no difference was evident between WF and NaF. A synergistic effect could have also occurred between the protective action of stannous and the release of fluoride from the glass ionomer, acting on the eroded enamel surface and lessening wear [31]. However, it should be noted that the different toothpastes had no effect on dentin interfaces, even though the erosion and abrasion cycles could have been more aggressive on dentin than on enamel due to histological differences [9].

The second null hypothesis was also rejected since there were differences in wear among the analyzed surfaces and interfaces the same toothpaste was used. The lowest wear was noted on the restorative materials (CR and RMGIC) followed by enamel and dentin. In another study, the same wear behavior was found by profilometry: more wear on enamel, followed by the glass ionomer and composite resin, especially when erosion was associated with abrasion [32]. This wear pattern was also observed for Salas et al. [33], where both restorative materials showed less loss than enamel, although microhardness was used to evaluate the percentage of wear after the erosive cycle. This study showed similar ERMGIC and RMGIC results when the SnF_2 toothpaste was aplied, supporting a synerystic effect between RMGIC and SnF_2 which may protect the enamel surface [31]. For demineralized enamel, the ionomeric material could promotes an increase in the pH of the demineralizing solution due to its buffer capacity and protects the substrate from mineral wear [31]. However, SnF_2 toothpaste, which claims to be anti-erosive, may have decreased effect when in contact with abrasive silica particles, since an ionic bond forms between the negative zeta potential of the silica particle and the stannous ion which is positive. This can decrease the concentration of the stannous ions available, consequently affecting the efficacy of its anti-erosive properties, as described above [15]. Thus, the similarity in wear values between ERMGIC and RMGIC may be more related to the effect of the ionomeric material on the adjacent enamel than the toothpaste itself.

The third null hypothesis of the study was also rejected differences in microhardness were found. The lowest hardness was found in RMGIC brushed with the SnF_2 toothpaste, probably due to the silica particles [15] and because RMGIC is more fragile than CR [32,34]. The dissolution of its matrix after acid erosion may have affected its mechanical properties [32,34]. In relation to the CR, no changes were found when the control and eroded sides were compared. This may be related to the use of nanoparticulate composites, which are considered more resistant to erosion [34,35].

Regarding the topograpghy images performed by AFM (Figure 2), the exact distinction of enamel prisms after erosion was not observed, perhaps the brushing action may have smoothed the surface roughness caused by citric acid [36]. In relation to dentin surface, there was a partial obliteration of dentinal tubules for the NaF and SnF_2 groups, corroborating with Poggio et al. [36]. It is known that obliteration could act against future acid attacks (Figure 2 e,f). CR had few effects of erosive/abrasive cycles probably due the matrix composition with the presence of aromatic rings in its chain, which become it more resistant and its inorganic particles which are distributed into the entire structure of the material, providing greater resistance to erosive-abrasive challenges [37]. On the other hand, the ionomeric material presented a very altered surface after the challenges with deep cracks, spaces between the particles with protrusion of the glass particles from ionomeric matrix. Guler et al. [38], investigating the effect of beverages with different pH on various resin-based restorative materials such as Z550 and Fuji II LC by AFM and SEM analysis found that the glass ionomer presented a damaged surface after erosive-abrasive challenges, while the composite resin showed no significant changes, regardless of the toothpaste used.

One limitation of this study was the presence of DOM in the dentin substrate. Schlueter et al. [26] have shown that profilometry analysis with DOM leads to an underestimation of the actual mineral loss. However, in the present study, the sample did not only contain a dentin block in which the DOM could be removed, since the dentin would have adhered to a restorative material and alteration of its structure could compromise the stability of the sample. In addition, it is speculated that the collagenase used to remove the DOM may cause some mineral precipitation due to the long immersion time in a calcium-rich solution, although it would be a very small amount [26]. Another limitation was the use of aritificial saliva which could reduce fluoride retention on surfaces in erosive protocols *in vitro* due to the absence of dental biofilm or salivary pellicle [6].

Future studies about the chemical composition of eroded dental substrates in relation to restorative materials are needed. Additionally, the action of these materials on eroded tooth tissue as well as the chemical changes resulting from the erosive/abrasive process in dental substrates and restorative materials should be studied.

2.6 Conclusion

Toothpastes with stannous fluoride and those without fluoride caused similar wear on dental surfaces.

Restorative materials were similar in wear for all toothpastes.

In relation to microhardness of restorative materials the resin-modified glass ionmer cement was negatively affected by the stannous-based toothpaste.

2.7 Acknowledgments

The authors thank the São Paulo Research Foundation (FAPESP; grant #2017/23360-3) and CAPES for the financial support 001.

2.8 References

[1] Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, Schmalz G, Shellis RP, Tveit AB, Wiegand A (2015) Consensus report of the european federation of conservative dentistry: erosive tooth wear-diagnosis and management. Clin Oral Investig 19:1557-1561. https://doi: 10.1007/s00784-015-1511-7

[2] Buedel S, Lippert F, Zero DT, Eckert GJ, Hara AT (2018) Impact of dentifrice abrasivity and remineralization time on erosive tooth wear in vitro. Am J Dent 31:29-33

[3] Caneppele TM, Jeronymo RD, Di Nicoló R, de Araújo MA, Soares LE (2012) In vitro assessment of dentin erosion after immersion in acidic beverages: surface profile analysis and energy-dispersive X-ray fluorescence spectrometry study. Braz Dent J 23:373–378

[4] Schlueter N, Glatzki J, Klimek J, Ganss C (2012) Erosive-abrasive tissue loss in dentine under simulated bulimic conditions. Arch Oral Biol 57:1176-82. https://doi: 10.1016/j.archoralbio.2012.04.001

[5] Assunção CM, Schlueter N, Rodrigues JA, Carvalho TS, Lussi A (2018) Do fluoride toothpastes have similar preventive effect in permanent and primary teeth a gainsterosive tooth wear? Int J Paediatr Dent 15. https://doi: 10.1111/ipd.12449

[6] Alghilan MA, Cook NB, Platt JA, Eckert GJ, Hara AT (2015) Susceptibility of restorations and adjacent enamel/dentine to erosion under different salivary flow conditions. J Dent 43:1476-1482. https://doi: 10.1016/j.jdent.2015.10.007

[7] Peutzfeldt A, Jaeggi T, Lussi A (2014) Restorative therapy of erosive lesions. Monogr Oral Sci 25:253-261. https:// doi: 10.1159/000360562

[8] Erdemir U, Yildiz E, Eren MM, Ozel S (2012) Surface hardness of different restorative materials after long-term immersion in sports and energy drinks. Dent Mater J 31:729–736.

[9] Ganss C, Lussi A, Schlueter N (2014) The histological features and physical properties of eroded dental hard tissues. Monogr Oral Sci 25:99-107. https://doi: 10.1159/000359939

[10] Zarella BL, Cardoso CA, Pelá VT, Kato MT, Tjäderhane L, Buzalaf MA (2015) The role of matrix metalloproteinases and cysteine-cathepsins on the progression of dentine erosion. Arch Oral Biol 60:1340-1345. https:// doi: 10.1016/j.archoralbio.2015.06.011

[11] Attin T, Koidl U, Buchalla W, Schaller HG, Kielbassa AM, Hellwig E (1997) Correlation of microhardness and wear in differently eroded bovine dental enamel. Arch Oral Biol 42:243–250. https://doi:10.1016/0003-9969(06)00073-2

[12] Aykut-Yetkiner A, Attin T, Wiegand A (2014) Prevention of dentine erosion by brushing with anti-erosive toothpastes. J Dent 42:856-861. https://doi: 10.1016/j.jdent.2014.03.011

[13] Cruz NV, Pessan JP, Manarelli MM, Souza MD, Delbem AC (2015) In vitro effect of low-fluoride toothpastes containing sodium trimetaphosphate on enamel erosion. Arch Oral Biol 60:1231-1236. htpps://doi: 10.1016/j.archoralbio.2015.05.010

[14] Ganss C, Marten J, Hara AT, Schlueter N (2016) Toothpastes and enamel erosion/abrasion - Impact of active ingredients and the particulate fraction. J Dent 54:62-67. https://doi: 10.1016/j.jdent.2016.09.005

[15] Ganss C, Möllers M, Schlueter N (2017) Do Abrasives Play a Role in Toothpaste Efficacy against Erosion/Abrasion? Caries Res 51:52-57. https://doi: 10.1159/000452867

[16] Pini NIP, Schlueter N, Sundfeld D, Semper Hogg W, Santos-Silva AR, Lopes MA, Aguiar FHB, Lovadino JR, Lima DANL (2018) Efficacy of stannous ions on enamel demineralization under normal and hyposalivatory conditions: a controlled randomized in situ pilot trial. Caries Res 51:543-553. https:// doi: 10.1159/000479041

[17] Lussi A, Carvalho TS (2015) Analyses of the erosive effect of dietary substances and medications on deciduous teeth. PLoS One 23:e0143957. https://doi: 10.1371/journal.pone.0143957

[18] Gallob J, Sufi F, Amini P, Siddiqi M, Mason S (2017) A randomised exploratory clinical evaluation of dentifrices used as controls in dentinal hypersensitivity studies. J Dent 64:80-87. https://doi: 10.1016/j.jdent.2017.06.009

[19] Lippert F, Arrageg MA, Eckert GJ, Hara AT (2017) Interaction between toothpaste abrasivity and toothbrush filament stiffness on the development of erosive/abrasive lesiosn in vitro. Int Dent J 67:344-350. https://doi: 10.1111/idj.12305

[20] Souza BM, Comar LP, Vertuan M, Fernandes Neto C, Buzalaf MA, Magalhães AC (2015) Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation in situ. Caries Res 49:499-507. https:// doi: 10.1159/000438466

[21] Pini NI, Lima DA, Lovadino JR, Ganss C, Schlueter N (2016) In vitro efficacy of experimental chitosan-containing solutions as anti-erosive agents in enamel. Caries Res 50:337-345. https://doi: 10.1159/000445758

[22] Briso AL, Caruzo LP, Guedes AP, Catelan A, Santos PH (2011) In vitro evaluation of surface roughness and microhardness of restorative materials submitted to erosive challenges. Oper Dent 36:397-402. https://doi: 10.2341/10-356-L

[23] Schlueter N, Lussi A, Tolle A, Ganss C (2016) Effects of erosion protocol design on erosion/abrasion study outcome and on active agent (naf and snf_2) efficacy. Caries Res 50:170-179. https:// doi: 10.1159/000445169

[24] Moda MD, Fagundes TC, Bresciani E, Briso ALF, Dos Santos PH (2019) Comparison of in vitro erosion protocols in bovine teeth to simulate natural erosion lesion: analysis of mechanical properties and surface gloss. J Appl Oral Sci 27:e20180107. https:// doi: 10.1590/1678-7757-2018-0107

[25] Schlueter N, Hara A, Shellis RP, Ganss C (2011) Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res 45 Suppl 1, 13-23. https:// doi: 10.1159/000326819

[26] Schlueter N, Jung K, Ganss C (2016) Profilometric quantification of erosive tissue loss in dentine: a systematic evaluation of the method. Caries Res 50:443-454.

[27] Gonçalves DFM, Briso ALF, Pini NIP, Moda MD, Parpinelli de Oliveira R, Santos PHD, Fagundes TC (2019) Effects of dentifrices on mechanical resistance of dentin and restorative materials after erosion and abrasion. J Mech Behav Biomed Mater 97:7-12. https:// doi: 10.1016/j.jmbbm.2019.05.006

[28] Lombardini M, Ceci M, Colombo M, Bianchi S, Poggio C (2014) Preventive effect of different toothpastes on enamel erosion: AFM and SEM studies. Scanning 36:401-410. https://doi: 10.1002/sca.21132

[29] Lechner BD, Röper S, Messerschmidt J, Blume A, Magerle R (2015) Monitoring demineralization and subsequent remineralization of human teeth at the dentin-enamel junction with atomic force microscopy. ACS Appl Mater Interfaces 7:18937-43. https://doi: 10.1021/acsami.5b04790

[30] Ganss C, Hardt M, Blazek D, Klimek J, Schlueter N (2009) Effects of toothbrushing force on the mineral content and demineralized organic matrix of eroded dentine. Eur J Oral Sci 117:255-260. https://doi: 10.1111/j.1600-0722.2009.00617.x

[31] Rolim FG, Sá AF, Silva-Filho GW, Brandim Ade S, Vale GC (2016) Effect of high-fluoride dentifrice on enamel erosion adjacent to restorations in vitro. Oper Dent 41:157-161. https://doi: 10.2341/14-292-L

[32] Yu H, Wegehaupt FJ, Wiegand A, Roos M, Attin T, Buchalla W (2009) Erosion and abrasion of tooth-colored restorative materials and human enamel. J Dent 37:913-922. https:// doi: 10.1016/j.jdent.2009.07.006

[33] Salas CF, Guglielmi CA, Raggio DP, Mendes FM (2011) Mineral loss on adjacent enamel glass ionomer cements restorations after cariogenic and erosive challenges Arch Oral Biol 56:1014-1019. https:// doi: 10.1016/j.archoralbio.2011.03.005

[34] Silva MA, Vitti RP, Sinhoreti MA, Consani RL, Silva-Júnior JG, Tonholo J (2016) Effect of alcoholicbeverages on surface roughness and microhardness of dental composi tes. Dent Mater J 35:621-626. https:// doi: 10.4012/dmj.2015-383

[35] Kaur S, Makkar S, Kumar R, Pasricha S, Gupta P (2015) Comparative evaluation of surface properties of enamel and different esthetic restorative materials under erosive and abrasive challenges: An in vitro study. Indian J Dent 6:72-80. https://doi: 10.4103/0975-962X

[36] Poggio C, Lombardini M, Vigorelli P, Ceci M (2013) Analysis of dentin/enamel remineralization by a CPP-ACP paste: AFM and SEM study. Scanning 35:366-374. https://doi: 10.1002/sca.21077

[37] Gajewski VE, Pfeifer CS, Fróes-Salgado NR, Boaro LC, Braga RR (2012) Monomers used in resin composites: degree of converstion, mechanical properties and water sorption/solubility. Braz Dent J 23:508-514.

[38] Guler S, Unal M (2018) The evaluation of color and surface roughness changes in resin based restorative materials with different contents after waiting in various liquids: an SEM and AFM study. Microsc Res Tech 81:1422-1433. https://doi: 10.1002/jemt.23104

Figures

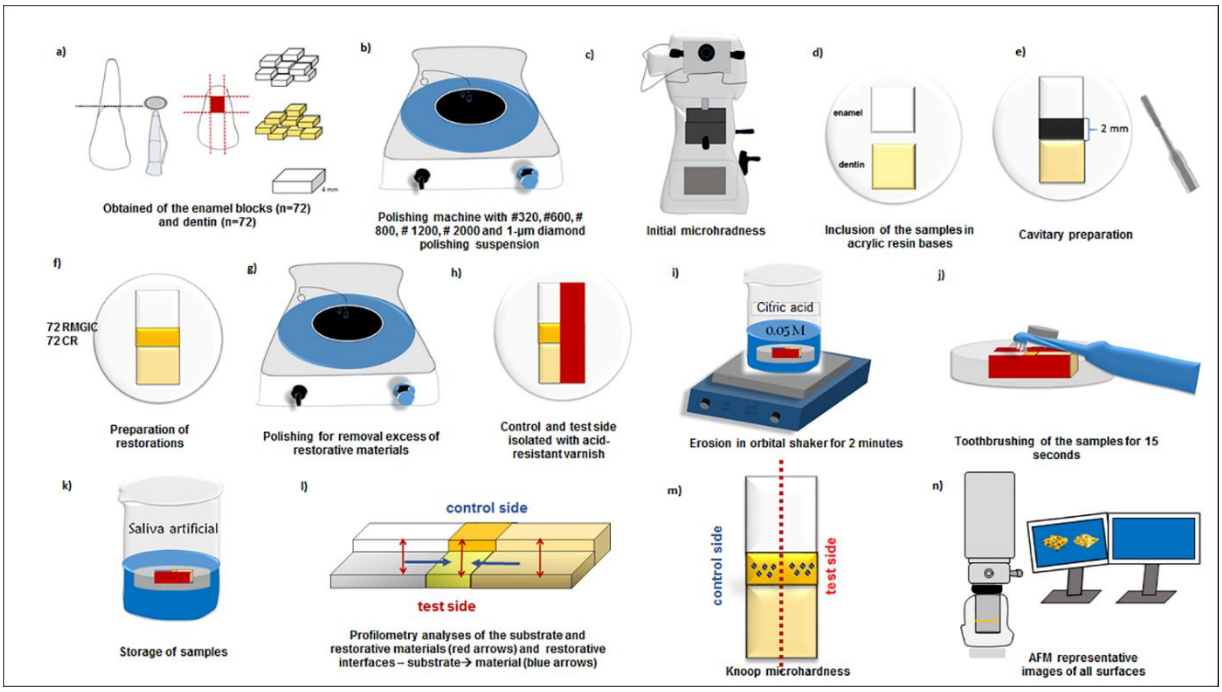


Figure 1. Study flowchart**.** (a) Obtaining 144 bovine incisors, 72 enamel and 72 dentin blocks (4 x 4 mm^2). (b) After, the specimens were polished in an automatic polishing machine. (c) The, was performed to surface microhardness analysis to select specimens. (d) Enamel and dentin blocks were included in acrylic base, being two specimens (enamel and dentin) in each base with a distance of 1 mm between them. (e) A cavity was prepared on the walls of the specimens, with a total distance of 2mm between them. (f) The restorations were applied. (g) The restorations were polished for the removal of excess of restorative material. (h) and the hemiface of each specimen/restoration set was covered with an acid-resistant varnish (i). The specimens were submitted to erosive (4x/day). (j) and abrasive (2x/day) challenges. (k) The specimens were storage in artificial saliva among the erosive cycles. (l) Analysis of profilometry was performed for evaluate wear obtained of dental substrates and restorative materials (red arrow) as well as restorative interfaces (blue arrow). (m) The restorative materials were submitted to surface microhardness analysis. (n) Atomic force microscopy (AFM) representative images of all surfaces.

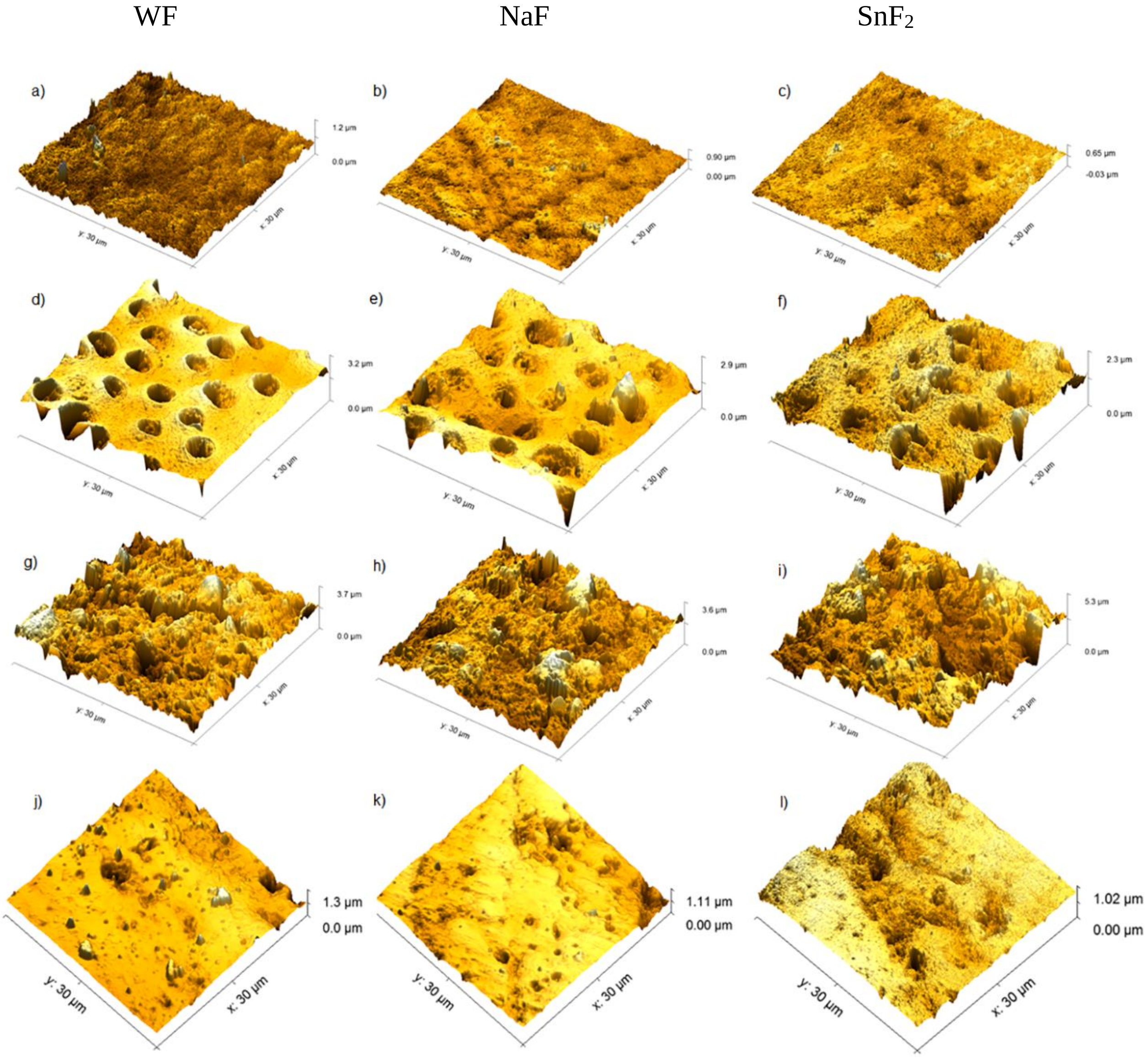


Figure 2. Representative AFM images (256 X 256 pixels) of enamel, dentin and restorative materials. a) Eroded enamel brushed with WF presented roughness values (Rms): 97.40 nm. b) Eroded enamel brushed with NaF presented roughness values (Rms): 67.17 nm. c) Eroded enamel brushed with SnF_2 presented roughness values (Rms): 52.38 nm. d) Eroded dentin brushed with WF presented roughness values (Rms): 358.8 nm. e) Eroded dentin brushed with NaF presented roughness values (Rms): 307.3 nm. f) Eroded dentin brushed with SnF_2 presented roughness values (Rms): 289.9 nm. g) Eroded RMGIC brushed with WF presented roughness values (Rms): 488.1 nm. h) Eroded RMGIC brushed with NaF presented roughness values (Rms): 470 nm. i) Eroded RMGIC brushed with SnF_2 presented roughness values (Rms): 788.6 nm. j) Eroded CR brushed with WF presented roughness values (Rms): 103.9 nm. k) Eroded CR brushed with NaF presented roughness values (Rms): 145.8 nm. l) Eroded CR brushed with SnF_2 presented roughness values (Rms): 124.2 nm.

Tables

Table 1. Materials used in this study

Material	Application mode	Composition	Manufacturer
Adaper Single Bond 2 (Adhesive system)	Apply one layer of adhesive, wait for 20s, air stream for 5s, and polymerize for 10s	BisGMA, HEMA, dimethacrylates, ethanol, water, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	3M ESPE St. Paul, MN, USA.
Filtek Z350 XT (color A2B) Batch:672912	Apply increments of 2 mm and polymerize for 20s each	Bis-GMA, UDMA, Bis-EMA, TEGDMA, PEGDMA, Zirconia and agglomerates of silica, camphorquinone	3M ESPE St. Paul, MN, USA.
Fuji II LC (color A3) Batch:17051316	GC conditioner was applied for 20s, rinsed and dried for 10s. 1 level scoop of powder to 2 drops of liquid was dispensed and mixed for 15-20s. The mixture was transferred to the centrix syringe	Powder: fluor-amino-silicate glass. Liquid: aqueous solution of polycarboxylic acid, TEGDMA and HEMA	GC Corporation, Tokyo, Japan.

Material	Type	Composition	Manufacturer
Curaprox Enzycal Zero (RDA-60)* Batch:442MHDEXP1121	Fluoride-free Toothpaste (WF)	Water, Sorbitol, Hydrated Silica, Glycerin, Steareth-20, Titanium Dioxide (Cl 77891), Aroma, Sodium Phosphate, Carrageenan, Sodium Chloride, Citric Acid, Sodium Benzoate, Potassium Thiocyanate, Glucose Oxidase, Amyloglucosidase, Lactoperoxidase .	Trybol AG, Neuhausen AM Rheinfall, Swiss.
Colgate Total 12 (RDA-70/80)*	Sodium Fluoride Toothpaste (NaF)	Sodium Fluoride (1450 ppm as NaF) Water, Triclosan, Sorbitol, Silica, Sodium Lauryl	Colgate-Palmolive, São Bernardo do

Batch:6184BR121R		Sulfate, PMV / MA Copolymer, Sodium Hydroxide, Saccharin Sodium, Titanium Dioxide	Campo, SP, Brazil.
Crest Pro-Health (RDA-155)* Batch:6039GF	Stannous Fluoride Toothpaste (SnF$_2$)	Stannous fluoride (1100 ppm F as SnF$_2$) Glycerin, Hydrated Silica, Sodium Hexametaphosphate, Propylene Glycol, PEG 6, Water, Zinc Lactate, Trisodium Phosphate, Sodium Lauryl Sulfate, Sodium Lauryl Sulfate, Carrageenan, Sodium Saccharin, Xanthan Gum, Blue 1	P&G, Cincinnati, USA.

Table 2. Mean (SD) of wear (µm) of dental substrates and restorative materials surfaces

	WF	NaF	SnF_2
ECR	4.53 (0.35) ^{Ab}	7.92 (0.34) ^{Bb}	5.03 (0.32) ^{Ab}
CR	0.13 (0.13) ^{Aa}	0.31 (0.17) ^{Aa}	0.33 (0.12) ^{Aa}
DCR	8.58 (0.47) ^{Ac}	14.53 (0.52) ^{Bc}	9.88 (0.38) ^{Ac}
ERMGIC	5.77 (0.24) ^{Ab}	6.97 (0.52) ^{Ab}	4.95 (0.38) ^{Ab}
RMGIC	0.96 (0.24) ^{Aa}	3.23 (0.36) ^{Aa}	1.78 (0.21) ^{Aab}
DRMGIC	10.15 (0.36) ^{Ac}	13.99 (0.44) ^{Bc}	9.64 (0.37) ^{Ac}

Upper case letters compare toothpastes into each surface. Lowercase letters compare surfaces into each toothpaste. There was no comparison between specimens restored with CR and RMGIC

ECR: enamel adjacent to composite resin; CR: composite resin; DCR: dentin adjacent to composite resin; ERMGIC: enamel adjacent to resin-modified glass ionomer cement; RMGIC: resin-modified glass ionomer cement; DRMGIC: dentin adjacent to resin-modified glass ionomer cement

Table 3. Mean (SD) of wear (µm) of restorative interfaces

	WF	**NaF**	**SnF_2**
Enamel/CR	-15.10 (0.79) ^{ABb}	-16.60 (0.89) ^{Ab}	-11.60 (1.13) ^{Bb}
Enamel/RMGIC	7.72 (0.45) ^{Ba}	8.08 (1.04) ^{Ba}	13.94 (0.59) ^{Aa}
Dentin/CR	-21.01 (0.75) ^{Ab}	-22.33 (1.56) ^{Ab}	-21.95 (1.33) ^{Ab}
Dentin/RMGIC	-11.74 (0.59) ^{Aa}	-10,08 (0.58)^{Aa}	-11.18 (0.77) ^{Aa}

Upper case letters compare toothpastes. Lowercase letters compare surfaces

Enamel/CR: enamel adjacent to composite resin; Enamel/RMGIC: enamel adjacent to resin-modified glass ionomer cement; Dentin/CR: dentin adjacent to composite resin; Dentin/RMGIC: dentin adjacent to resin-modified glass ionomer cement;

Table 4. Mean (SD) of surface microhardness (KHN – kg/mm^2), according to each toothpaste and restorative material

	WF	**NaF**	**SnF_2**
Control CR	74.1 (1.00) ^{Aa}	74.2 (1.44) ^{Aa}	73.5 (2.00) ^{Aa}
Eroded CR	73.3 (0.70) ^{Aa}	72.8 (1.66) ^{Aab}	71.1 (1.96) ^{Aa}
Control RMGIC	61.9 (1.25) ^{Bb}	66.7 (1.03) ^{Ab}	55.9 (1.24) ^{Cb}
Eroded RMGIC	47.8 (1.17) ^{Ac}	51.3 (1.88) ^{Ac}	34.9 (2.07) ^{Bc}

Upper case letters compare toothpastes. Lowercase letters compare surfaces

Capitulo 2

Synergism of different restorative materials and toothpastes in the nanomechanical properties and chemical composition of dental eroded substrates

Mariana Dias Moda[1], Paulo Henrique dos Santos[2], Nubia Inocencya Pavesi Pini[3], Leonardo Negri Furini[4], Carlos José Leopoldo Constantino[4], André Assmann[5], André Luiz Fraga Briso[1], Ticiane Cestari Fagundes[1]

[1] Department of Restorative Dentistry, Araçatuba School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil. Rua José Bonifácio 1193 Araçatuba, SP, Postal code 16015-050, Brazil.

[2] Department of Dental Materials and Prosthodontics, Araçatuba School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil. Rua José Bonifácio 1193 Araçatuba, SP, Postal code 16015-050, Brazil.

[3] Department of Restorative Dentistry and Prosthodontics, Ingá University Center UNINGÁ, Maringá, Paraná. Rod. PR 317, 6114 Parque Industrial 200, 87035-510, Brazil.

[4] Department of Physics, School of Technology and Applied Sciences, São Paulo State University (UNESP), Campus Presidente Prudente, Presidente Prudente, SP, Brazil.

[5] Department of Physics, Universidade Estadual de Ponta Grossa, UEPG, Ponta Grossa, PR, Brazil

***Corresponding author**

Ticiane Cestari Fagundes

Department of Restorative Dentistry

Araçatuba School of Dentistry, São Paulo State University (UNESP)

Street Address: José Bonifácio, 1193

Araçatuba, SP, Brazil

Postal code: 16015-050

Phone number: (+55)183636-3347

Email: ticiane.fagundes@unesp.br

MODA, M.D. **Synergistic action among different dentifrices and restorative materials on eroded enamel and dentin.** [Tese]. Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista “Júlio de Mesquita Filho”; Araçatuba, 2019.

3.1 Abstract

Objective: To evaluate nanomechanical properties and chemical composition of eroded restorative materials and dental surfaces using different toothpastes. *Materials and Methods:* One-hundred bovine blocks enamel (n=50) and dentin (n=50) were obtained to simulate restoration of cervical erosive lesions. Half of the specimens were restored with resin-modified glass ionomer cement (RMGIC), and the other half with composite resin (CR). One hemiface of each specimen was coated and submitted to erosive-abrasive cycles using the toothpastes (n=10): without fluoride (WF), sodium fluoride (NaF), and stannous fluoride (SnF_2). The specimens were analyzed for nanohardness (H) and elastic modulus (Er), chemical composition with energy-dispersive x-ray (EDS), and Raman microscopy. Data were analyzed by a two-way ANOVA and Tukey test ($\alpha = 0.05$). *Results:* NaF presented lower values of H for the DRMGIC-C with a statistical difference for WF ($p < 0.05$). SnF_2 resulted in lower values of Er for the ERMGIC-E and RMGIC-E, with statistical differences for the WF and NaF types of toothpaste ($p < 0.05$). WF showed lower Ca and P concentration for DCR-E than the other toothpaste types ($p < 0.05$). The phosphate and carbonate bands on the substrates showed minimal alterations after the erosive and abrasive cycles. Conclusion: Stannous-based toothpaste affected the elastic modulus on the enamel and ionomeric surfaces. Fluoride toothpastes increased Ca and P concentration of dentin adjacent of composite resin. Only the resin composite was capable of maintaining its nanomechanical properties and chemical composition after erosive-abrasive challenge regardless of the toothpaste. *Clinical relevance:* Stannous-based toothpaste was not able to protect the analyzed surfaces on nanomechanical and chemical properties.

Keywords: Abrasion, Composite resin, Erosion, Glass ionomer cement, Stannous ion.

3.2 Introduction[1]

ETW involves multiple factors and has occurred with increasing frequency over the past decade The etiology is related specially to eating habits due to the high consumption of acidic beverages [1,2] and may be associated with bulimia, anorexia, and gastroesophageal disorders [3]. Importantly, ETW occurs by an association between constant contact with acids and the mechanical forces from toothbrushing, contributing to the removal of surface tissue that is softened by acidic action [4]. Each of these factors exposes the dental elements to contact with acids, such as citric or hydrochloric acid. Acidic action on dental tissues may not be diagnosed early since it does not promote visible damage. However, as the erosive process advances, the dental substrate loss may occur and expose the dentin [5].

Furthermore, other factors may influence the erosive progression, such as the dissolution rate of dental substrates influenced by the presence of impurities in the substrate mineral content [6]. Thus, numerous studies have investigated the erosive dynamics considering different aspects such as composition of eroded dental tissues, distinct *in vitro* protocols to simulate erosive process [5,7,8], action of bioactive particles on eroded tissues [9] as well as the action of different toothpastes, rinses or varnishes with various active ingredients in order to minimize tooth loss [10,11,12].

Thus, this study aimed to compare types of toothpaste with different active compounds for nanomechanical properties and chemical composition of dental and restorative materials after erosive-abrasive cycles; because few studies in the literature have investigated the effect of different types of toothpaste in terms of those factors.

---

[1] Normalização segundo a revista Journal of Dentistry (ANEXO E).

The null hypotheses were that (1) different types of toothpaste would not affect the nanomechanical properties of the dental substrates and restorative materials after erosive-abrasive cycles, and (2) that different types of toothpaste would not affect the chemical composition of the dental substrates after erosive-abrasive cycles.

3.3 Materials and Methods

Experimental design

Two experimental factors were investigated in this *in vitro* study: First, toothpastes (WF) without fluoride, negative control; (NaF) sodium fluoride, positive control; and (SnF_2) stannous fluoride, and second the control and eroded surfaces (E) enamel, (D) dentin, (RMGIC) resin-modified glass ionomer cement, and (CR) composite resin. The characteristics of toothpaste types and restorative materials are shown in Table 1.

The response variables were nanomechanical properties (H) nanohardness and (Er) elastic modulus of all surfaces and the chemical composition of dental surfaces and restorative materials (EDS) using dispersive energy x-ray spectroscopy, and the chemical composition only of the dental surfaces using (R) Raman spectroscopy.

Specimen preparation

This study was previously approved by the local animal ethics committee (process # 00243-2018). Bovine incisors were stored in a 0.1 % aqueous solution of thymol for 30 days. A total of 50 enamel and 50 dentin blocks (4 × 4 × 2 mm) were obtained using a precision saw and a diamond disk (Isomet 1000; Buehler, Lake Bluff, IL, USA). The samples were then planed and flattened using silicon carbide papers (#320, #600, #1200, #2000) under constant irrigation and polished using a felt disc with 1 μm diamond paste (Arotec, Cotia, SP, Brazil). The blocks were sonicated to remove debris in distilled water for 15 minutes. These procedures left the enamel and dentin blocks with a thickness of 1 mm. The specimens were analyzed by Knoop microhardness (Micromet 5114 e OminiMet Software–Buehler, Lake Bluff, IL, USA) to standardize the samples [13] with enamel hardness between 320 to 360 KHN, and dentin hardness between 50 to 70 KHN. All specimens were stored in 100 % humidity until use.

Restorative procedures

Two specimens (one dentin and one enamel) were embedded in acrylic resin using a metal matrix with a 1 mm distance, for future restoration with different materials [14]. A cavity was prepared in the center of samples each block using a diamond tip (#1090, KG Sorensen, Barueri, SP, Brazil) operated at a high rotational speed and replaced after every fifth preparation. When the preparation was completed, the box-shaped cavity was (2 × 2 mm). The samples restored with composite resin were previously conditioned with phosphoric acid to 37% for 20s. Both cavities were filled with their respective restorative material according to the manufacturer’s instructions and then covered with a polyester strip. A glass slide was placed over the strip, and a static load of 0.53 kg was applied using a heavy glass slab to allow excess material to extrude over the top of the cavity margins, which ensured that the material was flush with the surface of the enamel and dentin [14]. Next, the glass slab was removed, and the materials were photocured through the polyester strip and glass slide using a light-curing unit with an irradiance of 1000 mW/cm^2 (Kavo, Joinville, SC, Brazil). Fifty samples were restored using CR (Filtek Z350 XT, 3M ESPE, St. Paul, MN, USA) and photocured for 20 seconds (Kavo, Joinville, SC, Brazil). Fifty other samples were restored using RMGIC (Fuji II LC, GC Corporation, Tokyo, Japan), photocured for 40 seconds, protected with petroleum jelly, and kept under humid conditions at 37 °C for seven days. After storage, the samples were polished as previously described for removal of excess material (#800, #1200, #2000, and felt disc).

A hemiface of each specimen was protected using an acid-resistant varnish (Colorama, São Paulo, SP, Brazil) to create the control and eroded sides [15].

The specimens were randomly assigned to the three experimental groups: (1) without fluoride (WF: Curaprox Enzycal Zero, Trybol AG, Neuhausen AM Rheinfall, Swiss), (2) sodium fluoride (NaF: Colgate total 12, Palmolive, Sao Bernardo do Campo, SP, Brazil.) and (3) stannous fluoride (SnF_2: Crest Pro-Health, P&G, Cincinnati, OH, USA).

Erosive-Abrasive Cycling

Specimens were subjected to five-days of erosive-abrasive cycles. Erosive cycles were performed four times per day, and abrasive cycles were applied after the first and last cycles each day. The samples were eroded by immersion in 250 mL of citric acid (PA; Merck, Darmstadt, Germany, pH = 3.2) for two minutes under agitation in an orbital shaker (Tecnal TE–420, Piracicaba, SP, Brazil) at 70 RPM. The toothpaste slurries were prepared with distilled water (1:3), and 2 milliliters of this solution was pipetted on the samples after the first and last erosive cycle; followed by the abrasive cycle with an electric brush using a circular motion (Oral-B Plak Control Ultra; Braun, Frankfurt, Germany), that weighed 200 grams, for 15 seconds, and immersed in the slurry for 2 minutes [11]. Each daily challenge was performed within a one-hour interval, and the samples were stored at 37 ºC in artificial saliva (1.5 mmol l-1 Ca(NO3)2.4H20; 0.9 mmol l-1 NaH2PO4.2H2O; 150 mmol l-1 KCl, 0.1 mmol l-1 buffer Tris; 0.03 ppm F; pH 7.0) [16]. At the end of the experimental period, the acid-resistant layer was removed, and the samples were stored under 100 % humidity.

Analyses of the nanohardness (H) and elastic modulus (Er)

The nanomechanical properties were measured using a nanohardness tester (UNAT, ASMEC, Zwick-Roell, Ulm, Germany). A Berkovich diamond tip was used at a load of 1000 µN and a standard trapezoidal load function of 5-2-5 seconds [17]. Three measurements were performed in each of following regions for each specimen: control and eroded dental surfaces adjacent to the restorative interface, RMGIC, and CR in the center of the restoration. In total, there were 18 indentations for each specimen. H and Er were calculated on load-displacement curves according to the following relationships [18]

$$H = Pmax / A$$

where PMAX is the maximum load and A is the projected contact area between the indenter tip and the specimen under the maximum load; and

$$Er = S \sqrt{\pi} / 2 \sqrt{A}$$

where S is the initial unloading stiffness, and A is the projected contact area between the indenter tip and the sample at maximum load.

Analyses of energy dispersive X-ray spectroscopy (EDS) and scanning electron microscopy (SEM)

The surface composition of dental substrates and restorative materials was obtained by X-ray EDS and SEM (EVO LS 15, Carl Zeiss, Oberkochen, Germany), and coated with gold through the equipment (Q150T, Quorum Technologies, Laughton, England). Three specimens from each group were selected for EDS analysis of the control and eroded surfaces of the dental substrates, and restorative materials (INCA x-act, Oxford Instruments, Concorde, New Hampshire, USA) over a defined area of 200 × 200 µm, using electronic-mode (20 kV) with 2000x magnification. A representative image of all groups was also obtained by SEM at 2000x magnification and 5000x magnifications [19,20].

Analysis of micro-Raman spectroscopy (R)

Raman measurements were performed using a spectrometer (Renishaw in-Via, Renishaw plc, Wotton-under-Edge, UK). Analyses were made at the molecular level using UV absorption spectrophotometer measurements of bands located between 150 and 1500 nm wavelengths. The excitation laser equipped with this apparatus was a He−Ne laser ($\lambda = 785$ nm), and 1200 lines per mm diffraction grating with exposure time was set at 10 seconds. Optical images were obtained using a microscope (Leica, Microsystems GmbH, Germany) coupled to the Raman spectrograph through a 50x objective lens and 500x magnification [21]. Chemical mapping of the surfaces was performed by comparing the integrated areas of the Raman peaks in arbitrary units (a.u.) (phosphate 960 cm^{-1}) (carbonate 1070 cm^{-1}) [22], using Grams/32 AI software (Galactic Industries Company, Salem, NH, USA).

Statistical Analysis

The statistical analyses were performed using Sigma Plot 12.5 software (Systat Software, San Jose, CA, USA). The data were analyzed for normality using a Shapiro-Wilk test. Nanomechanical properties data (H and Er) and chemical composition (EDS) of dental surfaces and restorative materials were analyzed with a two-way ANOVA repeated measurements and a Tukey post hoc test. Enamel, dentin, and restorative materials were considered separately. Data Raman integrated area peaks were submitted to two-way ANOVA repeated measurements and a Tukey post hoc test only for the dental surfaces. The level of significance was $\alpha = 0.05$.

3.4 Results

The nanomechanical properties (H and Er) are shown in Tables 2 and 3. There were significant differences only for DRMGIC-C, with lower H for NaF than WF ($p < 0.05$). When comparing the control surfaces using the same toothpaste, there were differences for WF toothpaste between ERMGIC-C and ECR-C and among RMGIC-C and CR-C for all toothpaste types ($p < 0.05$). The differences between restorative materials were also observed for eroded surfaces. Only surfaces that did altered after suffered by the erosion-abrasive cycling had RMGIC-E for NaF toothpaste and eroded CR-E for all types of toothpaste ($p > 0.05$).

Er values are shown in Table 3. NaF presented lower values with statistical differences for WF and SnF_2 for the ERMGIC-C ($p < 0.05$). However, WF showed lower values for ECR-C with a statistical difference for NaF and SnF_2 ($p < 0.05$). Furthermore, SnF_2 resulted in lower values of Er for the ERMGIC-E and RMGIC-E, with statistical differences for the WF and NaF types of toothpaste ($p < 0.05$). WF and SnF_2 types of toothpaste resulted in lower values of Er for the ECR-E, which was different from NaF ($p < 0.05$). When comparing control and eroded surfaces, there was a statistically significant decrease in the Er ($p < 0.05$), except for the CR surfaces that did not undergo erosive-abrasive cycling ($p > 0.05$).

Representative images from SEM are in Fig. 2. Since all eroded surfaces showed differences from the control, only images of the eroded surfaces are presented. Concerning the eroded enamel surfaces (Fig. 2A, 2B, and 2C), there were few differences among the various toothpaste types after the erosive-abrasive cycles. However, SnF_2 (Fig. 2C) showed the hastened formation. Regarding the eroded dentin surfaces, in addition to the differences found between control and eroded surfaces, larger dentinal tubules can be seen in the WF group (Fig. 2D), while partial obliteration of dentinal tubules with hastened formation was observed in NaF and SnF_2 toothpaste types (Fig. 2E and 2F, respectively). Considerable alterations were found on erosive surfaces for RMGIC (Fig. 2G, 2H, and 2I), irrespective of the toothpaste. Eroded CR surfaces showed minimal morphologic alterations for WF and NaF (Fig. 2J and 2K). However, SnF_2 (Fig. 2L) presented a grooved surface.

EDS analysis of the dental surfaces is presented in Fig. 3, and Fig. 4. The chemical elements found on dental surfaces were C (carbon), O (oxygen), Ca (calcium), P (phosphorus), Sn (stannous), Si (silica), and Cl (chlorine). However, only Ca and P were considered for these analyses. No differences were found for enamel surfaces among the types of toothpaste. Comparing enamel surfaces brushed with the same toothpaste, in specimens treated with WF and NaF lower Ca and P concentrations were observed for ECR-E; with SnF_2, this observation was found for ERMGIC-E. WF showed lower Ca and P concentration for DCR-E than the other toothpaste types ($p < 0.05$). Also, WF presented higher Ca and P concentration than NaF for DCR-C ($p < 0.05$). In relation to dentin surfaces using the same toothpaste, eroded dentin surfaces presented lower calcium and phosphorus concentrations than the control for all toothpaste types ($p < 0.05$). Sn was detected on some dentin surfaces brushed with SnF_2, and Si was presented in eroded dentin for all toothpaste types; however, no statistical analysis was performed for these elements because these ions were not observed in all specimens of the study.

EDS analysis of the restorative materials (RMGIC and CR) are presented in Fig. 5, and Fig. 6, respectively. RMGIC surfaces showed the presence of chemical elements: Ca, Si, F, Al (aluminum), and Sr (strontium). NaF and SnF_2 presented higher values than WF on the RMGIC-E for calcium ($p < 0.05$). SnF_2 presented higher fluoride values than WF and NaF on the RMGIC-C ($p < 0.05$). However, WF presented higher fluoride values than NaF on the RMGIC-E ($p< 0.05$). WF presented higher Al and Sr values than other toothpaste on the eroded surfaces ($p< 0.05$). In relation to surfaces using the same toothpaste, higher Ca values were found on the eroded than the control surfaces for NaF and SnF_2. However, the opposite occurred with F, Al, and Sr concentrations for SnF_2, and with Al, and Sr concentrations for NaF ($p< 0.05$). Regarding CR surfaces, the common chemical elements found were Si, and Zr (zirconia); eroded CR surfaces presented similar

Si and Zr concentrations in relation to the control surfaces ($p > 0.05$), regardless of toothpaste.

The common peaks and areas detected in both enamel and dentin were phosphate (960 cm^{-1}) and carbonate (1070 cm^{-1}) for the Raman analysis. There were no statistical differences among the toothpaste types for enamel surfaces in relation to phosphate and carbonate areas ($p > 0.05$). However, using only one toothpaste, ECR-E presented a lower phosphate area than SnF_2 ($p< 0.05$), ERMGIC-C showed lower carbonate area for NaF ($p < 0.05$). In relation to phosphate areas for dentin surfaces, SnF_2 showed higher area values than NaF ($p < 0.05$). Regarding dentin surfaces using only one toothpaste, control surfaces (DRMGIC and DCR) presented higher phosphate and carbonate area values than the eroded surfaces (DRMGIC and DCR), for all types of toothpaste ($p < 0.05$).

---

[1] Tabelas complementares referentes as análises de EDS (ANEXO C).

3.5 Discussion

Hardness analyses are one of the most widely used quantitative methods for measuring the mechanical properties of a substrate or material. [23]. There are distinct types of hardness depending on the indenter type, as well as for the load and penetration depth of the indenter. [23]. Depending on the substrate to be analyzed, and the degree of erosion of that tissue, the surface microhardness becomes inadequate, because the limits of indentation are unclear. Thus, measurement is inaccurate or impossible [23]. Nanohardness also produces small indentation regions, enabling the differentiation of areas that are intertubular, peritubular, or dentinal-tubular [26=24]. In the present study the dentin indentations were performed on the intertubular region. It was possible to analyze both the elastic deformation, which is transient and plastic deformation, which is permanent [18,23]. Nanoindentation also allows the calculation of the elastic modulus (Er), offering another parameter for the evaluation of the acid impact on substrates and restorative materials [23].

The first null hypothesis was rejected, since there were differences among the toothpaste types for nanomechanical properties, in particular for Er. One reason for differences found on control DRMGIC surfaces for H and control enamel surfaces for Er may be associated with the diffusion of citric acid or toothpaste slurry through the control surface that was isolated acid-resistant varnish since the blocks were previously selected by surface Knoop microhardness. This effect was observed in a previous study [27=25].

An interesting fact about nanohardness was that although there was no statistical difference among the enamel surfaces when using different toothpastes, enamel surfaces abrasioned with stannous-based toothpaste showed a hardness reduction of about 50% compared to other dentifrices. The reduced effect of the stannous toothpaste might be associated with the binding between negative zeta potential abrasive silica particles to positive stannous ions (Sn^{2+}) which may reduce the anti-erosive action of the toothpaste [10]. In the present study, regardless of the toothpaste employed, all eroded surfaces, except for the CR surface, showed a decrease in H and Er values. Thus, no toothpaste was able to maintain the nanomechanical properties, possibly because a protective layer was not formed. SEM images (Fig. 2A-2C) show enamel surfaces with notable irregularities and without the presence of a significant protective layer. A study that evaluated the application of NaF and TiF_4 varnishes concluded that NaF was not able to form a protective layer on enamel [26] Moreover, it is known that H and Er values may be affected depending on factors such as the region where indentation was performed [24]. It is worth noting that no differences among these toothpaste types were found in another study from our group, where ultramicroharness was used to evaluated dentin surfaces [25].

Regarding restorative materials, hardness tests allow indirect evaluation of the degree of monomers conversion to polymers (a material with higher hardness values has better polymerization conversion rate) [27]. In contrast, elastic modulus (Er) of an ideal restorative material should be slightly lower or similar to dentin, facilitating the transmission of adhesive interface forces [28]. In general, RMGIC was vulnerable to the erosive process, with a decrease in H and Er values, it´s important to highlight that the RMGIC indentations were performed on the polymeric matrix instead of inorganic paritcles. The ionomeric material naturally has lower hardness than CR, which was observed in another study [25]. In addition, the association of the erosive process with abrasion using toothpaste types with different abrasive levels seems to have accentuated the modification of its structure and contributed to the decrease of its mechanical values [25,29,30]; this association was more notable after brushing with stannous-based toothpaste. On the other hand, the CR presented higher values than glass ionomer cement in terms of mechanical properties, as was seen in other studies [29,30]. Besides, no effects on CR of erosive -abrasive cycles were noted independently of the toothpaste. This is likely associated with the composition of its organic matrix (BIS-GMA) and the arrangement or percentage of its nanoparticles [31].

Comparing the same type of surface when brushed with a single toothpaste, superior mechanical properties were found for ERMGIC compared to ECR in WF groups, except for the nanohardness of the eroded enamel. The effects of fluoride released only from RMGIC may act on the enamel surface when brushed with fluoride-free toothpaste [32].

The second null hypothesis was also rejected because there were differences in the chemical composition of the dental surfaces and restorative materials. EDS is widely used to investigate the chemical composition of surfaces, which uses a semi-quantitative or quantitative method to analyze substrates and materials [6,23,33]. Minerals from dental tissues are imperfect forms of hydroxyapatite; these imperfections result from the incorporation of ‘impure’ ion crystals from tissue fluids, as well as from mineral crystals during hard tissue formation [6]. Dental mineral tissues, when calcium deficient, such as carbonated hydroxyapatites, may contain some ions such as Na, K, Mg, Cl, Zn, Pb, Cu, and Al [6, 34]. It is known that this hydroxyapatite ion exchange can generate greater stress on enamel tissue, making it more susceptible to solubility [6]. Thus, it is possible to notice the presence of chemical elements of Na, Mg, Cl, and K, which corroborates the minerals detected through the EDS analysis in the present study.

Factors related to toothpaste types such as the type of fluoride compound, possible formation of precipitates on the surface, pH of the active agent, and duration and frequency of application are characteristics influencing the potential of chemical changes on the surfaces [33]. Regarding eroded enamel surfaces, lower Ca and P concentrations were found for all types of toothpaste, and is associated with the dissolution of hydroxyapatite. Thus, the loss of Ca and P ions after the erosive-abrasive cycle demonstrate that the toothpaste types did not prevent the dissolution of hydroxyapatite in relation to the control surface [35]. Furthermore, the Ca and P were more evident in enamel than dentin, corroborating with another study that investigated the chemical composition of eroded dental tissues and concluded that enamel naturally has a higher concentration of these compounds [6]. Another relevant fact was the absence of the Sn on the enamel surfaces on which the stannous-based toothpaste was applied, probably due to the fragility of SnF_2 that may have been removed after brushing [33]. Due to the instability of some compounds, a vehicle that can adhere to the dental surface is necessary such as fluoride gel and varnishes [33]; as well as the association of polymers with fluoride compounds, which can optimize the chemical bonding of these compounds to the dental surface [36]. NaF toothpaste seems have a potential effect on enamel adjacent to RMGIC, because no differences were found between control and eroded surfaces for Ca and P.

The presence of Sn was detected in a few eroded dentin specimens. Although it is an anti-erosive toothpaste, in this case, it seems to have acted more to obliterate the dentinal tubules (Fig. 2F) and act as a desensitizer. At least parts of the precipitates are loosely bound to the dentin surface and can be easily removed by brushing, since brushing reduced the protective effect of the test solutions in another study [33]. Lower tissue loss was observed in toothpaste types with lower pH values, higher fluoride concentration, lower Ca and P concentrations, larger solid particles, and higher surface wettability [37]. Dentinal tubule occlusion was also influenced by the presence of Sn^{+2} [37]. In present study, the pH values found were WF = 5.59, NaF = 7.24 and SnF_2 = 6.62, which are considered high pH values. This fact may also have contributed to the lower protective effectiveness of the toothpaste. In another study using EDS analysis, the efficacy of solutions contained SnF is related to the incorporation of Sn ions on the mineralized dentin when the organic portion is preserved on the subsurface. However, Sn precipitation occurred when the organic portion was removed from the surface [34]. Furthermore, higher Sn concentration was associated with higher fluoride ppm [34]. Another point to be highlighted is the presence of silica in most eroded dentin surfaces, for all toothpaste types. According to Ganns et al. [10], silica values of up to 10 % could be more harmful to surfaces than concentrations above this value. However, specific compositional information of the toothpastes studied were not provided by the manufactures, resulting in a limitation of the present study.

In relation to restorative materials, the ionomeric material was influenced by the action of the fluoride-based toothpaste types once the eroded RMGIC surfaces showed an increasing Ca and decreasing F for NaF and SnF_2 toothpaste types after erosive-abrasive cycling. This is probably due to ion exchange with the environment. This may be associated with the material's ability to stabilize the pH and at the same, time exhibit fluoride release to the environment [32]. In addition, NaF and SnF_2 promoted higher alterations on eroded surfaces, which may be compatible with SEM images (Fig. 2H and 2I), showing the greater changes suffered by the material after erosive-abrasive cycles. In contrast, CR showed a similar composition of Si and Zr (Fig. 2J-2L) after erosive-abrasive cycles, corroborating with nanomechanical properties that also remained constant. Guler et al. [38], investigating the effect of beverages with different pH and citric acid on various resin-based restorative materials such as Z550 and Fuji II LC using AFM and SEM analysis. They observed that the ionomeric group presented deep cracks and spaces between the particles while the CR showed no significant changes, concurring with the images obtained in this study. Fluoride-based toothpaste affected the structural composition of the ionomeric material, as seen in the SEM images (Fig. 2H and 2I), whereas the CR showed neither changes in the chemical composition nor significant morphological surface alterations (Fig 2J, 2K, and 2L).

Raman spectroscopy is an analytical technique capable of measuring the molecular composition and vibration of a substrate or material, providing information about chemical changes in samples [22]. In dentistry, that can be useful to analyze calcium fluoride formation in enamel, as well as a resin-to-dentin interface in restored teeth [39]. Previous studies have used phosphate (960 cm^{-1}) since that is indicative of the P-O stretch associated with hydroxyapatite [39]. Therefore, analysis of the concentration of phosphate within the enamel is a good indicator of the degree of mineralization [39]. In the face of an erosive process, phosphate release can be expected once the hydroxyapatite is dissolved. Beyond that, biological apatite is calcium deficient and contains substantial amounts of carbonate (1070 cm^{1}) [40]. The bands represent the intensity of the signal according to frequency, and the mathematical exploitation of this allows comparative and quantitative analysis. It is expected that phosphate is released in the erosive processes, resulting in a decrease in the intensity of this band [39]. In the present study, there were no differences among the types of toothpaste. However, phosphate areas of eroded enamel showed lower values than the control brushed with stannous toothpaste. Carbonate areas of eroded enamel presented lower values than the control brushed with sodium fluoride toothpaste, for other surfaces as well as teeth brushed with WF, no changes were found for phosphate and carbonate bands after erosion-abrasion cycles. No differences were found between intact and eroded enamel in extracted primary teeth either [40].

Furthermore, since the volumes involved were tiny, there could be an overestimation of the amount of phosphate released from the apatite crystal [40]. For dentin surfaces, there were differences between eroded and control surfaces, since decreased phosphate and carbonate were observed for all types of toothpaste. In other words, dentin surfaces were affected by the erosive-abrasive cycle, not by the action of the toothpaste.

Further *in situ* and *in vivo* studies are required to thoroughly analyze the mechanical and chemical alterations of composite resin and glass ionomer cement restorations in eroded enamel and dentin, since the presence of saliva and salivary pellicle influences the dissolution and abrasion behavior of dental, restorative materials surfaces, as well as the formations and stability of fluoride precipitates [41-43].

3.7 Conclusion

Regarding the nanomechanical properties, stannous toothpaste presented had a negative effect on elastic modulus of enamel and ionomeric eroded surfaces. However, the fluoride toothpastes had positive effect only on eroded dentin adjacent to composite resin, increasing Ca and P concentrations. The stannous toothpaste decreased the phosphate while the sodium toothpaste decreased carbonate on the eroded enamel. The composite resin showed unchanged after erosion for both nanomechanical and chemical properties regardless of toothpaste, while, ionomeric material presented some alterations in these properties.

3.8 Acknowledgments

This study was financially supported by CAPES 001.

3.9 References

1. Schlueter N, Amaechi BT, Bartlett D, Buzalaf MAR, Carvalho TS, Ganss C, Hara AT, Huysmans MDNJM, Lussi A, Moazzez R, Vieira AR, West NX, Wiegand A, Young A, Lippert F (2019) Terminology of erosive tooth wear: consensus report of a workshop organized by the ORCA and the cariology research group of the iADR. Caries Res 14:1-5. https://doi: 10.1159/000503308.

2. Coupal I, Soltysiak A (2017) Dental erosion in archaeological human remains: A critical review of literature and proposal of differential diagnosis protocol. Arch Oral Biol 84:50-57. https://doi: 10.1016/j.archoralbio.2017.09.011

3. Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, Schmalz G, Shellis RP, Tveit AB, Wiegand A (2015) Consensus report of the european federation of conservative dentistry: erosive tooth wear-diagnosis and management. Clin Oral Investig 19:1557-1561. https://doi: 10.1007/s00784-015-1511-7

4. Schlueter N, Glatzki J, Klimek J, Ganss C (2012) Erosive-abrasive tissue loss in dentine under simulated bulimic conditions. Arch Oral Biol 57:1176-82. https://doi: 10.1016/j.archoralbio.2012.04.001

5. Lussi A, Buzalaf MAR, Duangthip D, Anttonen V, Ganss C, João-Souza SH, Baumann T, Carvalho TS (2019) The use of fluoride for the prevention of dental erosion and erosive tooth wear in children and adolescents. Eur Arch Paediatr Dent Feb 14. https://doi: 10.1007/s40368-019-00420-0

6. Ganss C, Lussi A, Schlueter N (2014) The histological features and physical properties of eroded dental hard tissues. Monogr Oral Sci 25:99-107. https://doi: 10.1159/000359939

7. Shellis RP, Featherstone JD, Lussi A (2014) Understanding the chemistry of dental erosion. Monogr Oral Sci 25:163-179. https://doi: 10.1159/000359943

7. Schlueter N, Lussi A, Tolle A, Ganss C (2016) Effects of erosion protocol design on erosion/abrasion study outcome and on active agent (NaF and SnF2) efficacy. Caries Res 50:170-179. https:// doi: 10.1159/000445169

8. Moda MD, Fagundes TC, Bresciani E, Briso ALF, Dos Santos PH (2019) Comparison of in vitro erosion protocols in bovine teeth to simulate natural erosion lesion: analysis of mechanical properties and surface gloss. J Appl Oral Sci 7;27:e20180107. https://doi: 10.1590/1678-7757-2018-0107

9. Dündar A, Şengün A, Başlak C, Kuş M (2018) Effects of citric acid modified with fluoride, nano-hydroxyapatite and casein on eroded enamel. Arch Oral Biol 93:177-186. https://doi: 10.1016/j.archoralbio.2018.06.009

10. Ganss C, Möllers M, Schlueter N (2017) Do abrasives play a role in toothpaste efficacy against erosion/abrasion? Caries Res 51:52-57. https://doi:10.1159/000452867

11. Pini NI, Lima DA, Lovadino JR, Ganss C, Schlueter N (2016) In vitro efficacy of experimental chitosan-containing solutions as anti-erosive agents in enamel. Caries Res 50:337-345. https://doi:10.1159/000445758

12. Aykut-Yetkiner A, Attin T, Wiegand A (2014) Prevention of dentine erosion by brushing with anti-erosive toothpastes. J Dent 42: 856-861. https://doi: 10.1016/j.jdent.2014.03.011

13. Souza BM, Comar LP, Vertuan M, Fernandes Neto C, Buzalaf MA, Magalhães AC (2015) Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation *in situ*. Caries Res 49:499-507. https://doi: 10.1159/000438466

14. Alghilan MA, Cook NB, Platt JA, Eckert GJ, Hara AT (2015) Susceptibility of restorations and adjacent enamel/dentine to erosion under different salivary flow conditions. J Dent 43:1476-1482. https://doi:10.1016/j.jdent.2015.10.007

15. Francisconi LF, Honório HM, Rios D, Magalhães AC, Machado MA, Buzalaf MA (2008) Effect of erosive pH cycling on diferente restorative materials ando n enamel restored with these materials. Oper Dent 33:203-208. https://doi:10.2341/07-77

16. Cruz NV, Pessan JP, Manarelli MM, Souza MD, Delbem AC (2015) *In vitro* effect of low-fluoride toothpastes containing sodium trimetaphosphate on enamel erosion. Arch Oral Biol 60:1231-1236. https://doi:10.1016/j.archoralbio.2015.05.010

17. Strazzi Sahyon HB, Chimanski A, Yoshimura HN, Dos Santos PH (2018) Effect of previous photoactivation of the adhesive system on the color stability and mechanical properties of resin components in ceramic laminate veneer luting. J Prosthet Dent 20:631.e1-631.e6. https://doi:10.1016/j.prosdent.2018.06.014

18. Dos Santos PH, Karol S, Bedran-Russo AKB (2011) Long-term nano-mechanical properties of biomodified dentin–resin interface components. J Biomech 44:1691-1694. https://doi:10.1016/j.jbiomech.2011.03.030

19. Wiegand A, Schneider S, Sener B, Roos M, Attin T (2014) Stanility against brushing abrasion and protective effect of different fluoride compounds. Caries Res 48:154-162. https://doi:10.1159/000353143

20. Melo RA, Bispo ASL, Barbosa GAS, Galvão MR, de Assunção IV, Souza ROA, Borges BCD (2019) Morphochemical characterization, microhardness, water sorption, and solubility of regular viscosity bulk fill and traditional composite resins. Microsc Res Tech 82:1500-1506. https://doi: 10.1002/jemt.23315

21. Furini LN, Feitosa E, Alessio P, Shimabukuro MH, Riul A Jr, Constantino CJ (2013) Tuning the nanostructure of DODAB/nickel tetrasulfonated phthalocyanine bilayers in LbL films. Mater Sci Eng C Mater Biol Appl 33:2937-2946. https://doi: 10.1016/j.msec.2013.03.019

22. Toledano M, Cabello I, Osorio E, Aguilera FS, Medina-Castillo AL, Toledano-Osorio M, Osorio R (2019) Zn-containing polymer nanogels promote cervical dentin remineralization. Clin Oral Investig 23: 1197-1208. https://doi:10.1007/s00784-018-2548-1

23. Schlueter N, Hara A, Shellis RP, Ganss C (2011) Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res 45 Suppl 1, 13-23. https://doi:10.1159/000326819

24. Basting RT, Leme AA, Bridi EC, Amaral FL, França FM, Turssi CP, Bedran-Russo AK (2015) Nanomechanical properties, SEM, and EDS microanalysis of dentin treated

with 2.5% titanium tetrafluoride, before and after na erosive challenge. J Biomed Mater Res B Appl Biomater 103:783-789. https://doi:10.1002/jbm.b.33254

25. Gonçalves DFM, Briso ALF, Pini NIP, Moda MD, Parpinelli de Oliveira R, Santos PHD, Fagundes TC (2019) Effects of dentifrices on mechanical resistance of dentin and restorative materials after erosion and abrasion. J Mech Behav Biomed Mater 97:7-12. https://doi:10.1016/j.jmbbm.2019.05.006

26. Medeiros MI, Carlo HL, Lacerda-Santos R, Lima BA, Souza FB, Rodrigues JA, Carvalho FG (2016) Thickness and nanomechanical properties of protective layer formed by TiF4 varnish on enamel after erosion. Braz Oral Res 30:1. https://doi:10.1590/1807-3107BOR-2016

27. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, Biasotto M, Contardo L, Breschi L (2005) Degree of conversion and permeability of dental adhesives. Eur J Oral Sci. 113:525-530

28. Suzuki TY, Gomes-Filho JE, Gallego J, Pavan S, Dos Santos PH, Fraga Briso AL (2015) Mechanical properties of components of the bonding interface in different regions of radicular dentin surfaces. J Prosthet Dent 113:54-61. https://doi: 10.1016/j.prosdent.2014.07.009

29. Kaur S, Makkar S, Kumar R, Pasricha S, Gupta P (2015) Comparative evaluation of surface properties of enamel and different esthetic restorative materials under erosive and abrasive challenges: An *in vitro* study. Indian J Dent 6:172-180. https://doi: 10.4103/0975-962X.165047

30. Yu H, Wegehaupt FJ, Wiegand A, Roos M, Attin T, Buchalla W (2009) Erosion and abrasion of tooth-colored restorative materials and human enamel. J Dent 37:913-922. https://doi:10.1016/j.jdent.2009.07.006

31. Gajewski VE, Pfeifer CS, Fróes-Salgado NR, Boaro LC, Braga RR (2012) Monomers used in resin composites: degree of converstion, mechanical properties and water sorption/solubility. Braz Dent J 23:508-514

32. Rolim FG, Sá AF, Silva-Filho GW, Brandim Ade S, Vale GC (2016) Effect of high-fluoride dentifrice on enamel erosion adjacent to restorations *in vitro*. Oper Dent 41:157-161. https://doi:10.2341/14-292-L

33. Wiegand A, Schneider S, Sener B, Roos M, Attin T (2014) Stability against brushing abrasion and the erosion-protective effect of different fluoride compounds. Caries Res 48:154-162. https://doi:10.1159/000353143

34. Ganss C, Hardt M, Lussi A, Cocks AK, Klimek J, Schlueter N (2010) Mechanism of action of tin-containing fluoride solutions as anti-erosive agents in dentine - an *in vitro* tin-uptake, tissue loss, and scanning electron microscopy study. Eur J Oral Sci 118:376-84. https://doi: 10.1111/j.1600-0722.2010.00742.x

35. Vieira-Junior WF, Ferraz LN, Pini N, Ambrosano G, Aguiar F, Tabchoury C, Lima D (2018) Effect of toothpaste use against mineral loss promoted by dental bleaching. Oper Dent 43:190-200. https://doi:10.2341/17-024-TR

36. Bezerra SJC, João-Souza SH, Aoki IV, Borges AB, Hara AT, Scaramucci T (2018) Antierosive effect of solutions containing sodium fluoride, stannous chloride,and selecte d film-forming polymers. Caries Res 53:305-313. https://doi:10.1159/000493388.

37. João-Souza SH, Scaramucci T, Bühler Borges A, Lussi A, Saads Carvalho T, Corrêa Aranha AC (2019) Influence of desensitizing and anti-erosive toothpastes on dentine permeability: An *in vitro* study. J Dent 89:103176. https://doi: 10.1016/j.jdent.2019.07.014

38. Guler S, Unal M (2018) The evaluation of color and surface roughness changes in resin based restorative materials with different contents after waiting in various liquids: An SEM and AFM study. Microsc Res Tech 81:1422-1433. https://doi: 10.1002/jemt.23104

39. Gilchrist F, Santini A, Harley K, Deery C (2007) The use of micro-Raman spectroscopy to differentiate between sound and eroded primary enamel. Int J Paediatr Dent 17:274-280.

40. Osorio R, Toledano-Osorio M, Osorio E, Aguilera FS, Padilla-Mondéjar S, Toledano M (2018) Zinc and silica are active componentes to efficiently treat in vitro simulated eroded dentin. Clin Oral Investig 22:2859-2870. https://10.1007/s00784-018-2372-7

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

42. Joiner A, Schwarz A, Philpotts CJ, Cox TF, Huber K, Hannig M (2008) The protective nature of pellicle towards toothpaste abrasion on enamel and dentine. J Dent 36:360-368. https://doi: 10.1016/j.jdent.2008.01.010

43. Wiegand A, Meier W, Sutter E, Magalhães AC, Becker K, Roos M, Attin T (2008) Protective effect of different tetrafluorides on erosion of pellicle-free and pellicle-covered enamel and dentine. Caries Res 42:247-254. https://doi: 10.1159/000135669

Figures

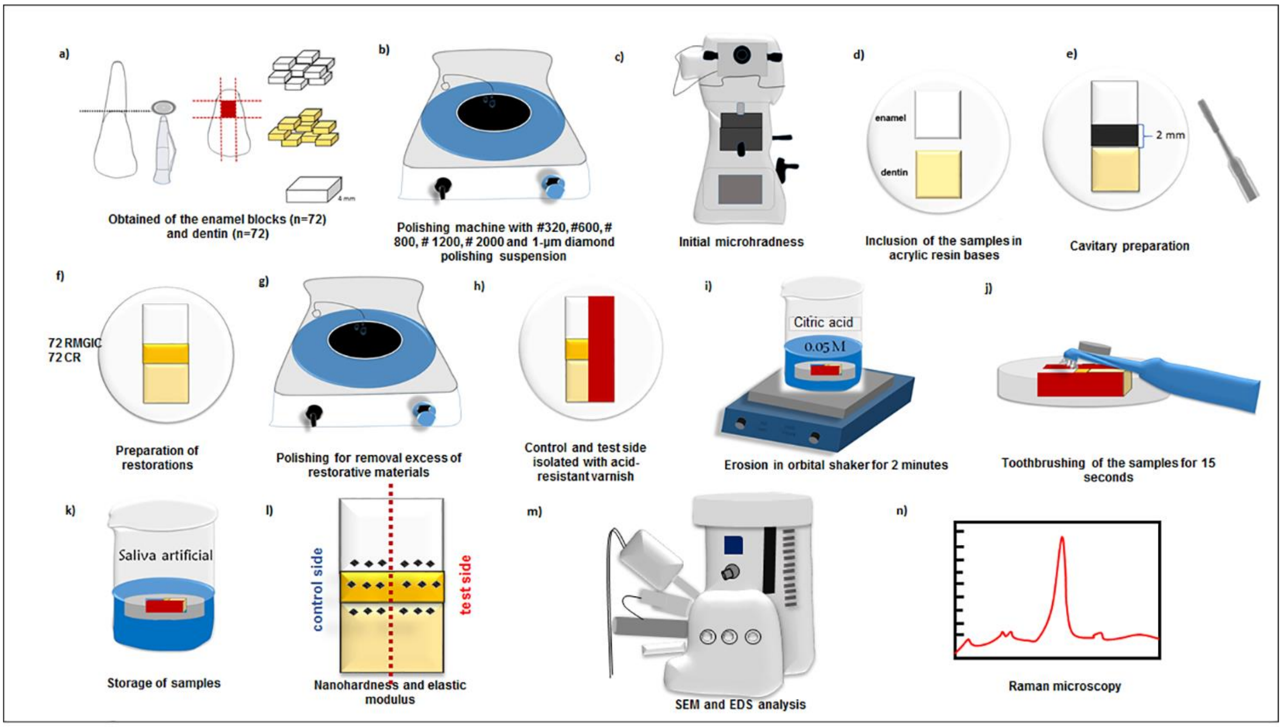


Figure 1. Study flowchart (a) Obtaining 120 bovine incisors, 60 enamel and 60 dentin blocks (4 x 4 mm^2). (b) After, the specimens were polished in an automatic polishing machine. (c) The, was performed to surface microhardness analysis to select specimens. (d) Enamel and dentin blocks were included in acrylic base, being two specimens (enamel and dentin) in each base with a distance of 1 mm between them. (e) A cavity was prepared on the mesial face of the specimens, with a total sharp box of 2 x 2 mm. (f) The restorations of RMGIC or CR were applied. (g) The restorations were polished for the removal of excess of restorative material. (h) The hemiface of each specimen/restoration set was covered with an acid-resistant varnish (i). The specimens were submitted to erosive (4x/day). (j) and abrasive (2x/day) challenges. (k) The specimens were storage in artificial saliva among the erosive cycles. (l) The dental substrates and restorative materials were submitted to nanohardness and elastic modulus analysis. (m) SEM/EDS analyses of the dental surfaces and restorative materials. (n) Raman spectroscopy analysis of dental surfaces

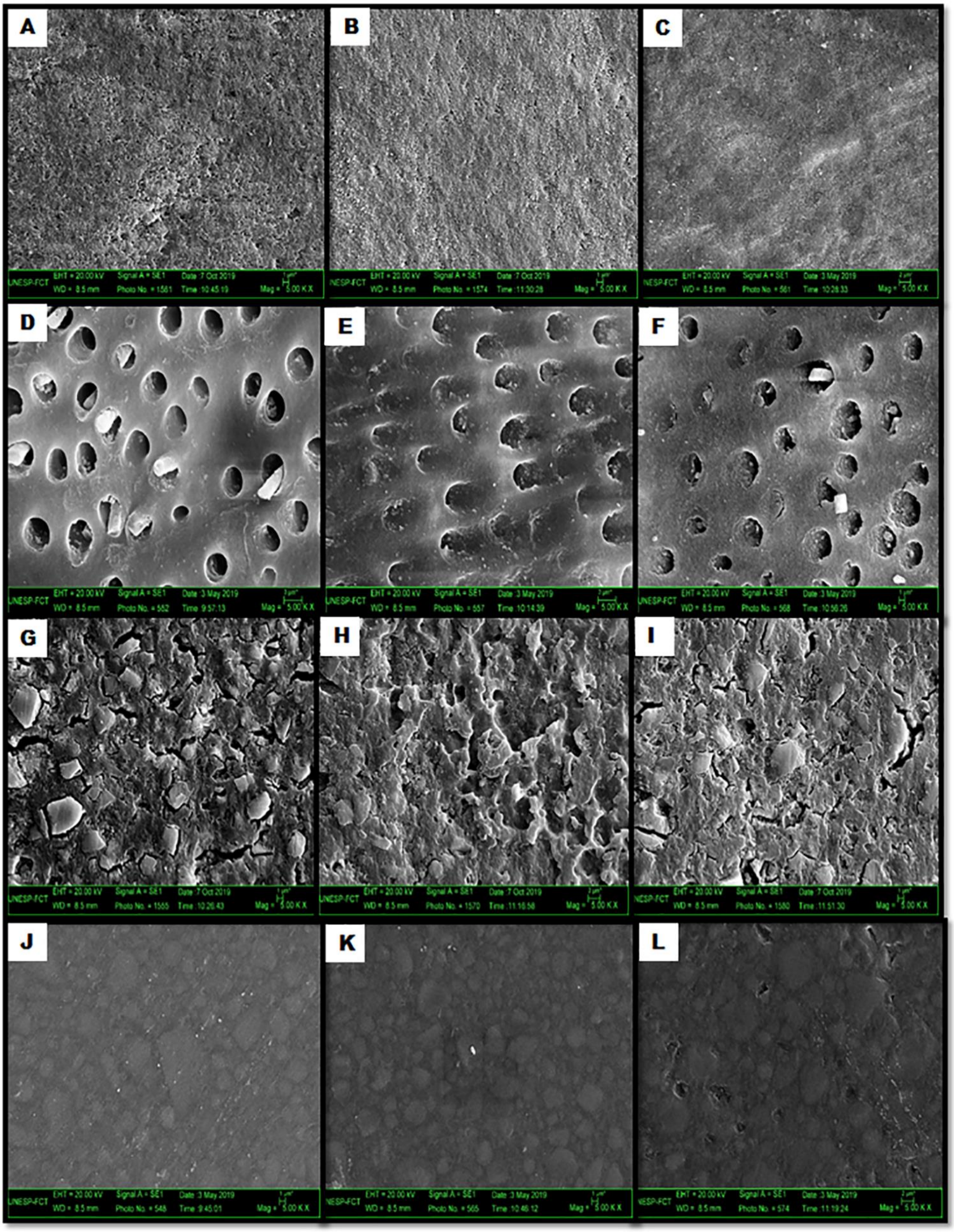
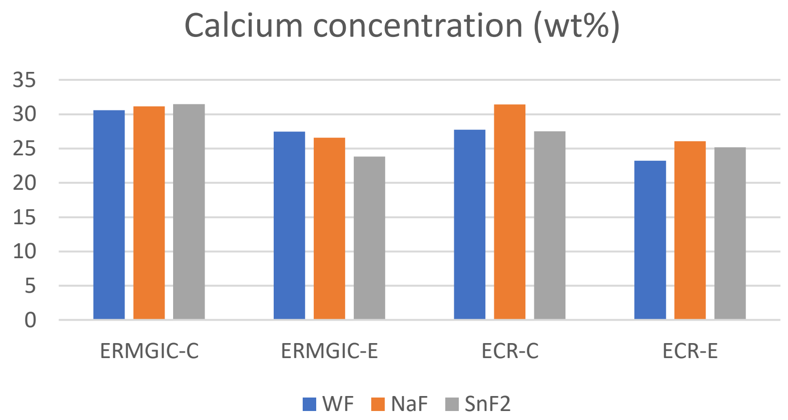

A
B
C
D
E
F
G
H
I
J
K
L

Figure 2. Representative SEM images of eroded surfaces (5,000 x). A: Eroded enamel surface brushing with WF toothpaste presented roughness. B: eroded enamel surface brushing with NaF toothpaste presented roughness. C: Eroded enamel surface brushing with SnF_2 toothpaste having mineral precipitation. D: Eroded dentin surface brushing with WF, dentinal tubules large and presence of odontoblastic processes. E: Eroded dentin surface brushing with NaF showed partial obliteration of the dentinal tubules. F: Eroded dentin surface brushing with SnF_2 also presented partial obliteration of the dentinal tubules. G: Eroded RMGIC surface brushing with WF showed some cracks. H: Eroded RMGIC surface brushing with NaF presented irregularities. I: Eroded RMGIC surface brushing with SnF_2 showed cracks and concavities. J: Eroded CR surface brushing with WF without alterations. K: Eroded CR surface brushing with NaF without alterations. L: Eroded CR surface brushing with SnF_2 showed a grooves.

Figure 3. Changes in enamel surfaces concentration [wt%] of the respective chemical elements by EDS analysis

a)



b)

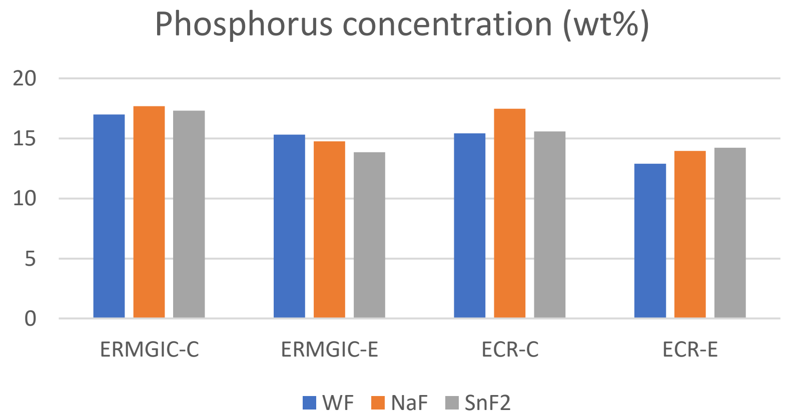
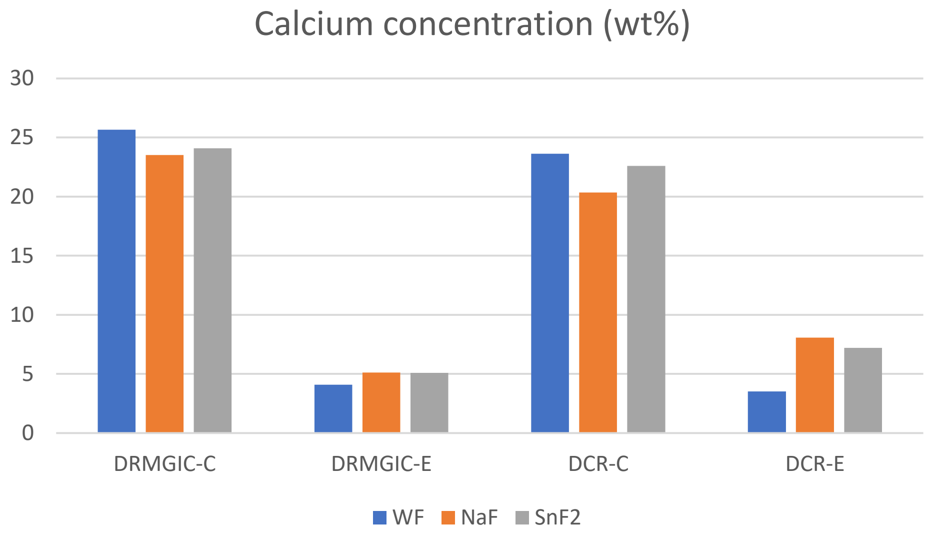


Figure 4. Changes in dentin surfaces concentration [wt%] of the respective chemical elements by EDS analysis

a)



b)

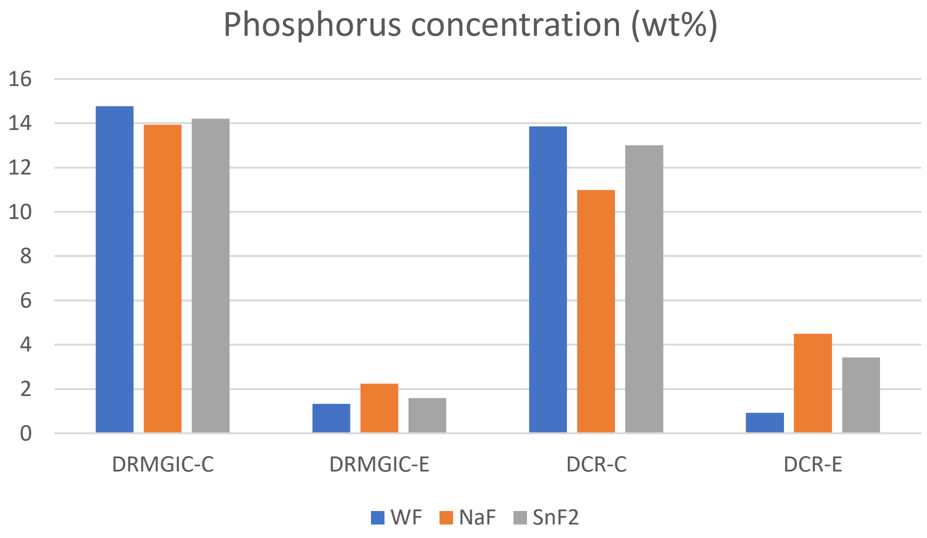


Figure 5. Changes in RMGIC surfaces concentration [wt%] of the respective chemical elements by EDS analysis

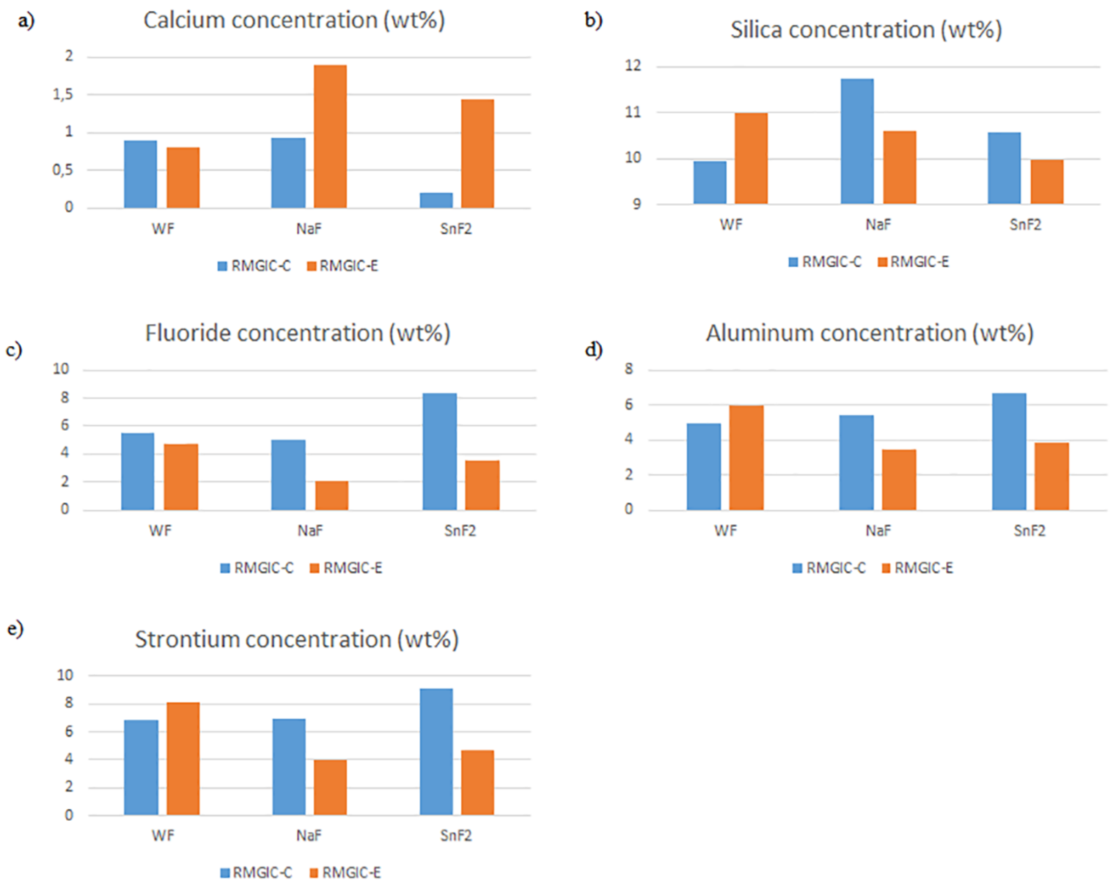
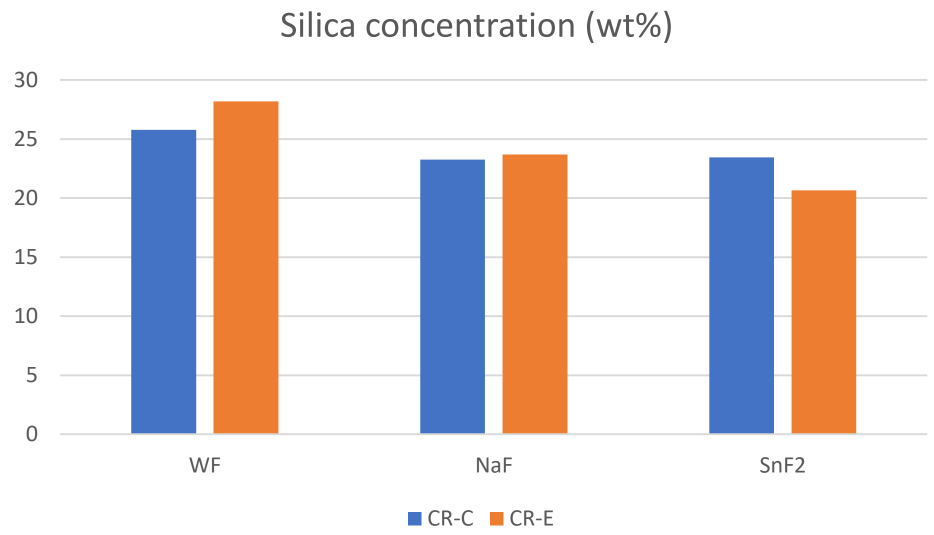


Figure 6. Changes in CR surfaces concentration [wt%] of the respective chemical elements by EDS analysis

a)



b)

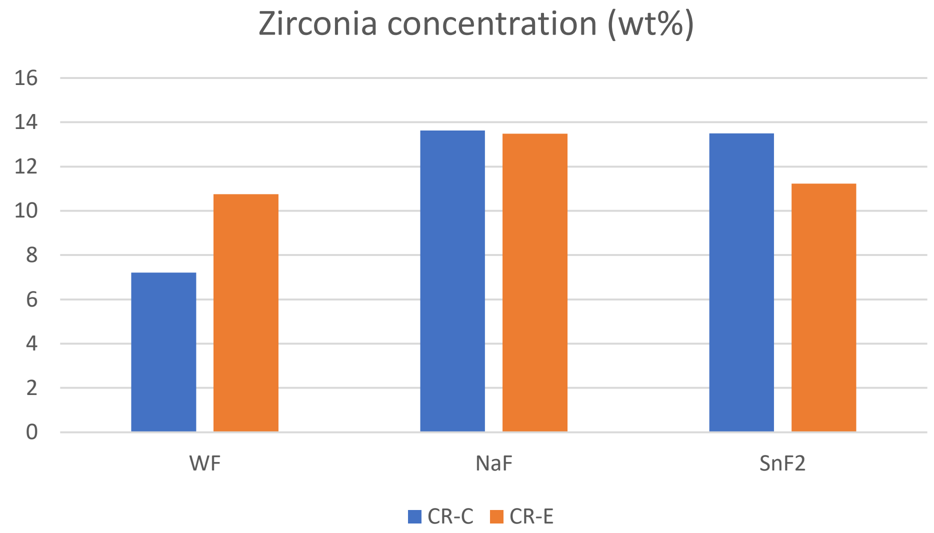


Table 1. Materials used in this study

Material	Application mode	Composition	Manufacturer
Adaper Single Bond 2 (Adhesive system)	Apply one layer of adhesive, wait for 20s, air stream for 5s, and polymerize for 10s	BisGMA, HEMA, dimethacrylates, ethanol, water, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	3M ESPE St. Paul, MN, USA.
Filtek Z350 XT (color A2B) Batch:672912	Apply increments of 2 mm and polymerize for 20s each	Bis-GMA, UDMA, Bis-EMA, TEGDMA, PEGDMA, Zirconia and agglomerates of silica, camphorquinone	3M ESPE St. Paul, MN, USA.
Fuji II LC (color A3) Batch:17051316	GC conditioner was applied for 20s, rinsed and dried for 10s. 1 level scoop of powder to 2 drops of liquid was dispensed and mixed for 15-20s. The mixture was transferred to the centrix syringe	Powder: fluor-amino-silicate glass. Liquid: aqueous solution of polycarboxylic acid, TEGDMA and HEMA	GC Corporation, Tokyo, Japan.

Material	Type	Composition	Manufacturer
Curaprox Enzycal Zero (RDA-60)* Batch:442MHDEXP1121	Fluoride-free Toothpaste (WF)	Water, Sorbitol, Hydrated Silica, Glycerin, Steareth-20, Titanium Dioxide (Cl 77891), Aroma, Sodium Phosphate, Carrageenan, Sodium Chloride, Citric Acid, Sodium Benzoate, Potassium Thiocyanate, Glucose Oxidase, Amyloglucosidase, Lactoperoxidase .	Trybol AG, Neuhausen AM Rheinfall, Swiss.
Colgate Total 12 (RDA-70/80)*	Sodium Fluoride Toothpaste (NaF)	Sodium Fluoride (1450 ppm as NaF) Water, Triclosan, Sorbitol, Silica, Sodium Lauryl	Colgate-Palmolive, São Bernardo do

Batch:6184BR121R		Sulfate, PMV / MA Copolymer, Sodium Hydroxide, Saccharin Sodium, Titanium Dioxide	Campo, SP, Brazil.
Crest Pro-Health (RDA-155)* Batch:6039GF	Stannous Fluoride Toothpaste (SnF_2)	Stannous fluoride (1100 ppm F as SnF_2) Glycerin, Hydrated Silica, Sodium Hexametaphosphate, Propylene Glycol, PEG 6, Water, Zinc Lactate, Trisodium Phosphate, Sodium Lauryl Sulfate, Sodium Lauryl Sulfate, Carrageenan, Sodium Saccharin, Xanthan Gum, Blue 1	P&G, Cincinnati, USA.

Table 2. Mean (SD) nanohardness (H) (GPa) values of surfaces and restorative materials using different toothpastes.

Control

Toothpaste	ERMGIC	ECR	RMGIC	CR	DRMGIC	DCR
WF	2.97 (0.45) ^{Aa}	2.66 (0.40) ^{Ab}	0.47 (0.20) ^{Ab}	0.69 (0.12) ^{Aa}	0.68 (0.15) ^{Aa}	0.63 (0.10) ^{Aa}
NaF	2.89 (0.73) ^{Aa}	2.96 (0.43) ^{Aa}	0.41 (0.19) ^{Ab}	0.67 (0.17) ^{Aa}	0.59 (0.12) ^{Ba}	0.61 (0.15) ^{Aa}
SnF_2	3.09 ± 0.83^{Aa}	2.98 ± 0.63^{Aa}	0.49 (0.21) ^{Ab}	0.70 (0.21) ^{Aa}	0.65 (0.13) ^{ABa}	0.67 (0.15) ^{Aa}

Eroded

Toothpaste	ERMGIC	ECR	RMGIC	CR	DRMGIC	DCR
WF	0.51 (0.17) ^{Aa*}	0.55 (0.22) ^{Aa*}	0.29 (0.09) ^{Ab*}	0.64 (0.08) ^{Aa}	0.05 (0.02) ^{Aa*}	0.10 (0.05) ^{Aa*}
NaF	0.52 (0.24) ^{Aa*}	0.50 (0.30) ^{Aa*}	0.34 (0.16) ^{Ab}	0.65 (0.18) ^{Aa}	0.08 (0.04) ^{Aa*}	0.06 (0.02) ^{Aa*}
SnF_2	0.27 (0.07) ^{Aa*}	0.23 (0.06) ^{Aa*}	0.25 (0.14) ^{Ab*}	0.63 (0.11) ^{Aa}	0.08 (0.03) ^{Aa*}	0.07 (0.02) ^{Aa*}

Upper case letters compare toothpastes in each control or eroded side. Lowercase letters compare surfaces separately ($p < 0.05$). *Statistical difference among the control and eroded surfaces.

ECR: enamel adjacent to composite resin; CR: composite resin; DCR: dentin adjacent to composite resin; ERMGIC: enamel adjacent to resin-modified glass ionomer cement; RMGIC: resin-modified glass ionomer cement; DRMGIC: dentin adjacent to resin-modified glass ionomer cement

Table 3. Mean (SD) elastic modulus (Er) (GPa) of surfaces and restorative materials using different toothpastes.

Control

Toothpaste	ERMGIC	ECR	RMGIC	CR	DRMGIC	DCR
WF	79.92 (7.45) ^{Aa}	64.74 (7.14) ^{Bb}	12.99 (3.38) ^{Aa}	13.60 (1.56) ^{Aa}	21.34 (3.56) ^{Aa}	18.70 (2.53) ^{Aa}
NaF	76.25 (14.51) ^{Ba}	82.37 (8.08) ^{Aa}	13.50 (3.07) ^{Aa}	13.31 (2.15) ^{Aa}	18.62 (2.97) ^{Aa}	18.26 (2.57) ^{Aa}
SnF_2	87.73 (14.86) ^{Aa}	75.22 (8.68) ^{Aa}	14.71 (4.26) ^{Aa}	14.26 (2.15) ^{Aa}	19.08 (3.90) ^{Aa}	19.70 (3.23) ^{Aa}

Eroded

Toothpaste	ERMGIC	ECR	RMGIC	CR	DRMGIC	DCR
WF	30.23 (8.63) ^{Aa*}	17.08 (8.22) ^{Bb*}	10.18 (2.37) ^{Ab*}	13.45 (1.51) ^{Aa}	1.54 (0.40) ^{Aa*}	2.65 (0.84) ^{Aa*}
NaF	34.52 (12.91) ^{Aa*}	34.59 (8.83) ^{Aa*}	10.38 (3.55) ^{Ab*}	13.52 (2.11) ^{Aa}	1.97 (0.61) ^{Aa*}	2.06 (0.79) ^{Aa*}
SnF_2	20.72 (9.90) ^{Ba*}	19.33 (3.26) ^{Ba*}	6.47 (1.14) ^{Bb*}	13.62 (1.50) ^{Aa}	2.36 (0.66) ^{Aa*}	1.99 (0.40) ^{Aa*}

Upper case letters compare toothpastes in each control or eroded side. Lowercase letters compare surfaces separately ($p < 0.05$). *Statistical difference among the control and eroded surfaces.

ECR: enamel adjacent to composite resin; CR: composite resin; DCR: dentin adjacent to composite resin; ERMGIC: enamel adjacent to resin-modified glass ionomer cement; RMGIC: resin-modified glass ionomer cement; DRMGIC: dentin adjacent to resin-modified glass ionomer cement

Table 4. Raman analysis in arbritary units (a.u.) of phosphate and carbonate for enamel

	Toothpaste	ERMGIC-C	ERMGIC –E	ECR-C	ECR-E
Phosfate	WF	21.480.48 Aa	21.233.25 Aa	22.910.76 Aa	18.867.15 Aa
	NaF	22.738.70 Aa	10.504.48 Aa	23.342.09 Aa	14.894.207 Aa
	SnF_2	22.704.72 Aa	9.544.844 Ab	23.826.80 Aa	16.608.54 Ab
Carbonate	WF	8.7105.55 Aa	7.811.1 Aa	9.147.602 Aa	7.592.61 Aa
	NaF	18.827.75 Aa	9.209.123 Aab	7.863.658 Aab	6.983.297 Ab
	SnF_2	19.452.03 Aa	7.437.626 Aa	9.779.156 Aa	7.191.421 Aa

Upper case letters compare toothpastes for each enamel surface. Lowercase letters compare each enamel surface to single toothpaste, $p< 0.05$.

surfaces

ECR: enamel adjacent to composite resin; CR: composite resin; DCR: dentin adjacent to composite resin; ERMGIC: enamel adjacent to resin-modified glass ionomer cement; RMGIC: resin-modified glass ionomer cement; DRMGIC: dentin adjacent to resin-modified glass ionomer cement

Table 5. Raman analysis in arbitrary units (a.u.) of phosphate and carbonate for dentin

	Toothpaste	DRMGIC-C	DRMGIC –E	DCR-C	DCR-E
Phosfate	WF	11.178.16 ABa	6.060.89 Ab	10.859.10 Aa	5.879.939 Ab
	NaF	9.703.929 Ba	6.533.032 Ab	9.935.627 Aa	5.687.787 Ab
	SnF_2	11.591.86 Aa	6.534.433 Ab	10.227.33 Aa	5.198.953 Ab
Carbonate	WF	4.741.671 Aa	2.126.61 Ab	4.642.99 Aa	1.749.055 Ab
	NaF	4.106.105 Aa	2.385.353 Ab	3.892.531 Aa	1.499.997 Ab
	SnF_2	52.757.41 Aa	21.21.655 Ab	42.673.69 Aa	13.324.13 Ab

Upper case letters compare toothpastes for each dentin surface. Lowercase letters compare each dentin surface to single toothpaste, $p < 0.05$.

surfaces

ECR: enamel adjacent to composite resin; CR: composite resin; DCR: dentin adjacent to composite resin; ERMGIC: enamel adjacent to resin-modified glass ionomer cement; RMGIC: resin-modified glass ionomer cement; DRMGIC: dentin adjacent to resin-modified glass ionomer cement

Anexo A – Referências da General Introduction

Alghilan MA, Cook NB, Platt JA, Eckert GJ, Hara AT. Susceptibility of restorations and adjacent enamel/dentine to erosion under different salivary flow conditions. J Dent. 2015 Dec;43(12):1476-82. doi: 10.1016/j.jdent.2015.10.007.

Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, Schmalz G, Shellis PR, Björg Tveit A, Wiegand A. Consensus report of the european federation of conservative dentistry: erosive tooth wear diagnosis and management. Swiss Dent J. 2016;126(4):342-346.

Ganss C, Hardt M, Blazek D, Klimek J, Schlueter N. Effects of toothbrushing force on the mineral content and demineralized organic matrix of eroded dentine. Eur J Oral Sci. 2009 Jun;117(3):255-60. doi: 10.1111/j.1600-0722.2009.00617.x.

Ganss C, Lussi A, Schlueter N. The histological features and physical properties of eroded dental hard tissues. Monogr Oral Sci. 2014;25:99-107. doi: 10.1159/000359939.

Ganss C, Marten J, Hara AT, Schlueter N. Toothpastes and enamel erosion/abrasion - impact of active ingredients and the particulate fraction. J Dent. 2016 Nov;54:62-67. doi: 10.1016/j.jdent.2016.09.005.

Ganss C, Möllers M, Schlueter N. do abrasives play a role in toothpaste efficacy against erosion/abrasion? Caries Res. 2017;51(1):52-57. doi: 10.1159/000452867.

Kumar S, Keeling A, Osnes C, Bartlett D, O'Toole S. The sensitivity of digital intraoral scanners at measuring early erosive wear. J Dent. 2019 Feb;81:39-42. doi: 10.1016/j.jdent.2018.12.005.

Marro F, De Lat L, Martens L, Jacquet W, Bottenberg P. Monitoring the progression of erosive tooth wear (ETW)using BEWE index in casts and their 3D images: A retrospective longitudinal study. J Dent. 2018 Jun;73:70-75. doi: 10.1016/j.jdent.2018.04.008.

Pini NIP, Schlueter N, Sundfeld D, Semper Hogg W, Santos-Silva AR, Lopes MA, Aguiar FHB, Lovadino JR, Lima DANL. Efficacy of Stannous Ions on Enamel Demineralization Under Normal and Hyposalivatory Conditions: A Controlled Randomized in Situ Pilot Trial. Caries Res. 2018 51:543-553. doi: 10.1159/000479041.

Sahyoun CC, Subhash HM, Peru D, Ellwood RP, Pierce MC. an experimental review of optical coherence tomography systems for noninvasive assess ment of hard dental tissues. Caries Res. 2019 Sep 18:1-12. doi: 10.1159/000502375.

Sakae LO, Bezerra SJC, João-Souza SH, Borges AB, Aoki IV, Aranha ACC, Scaramucci T. An in vitro study on the influence of viscosity and frequency of application of fluoride/tin solutions on the progression of erosion of bovine enamel. Arch Oral Biol. 2018 May;89:26-30. doi: 10.1016/j.archoralbio.2018.01.017.

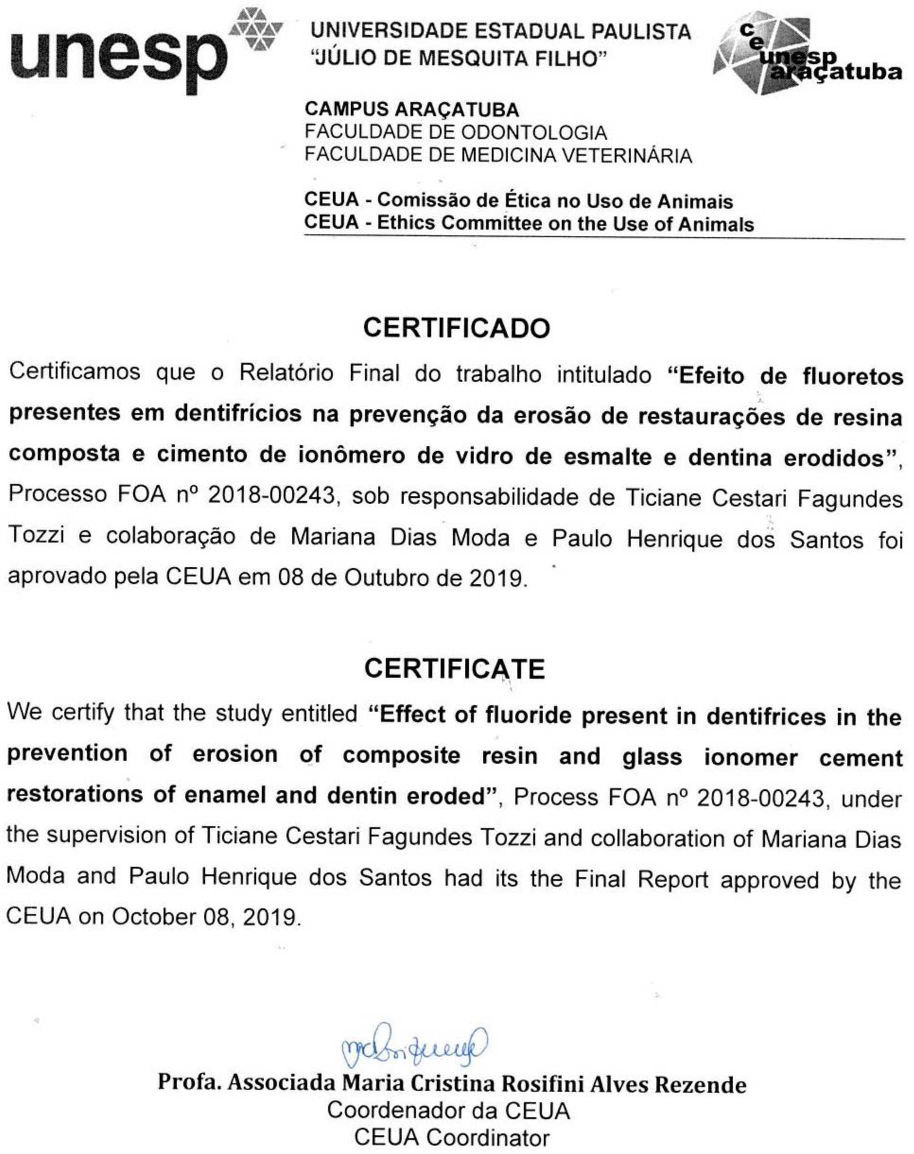
Schlueter N, Lussi A, Tolle A, Ganss C. Effects of erosion protocol design on erosion/abrasion study outcome and on active agent (naf and snf2) efficacy. Caries Res. 2016;50(2):170-9. doi: 10.1159/000445169.

Schlueter N, Amaechi BT, Bartlett D, Buzalaf MAR, Carvalho TS, Ganss C, Hara AT, Huysmans MDNJM, Lussi A, Moazzez R, Vieira AR, West NX, Wiegand A, Young A, Lippert F. Terminology of erosive tooth wear: consensus report of a workshop organized by the ORCA and the cariology research group of the IADR. Caries Res. 2019 14:1-5. https://doi: 10.1159/000503308.

Wiegand A, Schneider S, Sener B, Roos M, Attin T. Stability against brushing abrasion and the erosion-protective effect of different fluoride compounds. Caries Res. 2014;48(2):154-62. doi: 10.1159/000353143.

Young A, Thrane PS, Saxegaard E, Jonski G, Rölla G. Effect of stannous fluoride toothpaste on erosion-like lesions: an in vivo study. Eur J Oral Sci. 2006;114(3):180-3.

Anexo B - Certificado do Comitê de Ética no Uso de Animais (CEUA)



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Relatório Final do trabalho intitulado **"Efeito de fluoretos presentes em dentifrícios na prevenção da erosão de restaurações de resina composta e cimento de ionômero de vidro de esmalte e dentina erodidos"**, Processo FOA nº 2018-00243, sob responsabilidade de Ticiane Cestari Fagundes Tozzi e colaboração de Mariana Dias Moda e Paulo Henrique dos Santos foi aprovado pela CEUA em 08 de Outubro de 2019.

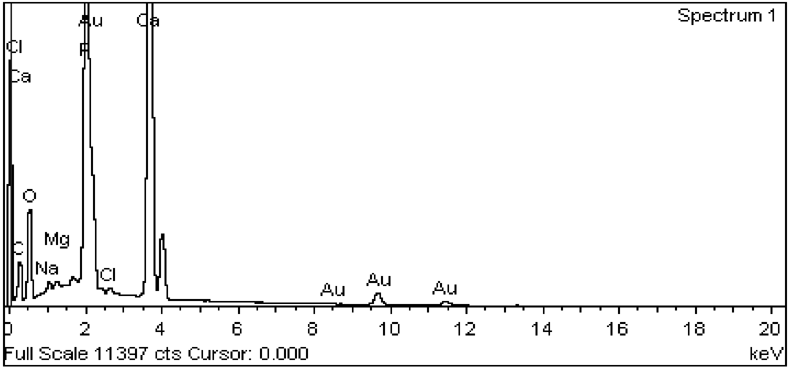
CERTIFICATE

We certify that the study entitled **"Effect of fluoride present in dentifrices in the prevention of erosion of composite resin and glass ionomer cement restorations of enamel and dentin eroded"**, Process FOA nº 2018-00243, under the supervision of Ticiane Cestari Fagundes Tozzi and collaboration of Mariana Dias Moda and Paulo Henrique dos Santos had its the Final Report approved by the CEUA on October 08, 2019.

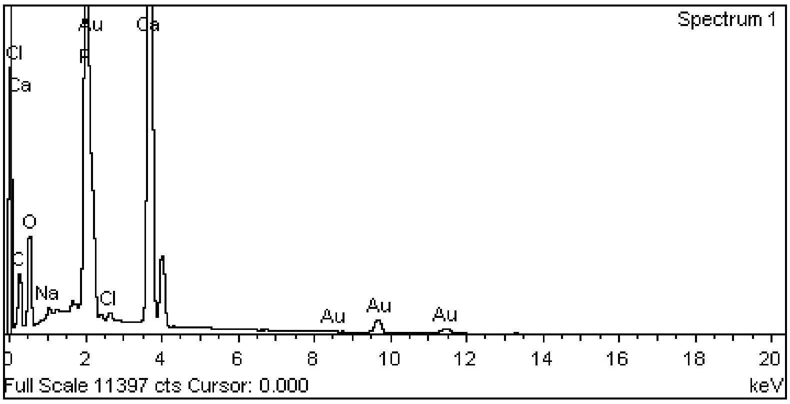
Profa. Associada Maria Cristina Rosifini Alves Rezende
Coordenador da CEUA
CEUA Coordinator

Anexo C- Espectroscopias representativas e tabelas complementares as análises de EDS

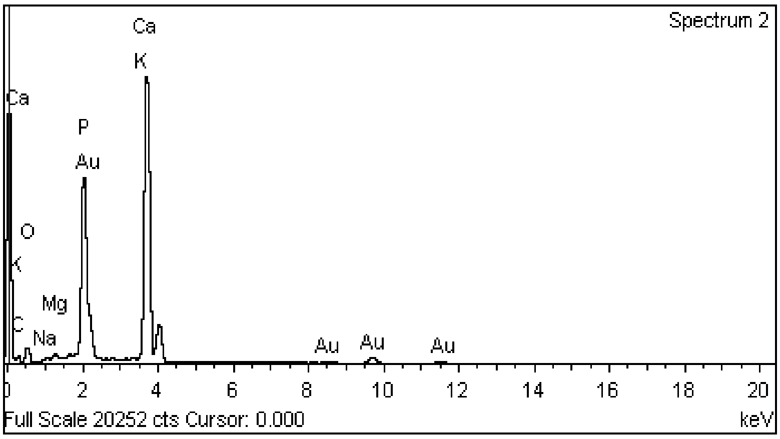
Graphic 1. Representative EDS spectroscopy for enamel surface



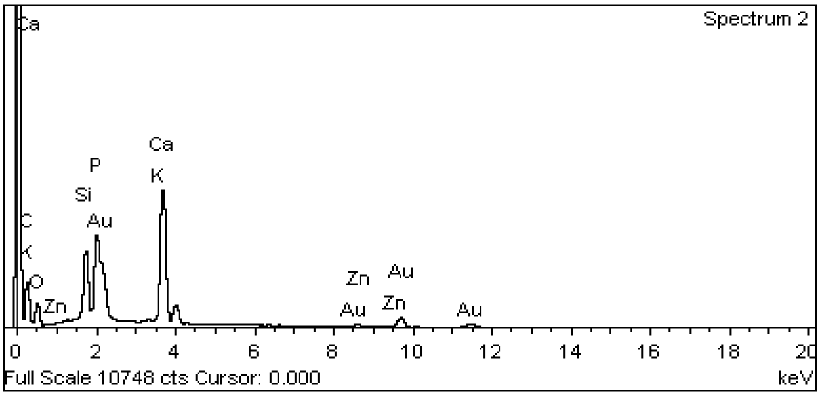
Graphic 2. Representative EDS spectroscopy for eroded enamel surface



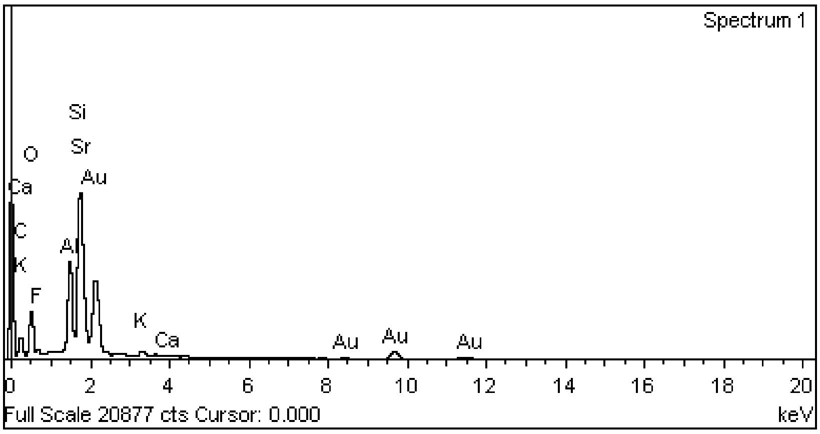
Graphic 3. Representative EDS spectroscopy for dentin surface



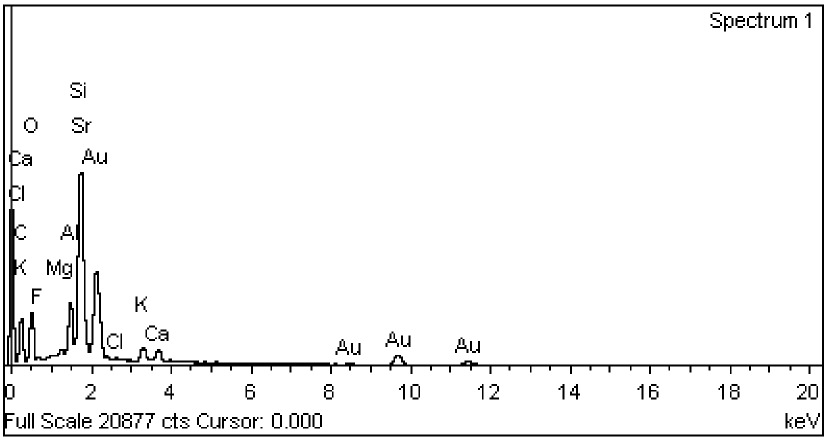
Graphic 4. Representative EDS spectroscopy for eroded dentin surface



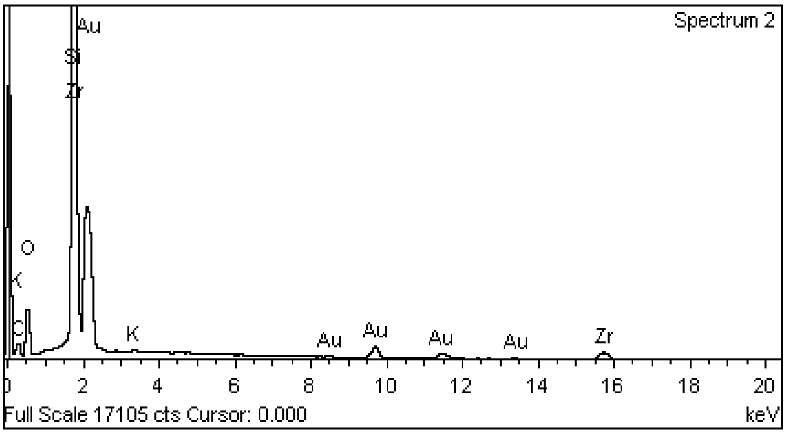
Graphic 5. Representative EDS spectroscopy for RMGIC surface



Graphic 6. Representative EDS spectroscopy for eroded RMGIC surface



Graphic 7. Representative EDS spectroscopy for CR surface



Graphic 8. Representative EDS spectroscopy for eroded CR surface

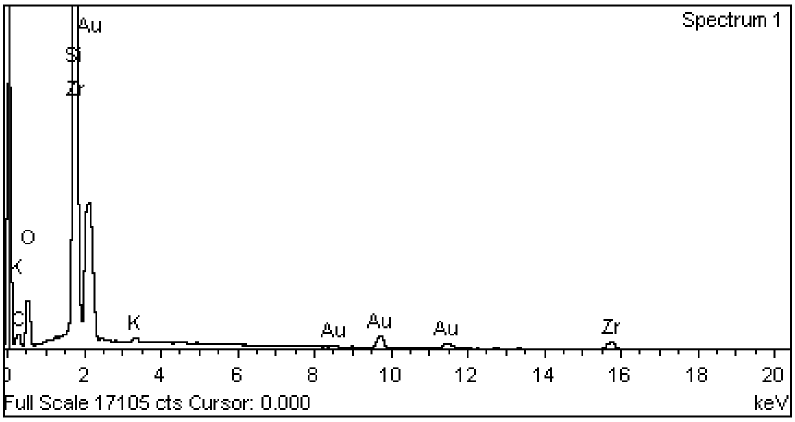


Table 1. Mean (Standard Deviation) of changes in enamel surfaces concentration [wt%] of the respective chemical elements by EDS analysis (200×200 µm)

	Toothpaste	**ERMGIC-C**	**ERMGIC –E**	**ECR-C**	**ECR-E**
Ca	WF	30.59 (4.55) Aa	27.46 (6.20) Aab	27.74 (4.51) Aab	23.24 (2.15) Ab
	NaF	31.13 (5.29) Aa	26.58 (6.07) Aa	31.42 (2.41) Aa	26.07 (2.71) Ab
	SnF_2	31.48 (1.48) Aa	23.85 (5.55) Ab	27.51 (7.05) Aab	25.19 (5.09) Aab
P	WF	17.00 (2.52) Aa	15.32 (2.88) Aab	15.44 (1.88) Aab	12.91 (0.55) Ab
	NaF	17.69 (1.79) Aa	14.76 (2.26)Aab	17.48 (0.96) Aa	13.96 (1.53) Ab
	SnF_2	17.32 (0.60) Aa	13.85 (2.62) Ab	15.60 (3.02) Aab	14.22 (2.64) Aab

Upper case letters compare toothpastes for each enamel surface. Lowercase letters compare each enamel surface to single toothpaste, $p < 0.05$.

ERMGIC-C: control enamel surface adjacent to resin-modified glass ionomer cement; ERMGIC-E: eroded enamel surface adjacent to resin-modified glass ionomer cement; ERC-C: control enamel surface adjacent to composite resin; ERC-E: eroded enamel surface adjacent to composite resin.

Table 2. Mean (Standard Deviation) of changes in dentin surfaces concentration [wt%] of the respective chemical elements by EDS analysis (200×200 µm)

	Toothpaste	**DRMGIC-C**	**DRMGIC –E**	**DCR-C**	**DCR-E**
Ca	WF	25.65 (1.91) Aa	4.09 (2.59) Ab	23.64 (1.29) Aa	3.51 (1.36) Bb
	NaF	23.51 (3.48) Aa	5.12 (2.56) Ab	20.36 (2.71) Ba	8.06 (3.05) Ab
	SnF_2	24.09 (1.39) Aa	5.09 (2.18) Ab	22.59 (2.38) ABa	7.20 (3.79) Ab
P	WF	14.77 (1.45) Aa	1.33 (2.24) Ab	13.86 (1.02) Aa	0.92 (1.42) Bb
	NaF	13.94 (1.77) Aa	2.24 (2.15) Ab	10.88 (3.08) Ba	4.50 (1.48) Ab
	SnF_2	14.21 (0.36) Aa	1.59 (2.50) Ab	13.01 (0.77) ABa	3.43 (2.84) Ab

Upper case letters compare toothpastes for each dentin surface. Lowercase letters compare each dentin surface to single toothpaste, $p < 0.05$.

DRMGIC-C: control dentin surface adjacent to resin-modified glass ionomer cement; DRMGIC-E: eroded dentin surface adjacent to resin-modified glass ionomer cement; DRC-C: control dentin surface adjacent to composite resin; DRC-E: eroded dentin surface adjacent to composite resin.

Table 3. Mean (Standard Deviation) of changes in RMGIC surfaces concentration [wt%] of the respective chemical elements by EDS analysis (200×200 µm)

	Toothpaste	**RMGIC-C**	**RMGIC –E**
Si	WF	9.94 (3.99) Aa	10.99 (1.75) Aa
	NaF	11.75 (3.22) Aa	10.61 (2.58) Aa
	SnF_2	10.58 (2.03) Aa	9.96 (1.35) Aa
Ca	WF	0.90 (0.89) Aa	0.80 (0.63) Ba
	NaF	0.94 (0.98) Ab	1.89 (0.85) Aa
	SnF_2	0.21 (0.19) Ab	1.44 (0.43) ABa
F	WF	5.49 (4.23) Ba	4.69 (0.59) Aa
	NaF	5.06 (4.78) Ba	2.09 (1.97) Ba
	SnF_2	8.30 (4.71) Aa	3.55 (1.33) ABb
Al	WF	4.98 (3.23) Aa	5.97 (1.00) Aa
	NaF	5.39 (1.55) Aa	3.46 (1.11) Bb
	SnF_2	6.65 (1.48) Aa	3.84 (1.53) Bb
Sr	WF	6.86 (4.35) Aa	8.09 (1.64) Aa
	NaF	6.99 (2.23) Aa	4.04 (1.81) Bb
	SnF_2	9.12 (0.65) Aa	4.65 (1.32) Bb

Upper case letters compare toothpastes for each dentin surfaces. Lowercase letters compare each dentin surface to single toothpaste, $p < 0.05$.

RMGIC-C: control resin-modified glass ionomer cement; RMGIC-E: eroded resin-modified glass ionomer cement.

Table 4. Mean (Standard Deviation) of changes in CR surfaces concentration [wt%] of the respective chemical elements by EDS analysis (200×200 µm)

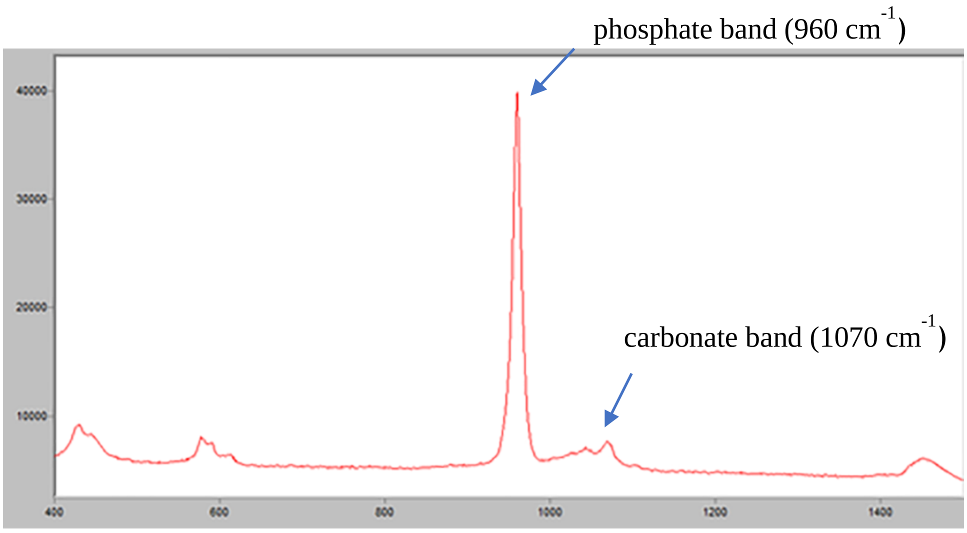
	Toothpaste	**CR-C**	**CR–E**
Si	WF	25.78 (1.49) Aa	28.19 (6.12) Aa
	NaF	23.26 (3.45) Aa	23.70 (4.54) Aa
	SnF_2	23.46 (2.27) Aa	20.67 (1.06) Aa
Zr	WF	7.21 (7.93) Aa	10.75 (8.36) Aa
	NaF	13.62 (3.66) Aa	13.48 (3.56) Aa
	SnF_2	13.50 (1.81) Aa	11.23 (0.66) Aa

Upper case letters compare toothpastes for each dentin surface. Lowercase letters compare each dentin surface to single toothpaste, $p < 0.05$.

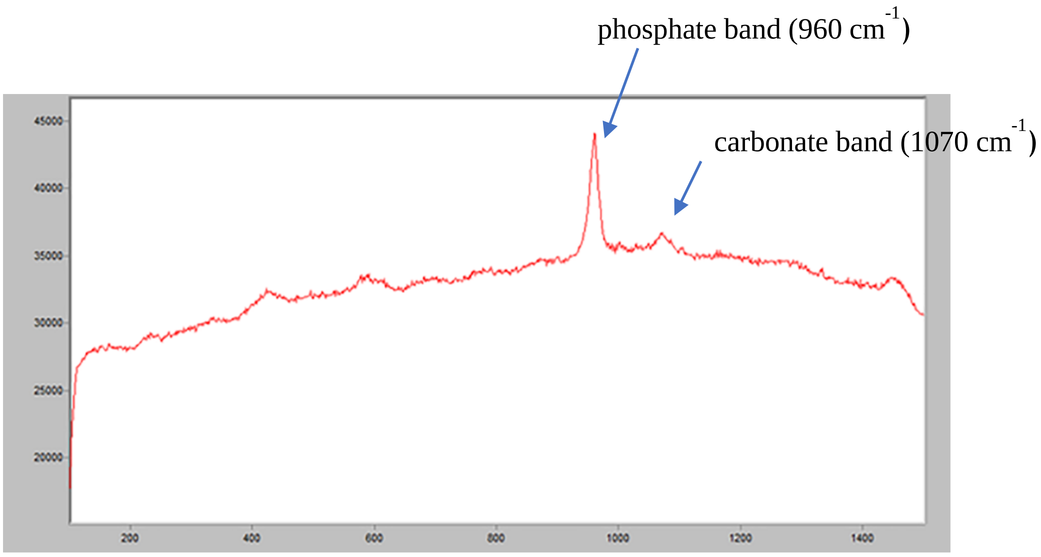
CR-C: control composite resin surface; CR-E: eroded composite resin surface.

Anexo D- Espectroscopias representativas de micro-Raman

Graphic 1. Representative micro-Raman spectroscopy for enamel surface showing phosphate (960 cm^{-1}) and carbonate (1070 cm^{-1}) bands.



Graphic 2. Representative micro-Raman spectroscopy for dentin surface showing the phosphate (960 cm^{-1}) and carbonate (1070 cm^{-1}) bands.



Anexo E - Normas das revistas selecionadas para a publicação dos artigos.

Capítulo 1

Periódico: Clinical Oral Investigations

https://www.springer.com/journal/784/submission-guidelines

Capítulo 2

Periódico: Journal of Dentistry

https://onlinelibrary.wiley.com/page/journal/10970029/homepage/forauthors.html