UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José dos Campos
Instituto de Ciência e Tecnologia

MARINA GULLO AUGUSTO

EFEITO ANTI-EROSIVO DE SOLUÇÕES CONTENDO DIFERENTES POLÍMEROS

2019

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Tese apresentada ao Instituto de Ciência e Tecnologia, Universidade Estadual Paulista (Unesp), Campus de São José dos Campos, como parte dos requisitos para obtenção do título de DOUTOR, pelo Programa de Pós-Graduação em ODONTOLOGIA RESTAURADORA.
Área: Dentística. Linha de pesquisa: Avaliação clínica e laboratorial de alterações da estrutura dental, de materiais e de técnicas de prevenção e tratamento em Dentística.

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Coorientadora: Profa. Dra.Taís Scaramucci

São José dos Campos
2019

Instituto de Ciência e Tecnologia [internet]. Normalização de tese e dissertação [acesso em 2019]. Disponível em http://www.ict.unesp.br/biblioteca/normalizacao

Apresentação gráfica e normalização de acordo com as normas estabelecidas pelo Serviço de Normalização de Documentos da Seção Técnica de Referência e Atendimento ao Usuário e Documentação (STRAUD).

Augusto, Marina Gullo
Efeito anti-erosivo de soluções contendo diferentes polímeros / Marina Gullo Augusto. - São José dos Campos : [s.n.], 2019.
105 f. : il.

Tese (Doutorado em Odontologia Restauradora) - Pós-Graduação em Odontologia Restauradora - Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, São José dos Campos, 2019.
Orientadora: Alessandra Buhler Borges
Coorientadora: Tais Scaramucci

1. Erosão dental. 2. Esmalte dental. 3. Polímeros. 4. Fluoreto de sódio. 5. Estanho. I. Borges, Alessandra Buhler, orient. II. Scaramucci, Tais, coorient. III. Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, São José dos Campos. IV. Universidade Estadual Paulista 'Júlio de Mesquita Filho' - Unesp. V. Universidade Estadual Paulista (Unesp). VI. Título.

Ficha catalográfica elaborada pela Biblioteca Prof. Achille Bassi e Seção Técnica de Informática, ICMC/USP
com adaptações - STATI, STRAUD e DTI do ICT/UNESP.
Renata Aparecida Couto Martins CRB-8/8376

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São José dos Campos, 3 de dezembro de 2019.

DEDICATÓRIA

Ao meu pai (in memoriam) por me ensinar desde cedo
a importância do conhecimento

AGRADECIMENTOS

Agradeço a **Deus** por me proteger, dar forças, saúde e coragem nesta jornada. Obrigada por me manter forte na busca pelos caminhos direcionados para o bem.

À **UNESP – Universidade Estadual Paulista "Júlio de Mesquita Filho"**, na pessoa da diretora do Instituto de Ciência e Tecnologia de São José dos Campos, Profa. **Rebeca di Nicoló**. Agradeço imensamente pelos dez anos de aprendizado que vivi nesta casa. A UNESP marcou a minha vida e sempre terá um lugar especial no meu coração.

Ao **Programa de Pós-Graduação em Odontologia Restauradora**, na pessoa do coordenador Prof. **Alexandre Luiz Souto Borges**. Nos meus anos na Pós-graduação fui representante discente no Conselho do Programa, na Comissão Permanente de Pesquisa e na Congregação, e assim pude acompanhar todo o trabalho necessário para se manter um programa de excelência. Por isso, agradeço a todos que dedicam seu tempo e conhecimento ao Programa, e desejo muitos e muitos anos de sucesso e conquistas.

À **CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior** pela concessão de bolsa de estudos no país. E pela concessão de bolsa de estudos no exterior, através do Programa de Doutorado-Sanduíche no Exterior (PDSE) – Código de Financiamento 001.

À **FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo** (Processo nº 2016/15755-5) pela concessão de bolsa de estudos no país e pelo apoio financeiro essencial para realização dessa pesquisa.

À minha orientadora Profa. **Alessandra Bühler Borges**. Agradeço infinitamente por todos os ensinamentos e oportunidades que me proporcionou nestes seis anos. Com você aprendi muito mais do que odontologia e ciência, e sempre levarei comigo seu exemplo de pessoa determinada e apaixonada pelo que faz. Desejo que nossos caminhos continuem seguindo juntos no futuro.

À minha coorientadora Profa. **Tais Scaramucci** cuja participação enriqueceu este e outros trabalhos. Obrigada pela parceria e boa vontade em todos os momentos.

To Prof **Nadine Schlueter**. *Thank you for the opportunity to learn with you. The period in Germany provided me a great professional and personal growth, and that was only possible due to your support, trust and encouragement. Moreover, I thank* **Julia Lang, Benedikt Luka, Benjamim Hofmann, Sybille Rau, Andrea and Anya** *for making my journey easier.*

Aos **professores do Departamento de Odontologia Restauradora do ICT-UNESP** com quem tive o prazer de conviver e aprender muito durante a minha pós-graduação. Em especial ao Prof. **Carlos Torres**, Profa. **Taciana Caneppele**, Prof. **Eduardo Bresciani,** Prof. **César Pucci,** Prof. **Sérgio Gonçalves e** Profa. **Filomena Huhtala** que foram fundamentais para a realização deste e de outros trabalhos, assim como foram responsáveis por grande parte do meu crescimento profissional. Obrigada pelos conselhos, correções e pela confiança em mim depositada.

Ao Prof. **Clóvis Pagani** por acreditar no meu potencial e pelos atendimentos inesquecíveis que realizamos juntos. Obrigada pelos ensinamentos, pelas oportunidades e pelos momentos divertidos que passamos juntos.

À Profa. **Idalina Vieira Aoki** por me abrir as portas do mundo da química da Poli-USP. Obrigada por esta oportunidade e por todo o suporte que ofereceu para a realização deste trabalho.

Aos Professores da banca, Profa. **Marília Buzalaf**, Prof. **Thiago Carvalho**, Prof. **Tiago Campos** e Profa. **Taciana Caneppele.** Fiquei muito honrada por aceitarem meu convite e disponibilizarem seu precioso tempo para correção deste trabalho. Considero vocês uma fonte de inspiração.

Ao Sr. **Carlos Alberto Guedes** por sempre receber as pessoas com um sorriso e não medir esforços para ajudar a todos que o procuram.

Às funcionárias do Departamento de Odontologia Restauradora, **Fernanda, Josiana e Liliane**, pelo suporte, amizade e disposição para ajudar.

Aos funcionários da Seção Técnica de Pós-graduação, **Bruno, Sandra e Carolina**, por serem sempre tão solícitos e gentis.

Ao meu namorado, melhor amigo e maior incentivador **Guilherme Schmitt de Andrade**. Faltam palavras para agradecer o que você significa na minha trajetória. Obrigada pelos incontáveis momentos de alegria que vivemos juntos e pelo suporte nos momentos difíceis. Se um dia eu sonhei com a felicidade, hoje a encontrei. Estendo os agradecimentos aos seus pais, **Vera e Marco**, pelo carinho que me dedicam e pelo incentivo sempre.

À minha mãe **Francisca Helena Gullo da Silva Augusto** que através da sua determinação e garra me ensinou a lutar pelos meus objetivos. Obrigada por todo o apoio nesta jornada. Meus dias são mais felizes na sua companhia e da Luli.

Ao meu pai **Álvaro Augusto Neto** *(in memoriam)* por ter sido a primeira pessoa que acreditou no meu futuro na área acadêmica. Te dedico esta conquista e todas as outras que virão. O destino acabou por nos separar precocemente, mas os seus ensinamentos estarão sempre vivos em mim. Saudades eternas!

À minha irmã **Stella Gullo Augusto** que mesmo morando do outro lado do mundo está sempre presente em minha vida. Obrigada por ser meu maior exemplo de coragem e positividade.

À minha amiga **Ingrid Fernandes Mathias Santamaria** que nunca mediu esforços para me apoiar e incentivar nos momentos alegres e nos difíceis com a mesma intensidade. Considero você uma irmã. Estendo os agradecimentos ao Prof. **Mauro Pedrini Santamaria** do qual tenho o privilégio de ser amiga e aluna. Obrigada pela parceria.

Aos meus irmãos árabes **Amjad e Aboud** que são um exemplo de lealdade e superação. Obrigada por fazerem com que eu me sentisse parte da família de vocês.

Às amigas **Cassia, Debora, Laura e Tania** pelos momentos juntas. A nossa amizade é um presente que ganhei da pós-graduação. Desejo que continuemos assim por muitos e muitos anos.

Aos amigos **Júlio, Ana Beatriz, Nathália, João Victor e Kiko** por tornarem os meus dias mais alegres e descontraídos. Obrigada pela parceria!

Aos meus alunos de iniciação científica **Luís Felipe, Giovanna e Beatriz**, através dos quais tive oportunidade de estar do outro lado da moeda. Na busca por ensiná-los, acabei aprendendo algo diferente com cada um. Obrigada!

Aos **voluntários que gentilmente doaram a saliva** utilizada neste estudo. Este trabalho não seria possível sem a colaboração de vocês.

À **Alexandra Elbakyan** pela sua coragem ímpar e iniciativa para permitir a popularização do conhecimento.

A **todos** que de alguma forma contribuíram para este trabalho.

"Os analfabetos do século XXI não serão aqueles que não conseguem ler e escrever, mas aqueles que não conseguem aprender, desaprender e reaprender"

Alvin Toffler

SUMÁRIO

Augusto MG. Efeito anti-erosivo de soluções contendo diferentes polímeros [tese]. São José dos Campos (SP): Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia; 2019.

RESUMO

O desgaste erosivo tem sido reconhecido como uma condição frequente nos últimos anos, principalmente devido a mudanças nos hábitos alimentares e comportamentais das populações em geral. Considerando a natureza irreversível desta condição, o diagnóstico precoce e a adoção de medidas preventivas são muito importantes. Dentre elas, a adição de polímeros a produtos de higiene bucal associados ou não a fluoretos apresenta-se como uma alternativa promissora, já que alguns polímeros apresentam compatibilidade com as estruturas dentais e capacidade de formação de um filme protetor. Este estudo foi subdivido em três artigos que visaram, através de diferentes abordagens, investigar o efeito anti-erosivo de polímeros formadores de filme, bem como o efeito da associação destes com fluoretos. O **primeiro artigo** consistiu em uma revisão da literatura sobre aspectos relacionados ao potencial de utilização dos polímeros para a prevenção da erosão dental. O **segundo artigo** consistiu em um estudo de varredura para verificar a capacidade de redução da dissolução da hidroxiapatita promovida por soluções contendo quatro polímeros (polioxirano, hidroxipropilmetilcelulose, pectina e um copolímero do polimetacrilato) associadas ou não com fluoreto de sódio -F (225 ppm F^-) e fluoreto de sódio + cloreto de estanho (800 ppm Sn^{2+}) - FS. A mensuração do potencial zeta da hidroxiapatita dispersa tratada com as soluções experimentais foi realizada a fim de complementar a análise. O **terceiro artigo** consistiu em um estudo de ciclagem erosiva/reendurecedora na presença de película adquirida que se propôs a investigar o potencial de remineralização, potencial de proteção, a perda superficial e a tensão superficial do esmalte após o tratamento com as soluções contendo o copolímero do polimetacrilato. Concluiu-se que a utilização de polímeros formadores de filme, associados ou não a fluoretos, constitui uma abordagem promissora para prevenção da erosão dental. Dentre os polímeros investigados, o copolímero do polimetacrilato é um promissor agente para ser adicionado à produtos de higiene bucal visando a prevenção dos desgastes erosivos.

Palavras-chave: Erosão dental. Esmalte dental. Polímeros. Fluoreto de sódio. Estanho.

Augusto MG. Anti-erosive effect of solutions containing different polymers [doctorate thesis]. São José dos Campos (SP): São Paulo State University (Unesp), Institute of Science and Technology; 2019.

ABSTRACT

Erosive wear has been recognized as a frequent condition in recent years, mainly due to changes in the dietary and behavioral habits of the general population. Considering the irreversible nature of this condition, early diagnosis and the adoption of preventive measures are very important. Among them, the addition of polymers to oral care products associated or not with fluorides is a promising alternative, as some polymers have compatibility with dental structures and ability to form a protective film. This study was subdivided into three articles that aimed, through different approaches, to investigate the anti-erosive effect of film-forming polymers, as well as the effect of their association with fluorides. The ***first article*** *consisted of a literature review about aspects related to the potential use of polymers to prevent dental erosion. The* ***second article*** *consisted of a scanning study to verify the ability to decrease the hydroxyapatite dissolution promoted by solutions containing four polymers (polyoxyrane, hydroxypropyl methylcellulose, pectin and a polymethacrylate copolymer) associated or not with sodium fluoride -F (225 ppm F^-) and sodium fluoride + tin chloride (800 ppm $Sn2^+$) -FS. Measurement of the zeta potential of dispersed hydroxyapatite treated with experimental solutions was performed to complement the analysis. The* ***third article*** *consisted of an erosive/rehardening cycling study in the presence of acquired pellicle that aimed to investigate remineralization potential, protection potential, surface loss and surface tension of the enamel after treatment with solutions containing the polymethacrylate. It was concluded that the use of film-forming polymers, associated or not with fluorides, is a promising approach for the prevention of dental erosion. Among the polymers investigated, the polymethacrylate copolymer is a promising agent to be added to oral hygiene products to prevent erosive wear.*

Keywords: Tooth erosion. Tooth enamel. Polymers. Sodium fluoride. Stannous.

1 INTRODUÇÃO

O surgimento de novos conceitos em Odontologia com ênfase nas propriedades preventivas dos fluoretos possibilitou um declínio substancial da prevalência da doença cárie, proporcionando um aumento na longevidade e integridade dos dentes (Marthaler, 2004). No entanto, o maior tempo de permanência dos dentes na cavidade bucal associado ao consumo crescente de bebidas e alimentos ácidos proporcionou um aumento na prevalência de desgaste erosivo nas últimas décadas, especialmente na população jovem (Jaeggi, Lussi, 2014).

O desgaste erosivo é o processo químico-mecânico que resulta em perda cumulativa de tecido dental não causado por bactérias (Schlueter et al., 2019). O início da condição erosiva no esmalte ocorre por meio de um processo chamado amolecimento (softening), que envolve a perda parcial do conteúdo mineral superficial do esmalte, levando ao aumento da rugosidade e a diminuição da dureza (Lussi et al., 2011). Em estágios mais avançados, o esmalte é completamente perdido levando à exposição da dentina subjacente e formando um defeito visível clinicamente (Ganss, Lussi, 2014). Em caso de continuidade dos episódios ácidos, pode-se perder estrutura dental a ponto de gerar dor, comprometimento pulpar, perda de função e estética (Peutzfeldt et al., 2014). Considerando a natureza irreversível desta condição, o diagnóstico precoce e a adoção de medidas preventivas e terapêuticas são muito importantes.

Para prevenir a erosão dental, diversas estratégias têm sido adotadas, incluindo a utilização de agentes remineralizantes à base de cálcio (De Alencar et al., 2014), irradiação com laser (Vlacic et al., 2007), aplicação de adesivos e/ou selantes de fissuras (Wegehaupt et al., 2013), uso de agentes inibidores de proteases (Buzalaf et al., 2012) e aplicação regular de produtos contendo fluoretos (Magalhaes et al., 2011).

Polímeros bioadesivos têm sido utilizados na indústria farmacêutica para aumentar o tempo de permanência de medicamentos no organismo. Do mesmo modo, a adição de polímeros em produtos de uso oral visa aumentar a substantividade dos ativos presentes na formulação (Zaman et al., 2010). Há evidências de que os polímeros interagem positivamente com compostos fluoretados, aumentando assim seu efeito protetor (Avila et al., 2017; Bezerra et al., 2019; White et al., 2011). Além disso, os polímeros bioadesivos, por si só, podem exibir algum efeito protetor contra dissolução ácida, provavelmente devido à sua interação com a superfície da hidroxiapatita, formando um filme capaz de reduzir a troca iônica entre a solução ácida e o substrato dental (Beyer et al., 2012).

Alguns polímeros têm sido testados como ingredientes ativos em enxaguantes bucais (Lei et al., 2014), dentifrícios (Carvalho, Lussi, 2014) e também como aditivos em bebidas ácidas (Scaramucci et al., 2011). Resultados promissores foram observados especialmente para caseína do leite (White et al., 2011), quitosana (Schlueter et al., 2014), polifosfato de sódio de cadeia linear (Hooper et al., 2007) e para a combinação entre carboximetilcelulose, goma xantana e copovidona (Gracia et al., 2010).

Diante desses resultados, podemos considerar que polímeros formadores de filme constituem uma opção interessante para serem utilizados em produtos de higiene bucal específicos para pacientes com alto risco ao desgaste erosivo. Sendo assim, é necessário que sejam desenvolvidos mais estudos testando a grande variedade desses agentes disponíveis no mercado, principalmente, os possíveis efeitos da combinação destes com fluoretos. Dentre as opções de polímeros a serem testados em enxaguantes bucais, podemos citar o polimetacrilato (Nielsen et al., 2011), polioxirano (Baig et al., 2004), hidroxipropilmetilcelulose (Baig et al., 2004), e a pectina (Beyer et al., 2012).

Há que se considerar ainda a interação dos polímeros com a película adquirida. Este filme orgânico é composto basicamente por proteínas salivares adsorvidas que revestem as estruturas da cavidade oral (Hara et al., 2006). A película atua como uma membrana seletiva que reduz a difusão de ácidos e consequentemente diminui a taxa de dissolução da hidroxiapatita, (Buzalaf et al., 2012; Hannig, Hannig, 2014). Há evidências de que componentes da dieta como a caseína, polifenóis e lipídios têm a habilidade de serem adsorvidos e incorporados pela película e consequentemente de melhorar seu potencial de inibição da erosão dental (Cheaib, Lussi, 2011; Hemingway et al., 2008; Kensche et al., 2013). A melhoria das propriedades protetoras da película adquirida através da modificação de sua composição pela interação com polímeros poderá trazer resultados promissores para a prevenção do desgaste erosivo.

Diante da necessidade de se proteger as estruturas dentais frente a desafios erosivos, se faz necessário investigar a capacidade de interação entre soluções à base de polímeros com a película adquirida visando determinar o potencial de tais soluções como agentes anti-erosivos, bem como o efeito da associação com o fluoreto de sódio associado ou não ao cloreto de estanho.

2 ARTIGOS

2.1 Artigo – Augusto MG, Scaramucci T, Campos TMB, Schlueter N, Borges AB. Polímeros formadores de filme para prevenção da erosão dental: uma visão geral / *Film-forming polymers for dental erosion prevention: an overview**

RESUMO

Objetivos: Esta revisão narrativa fornece uma visão geral sobre estrutura química de polímeros formadores de filme e os mecanismos envolvidos na interação destes com as superfícies dentais. A incorporação de polímeros em produtos de higiene bucal, bebidas ácidas e formulações de antiácidos visando a prevenção do desgaste erosivo também foi discutida. Fontes de dados: as bases de dados "Medline" e "Scopus" foram pesquisadas eletronicamente usando as principais palavras-chave: esmalte, polímero*, fluoreto* e erosão dental. O idioma foi restrito ao inglês. Conclusão: Os polímeros formadores de filme representam uma abordagem promissora para prevenir e controlar a erosão dentária. No entanto, estudos adicionais com condições clínicas mais relevantes são necessários para confirmar sua eficácia. Além disso, a padronização do desafio ácido, duração do tratamento e associação ao flúor é recomendada para permitir a comparação entre os estudos.

Palavras-chave: Esmalte. Dentina. Erosão dental. Polímeros. Fluoretos.

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*Artigo elaborado de acordo com as normas do Periódico *Journal of Dentistry*.

ABSTRACT

Objectives: This narrative review provide an overview regarding the chemical structure of film-forming polymers and the mechanisms related to their interaction with the tooth surfaces. The incorporation of polymers into oral care products, acid beverages and antacid formulations aiming to prevent the erosive tooth wear was also discussed. Data sources: "Medline" and "Scopus" databases were searched electronically using the main keywords: enamel, polymer, fluoride* and tooth erosion. Language was restricted to English. Conclusions: Film-forming polymers are a promising approach to prevent erosive tooth wear. However, additional studies using more relevant clinical conditions are needed to confirm this finding. Moreover, the standardization of the acid challenge, treatment duration and association to fluorides is recommended to allow comparisons between the studies.*

Keywords: Enamel. Dentin. Tooth erosion. Polymers. Fluorides.

1. Introduction

Over the recent decades, erosive tooth wear (ETW) has been recognized as a frequent condition, especially in the young population [1]. This condition has a multifactorial etiology, with acids from non-bacterial origin as the main element [2]. In early stages, frequent erosive challenges lead to a partial loss of the superficial mineral content from enamel and consequently there is a decrease of hardness [3]. With recurrent acid exposure and the association with mechanical factors, the softening is followed by a continuous layer-by-layer dissolution of enamel, leading to permanent loss of tooth structure [4]. Thus, the adoption of preventive measures is crucial to avoid further esthetic and functional damage, as well pain [5].

Besides the control of causative factors, some strategies have been proposed to prevent the progression of erosive tooth wear, as the use of products containing fluorides [6]. These products are extensively recommended and widely used in oral care, but as oppose to the caries lesion, in which fluoride-driven remineralization occurs in deeper levels, in erosion this effect is limited to the tooth surface [7,8], with the deposition of precipitates similar to calcium fluoride (CaF_2*) that act as a sacrificial layer during the acid episodes [6].*

Fluoridated products offer a degree of protection against ETW that is conceivably sufficient for most patients with average acid exposure [9,10]. However, for patients with high risk of ETW development, a more effective product is desirable. Thus, the addition of compounds, as stannous chloride ($SnCl_2$*), stannous fluoride (*SnF_2*) and titanium tetrafluoride (*TiF_4*) to oral care products has been investigated. Their metal cations can be incorporated into enamel and dentin structures, reducing their solubility [11,12]. In addition, they react with the phosphate groups and fluoride of the dental tissue surface, forming stable precipitates (stannous phosphate* *and titanium oxides, respectively), which create a layer more acid-resistant than pure CaF_2 [13,14]. However, the use of $SnCl_2$ is associated with some drawbacks, as tooth and tongue discoloration, bad taste, and mucosal desquamation [15], whereas the protective effect of products containing TiF_4 is highly depend on a very low pH [16], which is unfeasible for over-the-counter oral care products.*

The limitations of the current preventive strategies may have contributed to the increased incidence of ETW [17], emphasizing the need for seeking more effective agents [8]. Recently, the ability of polymers to interact with the surface of hydroxyapatite forming an acid resistant film has been investigated, but there is no consensus about their efficacy [18]. Thus, aiming to provide more information to head the search for promising anti-erosive polymers, this article provides an overview about the polymers structure, their mode of action, as well as the effect of their incorporation to oral care products, acid beverages, and antacid formulations, targeting the prevention of ETW.

"Medline" and "Scopus" databases were searched electronically using the main keywords: tooth, enamel, polymer, fluoride* and erosion. A hand search was also conducted to enlarge the literature investigation. Language was restricted to English.*

2. Polymers structure

The word polymer originates from Greek: poly (many) and mero (repetition units). Thus, polymers are organic or inorganic materials with high molar mass (over 10 thousand, reaching 10 million g/mol), containing a structure based on the repetition of small units [19]. According to their atomic content and synthesis, they are classified in:

- *Natural organic polymers: naturally synthetized, containing an organic backbone, e.g. wood, rubber, cotton, leather, silk, or even proteins, enzymes, starches, cellulose, and other substances;*
- *Natural inorganic polymers: naturally synthetized and found in ionic compounds and mineral salts, e.g. diamond and graphite;*
- *Artificial polymers: natural organic polymers that were artificially modified by chemical reactions, e.g. cellulose acetate, cellulose nitrate;*
- *Synthetic organic polymers: artificially synthetized, containing an organic backbone, e.g. polyethylene, polystyrene, and polymethacrylates;*
- *Synthetic inorganic polymers: artificially synthetized, containing an inorganic backbone, e.g. polyphosphoric acid.*

The polymerization reaction is responsible for the formation of a long polymeric molecule containing thousands of ***repeated units*** *bonded by covalent bonds. Figure 1 shows the example of ethylene, a molecule that can be combined with a catalyst to form polyethylene, one of the most important plastic materials today. To facilitate the representation of the polymer chemical structure, the repeated unit is placed in parenthesis with a subscripted "n" that represents the polymerization degree, which is the number of times in which the units are repeated.*

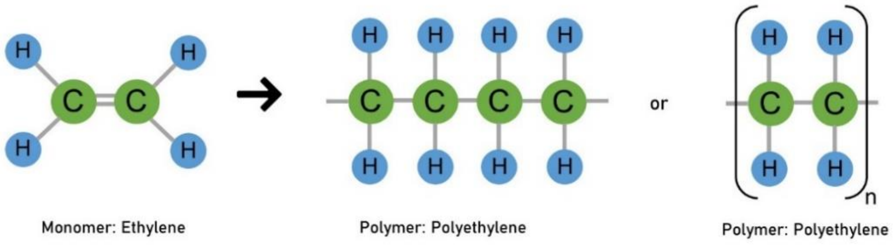

Fig. 1. *Illustration of the ethylene polymerization*

The molar mass (MM) of the polymer is calculated by the following formula: $MM_{polymer} = n \times MM_{monomer}$, *in which "n" represents the polymerization degree. However, during the polymerization reaction, polymeric chains with different lengths can be formed. Thus, the molar mass of a polymer is usually expressed as an average molar mass [20].*

The control of the polymerization reaction can produce a vast range of polymers with different molecular weights, and consequently, different physical properties [20], which can impact the formation of the polymeric film on the tooth surface. Previous findings showed that, under erosive conditions without mechanical impacts, a high molecular weight polymer showed better protective effect to enamel, but under chemo-mechanical challenges, a low molecular weight behaved better [21]. This may be due that the higher molecular weight polymer created thicker precipitates, which were then more able to prevent the underlying structure from direct contact with the acid. By the other side, a lower molecular weight polymer implies shorter chains of molecules and, as a consequence, more available binding sites for the retention and formation of multi-layers [21]. Further research investigating this interaction in detail remains necessary. One important aspect to considered in future studies is report the polymer molecular weight, allowing the comparison between them.

Besides variations in the molecular weight, different polymers can be obtained by combining some monomers with catalysts. Figure 2 shows that the combination of ethylene with ester radicals (R) can produce different polymers.

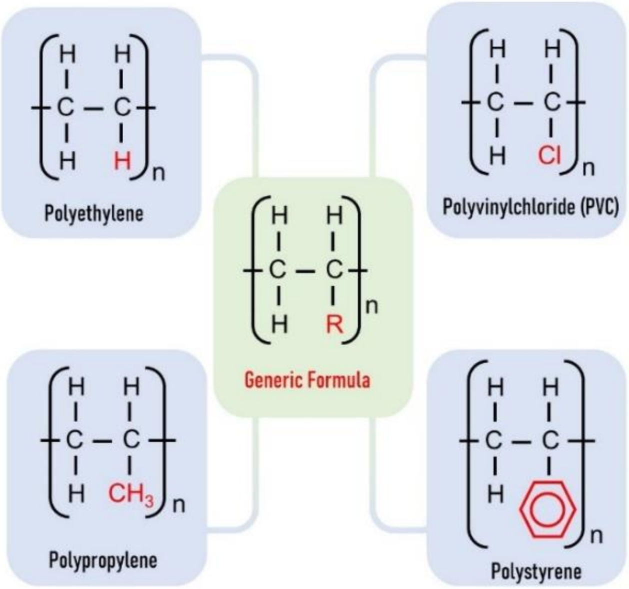

Fig. 2. *Representation of polymers that share the same generic formula.*

It is important to highlight that the polymers shown in Figure 2 are ***homopolymers,*** *since their respective repeated units are the same along the chain backbone. However, polymers with different repeated units in their structure are known as* ***copolymers****. Figure 3 shows the chemical structure of poly(methyl methacrylate) and two of its copolymers.*

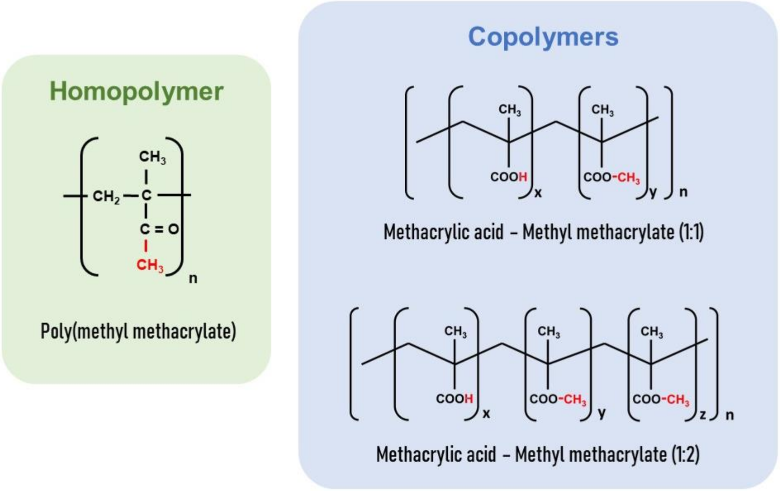

Fig. 3. *Representation of the chemical structure of poly(methyl methacrylate) and two of its copolymers. The proportion of methacrylic acid and methyl methacrylate in each repeated unit is given in parenthesis – (1:1) or (1:2).*

Considering only the polymers with different molecular weight, ester radicals (R), and their copolymers, it is evident that the number of potential polymers is vast. However, currently, only a small part of this wide range of possibilities has been studied, and an even a smaller amount is commercially available. In dentistry, the polymers have been extensively investigated as part of restorative materials, however, their use as anti-erosive agents is recent [18].

3. Mode of action of polymers on ETW prevention

The principle of forming a protective film on the tooth surfaces is not new, as the proteins of the acquired salivary pellicle do it naturally [22]. The acquired salivary pellicle acts as a selective membrane that prevents the direct contact of the acids with the underlying tissues [23]. In addition, the proteins from the basal layer of the acquired pellicle present binding sites for calcium and phosphate, maintaining a high concentration of these ions near the surface of the hydroxyapatite, thus reducing demineralization caused by acid episodes [24].

Statherin is one of the most important proteins of the acquired pellicle basal layer, being composed of a sequence of 43 amino acids [25]. However, only the polar portion of statherin can bind with the calcium ions from the hydroxyapatite, while the middle and tail regions are mobile and dynamic [26]. Thus, to optimize statherin binding to hydroxyapatite, synthetic proteins containing only the amino acid sequence observed in the most polar portion of the statherin were produced [27–29]. The results of in vitro studies showed that the modified statherin-like proteins can reduce demineralization by up to 60% compared to the original protein. Nonetheless, the difficulty and potentially high cost involved in the production of such proteins must be considered, being the use of polymers *containing specific chemical groups an interesting alternative to cope with this limitation.*

In the last decades, polymers with selected properties have been designed by the pharmaceutical industry to provide controlled release of medications and to increase their substantivity [30]. The so-called ***smart polymers*** *are designed to respond to specific changes in the environmental conditions as pH, light, temperature, magnetic field, and ionic strength [31]. In the same way, in dentistry, new polymers can be designed to present a high affinity with the hydroxyapatite, or the vast range of the previously designed polymers can be tested, representing a more cost-effective approach to form a protective film on the tooth surface. Nonetheless, to select the polymers with potential to establish ionic bindings with the hydroxyapatite, it is necessary to know the possible chemical interactions involved.*

In general, anionic molecules bind to calcium-sites and cationic molecules to phosphate-sites of the hydroxyapatite [32]. In a liquid medium (polar solvent), the dispersed hydroxyapatite particles acquire a superficial electric charge due to the electrostatic potential generated by the calcium and phosphate ions [33,34]. This surface charge will be compensated by an equal and opposite charge of counterions to maintain neutrality. Thus, on the interface between each particle and the liquid an ***electric double layer*** *is formed, which can be subdivided in: (1) inner layer or Stern layer – containing strongly bonded counterions; and (2) diffuse layer – containing loosely bounded counterions coexisting with ions with the same charge of particle [35]. The zeta potential corresponds to the electric potential on the plan (slipping plan) that separates the electric double layer from the bulk of the liquid (Fig. 4).*

The isoeletric point is the pH in which a molecule is electrically neutral [19]. Previous studies indicate that the isoeletric point of the *hydroxyapatite in deionized water is between 5 and 7 [36,37]. However, it must be considered that the zeta potential of the hydroxyapatite can be altered due to changes on pH,* Ca^{2+} *and* PO_4^{3-} *concentrations [32]. Thus, under conditions simulating the oral cavity, the hydroxyapatite was found to present a predominantly negative surface charge [33,38–40].*

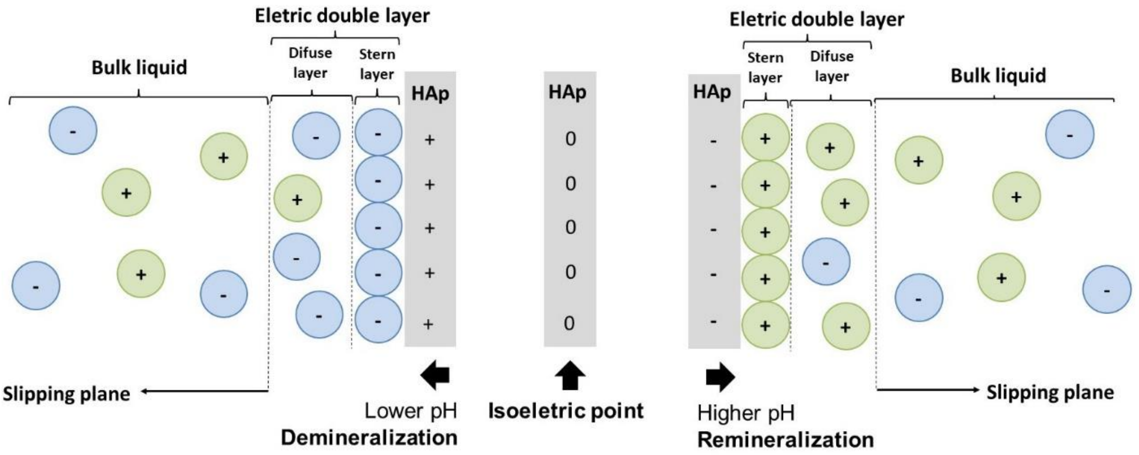

Fig. 4. *Representation of the electric double layer on the hydroxyapatite surface according to the pH of the solution.*

The negative surface charge of the hydroxyapatite in the oral cavity may favor the ionic interaction with cationic polymers, as some polymethacrylate copolymers [41] and chitosan [21]. However, considering the presence of polar proteins from the acquired salivary pellicle and the biofilm components, the polymer can also interact with positive sites, favoring the interaction with anionic polymers, as sodium polyphosphate [39], milk casein [42], carbopol [43] and pectin [44].

After the initial chemical interaction of the polymer with the tooth surface, two mechanisms may regulate the film formation. First, some polymers, as polymethacrylate copolymers, are able to precipitate due to changes on the pH [45], creating a deposit on the surface against further acid challenges [46]. Second, some polymers as alginates, casein and chitosan, are able to promote chain reticulation induced by exposure to *bivalent ions, as* Ca^{2+} *[47,48]. The cross-linking of polymer chains creates a gel-like structure that may protect the tooth surface. However, it is important to highlight that the characteristics of this structure is susceptible to the size (ion radius), amount of ions available, and ease of packing of the polymer chains around the ions [47]. Moreover, the ionic reticulation depends on the pH of the solution, since the charged functional groups of the polymer are influenced by the amount of* H^+ *in the solution [44].*

4. Polymers as active ingredients in oral care products

The technological improvements and the market competition promoted the formulation of a great variety of home-use oral care products, being observed a significant increase in their consumption in the last decades [49]. Fluoridated toothpastes and mouthrinses are common products of oral hygiene practices around the world, but as previously mentioned, a more effective product is desirable for patients with EWT.

A promising approach to prevent EWT seems to rely not only on the use of polymers, but also on their association with fluorides. The polymers added to oral care products can complement the protective effect of fluorides by binding to the phosphate sites (cationic polymers), or binding to calcium sites (anionic polymers) where fluoride was not bonded [43], thus preventing the underlying structure from direct contact with the hydrogen ions from the acid. Moreover, the polymers might react with fluoride, increasing its substantivity in the oral cavity [50].

The studies about the anti-erosive effect of toothpastes containing polymers are scarce, and most of them investigated the effect of chitosan, which was able to significantly reduce the surface loss caused by erosive/abrasive episodes [51–53]. Another study investigating the effect of independent variables on toothpaste formulations showed that the presence of Eudragit EPO, a polymethacrylate copolymer, was able to reduce the *erosion rate [41]. Although the addition of polymers to toothpastes is a promising approach to prevent ETW, further studies are needed to elucidate their mechanism of action, as well as their interaction with fluoride, salivary proteins and enamel [51].*

Another strategy investigated is the addition of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to mousses [54,55], gums [56,57], and varnishes [58] for erosion prevention. Promising results were found since CPP-ACP buffers free calcium and phosphate ion activity, maintaining a state of supersaturation with respect to tooth enamel that helps prevent demineralization and facilitates remineralization on erosion [59]. However, CPP-ACP appears to be less effective than fluoride in the control of dental erosion [18,60].

A higher number of studies investigating the addition of polymers to solutions can be found in the literature, possibly due to its simplified composition in comparison to toothpastes, in addition to be easier to investigate isolated variables. However, a great variability is noted in the experimental parameters used in these studies. Thus, in order to provide an overview of the available data, a compilation of such studies is presented in Table 1.

Table 1. Compilation of studies investigating the anti-erosive effect of polymer-based solutions

Study	***Type***	***Substrate***	***Acid challenge***	***Remineralizing solution***	***Anti-erosive treatment***				***Polymer effect***
					Polymers tested	***Concentration***	***Duration***	***Association with fluorides***	
Bezerra et al. [61], 2019	*In vitro*	*Enamel and dentin*	*0.3% citric acid – pH 2.6 (5 min, 4x/day, 5 days)*	*Collected human saliva*	*Gantrez MS-955* *Plasdone K-29/32* *PGA: Propylene glycol alginate* *CMC: Carboxymethylcellulose*	*1 g/l*	*2 min, 2x/day, 5 days*	*225 ppm F^- (NaF);* *225 ppm F^- (NaF) + 800 ppm Sn^{2+} ($SnCl_2$)*	*For enamel, Gantrez, Plasdone, and CMC exhibited an anti-erosive effect, and.PGA increased the protection of NaF. For dentin, only Gantrez reduced erosion.*
Beltrame et al. 2018 [62]	*In vitro*	*Dentin*	*0.5% citric acid – pH 2.3 (2 min, 6x/day, 5 days)*	*Mineral salt solution*	*Phosphorylated chitosan*	*5 g/l*	*2 min, 6x/day, 5 days*	*No*	*The treatment reduced erosive wear by approximately 32% in neutral and alkaline pH, when compared to the negative control.*
Avila et al. [43], 2017	*In vitro*	*Enamel*	*0.3% citric acid – pH 2.6 (2 min, 6x/day, 6 days)*	*Artificial saliva*	*Carbopol 980* *Carboxymethylcellulose* *Aristoflex AVC*	*1 g/l*	*1 min, 6x/day, 5 days*	*900 ppm F^- (NaF)*	*Carbopol 980 reduced the erosive wear magnitude to the same extent as the sodium fluoride.*
João-Souza et al. [63], 2017	*In situ*	*Enamel*	*1% citric acid – pH 2.4 (2 min, 6x/day, 5 days)*	*Human saliva*	*LPP: Sodium linear polyphosphate*	*20 g/l*	*2 min, 2x/day, 5 days*	*225 ppm F^- (NaF) + 800 ppm Sn^{2+} ($SnCl_2$)*	*The presence of LPP did not enhance the anti-erosive effect of the fluoridated solution.*
Pini et al. [21], 2016	*In vitro*	*Enamel*	*0.5% citric acid – pH 2.8 (2 min, 6x/day, 10 days)*	*Mineral salt solution*	*Chitosan with different molecular weight (150,350,400,450 kDa)*	*5 g/l*	*2 min, 2x/day, 10 days*	*500 ppm F^- (AmF) + 800 ppm Sn^{2+} ($SnCl_2$)*	*Under erosive conditions, the 450 kDa chitosan completely inhibited tissue loss, whereas under abrasive/erosive challenges the 150 and 350 kDa chitosan showed the best performance, reducing in ~60% the erosive wear compared to the negative control.*
Scaramucci et al. [64], 2016	*In vitro*	*Enamel and dentin*	*1% citric acid – pH 2.4 (5 min, 6x/day, 5 days)*	*Collected human saliva*	*Sodium linear polyphosphate*	*20 g/l*	*2 min, 3x/day, 5 days*	*225 ppm F^- (NaF);* *225 ppm F^- (NaF) + 800 ppm Sn^{2+} ($SnCl_2$)*	*The combination of NaF with $SnCl_2$ and/or LPP can protect the enamel against erosion. For dentin, neither toothbrushing nor the test solutions influenced the development of erosion*
Scaramucci et al. [39], 2015	*In vitro*	*Enamel and dentin*	*0.3% citric acid – pH 3.8 and 1% citric acid – pH 2.4 (5 min, 6x/day, 5 days)*	*Artificial saliva*	*Linear sodium polyphosphate* *Sodium pyrophosphate tetrabasic* *Sodium tripolyphosphate* *Sodium caseinate* *Bovine serum albumin*	*2 g/l and 20 g/l*	*2 min, 3x/day, 5 days*	*225 ppm F^- (NaF);* *225 ppm F^- (NaF) + 800 ppm Sn^{2+} ($SnCl_2$)*	*The addition of LPP and/or $SnCl_2$ can improve the fluoride solution protection against erosion of enamel but not of dentine*
Lei et al. [65], 2014	*In vitro*	*Enamel*	*1% citric acid – pH 3.8 (5 min, 1x, 1 day)*	*No*	*Synthetic amphiphilic diblock copolymer*	*0.06, 0.12, 0.25, 0.5 and 1 g/l*	*5 min, 1x*	*No*	*The treatment with the polymer decreased the mineral loss of hydroxyapatite by 36–46% compared to the untreated control, and protected the surface morphology of the enamel specimen following exposure to acid.*
White et al. [42], 2011	*In vitro*	*Enamel*	*0.3% citric acid – pH 3.2 (10 min, 9x, 1 day)*	*No*	*Casein* *CPP: Casein phosphopeptide* *GMP: Glycomacropeptide*	*5 g/l*	*10 min, 1x*	*300 ppm F^- (NaF)*	*Casein and NaF reduced enamel surface softening compared to the negative control, but CPP and GMP did not.*
Gracia et al. [66], 2010	*In vitro*	*Enamel*	*1% citric acid – pH 3.8 (5 min, 1x, 1 day)*	*No*	*Combination of 0.20% carboxymethylcellulose, 0.010% xanthan gum and 0.75% copovidone*	*-*	*1 min, 1x*	*300 mg/l fluoride*	*The treatment with the polymer significantly reduced the lesion deep and enhanced the delivery of fluoride to the surface of the lesion.*

Table 1 shows that promising results were obtained with the polymers tested in most of the studies. However, the different experimental parameters used can influence the obtained results and thus need to be considered carefully. Based on this review, the authors recommend variables to be considered in further studies investigating the addition of polymers to solutions. These recommendations should be understood as guidelines rather than as a standard design, although it has to be considered that a higher level of standardization is desirable to facilitate the comparison between the studies.

- ***Substrate:*** *the anti-erosive effect of the polymers can vary on enamel and dentin;*
- ***Acid challenge:*** *Higher concentrations of citric acid (1%) may be preferred than the lower ones (0.3%) to allow better discernment about the effects of the treatment with the polymers [39];*
- ***Remineralizing solution:*** *The efficacy of anti-erosion agents can be affected by protein interactions, thus the use of collected human saliva for in vitro studies is recommended [67] or preferably, conduction of in situ studies;*
- ***Anti-erosive treatment:*** *The binding affinity seems to be dependent on the polymer molecular weight [21], concentration, and pH of the solution [65];*
- ***Duration of the treatment:*** *a representative period of 1-2 minutes for testing solutions simulating mouthrinses should be preferable to not exceed the clinical condition;*
- ***Association to fluorides:*** *The polymers added to oral care products may complement the protective effect of fluoride [43], thus this association is recommended.*

5. Polymers as active ingredients in acidic beverages

Attempts to reduce the erosive potential of acidic beverages by supplementation with mineral agents have been reported, however, although successful, this approach present some drawbacks, mainly related to taste alterations, stability difficulties, and toxicity potential [68–71]. The use of food-approved polymers is a promising alternative to overcome these limitations [72].

The erosive potential of acid beverages was efficiently reduced by its modification with polymers under in vitro conditions. Promising in vitro results were found for xantham gum [73–76], carboxymethylcellulose [76], tripolyphosphate [76], polyphosphate [75], ovoalbumin [77], propylene glycol alginate [40,44], highly esterified pectin [40,44], gum arabic [40,44], casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [78] and linear sodium polyphosphate [39]. However, under in situ conditions, no protection was observed for linear sodium polyphosphate [79].

It is expected that the polymers could reduce erosive demineralization by adsorbing on the tooth surfaces, creating a protective film. The polymers can also increase the viscosity of acidic drinks, reducing the ion mobility in the solution, and consequently slowing down the dissolution kinetics [80]. Nevertheless, its efficacy is related to the polymer type, and contradictory results have been reported [81]. Additionally, factors as viscosity, stability, acceptance by consumers needs to be considered.

6. Polymers as active ingredients in antacid formulations

Patients with disorders like gastric reflux and bulimia commonly use antacid formulations. Recently, the pharmaceutical industry has added polymers to these products in order to enhance the retention of antacid formulations within the stomach, resulting in a longer duration of neutralization compared to conventional antacids [82]. An antacid containing alginate was shown to *suppress reflux after meals by creating a gel-like barrier that caps and displaces the acid pocket distal to the esophago-gastric junction [83].*

Alginate is a natural polysaccharide composed of α-d-mannuronic acid and β-l-guluronic acid. This polymer forms gel in the presence of various divalent cations, e.g. Ca^{2+}, Mg^{2+}*, by cross-linking the carboxylate groups of the guluronate groups on the polymer backbone [84]. Previous in vitro findings showed that rising with antacid suspension containing sodium alginate, sodium bicarbonate and calcium carbonate was effective to decrease both the enamel microhardness reduction and surface loss, which may be related to the viscoelastic polymer chain formed on the tooth surface [85,86].*

Furthermore, future studies should investigate if patients using such medications as a conventional antacid, not as a mouthrinse, present a lower susceptibility of ETW, since the polymer may reduce the ion mobility of the hydrochloric acid from the stomach and may adsorb on the tooth surface, behaving as protective agent.

7. Conclusions and future perspectives

This narrative literature review shows that film-forming polymers are a promising cost-effective approach to prevent and control dental erosion. However, additional studies using more relevant clinical conditions, mainly considering the acquired salivary pellicle, saliva composition and clearance, are needed to confirm their efficacy. Moreover, guidelines to standardize the acid challenges employed, the treatment duration and the association with fluoride (considering type and concentration) are necessary to allow comparisons between studies.

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2.2 Artigo – Augusto MG, Santos TA, Scaramucci T, Torres CRG, Aoki IV, Hara AT, Borges AB. Efeito de soluções a base de polímeros associadas a fluoretos e estanho na dissolução da hidroxiapatite / *Effect of polymer-based solutions associated to fluoride and stannous on hydroxyapatite dissolution**

RESUMO

Objetivo: Investigar o efeito de quatro polímeros (polioxirano, hidroxipropilmetilcelulose, pectina, e um copolímero do polimetacrilato -PMA) em duas concentrações (alta e baixa) associados ou não ao fluoreto de sódio -F (225 ppm F^-) ou fluoreto de sódio com cloreto de estanho (800 ppm de Sn^{2+}) -FS na dissolução da hidroxiapatita (HA). Métodos: A dissolução da hidroxiapatita após o tratamento com as soluções experimentais foi avaliado. Água deionizada foi o controle. Os cristais de hidroxiapatita pré-tratados foram adicionados a solução de ácido cítrico 0,3% (pH 3,8). Uma tituladora automática adicionou alíquotas de HCl 0.1N a velocidade de 28 µl/min, em um tempo de total de 5 minutos de reação. Os dados foram analisados com ANOVA 2-fatores e test t seguido pelo teste de Tukey (5%). Um teste adicional foi realizado para determinar o potencial zeta das partículas dispersas de HA tratadas com as soluções. Resultados: Para as soluções sem flúor, apenas o PMA reduziu significativamente a quantidade de dissolução de HA em comparação com a água deionizada (p = 0,0001). Os grupos tratados com as soluções contendo F e FS apresentaram menor dissolução de HA do que os tratados com as soluções sem flúor. PMA foi capaz de melhorar o efeito protetor de F, mas não afetou o FS. Não foram encontradas diferenças entre as concentrações testadas. Conclusão: Nas condições testadas, F e FS reduziram a dissolução da hidroxiapatita, e o efeito F foi melhorado pela combinação com PMA.

Palavras-chave: Erosão dental. Polímeros. Fluoretos. Cloreto de estanho.

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*Artigo elaborado de acordo com as normas do Periódico *Journal of Dentistry.*

ABSTRACT

Objective: To investigate the effect of four polymers (polyoxirane, hydroxypropylmethylcellulose, pectin, and a polymethacrylate copolymer -PMA) in two concentrations (low and high) associated or not with sodium fluoride -F (225 ppm F^-*) or sodium fluoride plus stannous chloride (800 ppm* Sn^{2+}*) -FS on hydroxyapatite (HA) dissolution. Methods: The HA dissolution after the treatment with the experimental solutions was evaluated. Deionized water was the control. The pre-treated HA crystals were added to a 0.3% citric acid solution (pH 3.8). A titrant machine added aliquots of 0.1N HCl at 28 µl/min rate, in a total reaction time of 5 minutes. Data were analyzed with 2-way ANOVA and Student t test followed by the post-hoc Tukey's test (5%). An additional test was performed to determine the zeta potential of the dispersed HA particles treated with the solutions. Results: For the "no fluoride" solutions, only PMA significantly reduced the amount of HA dissolution in comparison to deionized water (p=0.0001). The groups treated with solutions containing F and FS showed lower HA dissolution than those treated with solutions without fluorides. PMA was able to improve the protective effect of F, but it did not affect the FS. No differences were found between the concentrations tested. Conclusion: Under the conditions tested, F and FS reduced the hydroxyapatite dissolution, and the F effect was enhanced by the combination with PMA.*

Keywords: Tooth erosion. Polymers. Fluorides. Stannous chloride.

1. Introduction

Fluoride compounds are able to reduce dental erosion in enamel and dentin [5]. Sodium fluoride (NaF) promotes the deposition of precipitates similar to calcium fluoride (CaF2) on the tooth surfaces, which act as a sacrificial layer during the acid episodes [6]. This deposition can be favored by low pH, increased exposure time and the concentration of the solution [7]. Nevertheless, as sodium fluoride offers a limited protection against dental erosion, it could be associated with compounds containing metal cations, such as stannous chloride (SnCl2) [5]. Stannous can be incorporated into enamel and dentin structures, reducing their solubility [8,9]. In addition, it forms deposits on the tooth surface, creating an Sn-rich layer, which has shown to be more acid-resistant than pure CaF2 [10,11]. Unfortunately, the presence of stannous is associated to some drawbacks as bad taste, mucosal desquamation, as well as tooth and tongue discoloration [12].

In recent years, to further prevent/control ETW, the ability of film-forming polymers to protect the tooth has been evaluated, with promising results [13]. The anti-erosive effect of polymers is due to their affinity with dental structures and ability to form a protective film on enamel surface [14]. In addition, some polymers can interact with fluoride compounds, increasing their protection [15]. Promising results were found for casein [16], chitosan [17], sodium linear polyphosphate [18], propylene glycol alginate [19], carbopol [20], and the combination of carboxymethylcellulose, xanthan gum and copovidone [21]. However, a wide range of polymers is available to be tested as anti-erosive agents, creating an extensive field of research.

In general, anionic molecules are adsorbed on calcium-sites and cationic molecules on phosphate-sites of hydroxyapatite [22]. This interaction depends on intermolecular forces, which have their magnitude increased in the presence of polar groups [23]. But not only polar polymers can interact with the *hydroxyapatite (HA), some neutral molecules can acquire a slightly charged center when added to acid solutions, allowing a certain interaction with the charged sites at the enamel surface [24]. Another factor that may influence the adsorption of the polymers on the tooth surfaces is their molecular weight, being the adsorption favored by polymers with long chain length [25]. Thus, aiming to provide information to head the search for promising anti-erosive polymers, the present study investigated four polymers with different polarity and molecular weight: polyoxirane, hydroxypropylmethylcellulose -HPMC, pectin and a polymethacrylate copolymer -PMA.*

Polyoxirane is commonly used as a binder and mucoadhesive substance. Its ability to protect teeth against erosion has been previously registered in a patent [26], but further studies are necessary to confirm its protective effect. HPMC is a semi-synthetic polymer used by the food industry. It is known to reduce surface tension [27]. HPMC presents a similar structure to carboxymethylcellulose and ethylcellulose that presented promising anti-erosive potentials in previous investigations [28,29]. Pectin is a polymer obtained from the peel of citric fruits. It is an anionic polymer containing carboxylic (–COOH) and a carboxymethyl ($-COOCH_3$) *functional groups on its carbohydrate backbone. The ability of pectin to promote protection against erosion has been previously described [14,30]. However, opposite results were also reported [31], warring further investigations. Polymethacrylates (PMA) are synthetic polymers that can be designed to present specific chemical properties. The anti-erosive potential of different PMA copolymers was previously reported [26,32]. The copolymer investigated in this study (Eudragit E100) was designed to allow drug release in acidic environments [33]. It is a cationic polymer containing an amine* $[-NH(CH_3)_2]$ *functional group. An in vitro study showed that the presence of Eudragit E100 in a toothpaste formulation was effective to decrease the erosion rate [34], but there is no evidence supporting its effect when used in solution* *simulating mouthrinses.*

This study evaluated the ability of the four above mentioned polymers, combined or not with NaF or SnCl2, to inhibit HA dissolution. In addition, to provide a better understand of the interaction between the polymers and the HA surface, the analysis of the zeta potential of the dispersed HA particles treated with the solutions was performed.

2. Materials and Methods

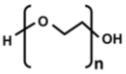
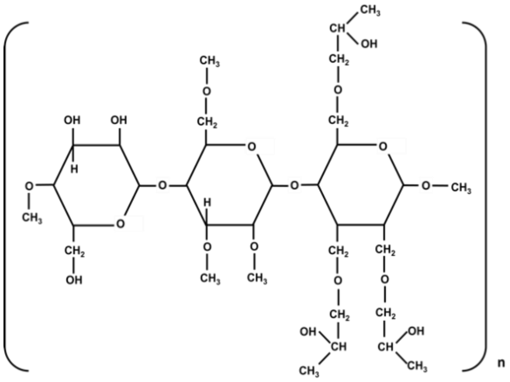
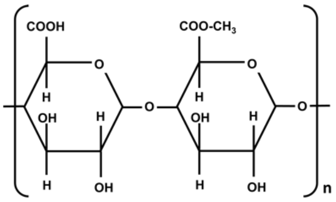
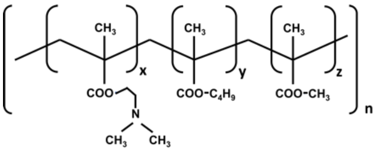
2.1 Study design

This study followed a factorial 9 × 3 design, considering the following experimental factors: polymers at 9 levels (no polymer, polyoxirane in low concentration, polyoxirane in high concentration, HPMC in low concentration, HPMC in high concentration, pectin in low concentration, pectin in high concentration, PMA in low concentration, and PMA in high concentration); and the type of fluoridated solution at 3 levels (no fluoride, sodium fluoride -F, and sodium fluoride + stannous chloride -FS). The solutions were tested by the hydroxyapatite dissolution test using pre-treated HA crystals in triplicate. The response variable was the amount of HA dissolution (in mg). The null hypothesis was that the different polymers tested, and the presence of fluoride would not influence the amount of HA dissolution.

2.2 Experimental solutions preparation

All solutions were freshly prepared prior the experiment. Table 1 shows the specifications of the polymers tested.

Table 1 – Specifications of the polymers tested

Polymer	***Polarity***	***Molecular weight (monomer)***	***Manufacturer***	***Molecular formula***	***Structural formula***
Polyoxirane (Polyox WSR-205)	*Non ionic*	*62 g/mol*	*Dow Chemical Company, Midland, MI, USA*	$C_2H_60_2$	
HPMC (Methocel F4 M Premium)	*Non ionic*	*748 g/mol*	*Dow Chemical Company, Midland, MI, USA*	$C_{32}H_{60}0_{19}$	
Pectin (GENU pectin 150 USA-SAG)	*Anionic*	*370 g/mol*	*CP Kelco, Limeira, SP, Brazil*	$C_{12}H_{18}0_{13}$	
Polymethacrylate (Eudragit E-100)	*Cationic*	*399 g/mol*	*Evonik Industries, Essen, Germany*	$C_{21}H_{37}N0_6$	

Previous data and the results of the viscosity assessment guided the choice of the concentrations of the polymers that were tested. All solutions presented a viscosity that was compatible with the viscosity of mouthrinses (up to 150 mPa.s based in a pilot study) when using 5 mg/ml (low concentration) and 20 mg/ml (high concentration), except HPMC, which presented a gel consistency at the higher concentration. Thus, only in this subgroup the high concentration was reduced to 10 mg/ml.

In the respective solutions, sodium fluoride (F) was added in the concentration of 0.05 g/l [35], whereas stannous chloride and gluconic acid sodium salt (stabilizing agent) were added in the concentrations of 1.28 g/l and

0.23 g/l, respectively [36]. For the PMA solutions with low and high concentrations, 0.5 g and 2 g of polymethacrylate were respectively dissolved in 4.5 ml and 18 ml of hydrated ethyl alcohol (92.8°), and then, deionized water was added until completing the final volume of 100 ml. The pH of all experimental solutions was adjusted to 4.5, using either 0.1 M KOH or 0.1 M HCl. In order to homogenize the solutions, they were mixed at 1500 rpm for 10 min in high speed vacuum mixer (SpeedMixer – FlackTek, Landrun, SC, USA).

2.3 Viscosity assessment

The dynamic viscosity of the solutions was determined using a vibroviscosimeter (SV-10, A&D, Toshima-ku, Tokyo, Japan). The results were obtained in duplicate, in a thermostatic bath at 25 ± 1 °C.

2.4 Measurement of hydroxyapatite dissolution

Twenty-five milligrams of hydroxyapatite crystals (Acros Organic, Geel, Belgium) were placed into 2 ml plastic tubes, and 1 ml of each experimental solution was added to it. Then, the tube was gently agitated for 60 s and centrifuged for 30 s, at 10,000 rpm. The supernatant was removed using a pipette. Thereafter, 1 ml of deionized water was added to the tube, followed by 60 s agitation. The tube was centrifuged again, and the supernatant removed. This washing procedure was repeated once, but at this time, only 0.9 ml of the supernatant was removed. The samples were then vortexed to disaggregate the hydroxyapatite and allow the hydroxyapatite dissolution analysis [15].

An automatic titrator (Hanna Instruments, Woonsocket, RI, USA) was used. The pre-treated hydroxyapatite crystals were added to a standard solution of 0.3% citric acid (pH adjusted to 3.8). Then, in order to maintain the pH constant, aliquots of the titrant solution (0.1N HCl) were added at 28 µl/min rate, in a total reaction time of 5 minutes.

After 5 min, the volume of HCl used was recorded and converted into the amount of hydroxyapatite dissolved (in mg), using the following formula [15]:

$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O$*, where, 1 mol of hydroxyapatite corresponds to 8 mols of* H^+.

The value of HA dissolution was obtained in triplicate for each experimental solution.

2.5 Determination of the zeta potential

The zeta potential of dispersed HA treated with the experimental solutions were measured by a zeta potential analyzer (Zetasizer Nano ZS90, Malvern Instruments, Malvern, UK), equipped with a Helium–Neon Laser of 633 nm and maximum of 4 mW. Electrophoretic light scattering (ELS) was used to measure the electrophoretic mobility of dispersed HA in 0.040 mol/L phosphate buffer (pH = 7.0), and this was later converted to zeta potential using the Helmholtz-Smoluchowski formula. Ten milligrams of pretreated HA were dispersed in 5 ml of the phosphate buffer assisted by 8 min under sonication in a common ultrasonic bath containing water. Samples were pipetted and introduced in the sample cell and examined at 20 °C. Five measurements were performed for each sample (after a minimum of 10 and a maximum of 40 runs) and the mean value recorded with its standard deviation [15].

2.6 Statistical analysis

For the hydroxyapatite dissolution test, two-way ANOVA was applied to evaluate the effect of the polymers and type of fluoridated solution. Post hoc pairwise comparisons were performed using the Tukey test, with a significance level of 5%. The comparison between the different concentrations of the polymers *was made by the Student t test. Minitab 17 Software (Minitab Inc, State College, PA, USA) was used for the calculations.*

3. Results

Table 2 shows the results of the viscosity assessment, in which is possible to observe that the solutions containing polyoxirane (in high concentration), HPMC (in both concentrations) and pectin (in high concentration) had their viscosity remarkably reduced when associated to FS, but this effect was not observed when they were associated to F.

Table 2 – Mean viscosity (SD) of the experimental solutions (mPa.s)

		No fluoride	***F***	***FS***
Deionized water		*1.18 ± 0.00*	*0.85 ± 0.01*	*0.86 ± 0.01*
Polyoxirane	*Low concentration*	*3.56 ± 0.04*	*4.25 ± 0.03*	*4.05 ± 0.03*
	High concentration	*43.4 ± 0.2*	*41.4 ± 0.1*	*3.01 ± 0.02*
HPMC	*Low concentration*	*18.9 ± 0.4*	*23.1 ± 0.2*	*3.31 ± 0.04*
	High concentration	*137 ± 2*	*123 ± 1*	*8.32 ± 0.01*
Pectin	*Low concentration*	*6.04 ± 0.03*	*4.65 ± 0.01*	*4.66 ± 0.08*
	High concentration	*70.4 ± 0.2*	*60.6 ± 0.4*	*27.3 ± 0.1*
PMA	*Low concentration*	*2.16 ± 0.06*	*1.83 ± 0.01*	*2.36 ± 0.03*
	High concentration	*3.68 ± 0.00*	*3.05 ± 0.01*	*3.78 ± 0.06*

Figure 1 shows the mean and standard deviation of HA dissolution in all subgroups, as well as the results of Tukey test for the interaction between factors. For the "no fluoride" solutions (N), only PMA significantly reduced the amount of HA dissolution in comparison to deionized water (p=0.0001). The solutions containing F and FS provided a lower HA dissolution than the polymers-only solutions. PMA was able to improve the protective effect of F, but it did not affect the FS. The presence of polyoxirane and HPMC reduced the anti-erosive effect of FS solution. The results of the Student t test showed no significant differences between the solutions containing the polymers in low and high concentration.

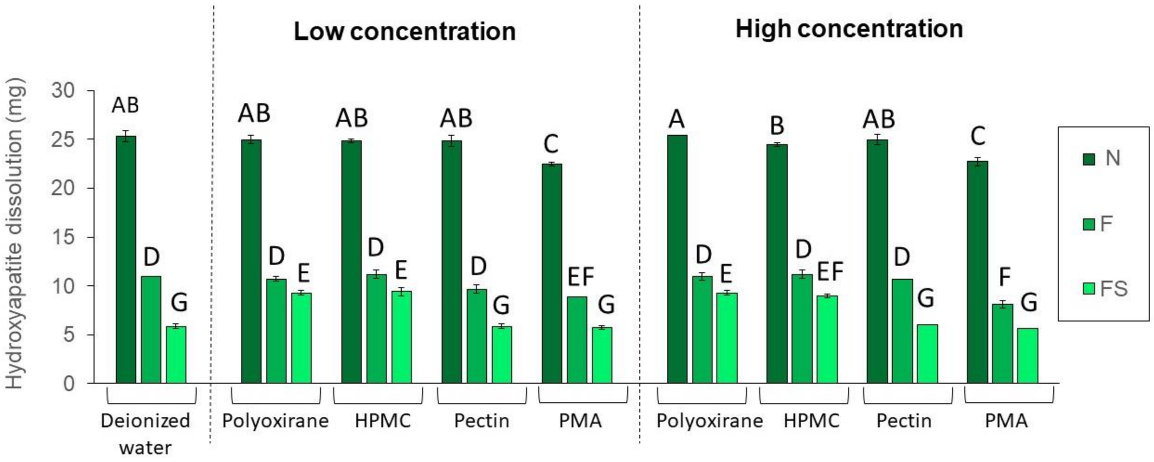


Fig 1. Mean and SD values for HA dissolution. Different letters indicate significant differences between the experimental solutions. Legend: N = no fluoride, F = with sodium fluoride, FS = with sodium fluoride and stannous chloride.

Table 3 shows the mean and standard deviation values of zeta potential for all subgroups. The HA was found to have a strong negative surface charge of -24.3 ± 2.01 mV. The treatment with the experimental solutions, resulted in small changes in its surface charge, except for the PMA groups, in which the HA became positive, supporting the adsorption of this molecule on HA surface.

Table 3 – Mean and standard deviation of zeta potential (mV) for all subgroups

		No fluoride	**F**	**FS**
Deionized water		*-24.3 (2.01)*	*-24.5 (0.75)*	*-27.7 (0.57)*
Polyoxirane	*Low concentration*	*-24.6 (2.83)*	*-32.1 (2.71)*	*-32.1 (1.52)*
	High concentration	*-23.6 (1.29)*	*-29.5 (1.27)*	*-33.8 (3.42)*
HPMC	*Low concentration*	*-24.6 (1.76)*	*-30.4 (1.85)*	*-32.2 (1.98)*
	High concentration	*-18.6 (1.51)*	*-24.9 (1.29)*	*-25.9 (0.96)*
Pectin	*Low concentration*	*-23.9 (1.95)*	*-25.0 (1.02)*	*-25.5 (1.37)*
	High concentration	*-22.5 (1.42)*	*-23.9 (1.94)*	*-26.6 (1.79)*
PMA	*Low concentration*	*2.5 (0.47)*	*9.4 (0.67)*	*12.8 (0.76)*
	High concentration	*4.5 (0.14)*	*8.6 (0.76)*	*9.4 (0.47)*

4. Discussion

The results of this study showed that the different polymers tested and the presence of fluorides significantly influenced the amount of HA dissolution. Thus, the null hypothesis was rejected.

In dentistry, the polymers have been extensively investigated as restorative materials, however, their use as anti-erosive agents is recent [13]. The number of potential polymers to be added to oral care products is vast. However, currently only a small part of this wide range of possibilities has been studied and an even smaller amount is commercially available [37]. The evaluation of the amount of HA dissolution after treatment with different solutions is a promising screening tool to refine the search for most promising polymers, and their optimal concentration.

Two mechanisms regulate the interaction between the polymers with the HA surface: chemical reactions [38] and physical adsorption [25]. The non-ionic polymers polyoxirane and HPMC do not interact strongly with F- or Sn^{2+}*, but they can entrap or encapsulate these ions in their polymeric and tridimensional structure conformation.*

Considering that physical adsorption is a weaker interaction, one can suppose that the centrifugation performed in the pre-treatment of the HA crystals may have reduced the protective effect derived from polymers that interact solely by physical adsorption. This could explain the lack of anti-erosive effect of polyoxirane and HPMC under the conditions of the present study. In addition, it was possible to observe that the addition of polyoxirane and HPMC reduced the protective effect of the FS solution. Possibly, many of the Sn^{2+} *ions were encapsulated by the polymers, avoiding the bonding of Sn2+ with the HA and reducing its protective effect [19].*

Pectin showed no protective effect against HA dissolution. This result may be related to fact that the protective effect of pectin is related to a gelatinization *process that blocks the ions H+ to achieve the HA surface. This process is dependent of the pH [30]. Thus, since the experimental solutions had their pH standardized in 4.5 to favor the* CaF_2 *deposition [7], probably the gelification process was not favored.*

The polymethacrylate was the only polymer able to provide a protective effect against HA dissolution by itself. The reaction between the secondary amine $[–NH^+(CH_3)_2]$ *present in the PMA molecule and the phosphate ions from the HA* $(H_2PO_4^-$ *and* $HPO_4^{2-})$ *may explain this result. The zeta potential analysis supports this assumption, since we observed that the HA treated with PMA acquired a positive charge, indicating the bonding with the polymer.*

The zeta potential of HA has been measured by several authors using different devices and methods [14,15,40,41]. On enamel, the electric double layer is formed due to the electrostatic potential generated by the calcium and phosphate ions present on the HA surface prevailing a net negative charge on it at neutral pH [40,42]. Coating the HA surface with a polymeric layer able to interact chemically with the HA particles and to change the zeta potential is an interesting strategy for the prevention of dental erosion, since the contact with the acid may be delayed by hindering H^+ *ions to reach the HA surface by the presence of an adsorbed polymer protective layer.*

PMA was also effective in improving the protective effect of fluoride. It may be speculated that whereas fluoride occupied the free positive sites (calcium sites) on the HA surface, PMA occupied the free negative sites (phosphate sites), resulting in a more homogenous protective layer [19].

Pectin and polymethacrylate did not alter the protective effect of the FS solution. That may be due to the fact that the molecular weight of F^- *(19 g/mol) and* Sn^{2+} *(119 g/mol) are lower than the polymers, then they first interacted with the charged sites of the HA molecule, leaving no polar sites available for larger and heavy molecules as the polymers.*

The addition of the polymers in a higher concentration did not provide a significant decrease in the erosive potential of the solutions. Similar results were observed in a previous study that tested the polymers in low (2 mg/ml) and high concentration (20 mg/ml) [15]. Although the use of the polymers in lower concentrations may be preferred in order to avoid possible toxic effects, we suggest that each polymer should have its own optimal concentration determined.

Some limitations of the present study should be considered since under in vivo conditions the presence of saliva, acquired pellicle, eating/drinking habits and other factors may influence the interaction of the polymers with the tooth surfaces [43]. Further studies in this area should be designed considering such conditions.

5. Conclusion

Under the conditions tested, it is possible to conclude that among the no fluoride solutions, only the ones containing PMA were able to provide protection against HA dissolution. The F and FS solutions significantly reduced the HA dissolution in comparison to the control. PMA improved the protective effect of the F solution, but this effect was nor observed when combining it with FS. No differences were found for the solutions containing the polymers in low and high concentration.

6. Acknowledgement

The authors would like to thank FAPESP (Sao Paulo Research Foundation, #2016/15755-5, and #2017/13799-8).

7. Disclosure Statement

The authors have no conflicts of interest to declare.

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2.3 Artigo – Augusto MG, Scaramucci T, Aoki IV, Torres CRG, Borges AB. Melhoria das propriedades anti-erosivas do flúor e do estanho através um copolímero do polimetacrilato / *Enhancement of fluoride and stannous anti-erosive properties by a polymethacrylate copolymer**

RESUMO

Objetivo: A combinação de polímero formadores de filme com soluções fluoretada é uma abordagem promissora para se prevenir e controlar o desgaste erosivo dental. Este estudo investigou se um copolímero do polimetacrilato (PMA) pode potencializar o efeito anti-erosivo de soluções contendo fluoreto de sódio F- (225 ppm F^-) e fluoreto de sódio associado ao cloreto de estanho -FS (800 ppm Sn^{2+}). Métodos: As soluções experimentais (F, FS, PMA, PMA+F, PMA+FS, e água deionizada-DW como controle) foram testadas na presença da película adquirida. Espécimes de esmalte (n=13/grupo) foram submetidos a um ciclo erosivo-reendurecedor que consistiu em imersão em saliva humana para formação da película adquirida (2h), solução de ácido cítrico 0.3% (5 min), saliva humana (1h), 4x/dia, por 5 dias. O tratamento com as soluções experimentais foi realizado 2x/dia, por 2 minutos. Os potenciais de reendurecimento (%Re) e de proteção (%Prot) dos tratamentos com as soluções foi através de microdureza Knoop, e a perda superficial (SL) através de perfilometria de contato. O ângulo de contato com o esmalte e o potencial zeta das partículas dispersas de hidroxiapatita (HA) também foram mensurados. Os dados foram avaliados utilizando-se ANOVA 2-fatores e teste de Tukey (5%). Resultados: A associação com PMA melhorou o %Re e o %Prot para W e F, mas não para FS. A associação com PMA reduziu significativamente a perda superficial: PMA+F = PMA+ FS < PMA < FS < F< DW. A presença de PMA também reduziu o ângulo de contato para as soluções. A HA apresentou forte carga superficial negativa após o tratamento com soluções DW, F e FS, enquanto que após o tratamento com as soluções contendo PMA, tornou-se positiva. Conclusão: O PMA tem potencial para aumentar o efeito anti-erosivo das soluções fluoretadas, sendo um agente promissor a ser adicionado aos produtos de higiene bucal para prevenção de desgaste erosivo dos dentes.

Palavras-chave: Esmalte. Erosão dental. Polímeros. Fluoretos. Cloreto de estanho.

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*Artigo elaborado de acordo com as normas do Periódico *Journal of Dentistry*

ABSTRACT

Objective: The combination of film-forming polymers with fluoridated solutions is a promising approach to prevent and control erosive tooth wear. This study investigated if a polymethacrylate copolymer (PMA) could potentiate the anti-erosive effect of solutions containing sodium fluoride -F (225 ppm F^-*) and sodium fluoride associated to stannous chloride -FS (800 ppm* Sn^{2+}*). Methods: The experimental solutions (F, FS, PMA, PMA+F, PMA+FS, and deionized water-DW as control) were tested in the presence of acquired salivary pellicle. Enamel specimens (n=13/group) were submitted to an erosion-rehardening cycle that consisted of immersion in human saliva for pellicle formation (2h), 0.3% citric acid solution (5 min), human saliva (1h), 4×/day, for 5 days. The treatment with the experimental solutions was carried out 2×/day, for 2 min. The rehardening (%Re) and protective (%Prot) potential of the treatment with the solutions were assessed by Knoop microhardness, and the surface loss (SL) by contact profilometry. The contact angle with enamel and the zeta potential of the dispersed hydroxyapatite (HA) particles treated with the solutions were also measured. Data were analyzed using 2-way ANOVA and Tukey tests (5%). Results: The association with PMA improved the %Re and the %Prot for W and F, but not to FS solution. The association with PMA significantly reduced the SL: PMA+F = PMA+ FS < PMA < FS < F< DW. The presence of PMA also reduced the contact angle with the enamel. The HA presented a strong negative surface charge after the treatment with DW, F and FS solutions, whereas after the treatment with the solutions containing PMA it became positive. Conclusion: PMA has potential to increase anti-erosive effect of the fluoridated solutions, being a promising agent to be added to oral care products for erosive tooth wear prevention.*

Keywords: Enamel. Tooth erosion. Polymers. Fluorides. Stannous chloride.

1. Introduction

Over the recent decades, erosive tooth wear (ETW) has been considered a growing condition, mainly due to changes in dietary and behavioral habits of populations [1]. Besides the control of causative factors, different strategies have been proposed to prevent ETW progression, such as the use of over-the-counter oral care products containing fluorides [2]. These products are commonly used worldwide, with a significant increase in their consumption in the last decades [3].

Monovalent compounds as sodium fluoride (NaF) and amine fluoride (AmF) promote the deposition of precipitates similar to calcium fluoride (CaF_2) *on the tooth surface, which will act as a sacrificial layer during the acid episodes [2]. However, fluoride is not as efficient for erosion prevention as for caries because to favor the mineralization in deeper levels it depends on the existence of a sheltered subsurface lesion [4,5]. Thus, the protective effect of fluorides on ETW is limited to the enamel surface. Moreover, the lower pH of the acids compared to bacterial acids contribute to reduce the efficacy of fluorides against erosion [6].*

Combining fluoride with metal cations as stannous (Sn^{2+}) *is an alternative to make the tooth surface more resistant to erosive demineralization [7,8]. Stannous can be incorporated into enamel and dentin structures, reducing their solubility [7,8]. In addition, it reacts with the phosphate, calcium and fluoride of the dental tissue, forming stable precipitates* [Sn_2OHPO_4, $Sn_3F_3PO_4$, $Ca(SnF_3)_2$] *[9], which create a more acid-resistant layer than pure* CaF_2 *[10,11]. However, the presence of stannous is associated to some drawbacks as bad taste, mucosal desquamation, as well as tooth and tongue discoloration [12].*

Considering the necessity of effective measures to prevent EWT, the ability of film-forming polymers has been investigated [13]. The anti-erosive potential of the polymers is due to their capability to interact with the hydroxyapatite surface, *forming a film that can reduce ion exchange between the acid and the dental substrate [14]. In addition, some polymers can interact with fluoride compounds, increasing their protection [15].*

Recently, the anti-erosive effect of mouthrinses [15–18], toothpastes [19,20] and acidic beverages [21–23] containing polymers has been investigated. However, a wide range of polymers is available to be tested, being necessary to consider their possible chemical interactions with the hydroxyapatite and the acquired salivary pellicle to produce an effective polymeric film on the tooth surface.

The polymethacrylate copolymer (PMA) investigated in this study (Eudragit E100, Evonik Industries, Essen, Germany) is a synthetic polymer designed to encapsulate medications in order to protect them from moisture, mask taste and odor, therefore being a non-toxic agent [24]. This polymer is completely soluble up to pH 5.0, when most of its amine functional groups are protonated $[–NH^+(CH_3)_2]$*. Under this condition, PMA presents a positive overall charge, behaving as a weak cationic polyelectrolyte. Considering that, under neutral pH, the hydroxyapatite* $[Ca_{10}(PO_4)_6(OH)_2]$ *presents a negative surface charge [15], the ionic interaction with Eudragit in its positivelly charged form would be favored.*

Thus, this study investigated the efficacy of the addition of a polymethacrylate copolymer to solutions simulating mouth rinses, as well as the effect of its association with sodium fluoride, and sodium fluoride plus stannous chloride in protecting the enamel against erosive challenges.

2. Material and Methods

2.1 Ethical aspects

For the collection and use of human saliva, the study protocol was approved by the local Ethics Committee in Research (CAAE: 59351516.8.0000.0077).

2.2 Study design

This study followed a 3 x 2 factorial design, considering the fluoridated solution at 3 levels (water as control, sodium fluoride, sodium fluoride plus stannous chloride) and the presence of the polymethacrylate copolymer at two levels (with PMA, without PMA). The solutions were evaluated in an in vitro erosion-rehardening model with bovine enamel specimens (n = 13). Knoop microhardness and surface loss were assessed. Additional tests were performed to determine the contact angle of the enamel treated with the experimental solutions, as well as the zeta potential of treated hydroxyapatite crystals. The null hypothesis tested was that the treatment with experimental solutions do not influence the rehardening potential, protective potential, surface loss, the contact angle with enamel and zeta potential of treated hydroxyapatite.

2.3 Specimens preparation

Fresh, non-damaged bovine incisors were collected for this study. The crowns were separated from the roots and stored into 0.1% thymol solution at 4 ºC until required. Seventy-eight cylindrical enamel specimens (3 mm in diameter) were obtained from the labial surface of the teeth, using a custom-made diamond-coated trephine mill adapted to a circular cutting machine. The enamel samples were embedded in acrylic resin (Extec Fast Cure Acrylic, Extec Corp, Enfield, CT, USA) and the resulting specimens were polished using SiC sandpapers in sequential grits of 1200, 2400, and 4000 (FEPA-P, nExtec, Enfield, CT, USA), under constant water irrigation for 30, 60 and 120 s, respectively. After each paper grit change, specimens were kept in an ultrasonic bath for 10 min, to *remove debris and abrasive grains. The specimens were examined under a stereomicroscope (Carl Zeiss – Stemi 2000 -20X) to ensure the absence of cracks or other surface defects [25]. The baseline surface microhardness (SMH) of all specimens was assessed using a Knoop Microhardness Tester (FM-700, Future-Tech, Tokyo, Japan) fitted with a 50 g load, for 10 s. Three indentations were performed, 100 µm apart from each other and averaged. The mean values were used to stratify the samples into groups.*

2.4 Groups division and preparation of the experimental solutions

The specimens were allocated into 6 groups (n = 13) according to the experimental solutions tested: Sodium fluoride (F), Sodium fluoride plus stannous chloride (FS), polymethacrylate copolymer (PMA), and the combinations PMA+F, PMA+FS. Deionized water – DW was the control.

The experimental solutions were freshly prepared prior the experiment. In the respective solutions, sodium fluoride (Sigma Aldrich, St Louis MO, USA) was added in the concentration of 0.497 g/l (225 ppm F^-*) [26], whereas stannous chloride (Sigma Aldrich Co.) and gluconic acid sodium salt (stabilizing agent - Sigma Aldrich Co.) were added in the following concentrations of 1.28 g/l (800 ppm* Sn^{2+}*) and 2.3 g/l, respectively [27]. The concentration of the polymethacrylate copolymer was determined based on the results obtained in a pilot study [28]. In the PMA groups, 1 g of the polymethacrylate copolymer (Eudragit E-100, Evonik Industries, Essen, Germany) was solved in 9 ml of hydrated ethyl alcohol 92.8*^{o}*, and then deionized water was added until completing the final volume of 50 ml. The pH of all experimental solutions, except DW, was adjusted to 4.5, using 0.1 M KOH or 0.1 M HCl. To avoid having the viscosity as a confounding factor, the dynamic viscosity of the solutions was determined using a vibroviscometer (SV-10, A&D, Toshima-ku, Tokyo, Japan). The results were obtained in duplicate, in a thermostatic bath at 25 ± 1* ^oC*.*

2.5 Saliva collection

Stimulated whole saliva was collected from twenty healthy volunteers without active caries, periodontal disease, or salivary dysfunction, who were not taking any medications. Previous to the saliva collection, the donors were instructed to brush their tooth with a fluoride-free dentifrice (Cocoricó, Bitufo, Sao Paulo, Brazil). The volunteers were instructed not to drink or eat 1 h before the collection. Saliva was collected in the morning under stimulation by chewing Parafilm M (Bemis Company, Inc, Neenah, Wisconsin, USA). The volunteers spited the saliva produced into ice-chilled tubes. After collection, the saliva was pooled and immediately centrifuged (10,000 rpm/4 ºC, 5 min). The supernatant was separated from the pellet and stored at -80 °C. The day before starting the experimental procedures, the amount of saliva needed was left to defrost overnight at 4 °C. Two hours before cycling, the saliva was left at room temperature [29].

2.6 Erosion-rehardening cycling protocol

In the beginning of the cycle, the specimens were immersed in human saliva during 2 h, at room temperature, to allow the salivary pellicle formation [30]. Then, they were submitted to the cycling protocol, consisting of 5 min immersion in 0.3% citric acid (natural pH ~ 2.6) and 60 min immersion in human saliva. This cycle was repeated 4 times per day, over 5 days. Treatment with the experimental solutions was performed 30 min after the first and the last erosive challenge, for 2 min. After the exposure, no rinse was performed, and the specimens were re-immersed into human saliva for the completion of the 30 min. The specimens were stored in human saliva during the overnight period. All experimental procedures were conducted at room temperature. Figure 1 shows the daily cycling sequence.

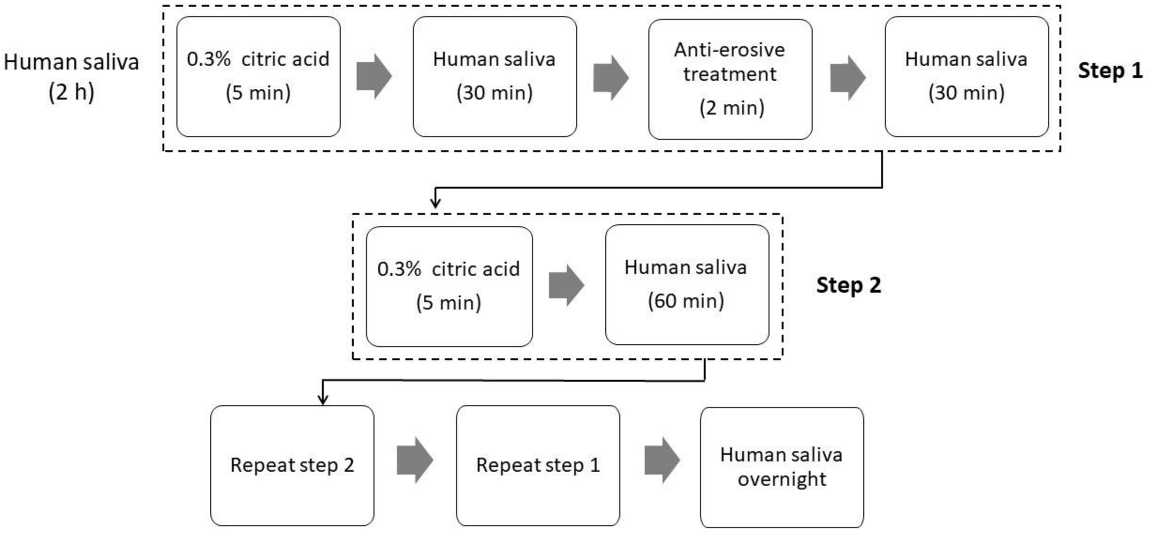

Fig. 1. *Illustration of the daily cycling sequence*

2.7 Surface microhardness analysis

In the first day of cycling, the surface microhardness (SMH) was measured for each specimen at three timepoints: after the first erosive challenge (E1), after the treatment with the experimental solutions (T) and after the second erosive challenge (E2). The reherdening potential of the solutions was calculated according to the formula: %Re = [(E1-T)/(E1)] × 100. The protective potential of the solutions was calculated according to the formula: %Prot = [(E1-E2)/(E1)] × 100 [16].

2.8 Surface loss assessment

Two parallel grooves were marked on the sides of the acrylic resin surface to create references for the superposition of the profiles. The specimens were placed in a custom-made setting device in order to allow the exact replacement of the samples during the obtention of the profiles, which was made by using a contact profilometer (MaxSurf XT 20, Mahr-Goettingen, Germany). The diamond stylus moved from the first reference area in the acrylic resin to the second one (4.2 mm long). Three baseline profile measurements were performed for each *specimen at intervals of 0.25 mm. At the end of the erosion-rehardening cycling, the final profiles were obtained using the same specifications described. The surface loss was calculated by matching the baseline and final profiles using a dedicated software (MarSurf XCR 20 4.50-07 SP3, 2011).*

2.9 Contact angle determination (goniometry)

Additional bovine enamel specimens were prepared for the contact angle determination. The coronary labial surfaces of the bovine incisors were ground flat using silicon carbide sandpaper P120 grit (Extec Corp., CT, USA) coupled to a circular polishing machine (Panambra, São Paulo, SP, Brazil) under constant water cooling, until an area of ~ 6 mm^2 *of enamel was exposed. The roots were removed using a diamond disc coupled to a low speed handpiece. The crowns were placed inside PVC rings and then embedded in acrylic resin (Jet Classico, Sao Paulo, SP, Brazil). The specimens were polished in the circular polishing machine using SiC sandpapers using the same sequential grits described previously.*

In order to avoid a potential confounding factor, the surface roughness of the specimens was assessed from profiles obtained using a contact profilometer (MaxSurf XT 20, Mahr). The diamond stylus moved 6 mm along the specimen's surface. Three measurements were performed for each specimen, with a distance of 0.25 mm between them. The mean surface roughness (Ra) values were determined with a cut-off value of 0.25 mm and a stylus speed of 0.1 mm/s. The Ra was used to stratify the specimens into 6 groups (n = 5) according to the experimental solution.

The specimens were immersed in the respective experimental solution for 2 min under agitation (60 rpm – Kline shaker, TS2000A, Biomixer). Then, they were air-dried for 15 s to prevent that the residual water of the experimental solutions could influence the contact angle measures [31].

The contact angle with the enamel was measured using the sessile drop technique. The specimens were placed on the base of the equipment and the angle between the needle and specimen surface was adjusted to 90°. A 0.5 µl deionized water drop was applied to the enamel. A pilot investigation determined that an initial pause of 10 s was needed to achieve equilibrium between the water and the surface. Then after this pause, the measurement of the contact angle was performed. Twenty images per second were obtained during 30 s. Image capture and contact angle processing were performed by software (One Attension, Biolin Scientific, Stockholm, Sweden). The average obtained by the right and left angles was considered for the analysis.

2.10 Determination of the zeta potential

The zeta potential of dispersed hydroxyapatite (HA) treated with the experimental solutions were measured by a zeta potential analyzer (Zetasizer Nano ZS90, Malvern Instruments, Malvern, UK), equipped with a Helium–Neon Laser of 633 nm and maximum of 4 mW. Electrophoretic light scattering (ELS) was used to measure the electrophoretic mobility of dispersed HA in 0.040 mol/L phosphate buffer (pH = 7.0), and this was later converted to zeta potential using the Helmholtz-Smoluchowski formula. Ten milligrams of pretreated HA were dispersed in 5 ml of the phosphate buffer assisted by 8 min under sonication in a common ultrasonic bath containing water. Samples were pipetted and introduced in the sample cell and examined at 20 °C. Five measurements were performed for each sample (after a minimum of 10 and a maximum of 40 runs) and the mean value recorded with its standard deviation [15].

2.11 Statistical analysis

The results indicated that the residuals were normally distributed and, by plotting against predicted values, the uniformity was checked. None of the *analysis of variance (ANOVA) assumptions were violated. Thus, two-way ANOVA was performed for %Re, %Prot, surface loss, contact angle and zeta potential data. Post hoc pairwise comparisons were performed using the Tukey test, with a significance level of 5%. Minitab 17 Software (Minitab Inc, State College, PA, USA) was used for the calculations.*

3. Results

The solutions presented viscosity values varying from 3.05 to 3.78 mPa.s, which is clinically compatible to a mouth rinse and similar to a commercial product (Elmex Erosion Protection – 1.20 mPa.s.). The rehardening potential (%Re) analysis showed significant differences for the presence of PMA and fluoride (p = 0.0001), but not for their interaction (p = 0.206). Table 1 shows the mean, standard deviation and results of the Tukey test. Lower values indicate decreases of microhardness, and therefore worse performance of the solutions. The %Re of F and FS were higher than the water, highlighting the rehardening effect of such solutions. The association with PMA improved the %Re of water and F, but not to FS solution.

The protective potential (%Prot) analysis showed significant differences for the presence of PMA, fluorides, and their interaction (p = 0.0001). Table 1 shows the mean, standard deviation and results of the Tukey test. Lower values indicate decrease of microhardness, and therefore worse performance of the solutions. The %Prot of F and FS were higher than the deionized water. The association with PMA improved the %Prot of DW and F, but not to FS solution. The %Prot of PMA+F and PMA+FS presented no significant differences.

Table 1 – Mean, standard deviation of surface microhardness, and results of the Tukey test for the rehardening (%Re) and protective (%Prot) potential.*

Experimental solutions	***Surface microhardness (KHN)***			$\%Re = \left(\frac{E1-T}{E1}\right)*100$		$\%Prot = \left(\frac{E1-E2}{E1}\right)*100$	
	$E1$	T	$E2$				
DW	237.47±11.97	226.03±16.06	150.45±18,51	-5.62±10.17	A	-55.44±16.99	A
F	240.24±10.62	251.71±9.31	195.11±9.89	4.53 ±3.25	B	-23.34±6.72	BC
FS	248.13±9.63	293.10±14.64	218.87±12.80	15.21±4.12	CD	-13.65±6.63	CD
PMA	242.84±12.19	253.38±15.17	189.74±15.42	3.98±5.55	B	-28.44±8.49	B
PMA+F	238.20±10.46	270.17±11.03	219.12±13.28	11.78±3.48	C	-9.08±8.20	D
PMA+FS	238.98±11.83	295.98±11.61	209.08±9.78	19.18±4.37	D	-14.46±6.65	CD

** Different letters in the columns indicate differences for the experimental solutions (p<0.05).*

The surface loss analysis showed significant differences for the presence of PMA, fluorides, and the interaction between them (p = 0.0001). Figure 2 shows the mean surface loss (SL), standard deviation and results of the Tukey test. The association with PMA significantly reduced the SL for deionized water, F, and FS solutions.

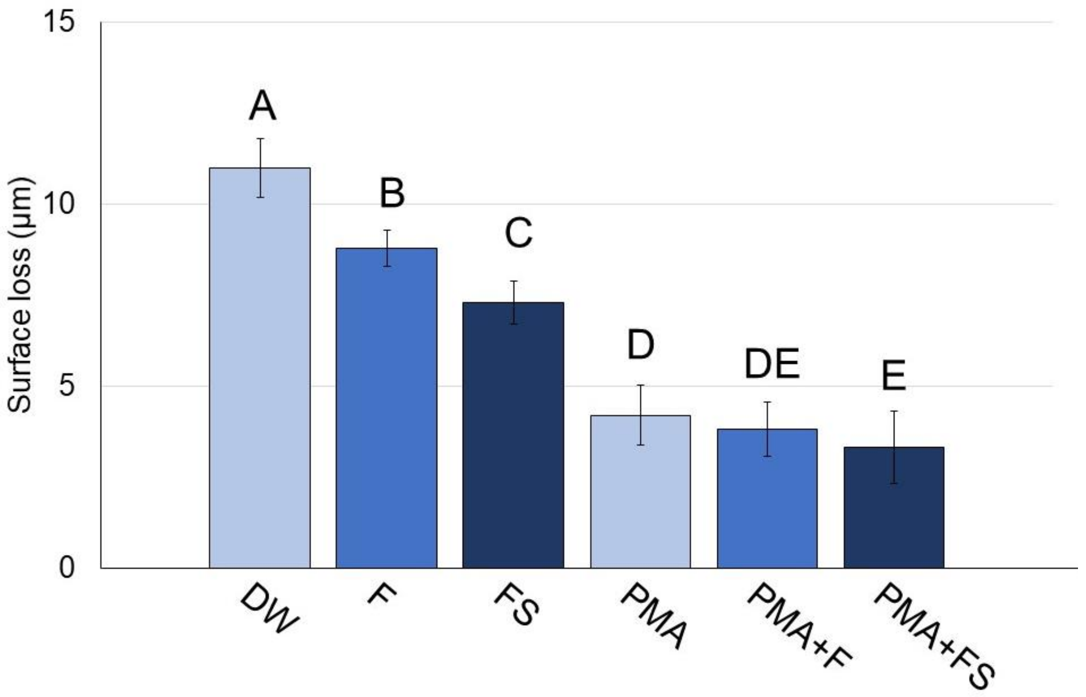

Fig.2. *Mean, standard deviation and results of the Tukey test (5%) for the surface loss results.*
** Different letters indicate differences among the treatments (p<0.05).*

The surface roughness analysis showed no significant differences between the groups (p = 0.987). The mean Ra of the groups varied from 0.0786 to 0.0834 µm. The contact angle analysis showed significant differences for the presence of PMA, fluorides, and their interaction (p = 0.0001). Table 2 shows the mean and standard deviation for surface roughness (Ra) and contact angle, as well as the results of the Tukey test for the contact angle. The presence of PMA significantly reduced the contact angle for deionized water, F, and FS solutions.

Table 2 – Mean, standard deviation of roughness and contact angle, and results of the Tukey test (5%).*

Experimental solutions	***Contact angle (degrees)***	
DW	*49.72±6.27*	*A*
F	*33.14±6.42*	*B*
FS	*32.47±4.98*	*B*
PMA	*22.79±6.51*	*C*
PMA+F	*21.81±5.92*	*C*
PMA+FS	*20.47±4.41*	*C*

** Different letters indicate differences for the experimental solutions (p<0.05).*

Table 3 shows the mean and standard deviation values of zeta potential for all subgroups. The HA was found to have a strong negative surface charge of -24.2 ± 1.7 mV. The treatment with the FS solution without PMA resulted a more negative surface charge than DW and F, whereas the treatment with the solutions with PMA changed the HA to positive, supporting the adsorption of this molecule on HA surface. In this group, F and FS solutions presented a stronger positive surface charge than DW.

Table 3 – Mean, standard deviation of zeta potential and results of the Tukey test (5%).*

Experimental solutions	***Zeta potential (mV)***	
DW	*-24.2±1.7*	*A*
F	*-24.0±0.8*	*A*
FS	*-27.6±0.6*	*B*
PMA	*4.47±0.14*	*C*
PMA+F	*8.74±1.01*	*D*
PMA+FS	*9.61±0.53*	*D*

** Different letters indicate differences for the experimental solutions ($p<0.05$).*

4. Discussion

The results of this study showed that the treatment with experimental solutions containing fluoride and PMA influenced all the variables analyzed. Thus, the null hypothesis was rejected.

The FS group presented higher %Re than the F group, indicating the incorporation of Sn to enamel and the formation of a Sn-rich layer [32]. The PMA group presented higher %Re than the deionized water group. The PMA molecule may have interacted via physical adsorption or chemical bonding with the negative sites of the enamel ($H_2PO_4^-$ and HPO_4^{2-}) and of the acquired salivary pellicle. The results of the zeta potential support this hypothesis since after the treatment with the solutions containing the polymer (PMA, PMA+F, PMA+FS), the HA became positive demonstrating the adsorption of this positive molecule on its surface. Moreover, the results of the goniometry show that the presence of PMA significantly reduced the contact angle and increased the hydrophilicity of the treated surface, further supporting that the charged molecules of the polymer were present on the enamel surface.

The group treated with PMA+F presented higher %Re than the group treated with F only. The interaction of PMA with the previously mentioned negative sites may have complemented the action of the fluoride, which bonded *with the positive sites from the enamel (Ca^{2+}) forming calcium fluoride-like particles [2]. However, one must to consider that the increased rehardening effect in the PMA groups is probably not due to a remineralizing effect, but to the hardness of the polymeric film layer deposited on the surface.*

The results of the %Prot suggest that the presence of PMA effective to protect the enamel surface against further acid challenges, and this protection can be improved by the combination with F and FS. However, in opposition to %Re, no significant difference was observed between the groups PMA+F and PMA+FS, corroborating to the hypothesis that the film formation on the PMA+FS group was impaired by the competition of PMA with Sn^{2+}. This may be related to the fact that this cationic form of PMA may have competed with Sn^{2+} for the same binding sites on the enamel and on acquired salivary pellicle proteins.

The SL analysis of the groups treated with fluoride solutions (without PMA) corroborates with the previous findings showing that sodium fluoride is able to reduce the tooth structure loss, and its combination with stannous contribute to improve the protection of the surface [10,11]. This is due to the ability of the stannous ions to be incorporated into enamel, reducing its solubility [7,8] and forming stable stannous precipitates on the tooth surface, which are more acid-resistant than CaF_2 [11,33].

The presence of PMA in the solutions showed that the polymer was effective in producing an acid resistant protective film on enamel surface, able to reduce the ETW progression. PMA is completely soluble up to pH 5.0, but when in a higher pH as the human saliva (pH ~ 6.5-6.9) [34], the polymer precipitates, creating a barrier against further acid challenges. In fact, at the end of the cycle, it was possible to observe at naked eye a very thin pellicle on specimens' surface. However, the formation of this pellicle seems to be due to a cumulative effect of the treatment. Possibly, after the initial adsorption of the PMA to enamel and to *the proteins of the acquired salivary pellicle, an acid-resistant multi-layer started be formed [35,36].*

However, some limitations of the current study should be pondered since the erosion-rehardening cycling protocol used does not consider the influence of the toothbrushing, the contact with the soft tissues, and the clearance action of the saliva. Thus, the observed effect of PMA needs to be confirmed under protocols simulating more real clinical conditions, being this a guide for future investigations.

The benefits of increasing the anti-erosive effect of sodium fluoride solution are related to the potential reduction of the unpleasant consequences of metallic compounds related to taste, soft tissue side-effects, and staining. PMA is biologically safe, since it is already used in several medications, and is cost-effective. Moreover, the use of other synthetic polymers, as PMA, for erosion prevention is encouraged, since specific functional groups can be added to their chain backbone [37], favoring the interaction with the tooth surfaces and the salivary acquired pellicle proteins.

5. Conclusion

Under the conditions tested, it is possible to conclude that PMA has potential to increase anti-erosive effect of the F and FS solution, being a promising agent to be added to oral care products such as mouth rinses for erosive tooth wear control.

6. Acknowledgement

The authors would like to thank FAPESP (Sao Paulo Research Foundation, #2016/15755-5, and #2017/13799-8).

7. Disclosure Statement

The authors have no conflicts of interest to declare.

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3 CONSIDERAÇÕES GERAIS

Com base nos artigos apresentados podemos concluir que os polímeros formadores de filme representam uma abordagem promissora e econômica para prevenir e controlar a erosão dentária. No entanto, são necessários estudos adicionais com condições clínicas mais relevantes para se confirmar sua eficácia. Além disso, a padronização do desafio ácido, da duração do tratamento e da associação com fluoretos são recomendados para permitir comparações entre os estudos.

A presença de polímeros em produtos de higiene bucal, em bebidas ácidas e em formulações antiácidas têm apresentado resultados positivos, porém uma maior quantidade de estudos investigando a adição de polímeros a soluções simulando enxaguantes bucais pode ser encontrada na literatura, possivelmente devido à sua composição simplificada que facilita a investigação de variáveis isoladas.

Dentre os quatro polímeros testados (polioxirano, hidroxipropilmetilcelulose, pectina e o copolímero do polimetacrilato -PMA) utilizando-se o modelo de dissolução da hidroxiapatita, o PMA apresentou a maior capacidade protetora, possivelmente devido a capacidade de sua amina secundária em se ligar aos íons fosfato da hidroxiapatita, criando uma barreira protetora. A análise do potencial zeta corrobora com esta hipótese, uma vez que foi observado que a hidroxiapatita tratada com PMA adquiriu uma carga positiva, indicando a interação com o polímero.

As soluções contendo fluoreto de sódio -F (225 ppm F^-) e fluoreto de sódio associado ao cloreto de estanho -FS (800 ppm Sn^{2+}) reduziram a dissolução da hidroxiapatita em comparação com a água deionizada, sendo a solução FS mais eficaz do que a solução F. Estes achados estão de acordo com a literatura atual que indica que o fluoreto de sódio promove a deposição de precipitados semelhantes ao fluoreto de cálcio (CaF_2) nas superfícies dentais, criando camada de sacrifício. Já o estanho pode se incorporar às estruturas dentais reduzindo sua solubilidade, e formar depósitos na superfície tornando-a mais ácido-resistente.

O PMA demonstrou capacidade de melhoria do efeito protetor do flúor. Acredita-se que o flúor ocupou os sítios de ligação positivos (Ca^{2+}) da superfície da hidroxiapatita e o PMA ocupou os sítios negativos (HPO_4^{2-}e $H_2PO_4^-$), resultando em uma camada protetora mais homogênea do que a formada após o tratamento com a solução contendo apenas PMA.

Por ter apresentado o resultado mais promissor dentre os polímeros testados na avaliação da dissolução da hidroxiapatita, as soluções contendo PMA foram avaliadas na presença da película adquirida utilizando-se um protocolo de ciclagem erosiva-reendurecedora. Foi observado que a associação com PMA melhorou o potencial de reendurecimento e o potencial de proteção da água deionizada e da solução contendo F, mas o mesmo efeito não foi observado para a solução FS. A associação com PMA reduziu significativamente a perda superficial e o ângulo de contato com o esmalte.

Apesar das limitações dos resultados obtidos no estudo in vitro, concluímos que a adição de PMA é uma abordagem promissora para se melhorar o efeito protetor de soluções fluoretadas contra a erosão dentária, o que pode ser explorado em futuras investigações.

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APÊNDICE A - Termo de consentimento livre e esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Caro (a) Senhor (a),

Eu, Alessandra Bühler Borges, Professora Adjunta do Departamento de Odontologia Restauradora do Instituto de Ciência e Tecnologia de São José dos Campos da Universidade Estadual Paulista (UNESP), à Av. Engº Francisco José Longo, 777 – São José dos Campos – SP, telefone para contato (12) 3947-9374, vou coordenar a pesquisa cujo título é “Efeito anti-erosivo de soluções contendo diferentes polímeros”.

O objetivo deste trabalho será avaliar o potencial anti-erosivo de soluções contendo diferentes polímeros formadores de filme sobre o esmalte dental. A hipótese de investigação será a de que as diferentes substâncias anti-erosivas não influenciam na dissolução da hidroxiapatita, assim como no desgaste e na microdureza do esmalte.

O presente estudo envolve o uso de saliva humana para permitir a simulação da condução bucal em condições laboratoriais. Serão coletados cerca de 50 ml de saliva, em tubos plásticos estéreis, no período da manhã, no Instituto de Ciência e Tecnologia (ICT – UNESP). O procedimento é simples, indolor e sem envolvimento de riscos aos voluntários.

Se houver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética (CEP) do ICT - UNESP, situado na Av. Engº Francisco José Longo, 777 – CEP 12245-000, em São José dos Campos – SP, telefone (12) 3947-9006, e comunique-se com Carlos Alberto Guedes. Informo que será garantida a liberdade da retirada do consentimento a qualquer momento e assim deixar de participar do estudo. Também não haverá custo nem pagamento pela colaboração

Acredito ter sido esclarecido (a) a respeito das informações que me foram citadas nesse documento, descrevendo o estudo a ser realizado e concordo em participar, sabendo quais os propósitos do estudo, os procedimentos a serem realizados, a garantias de confidencialidade e de esclarecimentos permanentes, e que minha participação não implicará em nenhuma despesa. Concordo em participar voluntariamente deste estudo e poderei retirar o meu consentimento a qualquer momento, antes ou durante a realização do mesmo, sem penalidade, prejuízo ou perda de qualquer benefício que possa ter adquirido.

São José dos Campos, ____ de _______________ de 20____

Nome do voluntário: ____
RG: ___________________________________ CPF: ________________________________
Endereço: ____

_____________________________ _____________________________
Assinatura do voluntário Assinatura do Pesquisador

APÊNDICE B – Detalhamento da Metodologia

1 Aspectos éticos

O presente estudo foi submetido ao Comitê de Ética em Pesquisa com Humanos do Instituto de Ciência e Tecnologia da Universidade Estadual Paulista (ICT-UNESP) por usar saliva que será coletada de voluntários. A realização da pesquisa foi aprovada através do parecer nº 1.734.862 (ANEXO A).

2 Preparo das soluções

Todas as soluções foram preparadas um dia antes do início de cada experimento. Todos os polímeros foram dissolvidos em água deionizada, exceto o polimetacrilato por ser solúvel apenas em solventes orgânicos (1 g de polimetacrilato dissolve em 9 ml de solvente). Assim, foi utilizado um protocolo baseado em um estudo anterior para solubilização de quitosana (Pini et al., 2016). Foi utilizado álcool etílico hidratado 92,8º para solubilização do polímero e em seguida adicionou-se água deionizada à solução até completar o volume final de 100 ml.

3 Padronização do pH das soluções

O pH das soluções utilizadas foi padronizado em 4,5 para eliminar um potencial fator de confusão na análise dos resultados. Para aumentar o pH foi utilizada solução de hidróxido de potássio (KOH) 0,1 M e para diminuir foi utilizada solução de ácido clorídrico (HCl) 0,1 M.

4 Mensuração da viscosidade dinâmica das soluções

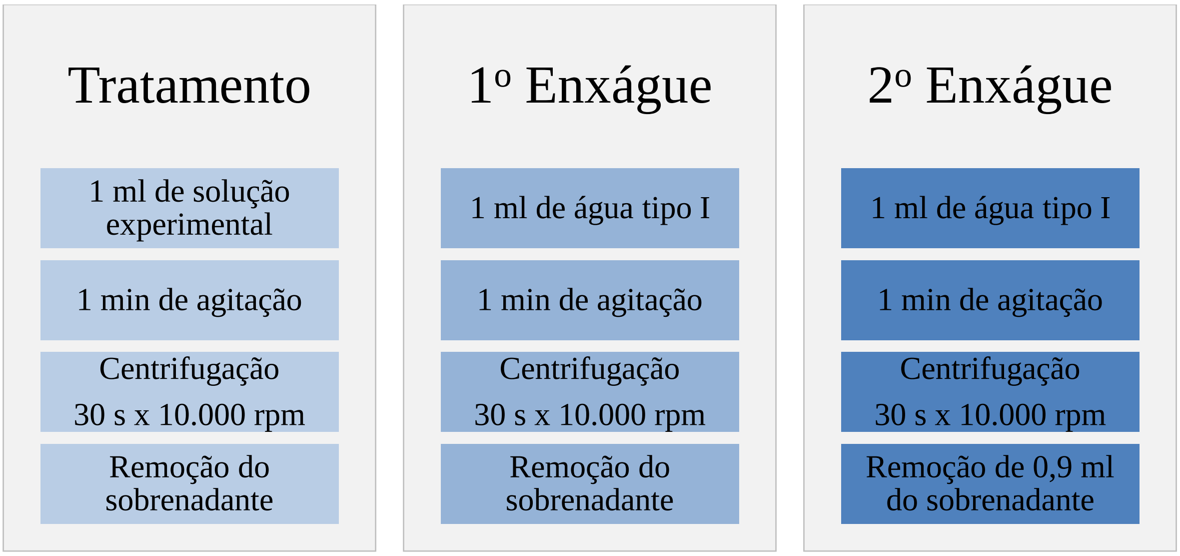
A viscosidade dinâmica das soluções foi determinada visando eliminar um potencial fator de confusão do efeito protetor das soluções (Sakae et al., 2018). Além de garantir uma medição adequada do potencial zeta, visto que este é diretamente influenciado pela viscosidade.

O teste foi conduzido no vibro viscosímetro (SV-10, A&D, Japão) do Departamento de Engenharia Química da Escola Politécnica da Universidade de São Paulo - USP, sob a supervisão da Profª Drª Idalina Vieira Aoki. A temperatura de medição foi padronizada em 25 °C.

5 Estudo de varredura através do teste de dissolução da hidroxiapatita

Foram pesados 25 mg de cristais de hidroxiapatita (Acros Organic, Geel, Bélgica) e colocados em tubos plásticos de 2 ml. Foi adicionado 1 ml de solução experimental a cada tubo utilizando-se uma pipeta automática. Os tubos foram agitados por 1 min e centrifugados por 30 s a 10.000 rpm. O sobrenadante foi removido e o primeiro enxágue foi realizado adicionando-se 1 ml de água deionizada aos tubos. Novamente eles foram agitados, centrifugados e o sobrenadante foi removido. Este procedimento de lavagem foi repetido mais uma vez, no entanto, no segundo enxágue apenas 0,9 ml do sobrenadante foi removido para permitir a desagregação da hidroxiapatita, como mostra a Figura 1 (Scaramucci et al., 2015).

Figura 1 – Pré-tratamento dos cristais de hidroxiapatita



Fonte: Elaborado pelo autor.

Os cristais de hidroxiapatita previamente preparados (25 mg) foram adicionados a 25 ml de solução padrão de ácido cítrico 0,3% (pH ajustado para 3,8 com solução de KOH) sob agitação magnética (100 rpm). Alíquotas de solução titulante (ácido clorídrico – HCl 0,1N) foram automaticamente adicionadas à velocidade de 28 µl/min, de modo que o pH da solução fosse constantemente mantido em 3,8 durante um tempo total de reação de 5 min. Para isto, foi utilizada uma tituladora automática (Hanna Instruments, Woonsocket, RI, EUA) (Figura 2).

Figura 1 – Tituladora automática

Fonte: Elaborado pelo autor.

Em seguida, o volume de HCl utilizado para manter o pH foi convertido na quantidade de hidroxiapatita dissolvida (mg), de acordo com a relação estequiométrica a seguir (Scaramucci et al., 2011):

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O,$$

* na qual 1 mol de hidroxiapatita corresponde a 8 mol de H^+

O teste foi realizado em triplicata para cada solução e a média foi considerada para o cálculo estatístico.

6 Mensuração do potencial zeta

Foram utilizados 25 mg de cristais de hidroxiapatita (Acros Organic, Geel, Bélgica), os quais foram pré-tratados com as soluções experimentais utilizando-se a mesma metodologia descrita no teste do pH-estacionário. Os cristais foram dispersos em 12,5 ml de tampão de fosfato dissódico/fosfato de potássio monossódico (pH = 7,0) (Neon Reagentes Analíticos, Suzano, SP, Brasil) e colocados em um banho ultrassônico durante 8 min. Em seguida, 2 ml desta suspensão foram colocados na cápsula do equipamento e examinados a 20 ^{o}C.

O potencial zeta da hidroxiapatita foi então obtido utilizando-se o aparelho Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK), equipado com um Laser de Hélio - Neônio de 633 nm e máximo de 4 mW (Figura 3). O espalhamento de luz eletroforética foi utilizado para se determinar a mobilidade da hidroxiapatita dispersa na solução tampão de fosfato a 0,04 mol/l e está foi convertida no potencial zeta utilizando-se a fórmula de Helmholtz – Smoluchowski.

Figura 3 – A) Equipamento Zetasizer, B) Cápsula do equipamento, C) Cápsula posicionada

Fonte: Elaborado pelo autor.

Cinco medidas foram realizadas para cada amostra (após um mínimo de 10 e máximo de 40 voltas) (Scaramucci et al., 2015). Os valores médios obtidos por cada amostra foram considerados para a análise estatística. A mensuração do potencial zeta foi conduzida no Departamento de Engenharia Química da Escola Politécnica da Universidade de São Paulo - USP, sob a supervisão da Profa. Idalina Vieira Aoki.

7 Avaliação do efeito anti-erosivo das soluções contendo um copolímero do polimetacrilato sobre o esmalte

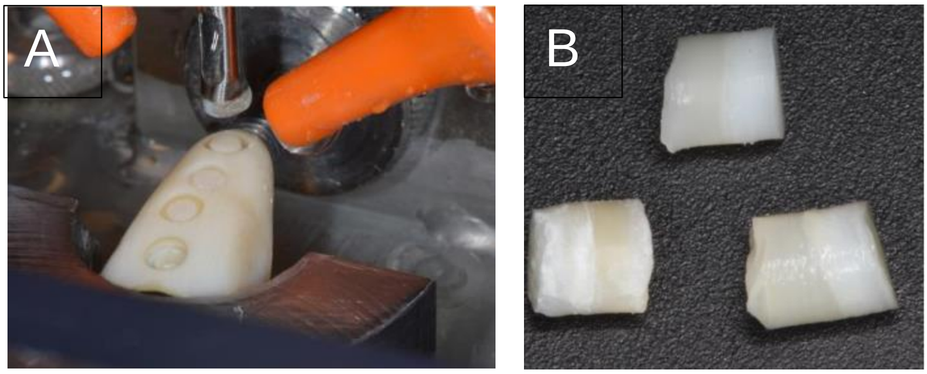
Como as soluções experimentais contendo o copolímero do polimetacrilato investigado apresentaram o maior potencial de reduzir a dissolução da hidroxiapatita, elas foram investigadas através de um modelo de ciclagem erosiva/remineralizadora na presença de película adquirida

7.1 Obtenção de amostras de esmalte bovino

Foram selecionados incisivos bovinos hígidos recém-extraídos de animais abatidos em frigorífico. Os dentes foram mantidos em solução de timol a 0,1%, com pH 7,0 até o preparo dos espécimes. Para separar a porção coronária da porção radicular, foi realizado um corte perpendicular ao longo eixo do dente na altura da junção cemento-esmalte, utilizando-se disco diamantado acoplado à peça reta.

As coroas foram fixadas à base de uma cortadora de amostras circulares com a face vestibular voltada para cima. Foram obtidas amostras cilíndricas de esmalte bovino utilizando-se uma broca diamantada trefina com 3 mm de diâmetro interno (Borges et al., 2012), como mostra a Figura 4.

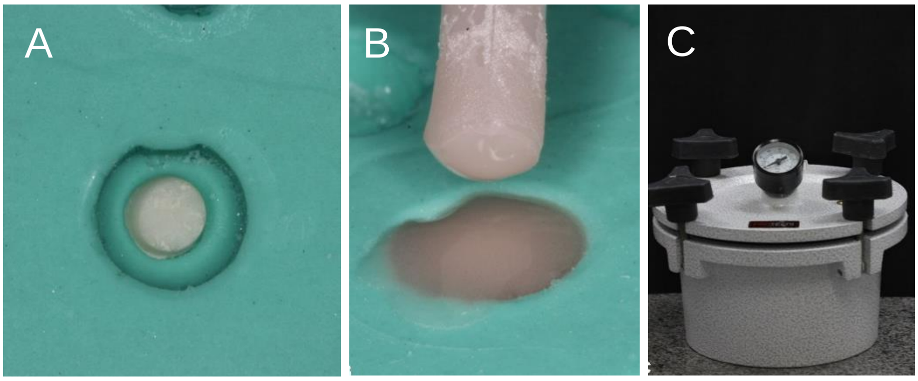
Figura 4 – Obtenção de amostras cilíndricas de esmalte bovino. A) Coroa bovina posicionada na cortadora circular. B) Amostras obtidas



Fonte: Elaborado pelo autor.

Obtidos os cilindros de esmalte estes foram embutidos em resina acrílica autopolimerizavél (JET- São Paulo, Brasil). Para essa etapa foi utilizada uma matriz de silicone com 6 mm de diâmetro e 3,1 mm de profundidade, sendo que no interior desta existia uma outra cavidade em segundo nível, com 3 mm de diâmetro e 0,1 mm de profundidade, onde o espécime de dentina foi posicionado com sua porção externa voltada para o interior da matriz. A matriz também apresentava uma concavidade em um dos lados, para que o espécime seja posicionado adequadamente no momento das leituras de perfil. Após a inserção da resina acrílica os espécimes foram levados a uma polimerizadora de pressão, onde seguindo as normas do fabricante foi preenchida com a água até o nível estabelecido e mantida em pressão por 10 minutos, em seguida foi despressurizada e aguardou-se 5 minutos antes de abri-la (Figura 5).

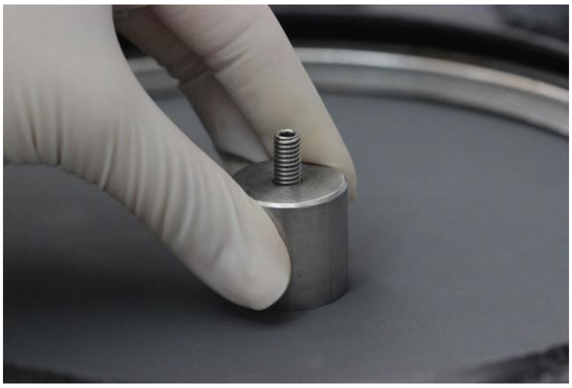
Figura 5 – A) Amostra de dentina sendo posicionada na matriz de silicone; B) Inserção da resina acrílica com pipeta Pasteur; C) Polimerizadora de pressão.



Fonte: Elaborado pelo autor

Após a completa polimerização dos espécimes, as suas bases foram aplainadas utilizando-se uma lixa de carbeto de silício de granulação P600 acoplada à politriz circular motorizada (Polipan-U, Panambra Zwick, São Bernardo do Campo, SP, Brasil) sob constante irrigação. Um dispositivo de metal para segurar os espécimes. Em seguida, foi realizado o polimento da superfície dos espécimes que tinha a amostra de esmalte utilizando-se lixas de carbeto de silício de granulação 1200, 2400 e 4000 (Extec, Enfield, CT, EUA) durante 30, 50 e 120 segundos respectivamente, seguindo-se as mesmas especificações previamente descritas (Figura 6).

Figura 6 – Dispositivo de metal utilizado no polimento das amostras posicionado sobre a politriz circular

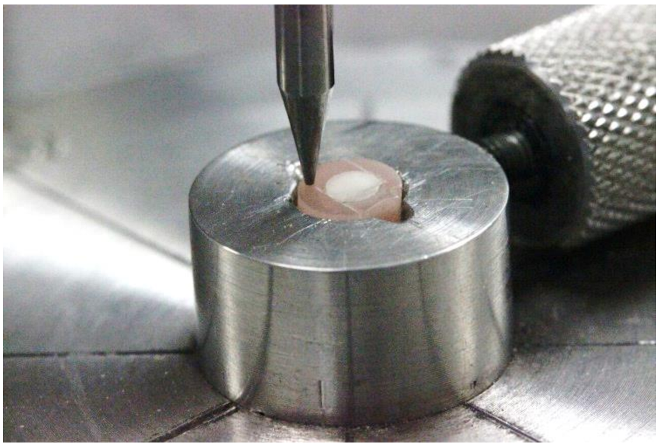


Fonte: Elaborado pelo autor

Para remover quaisquer resíduos deixados após o polimento com cada lixa, os espécimes foram colocados em um aparelho de ultrassom (Ultrasonic Cleaner, Odontobrás, Ribeirão Preto, SP, Brasil) contendo água deionizada durante 10 minutos.

Posteriormente, foram realizados dois riscos paralelos na superfície dos espécimes sobre a resina acrílica e lateralmente à superfície de esmalte, utilizando dispositivo projetado em uma pequena mesa de coordenadas XY, com uma base giratória, contendo uma ponta de aço (Figura 7). Estas marcações paralelas serviram como guias para a sobreposição dos perfis obtidos na análise perfilométrica.

Figura 7 – Confecção dos riscos paralelos utilizados como guias para a análise perfilométrica



Fonte: Elaborado pelo autor

Os espécimes foram mantidos em ambiente com umidade relativa 100% até o momento do uso. As amostras foram randomizadas com base na microdureza inicial (KHN1) que será descrita a seguir e aleatoriamente divididas em seis grupos (n = 13), de acordo com a solução experimental avaliada:

- Água deionizada;
- F – solução de fluoreto de sódio (225 ppm F^-);

- FS – solução de fluoreto de sódio (225 ppm F^-) + cloreto de estanho (225 ppm F^- + 800ppm Sn^{2+});
- PMA – solução contendo o copolímero do polimetacrilato investigado
- PMA + F
- PMA + FS

7.2 Coleta de saliva humana

Para coleta de saliva humana, foram selecionados 20 voluntários de ambos os gêneros, maiores de 18 anos, saudáveis, livres de cáries, doença periodontal e sem histórico de xerostomia. Todos os indivíduos foram esclarecidos quanto à proposta da pesquisa, e assinaram um Termo de Consentimento Livre e Esclarecido (APÊNDICE A).

Antes da coleta, os voluntários foram analisados quanto ao fluxo salivar estimulado que deveria ser maior ou igual a 1 ml/min e quanto fluxo salivar não estimulado que deveria ser maior ou igual a 0,2 ml/min (Bardow et al., 2001). A técnica de coleta para determinação dos fluxos foi a mesma descrita em Scaramucci et al. 2012. Os voluntários que se encaixaram nesses parâmetros receberam instruções para se alimentarem normalmente pela manhã e realizarem escovação dental com dentifrício sem flúor (Cocoricó, Bitufo, São Paulo, Brasil) antes da coleta, que ocorreu duas horas após o café da manhã.

Foi realizada estimulação do fluxo salivar com mastigação de Parafilm® M (Bemis Company, Inc, Neenah, Wisconsin, EUA). A saliva coletada foi armazenada em tubos Falcon resfriados. Na sequência, esta foi filtrada, centrifugada (10000 rpm/4ºC por 5 min) e permaneceu congelada (-80ºC) até o momento de uso (Masson et al., 2013).

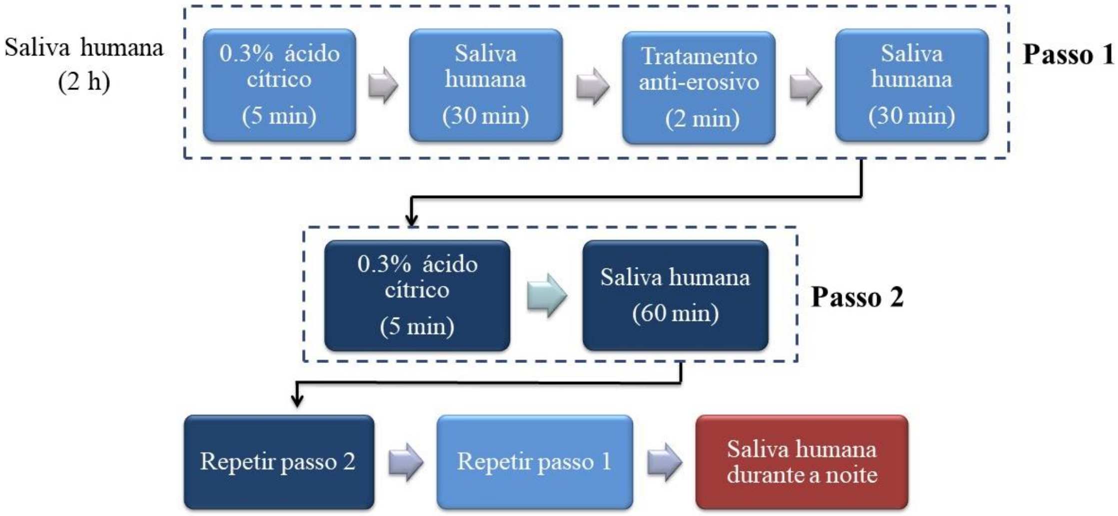
Para formação da película adquirida, a saliva foi descongelada à temperatura ambiente. Antes do início de cada ciclo diário, os espécimes ficaram imersos durante 2h em 3 ml de saliva sob agitação constante (Cheaib, Lussi, 2011). Em seguida, os espécimes foram lavados com água ultra-purificada para remoção das proteínas não adsorvidas.

7.3 Ciclagem erosiva/remineralizadora

A ciclagem foi realizada durante cinco dias, nos quais foram realizados quatro episódios erosivos por dia. Os espécimes foram imersos durante 5 minutos em solução de ácido cítrico a 0,3% (pH natural = 2,6), lavados com água deionizada por 20 s e estocados por 60 min em saliva humana

A exposição às soluções anti-erosivas foi realizada 30 min após o primeiro e o último episódio erosivo do dia, durante 2 min (Figura 8). Após a exposição, os espécimes não foram enxaguados, apenas reimersos na saliva humana por mais 30 min. Após a realização dos ciclos diários, os espécimes foram estocados em saliva humana até o dia seguinte. O experimento foi conduzido à temperatura ambiente.

Figura 8 – Ilustração representando a ciclagem erosiva durante um dia



Fonte: Elaborado pelo autor.

7.4 Ensaio de microdureza

O ensaio de microdureza objetivou avaliar os efeitos dos episódios erosivos e dos tratamentos sobre o esmalte (Lussi et al., 1993) e neste estudo foi realizado em quatro momentos:

I (inicial): Antes da ciclagem dos espécimes, a fim de se obter a microdureza inicial do esmalte sadio para realizar a randomização dos grupos.

E1: Após a primeira imersão em solução ácida (5 min) para se obter o valor de microdureza após desmineralização inicial.

T: Após o primeiro tratamento e imersão em saliva, para se avaliar o reendurecimento do esmalte.

E2: Após o segundo desafio erosivo do primeiro dia, para se avaliar o potencial efeito protetor das soluções testadas.

Os valores de microdureza foram obtidos com indentador Knoop montado em microdurômetro (FM-700, Future-Tech, Tókio, Japão), com aplicação de carga de 50 g durante 10 s (Borges et al., 2012). Foram realizadas três indentações na parte inferior dos espécimes (de forma a não interferir com a leitura do perfil), com distância de 100 μm entre elas. Foi utilizada a média dos valores obtidos nas três indentações. Os espécimes que apresentaram os valores de microdureza inicial discrepantes em 10% foram descartados e substituídos.

Com base nos valores de microdureza do esmalte obtidos, foi realizado o cálculo do porcentual de remineralização (%Re), utilizando-se a equação a seguir:

$$\%Re = \left(\frac{E1 - T}{E1}\right) * 100$$

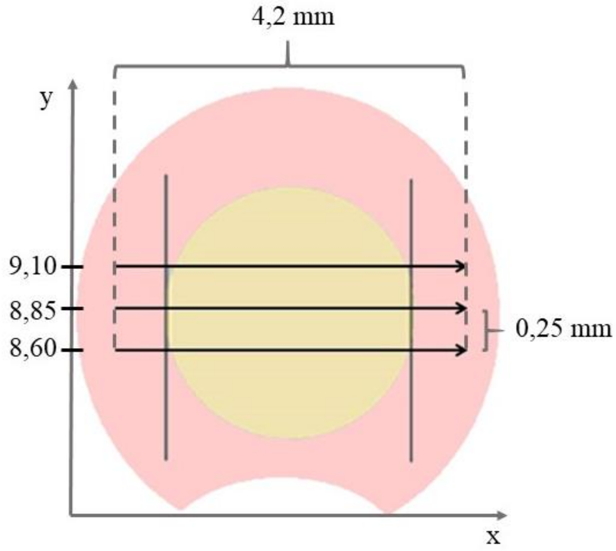
Também foi realizado o cálculo do porcentual de proteção (%Prot), utilizando-se equação a seguir:

$$\%Prot = \left(\frac{E1 - E2}{E1}\right) * 100$$

7.5 Mensuração da perda superficial

Para cada espécime foram obtidos três perfis, cada um referente a uma coordenada de medição (8,60; 8,85 e 9,10). Os perfis foram obtidos realizandose três varreduras com extensão de 4,2 mm e 0,25 mm de distância entre elas. Após a ciclagem, foram obtidos três perfis nos mesmos locais das medidas iniciais (Figura 9).

Figura 92 – Imagem representativa da região dos perfis



Fonte: Elaborado pelo autor.

Para a mensuração da quantidade de perda superficial em cada espécime os perfis iniciais e finais foram sobrepostos utilizando-se o Software Mahr Surf XCR 20 4.50-07 SP3 e os riscos pré-realizados como guias.

7.6 Mensuração do ângulo de contato - goniometria

Foram selecionados incisivos bovinos hígidos recém-extraídos de animais abatidos em frigorífico. Os dentes foram mantidos em solução de timol a 0,1%, com pH 7,0 até o preparo dos espécimes. As faces vestibulares coronárias foram desgastadas com lixa de carbeto de silício de granulação 120 (Extec Corp, CT, EUA), acoplada à politriz circular (DP-10, Panambra, São Paulo, SP, BR) sob refrigeração constante com água, até a exposição de uma área de aproximadamente 6 mm^2 de esmalte. Foi realizado um corte perpendicular ao longo eixo do dente a distância de 2 mm da junção cemento-esmalte, utilizando-se um disco diamantado perfurado (American Burrs, Porto Alegre, RS, Brasil) acoplado à peça reta. A face vestibular previamente desgastada de cada dente bovino foi colocada voltada para baixo em um molde de silicone e foi vertida resina acrílica incolor ativada quimicamente (Jet-Clássico, São Paulo, SP, Brasil). Após o término da polimerização, a face de esmalte foi lixada durante 1 minuto com lixas de carbeto de silício (Extec Corp, CT, EUA) de granulação P1200, P2400 e P4000 por 30, 60 e 120 s respectivamente utilizando-se um dispositivo planificador acoplado à politriz circular. A cada troca de lixa os espécimes receberam um banho em cuba ultrassônica contendo 200 ml de água deionizada para se remover os grãos abrasivos e detritos de esmalte.

Para permitir a estratificação dos espécimes em grupos homogêneos, foi realizada a análise da rugosidade inicial das amostras. Os perfis de cada espécime foram obtidos utilizando um perfilômetro de contato (MarSurf XT 20, Mahr-Goettingen, Alemanha). A ponta do aparelho varreu uma distância de 4,2 mm. Três medidas de perfil foram realizadas para cada amostra, com uma distância de 0,25 mm entre elas. Os valores de rugosidade foram obtidos a partir destes perfis. O valor da rugosidade (Ra) de cada espécime foi determinado com um *cut-off* de 0,8 mm e um comprimento transversal de 0,25 mm. A média dos valores de Ra dos três perfis foi calculada e utilizada para a estratificação em grupos homogêneos.

Para a realização da goniometria foi utilizada a técnica da gota séssil, a qual prevê a deposição de um líquido purificado sobre a superfície de um sólido (esmalte) com o auxílio de uma microseringa. Para obtenção do ângulo de contato inicial, as amostras ficaram imersas durante 2 min em água ultrapurificada (tipo I) para hidratação e em seguida foram levemente secas com papel absorvente. As amostras foram então colocadas na base do equipamento e a angulação foi ajustada de modo que a agulha da microseringa e a superfície do esmalte formassem um ângulo de 90º. Foi aplicada uma gota de 0,5 µl de água tipo I sobre a superfície do esmalte e foram realizadas leituras com tempo total de 30 s, com pausa inicial de 10 s, sendo obtidas 20 imagens por segundo. A captação das imagens e o processamento dos ângulos de contato foi realizado por um software (One Attension, Biolin Scientific, Estocolmo, Suécia), sendo considerada a média obtida pelos ângulos direito e esquerdo. Cada amostra foi medida uma única vez.

Para obtenção do ângulo de contato final, as amostras foram imersas nas respectivas soluções anti-erosivas durante 2 min sob leve agitação horizontal (Agitador de Kline, TS2000A, Biomixer). Em seguida, foram realizados os procedimentos de secagem e mensuração do ângulo de contato como descrito previamente.

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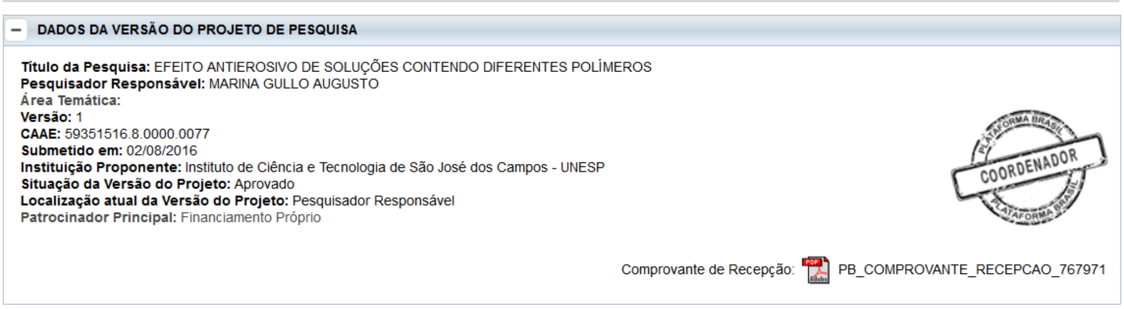
ANEXO A – Comprovante de aprovação no Comitê de Ética em Pesquisa com Humanos do Instituto de Ciência e Tecnologia da Universidade Estadual Paulista (ICT-UNESP)



DADOS DA VERSÃO DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO ANTIEROSIVO DE SOLUÇÕES CONTENDO DIFERENTES POLÍMEROS
Pesquisador Responsável: MARINA GULLO AUGUSTO
Área Temática:
Versão: 1
CAAE: 59351516.8.0000.0077
Submetido em: 02/08/2016
Instituição Proponente: Instituto de Ciência e Tecnologia de São José dos Campos - UNESP
Situação da Versão do Projeto: Aprovado
Localização atual da Versão do Projeto: Pesquisador Responsável
Patrocinador Principal: Financiamento Próprio

Comprovante de Recepção: PB_COMPROVANTE_RECEPCAO_767971

ANEXO B – Comprovante do Termo de Cessão de Direitos sobre Propriedade Intelectual à UNESP

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Reitoria

TERMO DE CESSÃO DE DIREITOS SOBRE PROPRIEDADE INTELECTUAL

Cedentes: 1. ALESSANDRA BÜHLER BORGES, brasileira, casada, professora universitária, inscrita no CPF/MF sob o nº 028.998.827-67, portadora do documento de identidade RG nº 09234455-5, SSP/SP, residente em São José dos Campos (SP), na Av. São João, 500, apto 174A, Jardim Esplanada, CEP 12.242.840; **2. MARINA GULLO AUGUSTO**, brasileira, solteira, cirurgiã-dentista, inscrita no CPF/MF sob o nº 383.389.718-50, portadora do documento de identidade RG nº 44968490-8, SSP/SP, residente em São José dos Campos (SP), na Rua Ruivo, nº 143, apto 203, Jardim Aquarius, CEP 12246-130. **3. CARLOS ROCHA GOMES TORRES**, brasileiro, casado, professor universitário, inscrito no CPF/MF sob o nº 185.793.408-37, portador do documento de identidade RG nº 54196, SSP/SP, residente em Jacarei (SP), na Rua Santa Cruz, 113, apto 166, Jardim Califórnia, CEP 12305-600. **4. TAÍS SCARAMUCCI FORLIN**, brasileira, casada, professora universitária, inscrita no CPF/MF sob o nº 301.153.988-05, portadora do documento de identidade RG nº 32.615.965-4, SSP/SP, residente em São Paulo (SP), na Av. Armando Ferrentini 320, apto 82, Aclimação, CEP 04103-030.

Cessionária: UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" - UNESP, autarquia estadual de regime especial, criada pela Lei nº 952 de 30.01.1976, devidamente inscrita no CNPJ/MF sob o nº 48.031.918/0001-24, com sede na Rua Quirino de Andrade, 215, Centro, São Paulo (SP), CEP 01.049-010.

Pelo presente instrumento, nesta e na melhor forma de direito, os Cedentes autorizam a Cessionária a depositar o pedido de patente intitulado **"EFEITO ANTIEROSIVO DE SOLUÇÕES CONTENDO UM COPOLÍMERO DO POLIMETACRILATO"** junto ao Instituto Nacional da Propriedade Industrial, cedendo todos os direitos patrimoniais a ele relativos na forma e para os fins do disposto na Lei 9.279 de 14.05.1996 e Lei 8.666 de 21.06.1993, Artigo 111, a título gratuito, sem qualquer restrição quanto à forma, tempo ou lugar, desde já ficando autorizadas quaisquer alterações que venham a ser consubstanciadas em futuras atualizações, modificações ou derivações tecnológicas.

Por ser a expressão da verdade, este documento é firmado na presença de duas testemunhas que também o assinam.

São Paulo, 29 de MARÇO de 2018.

Cedentes:

ALESSANDRA BÜHLER BORGES

MARINA GULLO AUGUSTO

CARLOS ROCHA GOMES TORRES

Tais Scaramucci
TAÍS SCARAMUCCI FORLIN

Cessionária:

UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" – UNESP

Testemunhas:

1. Keyla Santos Bento
CPF/MF: 323.669.268-55

2. Sabrina Paduan
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