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Mariana Emi Nagata

EFEITO DE GÉIS FLUORETADOS SUPLEMENTADOS COM NANOPARTÍCULAS DE TRIMETAFOSFATO DE SÓDIO SOBRE A REMINERALIZAÇÃO DE LESÕES DE CÁRIE E SOBRE O DESGASTE DENTAL EROSIVO IN VITRO

ARAÇATUBA - SP

2018

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Tese apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para a obtenção do título de Doutor em Ciência Odontológica – Área Saúde Bucal da Criança.

Orientador: Prof. Dr. Juliano Pelim Pessan
Coorientador: Prof. Dr. Alberto Carlos Botazzo Delbem
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Mariana Emi Nagata

Dedicatória

Dedicatória

Dedico este trabalho

Aos meus pais, Hiromitsu e Clara

Pelo apoio em todos os momentos difíceis, pelo exemplo de dedicação e perseverança e pelos constantes incentivos para que me tornasse uma pessoa melhor. Por me ensinarem com o mais sincero e verdadeiro amor a importância da honestidade e humildade. Meu amor e admiração por vocês são infinitos!

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Agradecimentos especiais

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Pela minha vida, saúde, pelos meus pais, pelos meus irmãos, pelo meu sobrinho, pelas pessoas maravilhosas que me destinou conhecer e por me iluminar nas decisões e momentos mais difíceis. Sua presença em minha vida me conferiu segurança e amparo para que eu seguisse pelos melhores caminhos.

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"Há muros que só a paciência derruba. E há pontes que só o carinho constrói"

Cora Coralina

Mariana Emi Nagata

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Agradecimentos

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"Nada do que vivemos tem sentindo, se não tocarmos o coração das pessoas."

Cora Coralina

Mariana Emi Nagata

Epígrafe

"Mesmo quando tudo parece desabar, cabe a mim decidir entre rir ou chorar, ir ou ficar, desistir ou lutar; porque descobri, no caminho incerto da vida, que o mais importante é o decidir"

Cora Coralina

Mariana Emi Nagata

Resumo

NAGATA, M.E **Efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio sobre a remineralização de lesões de cárie e sobre o desgaste dental erosivo *in vitro.*** 2018. 106 f. Tese (Doutorado em Ciência Odontológica, área de Saúde Bucal da Criança) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2018.

O presente estudo avaliou o efeito de géis fluoretados suplementados com Trimetafosfato de Sódio (TMP) sobre a remineralização de lesões de cárie artificial e sobre o desgaste erosivo do esmalte dental bovino *in vitro*. Para o 1º capítulo, blocos de esmalte (*n*=168) com lesões de cárie artificiais foram analisados por dureza de superfície (DS) e aleatoriamente divididos em 7 grupos (*n*=24/grupo), de acordo com os géis testados: Placebo (sem F/TMP), 4500 μg F/g (4500F), 9000 μg F/g (9000F), 4500F+2,5% TMP nanoparticulado (2,5% Nano), 4500F+5% TMP nanoparticulado (Nano 5%), 4500F+5% TMP microparticulado (Micro 5%) e 12300 μg F/g (Acidulado). Os blocos foram tratados uma única vez com os géis (1 minuto) previamente à ciclagem de pH (6 dias). Em seguida, foram determinadas a porcentagem de recuperação de DS (%RDS), a área integrada da lesão de subsuperfície (ΔKHN) e o conteúdo de flúor fortemente-ligado (F), CaF_2, cálcio (Ca) e fósforo (Pi) formado (após a aplicação dos géis) e retido no esmalte (após a ciclagem de pH). Os dados foram submetidos a ANOVA e teste de Student-Newman-Keuls ($p<0,05$). Os grupos 2,5% Nano e 5% Micro alcançaram %RDS semelhante aos géis 9000F e Acidulado. Para ΔKHN, os maiores valores foram observados para os grupos Placebo e 5% Nano, e os menores, para 2,5% Nano, 5% Micro, 9000F e Acidulado. Todos os grupos tiveram valores semelhantes de CaF_2 retido, exceto Placebo e Acidulado. Um aumento nas concentrações de Ca foi observado para os grupos com TMP nanoparticulado. Em relação ao Pi formado e retido, os grupos com TMP foram semelhantes ao 9000F e ao Acidulado. No 2º capítulo, blocos de esmalte (*n*=140) foram aleatoriamente distribuídos em 7 grupos, utilizando os mesmos géis e modo de aplicação descritos no 1º capítulo. Metade da superfície dos blocos foi protegida com esmalte ácido-resistente (área controle), expondo a outra metade ao tratamento com os géis e ao desafio erosivo (ERO) ou erosivo+abrasivo (ERO+ABR). Após a aplicação dos géis, todos os blocos (n=20/grupo) foram submetidos a ERO (imersão em ácido cítrico 0,05 M, pH 3,2, 90 segundos, 4 vezes/dia, 5 dias, sob agitação), enquanto metade dos blocos (n=10/grupo) foi adicionalmente submetida a escovação (15 segundos) após cada desafio erosivo (ERO+ABR). Os blocos foram analisados por perfilometria e dureza em secção longitudinal (perda da dureza em profundidade - ΔKHN). Os dados foram submetidos a ANOVA e teste de Fisher ($p<0,05$). Para ERO, o desgaste do esmalte associado a 2,5% Nano, 5% Nano e Acidulado foi significantemente menor que 4500F, enquanto que para ERO+ABR o menor desgaste de esmalte foi observado para 5% Nano. Entre os géis com TMP, os menores valores de ΔKHN foram observados para 2,5% Nano para ERO. Os resultados permitem concluir que a adição de TMP nanoparticulado a 2,5% ou TMP microparticulado a 5% ao gel 4500F aumentou significativamente a remineralização de lesões artificiais de cárie *in vitro*. Quanto ao efeito sobre o desgaste dental erosivo, a adição de 5% de TMP nanoparticulado ao gel 4500F produziu efeitos protetores superiores quando comparado ao TMP microparticulado.

Palavras–chave: Esmalte dentário, Flúor, Fosfatos, Desmineralização do dente, Erosão dentária.

Abstract

NAGATA, M.E **Effect of fluoride gels supplemented with nano-sized Sodium Trimetaphosphate on the remineralization of caries lesions and erosive dental wear *in vitro*.** 2018. 106 f. Tese (Doutorado em Ciência Odontológica, área de Saúde Bucal da Criança) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2018.

The present study evaluated the effect of fluoride gels supplemented with sodium trimetaphosphate (TMP) on the remineralization of caries-like lesions and on erosive wear of bovine enamel *in vitro*. For the first chapter, enamel blocks (n=168) with caries-like lesions were evaluated by surface hardness (SH), and randomly divided into 7 groups (n=24/group), according to the tested gels: (a) Placebo (no F/TMP), 4,500 μg F/g (4500F), 9,000 μg F/g (9000F), 4,500F+2.5% nano-sized TMP (2.5% Nano), 4,500F+5% nano-sized TMP (5% Nano), 4,500F+5% micrometric TMP (5% Micro) and 12,300 μg F/g (Acid gel). Gels were applied on the blocks only once (1 minute) with the gels prior to the pH-cycling regimen (6 days). Following, the percentage of SH recovery (%SHR), integrated subsurface hardness area (ΔKHN), and firmly-bound fluoride (F), CaF_2, calcium (Ca) and phosphorus (Pi) formed (after gels application) and retained (after pH cycling) in/on enamel were determined. Data were submitted to ANOVA and Student-Newman-Keuls test ($p<0.05$). The 2.5% Nano and 5% Micro groups reached %SHR similar to the 9000F and acid gel. For ΔKHN, the highest values were observed for the Placebo and Nano 5% groups, and the lowest, for 2.5% Nano, Micro 5%, 9000F and Acid gel. All groups had similar values of CaF_2 retained on enamel, except Placebo and Acid gel. An increase in Ca concentrations was observed for the groups treated with nano-sized TMP. Regarding Pi formed and retained, groups treated with TMP were similar to 9000F and Acid gels. In the second chapter, the enamel blocks (n=140) were randomly divided in 7 groups, using the same gels and mode of application described in the first chapter. Half of the blocks' surface was protected with acid-resistant varnish (control area), exposing the other half to the treatment with gels and to erosive (ERO) or erosive+abrasive (ERO+ABR) challenges. After treatment with the gels, all blocks (n=20/group) were submitted to ERO (immersion in 0.05 M citric acid, pH 3.2, 90 seconds, 4 times/day, 5 days, under agitation), while half of the blocks (n=10/group) was additionally subjected to brushing (15 seconds) after each erosive challenge (ERO+ABR). The blocks were evaluated by profilometry and cross-sectional hardness (integrated hardness loss in depth - ΔKHN). Data were submitted to ANOVA and Fisher's test ($p<0.05$). For ERO, enamel wear associated with 2.5% Nano, 5% Nano and Acid gels was significantly lower than 4500F, whereas for ERO+ABR the lowest enamel wear was observed at 5% Nano. Among the TMP gels, the lowest ΔKHN values were observed at 2.5% Nano under ERO conditions. The results allow to conclude that the addition of 2.5% nano-sized TMP or 5% micrometric TMP to the 4500F gel significantly increased the remineralization of artificial caries lesions in vitro. As for the effect on erosive tooth wear, the addition of 5% nano-sized TMP to the 4500F gel produced superior protective effects when compared to micrometric TMP.

Key-words: Dental enamel, Fluoride, Phosphates, Tooth demineralization, Dental erosion

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Lista de abreviaturas e símbolos

LISTA DE ABREVIATURAS E SÍMBOLOS

ANOVA	Análise de Variância
Ca	Cálcio/Calcium
CaF_2	Fluoreto de cálcio
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico/National Council for Scientific and Technological Development
°C	Graus Celsius
DS	Dureza de superfície
DP	Desvio padrão
ERO	Erosão
ERO+ABR	Erosão+Abrasão
F	Fluoreto
FA	Firmly-bound F/ F fortemente ligado
g	Grama
h	Hora (s)
HCl	Ácido Clorídrico
H_2O	Água
Kgf/mm^2	Kilograma força por milímetro quadrado
KHN	Knoop Hardness Number/Número de Dureza Knoop
L	Liter/Litro
Log_{10}	Logaritmo na base 10
mL	Mililitro
M	Molar
mm	Milímetro
Min.	Minuto
Mg	Miligrama
mV	Milivoltagem/milivolt
NaOH	Hidróxido de Sódio
Nm	Nanômetro
µg	Micrograma
µg/g	Micrograma por grama
µg F/g	Micrograma de fluoreto por grama
µg/mL	Micrograma por mililitro
µL	Microlitro

p	Probabilidade
pH	Potencial Hidrogeniônico
Pi	Fósforo inorgânico
ppm	Parte por milhão
Re>Des	Remineralização maior Desmineralização
SD	Standard Deviation
SH	Surface hardness
%SHR	Percentage of Surfasse Hardness Recovery
TISAB	Total Ionic Strenght Adjustment Buffer/Tampão de Ajuste da Força Iônica Total
TMP	Sodium Trimetaphosphate/Trimetafosfato de sódio
TMPmicro	Trimetafosfato de sódio microparticulado
TMPnano	Trimetafosfato de sódio nanoparticulado
UNESP	Universidade Estadual Paulista/São Paulo State University
ΔKHN	Integrated loss of subsurface hardness/ Perda dureza integrada de subsuperfície/ Integrated hardness loss in depth/ Perda integrada da dureza em profundidade

SUMÁRIO

Introdução geral

INTRODUÇÃO GERAL

As alterações na saúde bucal repercutem no bem-estar e na qualidade de vida das crianças (Abanto et al., 2017; Martins et al., 2017), razão pela qual alternativas para se eliminar ou diminuir a prevalência e severidade das enfermidades bucais tem sido intensivamente estudadas. Entretanto, mesmo com os avanços odontológicos e estratégias preventivas, a cárie dentária ainda continua sendo um importante problema de saúde pública em nível mundial (Kassebaum et al., 2015). Na maioria dos países, a distribuição e severidade desta doença dependem de fatores socioeconômicos e ambientais, o que coloca em maior risco os indivíduos e grupos populacionais mais vulneráreis (Retnakumari & Cyriac et al., 2012).

A cárie dentária é uma doença causada pelo desequilíbrio no processo dinâmico de desmineralização e remineralização do esmalte dentário, resultante do metabolismo microbiano sobre a superfície dentária, levando a alterações no pH que podem levar a uma perda mineral ao longo do tempo e, subsequentemente, à formação de cavidade (Kid, 2011). Embora nas últimas décadas tenha sido observada uma redução significativa na prevalência desta doença em vários países (Lagerweij & Loveren, 2015), a cárie não tratada em dentes permanentes é mundialmente considerada a condição bucal mais prevalente e afeta 2,4 bilhões de pessoas. Em dentes decíduos, é a décima condição de saúde mais prevalente, afetando 621 milhões de crianças em todo o mundo (Kassebaum et al., 2015). No Brasil, dados do Projeto de Saúde Bucal 2010 mostraram que 56,5% das crianças aos 12 anos de idade têm pelo menos um dente permanente com experiência de cárie, o que representa, aproximadamente, 1,7 milhões de crianças (Freire et al., 2013).

Paralelamente, em função de mudanças nos hábitos alimentares, comportamentais, e nas práticas de higiene, outras alterações dentárias têm se destacado nos últimos anos, cuja etiologia não depende diretamente de microrganismos e da condição de higiene bucal do paciente, sendo denominadas de lesões não cariosas. Uma delas, a erosão dentária, é definida pela dissolução da estrutura dentária provocada pela ação de ácidos, por meio de um processo químico sem envolvimento bacteriano (West & Joiner, 2014). O processo de erosão ocorre quando a fase aquosa ao redor do esmalte está subsaturada com relação ao mineral do dente. Portanto, a erosão resulta de uma exposição contínua à um meio ácido, o que reduz a microdureza da superfície remanescente, tornando-a mais propensa aos impactos mecânicos (Wiegand & Attin, 2003; Lussi et al., 2011). A etiologia da erosão é multifatorial, o que torna esta condição ainda mais complexa. Dentre os fatores extrínsecos, destacam-se aspectos relacionados ao estilo de vida contemporâneo das crianças, como hábitos alimentares (ingestão de alimentos e bebidas ácidas) e medidas de higiene bucal. Além destes, fatores intrínsecos, como distúrbios alimentares (bulimia, problemas gastroesofágicos) também são capazes de interferir na severidade do desgaste erosivo (Zero & Lussi, 2006; Lussi & Carvalho, 2014; Lussi & Helwig et al., 2014). A prevalência em crianças e adolescentes brasileiros varia de 20% a 51% (Gurgel et al., 2011; Vargas-Ferreira et al., 2011; Murakami et al., 2015; Salas et al., 2017), sendo os incisivos superiores os dentes mais afetados e a maioria das lesões, restritas ao esmalte (Aguiar et al., 2014).

Os produtos fluoretados, principalmente os de aplicação profissional, têm sido amplamente utilizados para a prevenção e tratamento da cárie dentária, devido à formação de precipitados de CaF_2 na superfície do esmalte, os quais atuam como reservatórios de liberação lenta de flúor (Buzalaf et al., 2011). Por outro lado, a ação do F para prevenir ou mesmo minimizar o efeito da erosão dentária tem sido muito questionada. Na erosão, a desmineralização ocorre principalmente nas camadas mais superficiais do esmalte, enquanto que o processo relacionado à cárie dentária envolve desmineralização na subsuperfície do esmalte, com a camada mais externa intacta. Quando a erosão dentária ocorre, a superfície amolecida é facilmente removida por fatores mecânicos, como a abrasão resultante da escovação, não havendo tempo suficiente para o flúor atuar por meio da remineralização. Desta forma, o efeito predominante de produtos fluoretados frente a um desafio erosivo é a proteção da superfície contra a perda mineral ao invés da sua remineralização (Magalhães et al., 2011).

Dentre os veículos de aplicação profissional, merecem destaque os géis fluoretados, devido ao baixo custo, facilidade operacional e aceitabilidade por parte dos pacientes (Gao et al., 2016). A aplicação de géis fluoretados normalmente é feita com pincéis, sob isolamento absoluto, ou utilizando moldeiras, as quais devem ser mantidas na boca da criança durante 2 a 10 minutos (Marinho et al., 2015). Durante esse procedimento, é grande o risco de ingestão e consequente toxicidade aguda, principalmente por crianças pequenas, razão pela qual o uso de géis F não é geralmente recomendado para crianças menores de seis anos (Weyant et al., 2013; Marinho et al., 2015).

Considerando os amplos benefícios da aplicação profissional de flúor no tratamento e prevenção da cárie e da erosão dentária, a busca por estratégias que visem aumentar a eficácia clínica desses produtos sem aumentar os efeitos adversos em crianças pequenas tem se intensificado nos últimos anos (Pancote et al., 2014; Danelon et al., 2014). O uso de fosfatos inorgânicos tem sido uma estratégia potencial quando adicionado a produtos fluoretados de uso caseiro e aplicação profissional, havendo forte evidência a partir de estudos *in vitro* e *in situ* sobre o efeito de produtos suplementados com Trimetafosfato de Sódio (TMP) nos processos de des- e re-mineralização do esmalte, bem como na redução do desgaste dental erosivo (Moretto et al., 2013; Danelon et al., 2014; Manarelli et al., 2015). Com relação a géis fluoretados, uma série de estudos demonstrou que a adição de TMP a um gel com concentração reduzida de F (4500 ppm F) aumentou significativamente os efeitos preventivos e terapêuticos deste em comparação a um gel de mesma concentração de F, sem TMP, atingindo valores semelhantes aos obtidos para géis contendo 9000 ppm F (neutro) e 12.300 ppm F (acidulado), em modelos *in vitro* e *in situ* para a cárie e erosão dental (Danelon et al., 2013, 2014; Pancote et al., 2013; Akabane et al., 2018). O TMP se adsorve à superfície do esmalte, agindo como uma barreira à difusão ácida, reduzindo as trocas minerais entre o meio e o esmalte, além de facilitar a difusão dos íons cálcio e fósforo para o interior do esmalte (Moretto et al., 2013; Manarelli et al., 2014; Takeshita et al., 2016).

Na busca por aumentar ainda mais o sinergismo entre o F e o TMP, estudos recentes avaliaram o uso de TMP nanoparticulado adicionado a um dentifrício convencional (1100 ppm F) o qual reduziu significativamente a desmineralização do esmalte quando comparado ao dentifrício suplementado com o TMP micrométrico (Danelon et al., 2015). Além disso, este mesmo dentifrício (1100 ppm F + 3% TMP nanoparticulado) promoveu um efeito protetor contra o desgaste erosivo do esmalte dentário de forma semelhante ao observado para um dentifrício contendo 5000 ppm F (Danelon et al., 2017). Esses resultados podem ser explicados pela maior reatividade das nanopartículas com o esmalte dental, devido à redução de tamanho da partícula e consequentemente o aumento da adsorção do TMP ao esmalte (Danelon et al., 2015).

Considerando os resultados promissores observados para dentifrícios e a ausência de estudos avaliando os efeitos do TMP nanoparticulado quando adicionado a produtos de alta concentração de F, seria interessante avaliar os efeitos desta associação tanto sobre a cárie dentária, como sobre o desgaste erosivo do esmalte. Com base nos resultados supracitados, é possível que o uso de nanopartículas de TMP promova um efeito sinérgico adicional em comparação ao TMP microparticulado, o que poderia ter importantes implicações para a prática clínica. Dessa forma, o objetivo do presente estudo foi avaliar o efeito de géis fluoretados suplementados com nanopartículas de TMP sobre a remineralização de lesões artificiais de cárie e sobre o desgaste erosivo do esmalte dental bovino *in vitro* (Anexo A).

Para abordar o tema proposto, o estudo será apresentado em dois capítulos, conforme descrito abaixo:

- Capítulo 1: **"Effect of fluoride gels with nano-sized sodium trimetaphosphate on the remineralization of caries lesions *in vitro***"*

- Capítulo 2: **"*In vitro* effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate on enamel erosive wear"***

* artigos formatados de acordo com as normas do periódico Journal of Dentistry (Anexo B).

Considerações finais

CONSIDERAÇÕES FINAIS

Os resultados do estudo apresentado no Capitulo 1 permitem concluir que a associação entre TMP e os géis fluoretados (4500 μg F/g + TMP micro 5% ou TMP nano 2,5%) resultou em um aumento significativo na remineralização das lesões de cárie artificiais quando comparado com o gel (4500 μg F/g) sem TMP. Além disso, os mesmos géis suplementados promoveram efeito remineralizador semelhante aos observados para os dois controles positivos (gel acidulado e 9000 μg F/g), demonstrando ser possível reduzir a concentração de F e manter a efetividade do produto em padrão semelhante às formulações convencionais. Entretanto, apesar do gel contendo TMPnano na concentração de 2,5% ter promovido bons resultados, o mesmo não promoveu efeito adicional em relação ao TMP microparticulado a 5%, o que não justificaria o uso de nanopartículas na produção dos géis. Cabe ressaltar, no entanto, que o estudo foi desenvolvido sob um modelo *in vitro* de curta duração, envolvendo apenas uma única aplicação dos géis (1 min) e sem envolver a exposição diária a um dentifrício fluoretado. Assim, sugere-se que mais estudos com protocolos *in vitro* e *in situ* sejam desenvolvidos, para que o efeito dos tratamentos a longo prazo seja também avaliado, sob condições que melhor reproduzam as intrabucais. Quanto aos resultados apresentados no Capítulo 2, estes permitem concluir que os géis suplementados com TMP, principalmente sob a forma de nanopartículas, promoveram um marcante efeito protetor contra o desgaste erosivo do esmalte, especialmente considerando o protocolo agressivo do estudo e a realização de uma única aplicação dos géis. Quanto ao desafio erosivo, todos os géis suplementados promoveram maior efeito protetor em comparação ao gel sem suplementação (4500 μg F/g), atingindo níveis semelhantes ao do controle positivo (9000 ppm F) e da formulação comercial. Já para desafios erosivos seguido de abrasão, o gel que promoveu o melhor efeito protetor foi o suplementado com TMPnano à 5%, o qual foi superior em relação a todos os grupos testados, incluindo o controle positivo e formulação comercial.

Diante dos resultados obtidos nos dois capítulos, considera-se que a suplementação com nanopartículas de TMP promoveu resultados promissores. Confirmou-se mais uma vez o sinergismo existente entre o F e o TMP em relação à cárie e a erosão dentária e permitiu ainda levantar teorias sobre o mecanismo de ação deste nanocomposto. Quanto a este último aspecto, merece destaque o fato de o gel suplementado com TMPnano a 5% ter promovido o pior efeito remineralizador em lesões de cárie artificiais (atingindo níveis semelhantes aos observados ao gel placebo), mas o maior efeito protetor sobre o desgaste erosivo do esmalte. Uma possível explicação para esse resultado pode estar relacionada aos diferentes protocolos e tipos de lesões estudadas em cada capítulo. No primeiro capítulo, a metodologia empregada induziu a remineralização de lesões de cárie artificiais, de forma que o TMP a uma maior proporção molar com o flúor (4500F + Nano 5%) não garantiu adequada remineralização das lesões de cárie possivelmente pela competição dos dois princípios ativos pelos mesmos sítios de ligação na estrutura dentária. Por outro lado, no segundo capítulo, o protocolo envolveu a desmineralização do esmalte por ação de ácido cítrico, seguida ou não de abrasão por escovação, o que possibilitou especular que, em condições mais extremas (pH abaixo de 4,0), o TMP em uma maior proporção molar com o flúor reduziu a perda mineral de forma mais eficaz do que promoveu a remineralização do esmalte dentário. Dessa forma, consideramos que os resultados promissores obtidos no presente estudo podem servir como base para a execução de futuros ensaios laboratoriais, com a finalidade de se determinar a proporção molar que tenha o melhor efeito sinérgico tanto para a cárie como sobre a erosão dentária.

Anexos

ANEXOS

ANEXO A

REFERÊNCIAS INTRODUÇÃO GERAL

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ANEXO B

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