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

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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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"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

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

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).

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

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Declaration of interest

The authors declare no conflict of interest that may affect the manuscript judgment.

Abstract

Objective: to evaluate the effects of fluoride (F) gels supplemented with TMP in conventional (TMPmicro) or nano-sized (TMPnano) particles on the remineralization of caries-like lesions *in vitro.* **Methods:** Bovine enamel blocks (n=168) were selected by surface hardness (SH) after induction of subsurface lesions, and randomly divided into seven groups (n=24/group): Placebo (without F/TMP), 4,500 µg F/g (4500F), 9,000 µg F/g (9000F), 4500F + 5% TMPmicro (5% Micro), 4500F + 2.5% TMPnano (2.5% Nano), 4500F + 5% TMPnano (5% Nano) and 12,300 µg F/g (Acid gel). The gels were applied in a thin layer for one minute. Half of the blocks were submitted to pH cycling for 6 days, while the remaining specimens were used for loosely- (CaF_2) and firmly-bound (FA) fluoride analysis. The percentage of surface hardness recovery (%SHR), cross-sectional hardness (ΔKHN), CaF_2, FA, Calcium (Ca) and Phosphorus (Pi) on/in enamel were determined. Data (log10-transformed) were submitted to ANOVA and Student-Newman-Keuls test ($p<0.05$). **Results:** A dose-response relationship was observed between F concentrations in the gels without TMP and %SHR and ΔKHN. The 2.5% Nano and 5% Micro reached %SHR similar to 9000F and Acid gels. For ΔKHN, the Placebo and 5% Nano had the highest values, with the lowest observed for 5% Micro, 2.5% Nano, 9000F and Acid gels. All groups had similar values of CaF_2 retained, except Placebo and Acid gel. An increase in Ca concentrations was observed for nano-sized TMP groups. Regarding Pi, both formed and retained, TMP groups were similar to 9000F and Acid. **Conclusion:** the addition of 2.5% nano-sized or 5% micrometric TMP to low-fluoride gels lead to enhanced remineralization of artificial caries lesions *in vitro*.

Clinical Significance: the addition of TMP to a low-fluoride gel significantly enhanced its remineralizing effect to levels similar to those achieved by conventional formulation. Since this was attained by using 2- to 3-fold lower fluoride concentrations, this gel could be a safer alternative in the clinical practice, especially for young children.

Introduction

Caries-preventive programs are focused on children and usually considered as a priority for dental public health, being less expensive than its treatment [1]. Topically applied fluoride (F) products, especially at high concentrations, have been used as a low-cost and easily operated measure for the prevention and treatment for caries lesions [2], among which the most commonly used are solutions, gels and varnishes [3]. Because of the risk of overingestion and consequent acute toxicity, the use of F gels is not generally recommended for children younger than six years old [4]. Considering the extensive benefits and wide use of these products, the search for strategies aiming to increase their clinical effectiveness without increasing possible side-effects in young children is highly desirable.

There is considerable *in vitro* and *in situ* evidence on the efficacy of fluoridated products supplemented with phosphate salts on enamel de- and re-remineralization processes, including Sodium Trimetaphosphate (TMP) [5,6], Sodium Hexametaphosphate (HMP) [7,8] and Calcium Glycerophosphate (CaGP) [9,10]. Such laboratory evidence has been recently confirmed in a randomized clinical trial, showing that the supplementation of a low-F (500 ppm F) toothpaste with micrometric TMP or CaGP resulted in lower or similar caries progression, respectively, when compared with a conventional dentifrice formulation (1100F) [11].

Furthermore, recent studies reported the use of nano-sized TMP or HMP further enhanced the effects of F on enamel de- and re-mineralization when added to dentifrices [12,13]. Such additional effects could be explained by the high ratio of surface area to volume, as well as a high percentage of atoms on the surface compared to larger particles, which makes nano-sized particles more reactive [13].

Despite the promising results obtained for dentifrice formulations described above, no evidence is available for F vehicles for professional application supplemented with nano-sized phosphate salts. Considering the relevance of this issue for clinical practice, the present study aimed to evaluate the effects of F gels supplemented with TMP in conventional (TMPmicro) or nano-sized (TMPnano) particles on the remineralization of caries-like enamel lesions *in vitro*. The null hypothesis was that fluoride gels supplemented with nano-sized

TMP have a similar ability to promote enamel remineralization compared to their counterpart supplemented with micrometric TMP.

Material and Methods

Experimental Design

Bovine enamel blocks (4×4 mm, n = 168) were obtained from the flattest portion of the vestibular face of the crowns. The blocks were selected by surface hardness (SH) and caries-like lesions was induced. Following, they were randomly divided into 7 experimental groups (n = 24/group) according to the gels tested: (a) Placebo (without F or TMP), (b) 4,500 μg F/g (4500F), (c) 9,000 μg F/g (9000F), (d) 4500F plus 5% micrometric TMP (5% Micro), (e) 4500F plus 2.5% nanosized TMP (2.5% Nano), (f) 4500F plus 5% nano-sized TMP (5% Nano) and (g) 12,300 μg F/g (acid gel) . The blocks were treated once with the respective gels prior to a pH cycling regimen (6 days). SH, integrated area of the subsurface lesion (ΔKHN), enamel F, Ca and Pi concentrations, as well as CaF_2 formed (after application of the gels) and retained (after pH cycling) in/on enamel were determined (Anexo C e D).

Synthesis and characterization of nano-sized (TMP) particles

The synthesis and characterization of nano-sized TMP was carried out at Federal University of São Carlos based on Danelon et al.,[13]. To prepare nano-sized TMP, 70 g of pure (micrometric) sodium trimetaphosphate ($Na_3O_9P_3$, Aldrich, purity ≥95% CAS 7785- 84-4) was ball milled using 500 g of zirconia spheres (diameter of 2 mm) in 1 L of isopropanol. After 48 h, the resulting powder was separated from the alcoholic media and ground in a mortar. The powder crystallinity was characterized by X-ray diffraction (XRD) using a Rigaku Dmax 2500 PC difractometer in the 2ϴ range from 10º to 80º with a scanning rate of 28/min. The coherent crystalline domains (crystallite size) were estimated using the Scherrer equation: $L = \lambda K / B \cos\Theta_B$ where L is the linear dimension of a monocrystalline nano-particle, λ is the wavelength of the incident X-ray, B is the diffraction line width of the diffraction peak, Θ_B is the Bragg angle obtained from the XRD pattern, and K is a numerical constant which value is 0.9. The scanning electron microscopy (SEM) images were collected using a Philips XL-30 FEG.

The micrometric TMP particle size was 450 nm reaching the approximate size of 22.7 nm (nanoparticle).

Gel formulation and determination of fluoride in products

The experimental gels were produced in the laboratory of Pediatric Dentistry from Araçatuba Dental School, using the following ingredients: carboxymethylcellulose (Sigma-Aldrich Co., St. Louis, MO, USA), sodium saccharin (Vetec, Duque de Caxias, Rio de Janeiro, Brazil), glycerol (Merck, Darmstadt, Germany), peppermint oil (Synth), and water. Formulations without F (Placebo) and containing F (NaF - Merck®, Germany) in the concentrations of 4,500 and 9,000 µg F/g were obtained. Micrometric TMP (Sigma-Aldrich Co., St. Louis, MO, USA) was added at a concentration of 5%, and nano-sized TMP at concentrations of 2.5% and 5% to the 4,500 µg F/g gel. A commercial acidic gel was used as a positive control (12,300 µg F/g, acid gel, pH=4.5, DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil). The F concentration in the gels was determined using a specific electrode for the F ion (9609 BN; Orion Research Inc., Beverly, MA, USA) attached to an ion analyzer (Orion 720 A+; Orion Research Inc.) and calibrated with standards containing 0.125–2.000 µg F/g. Approximately 100-110 mg of each product was dissolved in deionized water and transferred to a volumetric flask. The volume was then adjusted to 100 ml using deionized water. For each product, three dilutions were prepared, which were analyzed in duplicate, after buffering with total ionic strength adjustment buffer II (TISAB II) [14].

Induction of artificial caries lesions

To induce caries-like lesions, all surfaces of each specimen, except the enamel surface, were coated with acid-resistant varnish, and subsurface enamel demineralization was produced by immersing each block individually in 32 mL of a solution with 1.3 mmol/L Ca and 0.78 mmol/L P in 0.05 mol/L acetate buffer, pH 5.0; 0.03 ppm F, for 16 h at 37 °C [15,16]. The blocks were submitted to post-demineralization surface hardness measurement (SH_1), by producing five indentations spaced 100-µm apart the five initial ones (SH) (Anexo E).

Treatment with gels and pH cycling (Re>Des)

In the first day of pH cycling, a thin gel layer was applied so that the exposed area of enamel was completely covered by the gel using a cotton swab for 1 min. After treatment, the gel was removed and blocks were washed with deionized water and gently dried with absorbent paper. The specimens were individually subjected to a pH cycling regimen at 37 °C for 6 consecutive days. Each cycle alternated between the remineralizing (RE) solution (1.1 mL/mm^2; 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/ L KCl in cacodylate buffer 20 mmol/L, 0.05 ppm F, pH 7.0) and the demineralizing (DE) solution for cariogenic challenges (2.2 mL/mm^2; 2.0 mmol/L Ca and P, in acetate buffer 75 mmol/L, 0.04 ppm F, pH 4.7) [15,16] (Anexo F).

Hardness analysis

The surface hardness analysis was determined before the experiments (SH), after enamel demineralization (SH_1), as well as after pH cycling (SH_2) using a hardness tester (Buehler, Lake Bluff, USA and Mitutoyo Corporation, Kanagawa, Japan) and a Knoop diamond indenter, under a 25 g load for 10 s [13]. Five indentations spaced 100-μm apart were made at the center of the enamel surface for determining initial surface hardness (SH) and also after artificial caries lesions were induced (SH_1), spaced 100-μm apart from SH. After the experimental periods, five other indentations were made (SH_2) spaced 100-μm apart and from SH_1. The percentage of surface hardness recovery (%SHR) was calculated using the following formula: $\%SHR=[(SH_2-SH_1)/(SH-SH_1)]\times100$. For cross-sectional hardness measurements, enamel blocks were longitudinally sectioned through their center and embedded in acrylic resin with the cut face exposed and polished. A sequence of 14 indentations at different distances (5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 μm) from the enamel surface were created in the central region, using the above mentioned microhardness tester, with a Knoop diamond indenter under a 5 g load for 10 s (Buehler, Lake Bluff, USA) [17,18]. The integrated hardness area (KHN×μm) for the lesion into sound enamel was calculated using the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated hardness area for sound enamel to obtain the integrated recovery of subsurface hardness (ΔKHN) [17,18] (Anexo G).

Determination of CaF_2-like concentrations (formed and retained)

The concentration of CaF_2 in enamel was determined immediately after the application of the experimental gels (CaF_2 formed) and after the pH cycling (CaF_2 retained). The blocks were measured with a Mitutoyo CD -15B digital caliper (Mitutoyo Corp., Japan) obtaining the surface area of the specimens. The surface of each specimen, except for the enamel, was coated with wax, and subsequently immersed in 0.5 ml of 1.0 mol/l KOH solution for 24 h under constant agitation. After, the solution was neutralized with 0.5 ml of 1.0 mol/l HCl and buffered with 1.0 ml of TISAB II. An ion analyzer (720A; Orion Research, USA) and a combined ion-selective electrode (9609 BN; Orion Research, USA) previously calibrated with standards of 0.0625, 0.125, 0.250, 0.500, and 1.0 µg F/ml were used. Data were obtained in mV and were converted to microgram F per square centimeter using Microsoft Excel (Anexo H).

Fluoride, Calcium, and Phosphorus Content in Enamel (formed and retained)

The other halves of the blocks were sectioned again (2 × 2 mm), and an enamel biopsy was performed [19,20]. The blocks were fixed to a mandrel and attached to the top of a modified microscope with a micrometer (Pantec, São Paulo, Brazil) to measure the depth. Self-adhesive polishing disks (13 mm in diameter), with 400-grit silicon carbide (Buehler), were fixed to the bottom of polystyrene crystal tubes (J-10, Injeplast, São Paulo, Brazil). A 50-µm-deep layer of each enamel block was removed [9,18], and 0.8 ml HCl 0.5 mol/l was added to the resulting enamel powder. The tubes were kept under agitation during 60 min, and 0.5 ml NaOH 0.5 mol/l was then added, following a protocol modified from Danelon et al. [13]. For F analysis, samples were buffered with TISAB II and analyzed with an ion-specific electrode (Orion 9609) connected to an ion analyzer (Orion 720+). A 1:1 ratio (TISAB:sample) was used. Calcium analysis was performed using the Arsenazo III colorimetric method [21]. Inorganic phosphorus was measured according to Fiske and Subbarow, 1925 method [22] (Anexo I).

Statistical analysis

SigmaPlot 12.0 software was used for statistical analysis, and the significance level was set at 5%. Data analysis considered the values of SH, SH_1,

%SHR and ΔKHN, as well as CaF_2, F, Ca and P content in/on enamel before (formed) and after (retained) the pH cycling, and as variation factor, the experimental gels. The results were log_{10}-transformed and passed the normality test (Shapiro-Wilk) and homogeneity test (Bartlett). Data were analyzed to one-way ANOVA (SH, SH_1, %SHR and ΔKHN) or two-way ANOVA (enamel CaF_2, F, Ca and P), followed by Student-Newman-Keuls test.

Results

Mean fluoride concentrations (SD, µg F/g) in the placebo, 4500F, 9000F, 2.5% Nano, 5% Nano, Micro 5% and Acid gel groups were 19.8 (2.91), 4339.9 (96.7), 9120.7 (114.4), 4383.2 (180.3), 4,093.9 (224,6), 4148,7 (93.2) and 13136.9 (995.07), respectively. Mean (SD) initial surface hardness was 366.3 KHN (5.1), while mean (SD) surface hardness after demineralization (SH1) was 58.9 KHN (12.6), with no statistical difference among the groups after the random allocation (p= 0.080).

A dose-response relationship was observed between fluoride concentrations in the experimental gels without TMP and %SHR (Placebo<4500F<9000F=acid gel). The lowest %SHR values were observed for placebo, 4500F and 5% Nano, which were significantly different from the other groups. Conversely, 5% Micro, 2.5% Nano, 9000F and Acid gel groups had the highest %SHR values, without significant differences among them. In terms of subsurface lesion area (ΔKHN), a similar pattern of that seen for %SHR was observed, with the placebo and 5% Nano gels (p=0.953) presenting significantly higher ΔKHN values when comparing with the remaining groups without significant differences among 5% Micro, 2.5% Nano, 9000F and acid gel groups. The addition of 5% TMPnano to 4500F led to results similar to those attained by the placebo gel (Table 1).

The acid gel promoted the highest CaF_2-like levels among all groups after topical application. After pH cycling, all groups had similar CaF_2-like concentrations, except for the placebo and acid gel. The concentration of formed F in enamel was similar among the 9,000 and TMP groups. The amount of F retained in enamel significantly increased after remineralization in all groups ($P< 0.05$) (Table 2).

As for enamel Ca concentrations, a marked increase was observed after pH cycling for groups treated with nano-sized TMP, in contrast to the other groups ($p<0.05$). Regarding Pi formed and retained in enamel, groups treated with TMP showed similar values to 9000F and acid gel. Pi levels increased after pH cycling, despite it was only significant for the 4500F, 5% Micro, 2.5% Nano, 5% Nano and Acid gel ($p<0.05$). Other comparisons are shown in Table 2.

Discussion

The addition of inorganic phosphate salts to topically applied F products has been proposed to improve the efficacy in the prevention/treatment of early caries lesions (*i.e.,* white spot lesions), at the same time as reducing F levels in the formulation, in order to minimize the possibility of acute side-effects. *In vitro* and *in situ* studies demonstrated that micrometric TMP associated with fluoridated gels significantly enhanced the remineralization of artificial caries lesions [23], and prevented enamel demineralization [5, 25]. The present study showed that the addition of TMP to a low-F gel (4500F) resulted in a higher remineralizing capacity then its counterpart without TMP, achieving levels similar to those of a conventional neutral (9,000 ppm F) and acid (12,300 ppm F) gels. Nonetheless, no additional benefit was achieved from the use of nano-sized TMP when compared with micrometric particles, thus leading to the acceptance of the study's null hypothesis.

The current methodology and the product tested were chosen based on promising results observed for dentifrices associated with nano-sized TMP and HMP particles. Danelon et al. [26] observed *in vitro* that treatment with a 1100 ppm F toothpaste supplemented with 3% TMPnano reduced mineral loss in ~44% compared to its micrometric counterpart. In an *in vitro* study, Dalpasquale et al. [11] showed that the addition of 0.5%HMPnano to a 1100 F toothpaste significantly enhanced its effects against enamel demineralization when compared to its counterpart without HMPnano. Furthermore, the addition of 0.05% nano-sized TMP to a low-F toothpaste (250 µg F/g) promoted significantly lower ΔKHN among all groups, including a 1100 ppm F toothpaste [27].

In the present study, while 2.5% TMPnano, 5%TMPmicro, 9,000F and acid gel promoted similar %SHR and ΔKHN, the addition of 5% TMPnano to 4500F resulted in ΔKHN value similar to those achieved by the Placebo formulation. These findings seem to confirm that the TMP:F molar ratio has a strong influence on the resulting effect against enamel demineralization [26,28-30]. TMPnano concentrations tested in the present study (2.5% and 5%) were based on TMP/F ratio from previous studies which reported the addition of micrometric particles of TMP at 5% to a 4,500 ppm F gel promoted a significantly higher effect against enamel demineralization and on the remineralization of caries-like lesions when compared with its counterpart without TMP [5,23,25]. Furthermore, the studies with nano-sized TMP/HMP in dentifrices were decisive for the choice of 2.5% TMPnano, since it was expected that a lower concentration of nano-sized TMP (compared with micrometric particles) could result in greater efficacy, similarly as observed in previous studies [12,27].

Despite most clinical studies have adopted professional topically gel application time ranging from 2 to 10 min [4] the present study used the time of 1 min based on Delbem et al. [18] and Villena et al. [31]. In the latter, the results demonstrated that the Acidulated Phosphate Fluoride application for either 1 or 4 min is equally effective in increasing enamel F concentrations, and in reducing enamel demineralization. Another recommendation of gel application is refraining from eating and drinking for at least 30 min after application [4]. In the present study, however, the blocks were washed with deionized water immediately after application, based on previous findings demonstrating that water rinsing after professionally applied topical fluoride gel or foam will have no adverse effect on the therapeutic benefit of the treatment [18,32]. In addition, this step was adopted to avoid the contamination of cycling solutions with the gels treatment and ensure that no trace of gel remained on the block surface which could alter study results.

The amount of calcium fluoride (CaF_2) formed after application of high fluoride concentrations is paramount for achieving maximum preventive and therapeutic effects, since this layer act as an efficient source of free fluoride and calcium ions during cariogenic challenges. The acid gel group had the highest CaF_2-like concentrations formed (after topical application), followed by 9000F and 4500F groups, confirming that CaF_2 formation could be enhanced by increasing the fluoride concentration of the topical agent and/or lowering the pH of the topical agent [33]. After pH cycling, all groups had similar concentrations of retained

CaF_2-like, except the placebo and acid gel groups. The results suggest that the effect of TMP was not related to the deposition of calcium fluoride (CaF_2) on the enamel, and this reduction might be due to the increase in Ca and F retention on the TMP molecules that are adsorbed to enamel [6], instead of the deposition of CaF_2 globules on the enamel surface

Nevertheless, for firmly-bound F (formed), similar values were observed among the 9000F and TMP groups, and after pH cycling, F concentration significantly increased in all groups ($p<0,05$), which is not in line with the findings of Manarelli et al. [6], in a study with fluoridated varnishes. Also for Ca concentrations in the enamel, a marked increase was observed after pH cycling for nano-sized TMP groups, in contrast with groups not supplemented with TMP. Regarding Pi results, groups treated with TMPmicro/nano showed values similar to 9000F and acid gel. Moreover, the addition of TMP to 4,500F gel had an effect on enamel mineral composition. Despite the 2- to 3-fold difference in the F content between TMPmicro/nano groups and 9,000F/acid gel, similar F (formed), Ca and Pi values were observed in the enamel treated with these gels, which confirm the results obtained for previous studies with dentifrices [27,28,34,35].

In the present study, while TMPnano at 5% increased the amount of F (formed), Ca and Pi in the enamel, on the other hand it promoted the lowest %SHR among the fluoridated gels, and similar ΔKHN when compared with the placebo gel. It could be suggested that the addition of large percentage of TMPnano would supersaturate the enamel surface, and since the adsorption of polyphosphates to enamel occurs rapidly after exposure and is followed by the adsorption of F [36], an appropriate molar proportion between TMP and F must be used for optimizing the anticaries action. The suggested molar proportion of TMP/NaF is between 1.24:1 and 3.72:1 [29]. For high-fluoride products (as gels), the maximum effect on enamel demineralization was achieved with the 4,500F + 5% TMPmicro (TMP: NaF/0.7:1) [5].

Although the conditions of *in vitro* models can be carefully controlled and have the ability to show a dose-response relationship to different levels of fluoride in demineralization and remineralization process, the main role of *in vitro* methods is to provide information about mode of action of new compounds and facilitate the generation of sufficient quantitative data to give the investigator confidence to properly design clinical trials [37]. Under tested conditions of this *in vitro* study, it can be concluded that the addition of TMP to low-fluoride gels lead to enhanced remineralization of artificial caries lesions *in vitro*, despite no additional benefit was attained by the use of nano-sized particles.

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Table 1. Mean (SD) percentage of surface hardness recovery (%SHR) and integrated loss of subsurface hardness (ΔKNH) according to groups.

Groups	%SHR	ΔKHN
Placebo	14.9 (3.5)[a]	5795.9 (1176.7)[a]
4500F	24.2 (6.6)[b]	4873.2 (955.3)[b]
9000F	29.8 (8.4)[c]	3661.5 (646.1)[c]
5% Micro	34.8 (6.7)[c]	3063.8 (959.8)[c]
2.5% Nano	35.3 (9.1)[c]	3136.4 (884.2)[c]
5% Nano	19.7 (5.8)[d]	5773.2 (1070.2)[a]
Acid gel	36.9 (6.0)[c]	3132.5 (789.2)[c]

Distinct superscript lowercase letters indicate statistical significance in each column (Student –Newman Keuls test, *n*=12, $p<0.05$). Data were $\log_{10}$-transformed for the statistical analysis.

Table 2. Mean (SD) CaF_2, firmly bound fluoride, Ca and Pi formed (F) and retained (R) in enamel after gels treatment and pH-cycling according to groups.

Grs	CaF_2, µg/cm^2		Fluoride, µg/cm^2		Ca		Pi	
	F	R	F	R	F	R	F	R
Placebo	$0.8^{A,a}$	$3.1^{B,a}$	$0.46^{A,a}$	$0.84^{B,a}$	$701.9^{A,a}$	$812.4^{A,ab}$	$339.6^{A,abcde}$	$370.3^{A,a}$
	(0.1)	(0.4)	(0.07)	(0.17)	(102.9)	(195.8)	(107.7)	(198,3)
4500F	$35.0^{A,bc}$	$5.0^{B,bcd}$	$0.51^{A,a}$	$1.57^{B,bcde}$	$502.9^{A,a}$	$978.7^{B,b}$	$300.3^{A,ab}$	$541.1^{B,bc}$
	(7.6)	(1.3)	(0.16)	(0.50)	(201.5)	(346.3)	(72.4)	(125,9)
9000F	$63.0^{A,d}$	$5.8^{B,cd}$	$0.73^{A,bc}$	$1.64^{B,cde}$	$988^{A,b}$	$944.7^{A,ab}$	$368.8^{A,bcdef}$	$434.8^{A,a}$
	(8.7)	(1.3)	(0.17)	(0.35)	(239.9)	(196.0)	(90.0)	(94.1)
5% Micro	$30.9^{A,bce}$	$5.2^{B,bcd}$	$0.64^{A,bcde}$	$1.40^{B,bcde}$	$1361.2^{A,c}$	$1340.2^{A,cd}$	$488.7^{A,f}$	$625.0^{B,cde}$
	(4.3)	(0.8)	(0.13)	(0.27)	(357.0)	(416.9)	(106.5)	(143,9)
2.5%Nano	$32.6^{A,ce}$	$6.0^{B,d}$	$0.75^{A,c}$	$1.68^{B,e}$	$1075.9^{A,b}$	$1502.2^{B,d}$	$454.3^{A,ef}$	$674.2^{B,de}$
	(7.5)	(1.4)	(0.23)	(0.39)	(366.7)	(402.4)	(181,8)	(181,8)
5% Nano	$37.1^{A,bc}$	$5.9^{B,d}$	$0.69^{A,bce}$	$1.74^{B,cde}$	$684.9^{A,a}$	$1369.5^{B,d}$	$454.3^{A,def}$	$712.0^{B,e}$
	(6.7)	(1.4)	(0.16)	(0.32)	(151.3)	(403.8)	(106.5)	(161,2)
Acid gel	$198.7^{A,f}$	$9.4^{B,e}$	$1.84^{A,f}$	$5.54^{B,f}$	$1384.7^{A,c}$	$1296.6^{A,cd}$	$448.9^{A,cdef}$	$578.7^{B,cde}$
	(27.0)	(2.1)	(0.59)	(1.94)	(303.2)	(217.7)	(105.2)	(69.6)

Different lowercase superscript letters show significant difference among groups in each analysis. Different uppercase superscript letters indicate differences between CaF2, F, Ca and Pi (formed and retained) within each group. Data (log10-transformed) were submitted the Student-Newman-Keuls' method, n =12 ($p < 0.05$).

In vitro effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate on enamel erosive wear

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Declaration of interest

The authors declare no conflict of interest that may affect the manuscript judgment.

Abstract

Objective: to assess *in vitro* the protective effect of fluoride (F) gels supplemented with micrometric or nano-sized sodium trimetaphosphate (TMPmicro and TMPnano, respectively) against enamel erosion*.* ***Methods*****:** Bovine enamel blocks (n=140) were selected by surface hardness (SH), and randomly divided into seven groups (n=20/group): Placebo (without F/TMP), 4,500 µg F/g (4500F), 9,000 µg F/g (9000F), 4500F plus 2.5% TMPnano (2.5% Nano), 4500F plus 5% TMPnano (5% Nano), 4500F plus 5% TMPnano (Micro 5%) and 12,300 µg F/g (Acid gel). Blocks were treated once during one minute with the gels, and submitted erosive (ERO, n=10/group) or erosive plus abrasive (ERO+ABR, n=10/group) challenges 4 times/day, for 90 seconds each challenge (under reciprocating agitation), during consecutive 5 days. Blocks were analyzed by profilometry, and by surface (SH) and cross-sectional hardness (∆KHN). Data were submitted to two-way ANOVA followed by Fisher's test ($p<0.05$). ***Results:*** For ERO, both gels supplemented with TMPnano promoted enamel wear significantly lower than Placebo and 4500F, while for ERO+ABR, the lowest enamel wear was achieved by the use of 4500F+5% Nano. For ERO, significantly lower softening was observed for enamel treated with 4500F+5% Micro and 4500F+2.5% Nano, while for ERO+ABR, lower softening was observed for Placebo and 9000F groups. Among the TMP-containing gels, the lowest ∆KHN values were observed for 4500F+2.5% TMPnano under ERO condition. ***Conclusion:*** the addition of 5% nano-sized TMP to a low-fluoride gel produced superior protective effects for enamel under ERO and ERO+ABR conditions, when compared with micrometric TMP.

Clinical Significance: the supplementation of low-F gels with TMP was shown to significantly improve their effects on erosive wear, and the use of nano-sized TMP further enhances this protective action.

Introduction

Prevalence data shows that erosive tooth wear has become a common and increasing condition especially in younger age groups [1-5]. Since this condition is a lifelong cumulative process, both primary and permanent teeth are affected [6,7], and changes in nutritional habits over the last decades, such higher frequency and consumption of acidic foods and soft drinks, could be significantly associated with the prevalence of erosive wear [2,8]. Furthermore, when the softened enamel (caused by exposure to acids) suffers mechanical stress, as that occurred during toothbrushing, the progression of wear further increases, whereas the eroded enamel is more susceptible to abrasive wear [1,9,10].

Fluoridated products have been widely used for the prevention and treatment of dental caries, due to the formation of CaF_2 precipitates on the tooth surface. However, the role of fluoride in erosion therapy has long been questioned [11,12]. To ensure enamel protection in erosive wear, the CaF_2-like layer deposited on enamel should be thick enough to form a physical barrier able to protect the underlying enamel against acid challenges [12]. Most fluoridated products with monovalent fluorides at low or medium concentrations and at neutral pH show a limited preventive/therapeutic effect against dental erosion, while high-concentration, acidic products and polyvalent fluoride formulations provide a higher benefit [11].

As mentioned above, the high prevalence of erosive tooth wear calls for further research into the implementation of adequate preventive measures, associated with professional orientation to reduce the frequency of ingestion of acid foods and beverages [13]. Whereas the use of fluoride alone shows a slight protective effect against erosion/abrasion, recent studies have focused on the association of fluoride to other protective agents, including inorganic phosphates (Sodium Trimetaphosphate/TMP and Sodium Hexametaphosphate/HMP) to enhance the effects of fluoridated vehicles against erosive challenges [14-19].

The supplementation of a fluoridated dentifrice with micrometric Sodium Trimetaphosphate was studied by Moretto et al., [14]. On *in vitro* conditions they observed that 5,000 µg F/g and 500 µg F/g plus 3% TMP dentifrices had a greater protective effect when compared with a conventional one (1,100 µg F/g), under both ERO and ERO+ABR conditions. Besides that, the protective effect was also observed in mouthrinse [16], varnish [15] and gel [18]. As an attempt to further enhance the effects of TMP, a recent investigation assessed the impact of nano-sized TMP added to a fluoridated dentifrice against erosive challenges, concluding that the addition of 3% TMPnano to a 1100 ppm F dentifrice promoted a protective effect against enamel erosive wear similar to those seen for the 5000 ppm F dentifrice [20]. These results could be explained by the higher reactivity of TMPnano, due to reduction in size and increased the adsorption on enamel [21].

Considering the aforementioned data and the absence of studies focusing on the supplementation of gels with nano-sized TMP, the aim of this study was to assess *in vitro* the protective effect of these gels against enamel erosion. The null hypothesis was that fluoride gels supplemented with nano-sized TMP have a similar protective effect to its counterpart supplemented with micrometric TMP.

Materials and Methods

Experimental design

Bovine enamel blocks (n=140) were selected by surface hardness (SH), and randomly divided into 7 experimental groups (n=20/group), according with the gels tested: (a) Placebo (without F/TMP), (b) 4,500 µg F/g (4500F), (c) 9,000 µg F/g (9000F), (d) 4500F plus 2.5% nanosized TMP (2.5% Nano), (e) 4500F plus 5% nano-sized TMP (5% Nano), (f) 4500F plus 5% micrometric TMP (Micro 5%) and (g) 12,300 µg F/g (Acid gel). Blocks were treated once during one minute with the gels, and submitted erosive (n=10) or erosive plus abrasive (n=10) challenges 4 times/day, for 90 seconds each challenge (under reciprocating agitation), during consecutive 5 days. Erosive wear was evaluated by profilometry, and mineral loss was analyzed by the SH and cross-sectional hardness (Anexo J).

Enamel blocks preparation

Bovine central lower incisors were obtained and stocked in neutral formalin solution at 2% (pH 7.0) for 30 days. Enamel blocks (4 × 4 mm) were obtained from the flattest portion of the crowns and fixed in an acrylic resin discs using sticky wax. Following, the dentine and enamel were properly polished to remove around of 200 µm of the enamel surface. SH was determined by performing 5 impressions in the central region of the blocks surface (Knoop diamond, 25 g, 10 s; Buehler, Lake Bluff, USA). Blocks with mean hardness between 330.0 and 380.0 kgf/mm^2 were selected. During all procedures, the blocks were maintained in humidified atmosphere with 2% formaldehyde at pH 7.0 [22].

Synthesis and characterization of nano-sized (TMP) particles

The synthesis and characterization of nano-sized TMP was carried out at Federal University of São Carlos. The micrometric TMP particle size was 450 nm reaching the approximate size of 22.7 nm (nanoparticle). To prepare nano-sized TMP, 70 g of pure (micrometric) sodium trimetaphosphate ($Na_3O_9P_3$, Aldrich, purity ≥ 95% CAS 7785-84-4) was ball milled using 500 g of zirconia spheres (diameter of 2 mm) in 1 L of isopropanol. After 48 h, the powder was separated from the alcoholic media and ground in a mortar. The powder crystallinity was characterized by X-ray diffraction (XRD) using a Rigaku Dmax 2500 PC difractometer in the 2θ range from 10 to 80º with a scanning rate of 2º/min. The coherent crystalline domains (crystallite size) were estimated using the Scherrer equation:

$$L = \frac{K\lambda}{B\cos\theta_B}$$

where L is the linear dimension of a monocrystalline nanoparticle, λ is the wavelength of the incident X-ray, B is the diffraction line width of the diffraction peak, θ_B is the Bragg angle obtained from the XRD pattern, and K is a numerical constant which value is 0.9 [21].

Gel formulation and determination of fluoride in the products

Experimental gels were prepared in a laboratory with the following components: carboxymethylcellulose, sugar, glycerin, peppermint oil, deionized water. Fluoride were added to the gels at concentrations of 4500 and 9000 µg F/g (as NaF, Merck, Darmstadt, Germany). To the 4500 µg F/g gel, sodium trimetaphosphate (TMP, Sigma-Aldrich Co., St. Louis, MO, USA) were added at concentration of 5% (micrometric particles), as well as 2.5% and 5% (nano-sized particles). A gel without F and TMP (placebo) was also prepared with the same basic formulation and serve as negative control. Also, a commercial acid gel containing 12,300 µg F/g (as NaF, DFL Industry and Trade SA, Rio de Janeiro, RJ, Brazil) was included and served as a commercial control. F concentrations in the gels were determined using specific electrode for F ion (9609 BN - Orion) and ion analyzer (720A - Orion Research, USA), previously calibrated with five standards containing 0.125 – 2.000 µg F/g, as previously reported [23], in solutions obtained from the dilution of 100-110 mg of each gel in 100 mL of deionized water, after buffering with TISAB II. The data obtained in millivolt were converted to µg F/mL.

Treatment of the blocks and erosive challenges

Half of the surfaces of each block was protected with acid-resistant nail varnish (control area), so that only half of their surfaces (test area) was subjected to treatment with the gels and to the ERO or ERO+ABR challenges. The gels were applied in a thin layer for one minute only in the first day after, the gels were removed and blocks were washed with deionized water and dried with absorbent paper. Following the treatment, the blocks were submitted to ERO (n=10) or ERO+ABR (n=10) challenges. For 5 days, the enamel blocks were subjected to ERO by immersion of blocks in 0.05 M citric acid (1%), pH 3.2, for 90 seconds, 4 times/day, followed by a remineralizing period of 2 h among erosive challenges (immersion in unstirred artificial saliva, pH 7.0). Demineralization procedures were performed on a shaking plate performing reciprocating and constant movement frequency (35/min) [24]. ERO+ABR were produced on half of the blocks by using a mechanical brushing machine (250 g axial load, 5 strokes/s; Elquip Maq Escovação, São Carlos, Brazil) using a slurry of a placebo (F-free) dentifrice, immediately after the immersion in citric acid, during 15 seconds. The other half of the blocks (ERO only) was immersed in the placebo dentifrice slurry for 15 seconds after each erosive challenge [18] (Anexo K).

Analysis of enamel wear

At the end of the 5-day experimental period, the nail varnish was carefully removed from the control areas of each block with acetone-soaked cotton wool, prior to analysis of surface wear. Enamel loss was determined in relation to the reference surfaces by profilometry (Surftest SJ 401 – Mitutoyo American Corpora- tion), by scanning the surface of each block from the reference surfaces (control) across the exposed surfaces. The mean value of 5 readings was calculated for each block [17] (Anexo L).

Analysis of surface and cross-sectional hardness

Surface hardness was determined prior to the experiment (SH), and after ERO or ERO+ABR (SH_1), using the micro hardness Micromet 5114 hardness tester (Buehler, Lake Bluff, USA and Mitutoyo Corporation, Kanagawa, Japan) and the Buehler OmniMet software (Buehler, Lake Bluff, United States), load of 25 g for 10 sec. Five indentations spaced 100 µm from each other were performed for SH_1 and SH_2, at a distance of 100 µm from SH_1 and SH_2. After that, enamel blocks were cross-sectioned at the center, and half of each block were included in acrylic resin and subsequently polished. For cross-sectional hardness, the same microhardness tester was employed, using a load of 5 g, for10 s. A sequence of 9 prints at distances of 5, 10, 15, 20, 25, 30, 40, 50 and 70 µm from the external enamel surface was performed in the center of blocks, for both the control and the test areas. The integrated hardness area (KHN × µm) of the lesion up to the intact enamel was calculated using the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated area of intact enamel hardness, thus achieving the integrated hardness loss (ΔKHN) [19] (Anexo M).

Statistical analysis

For the statistical analysis, SH, SH_1, ΔKHN and enamel wear (µM) were considered as the response variables, and the type of experimental gels and the erosive challenges (ERO, ERO+ABR) were considered as the variation factors. The results were submitted to normality test (Shapiro-Wilk) and homogeneity test (Bartlett). The data were then analyzed by two-way ANOVA followed by Fisher's test, assuming a 5% significance level. Analyses were performed using SigmaPlot software, version 12.0.

Results

Mean fluoride concentrations (SD, µg F/g) in the placebo, 4500F, 9000F, 2.5% Nano, 5% Nano, 5% Micro and acid gel were 24.1 (4.18), 4,059.7 (56.2), 8,4307 (237.6), 4,383.2 (180.3), 4,093.9 (224.6), 4,148.7 (93.2) and 13,136.9

(995.07), respectively. The initial mean value of surface hardness (SHi) considering all groups was 360.1 (8.6) kgf/mm^2.

Lower values of enamel wear were observed for ERO than ERO+ABR challenge ($p < 0.001$). For ERO, similar enamel wear was observed among 9000F, 2.5% Nano, 5% Nano, 5% Micro and acid gel, followed by 4500F and Placebo groups. For ERO+ABR, the addition of 5% nano-sized TMP to the 4500F gel promoted the lowest enamel wear among all experimental groups (Table 1).

ERO promoted higher enamel surface softening than ERO+ABR ($p < 0.001$). For ERO, lower softening was observed for enamel treated with 5% Micro and 2.5% Nano, while for ERO+ABR, lower softening was observed for Placebo and 9000F groups (Table 1).

As for ΔKHN, a dose-response relationship was observed among Placebo, 4500F and 9000F, despite significant differences were not observed between 4500F and 9000F (ERO), and between Placebo and 4500F (ERO+ABR). Among the TMP-containing gels, the lowest ΔKHN values were observed for 2.5% Nano under both conditions, despite this difference was only significant for ERO (Table 1).

Discussion

The use of inorganic phosphates to enhance the effect of fluoridated products have been extensively reported by recent investigations, and this association was shown to be effective in minimizing demineralization and boosting remineralization of dental enamel, as well as in reducing erosive enamel wear [14,15, 25-28]. Such effects were also shown to be further enhanced when TMP was added as nano-sized particles to fluoridated toothpastes, [20,21,29], but no data was available for vehicles at higher fluoride levels, including varnishes and gels. The present study showed that the supplementation of fluoridated gels with nano-sized TMP have a higher ability to reduce enamel erosive wear compared to their counterpart supplemented with micrometric TMP, which was also shown to be dependent on the concentration of nano-sized TMP and on the type of challenge (ERO or ERO+ABR). Therefore, the null hypothesis was partially rejected.

Traditional fluoridated formulations have a well-established protective effect against enamel demineralization due to the formation of a CaF_2 layer.

However, opposed to caries (*i.e.*, subsurface lesions), in erosion the demineralization occurs mainly in the outermost layers of enamel, so that the CaF_2 precipitates become highly soluble during acid challenges. Hence, despite a protective effect against erosion is achieved by conventional fluoridated products, they have a limited action, especially considering consecutive erosive challenges [11,30]. In this sense, the addition of new remineralizing compounds has shown promising results against enamel erosive wear. Pancote et al., 2014 [18] observed that a low-F gel (4500F) associated with TMP on enamel subjected to ERO and ERO+ABR, led to lower enamel wear compared to the 9000F and the acid gel, under both conditions. Notwithstanding, mechanisms of action of TMP have not been completely elucidated. TMP seems to adsorb on the enamel surface, acting as a barrier to acid diffusion, prompting the formation of a more stable mineral, what ultimately affects the de-remineralization processes [15,28,31]. Furthermore, the effects of TMP alone are known to be minimal, so that optimum synergistic effects are only achieved when fluoride and TMP are co-administered at an appropriate ratio [31,32].

In the present study, for ERO the association of 4500F with micrometric (5%) and nano-sized (2.5% and 5%) TMP promoted enamel wear similar to those achieved by 9000F and Acid gel. The discrepancy between the current results and the data reported by Pancote et al. (2014) [18] is likely to be due to the different protocols employed. While in the above-mentioned study, the acid challenges were produced by static immersion in the acid solutions, the present protocol included a reciprocating and constant movement during the demineralization procedure, in order to avoid saturation of the media around the enamel surface [24]. Shlueter et al., 2016 [24] evaluated the impact of erosive factors (concentration, pH and movement type of acid) on the dimension of tissue loss and on efficacy of active agents used as anti-erosive/anti-abrasive therapeutics. The authors observed that dynamic conditions play an important role in terms of the magnitude of tissue loss. During demineralization, even assuming a mild movement, it leads to an exchange of the acid solution, providing new hydrogen ions that increase the rate of enamel dissolution.

Although non-fluoridated dentifrices promote higher wear on eroded enamel than fluoridated dentifrice [33], and the use of the latter is closer to clinical conditions [9], the present investigation used a Placebo dentifrice (non-F) slurry to perform toothbrushing on the specimens considering the importance to evaluate the effects of the gels alone, and not the combination of treatments (F dentifrice + gel). For ERO+ABR, the lowest enamel wear was observed for 5% Nano when compared with all groups. Brushing with a non-F dentifrice significantly increased tissue loss in all groups, and a relative protective effect was only observed for both gels supplemented with nano-sized TMP, since no significant difference was observed among 4500F, 9000F, 5% Micro and Acid gel when compared with Placebo.

As for SH analysis, contradictory findings were observed in the literature compared to this investigation. In the present study, ERO promoted significant higher enamel softening than Ero+Abr, which is in line with studies assessing the effects of a TMP-containing dentifrice [14] and varnishes [15] on ERO and ERO+ABR. On the other hand, an inverse trend (*i.e.*, lower SH values were observed for ERO+ABR) in a study assessing the effects of a low-F gel supplemented with sodium hexametaphosphate on ERO and ERO+ABR [19]. At first glance, in advanced stages of erosion it was expected that a marked enamel softening would be observed by the sum of ERO and ABR; however it should be considered that the softened enamel caused by the erosive challenge was mechanically removed during toothbrushing (ABR), thus exposing a more mineralized (therefore, harder) enamel. This may also explain the different pattern observed for the Placebo gel, which showed the highest enamel wear and the highest surface hardness after ERO+ABR. Since this trend had not been previously observed, studies with different protocols could be useful in elucidating this point. Considering the softening caused by enamel dissolution after erosive challenges, surface hardness analysis is a technique widely used to investigate mineral changes in erosion models. Despite surface hardness measurement is a simple method, it is not suitable for highly eroded surfaces, in which the indentations are not clearly defined, thus precluding the measurement [24,34].

In contrast with the pattern observed for SH, ∆KHN showed a clearer dose-response relationship amongst the non-supplemented groups (Placebo > 4500F > 9000F = Acid) for ERO. In addition, among TMP groups the 2.5% Nano had a marked effect on ∆KHN after ERO, being ~46% lower than Micro 5% and Nano 5% groups, and ~71% lower when compared to its counterpart without TMP (4500F). Although 9000F and Acid gel had two- and three-fold higher F concentrations, respectively, than TMP supplemented gels, the ∆KHN for Nano 2.5% was twice as low. Moreover, despite the ERO+ABR challenge was more aggressive, the TMP groups (2.5% Nano, 5% Nano and 5% Micro) were able to remain similar to high fluoride concentration groups (9000F and Acid). For ERO+ABR, Placebo and 4500F did not differ between them and the remaining groups were similar to each other (Placebo = 4500F < 9000F = Nano 2.5% = Nano 5% = Micro 5% = Acid).

A substantial number of methodologies both *in vitro* and *in situ* have been proposed, and these are useful tools for the study of erosion at different stages [35,36]. Authors recommend variables (type of dental hard tissue, duration of the cycles, type of demineralization solution, brushing force, type of toothpaste, association or not with toothbrushing, use of artificial/natural saliva) to be considered in erosion/abrasion protocols, in order to conduct the study in feasible and practicable ways. These variables are important for the standardization of methodologies, which would facilitate comparison of study outcomes [9].

In general, considering all the variables analyzed, both gels supplemented with TMPnano showed the most promising results, especially the 5% Nano, which presented the greatest preventive effect for enamel under erosive conditions, followed by abrasion by brushing, when compared with micrometric TMP.

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Table 1 – Mean (SD) values of wear, percentage of surface hardness loss (%SHL), and integrated hardness loss in depth of the enamel (∆KHN) according to conditions of challenge (Ero and Ero+Abr) and groups

Groups	Wear(µm)		%SHL (kgf/mm^2)		∆KHN (Kgf/mm^2 x µm)	
	Ero^{A}	Ero+Abr^{B}	Ero^{A}	Ero+Abr^{B}	Ero	Ero+Abr
Placebo	11.5(1.1)^{a}	13.3(1.9)^{a}	-93.3(1.3)^{a}	-71.6(4.2)^{a}	2254.7 (485.6)^{A,a}	2004.2 (560.0)^{A,a}
4500F	10.3(0.8)^{b}	12.8(1.5)^{a,b}	-92.4(1.8)^{a,b}	-77.3(4.7)^{b,d}	1513.9(366.2)^{A,b}	1830.9(589.5)^{A,a}
9000F	9.6(0.8)^{b,c}	12.1(1.3)^{a,b}	-90.1(1.8)^{b,d}	-72.3(4.3)^{a,c,d}	1165.2(442.7)^{A,b,c}	698.6(225.3)^{B,b}
2.5% Nano	8.8(0.9)^{c}	12.0(1.4)^{b}	-86.2(1.9)^{c,d}	-75.0(4.5)^{c,d}	437.1(225.1)^{A,d}	484.6(247.0)^{A,b}
5% Nano	8.9(1.1)^{c}	10.6(1.1)^{c}	-90.5(2.2)^{b,d}	-78.7(4.2)^{b}	811.3(302.0)^{A,c}	822.3(356.0)^{A,b}
5% Micro	9.5(0.8)^{b,c}	12.9(1.8)^{a,b}	-87.7(3.0)^{d}	-76.4(3.9)^{b,c,d}	810.7(401.3)^{A,c}	492.4(184.6)^{A,b}
Acid gel	8.7(1.2)^{c}	12.5(1.8)^{a,b}	-91.2(2.2)^{a,b}	-75.1(3.4)^{d}	1191.1(591.6)^{A,b}	654.5(476.1)^{B,b}

Mean (SD), n = 10. Upper and lowercase letters indicate significant differences between challenges and among treatments, respectively (ANOVA 2-way, Fisher test; $p < 0.05$).

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

JOURNAL OF DENTISTRY
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[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
Reference to a website:
[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
Reference to a dataset:
[dataset] [5] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1.
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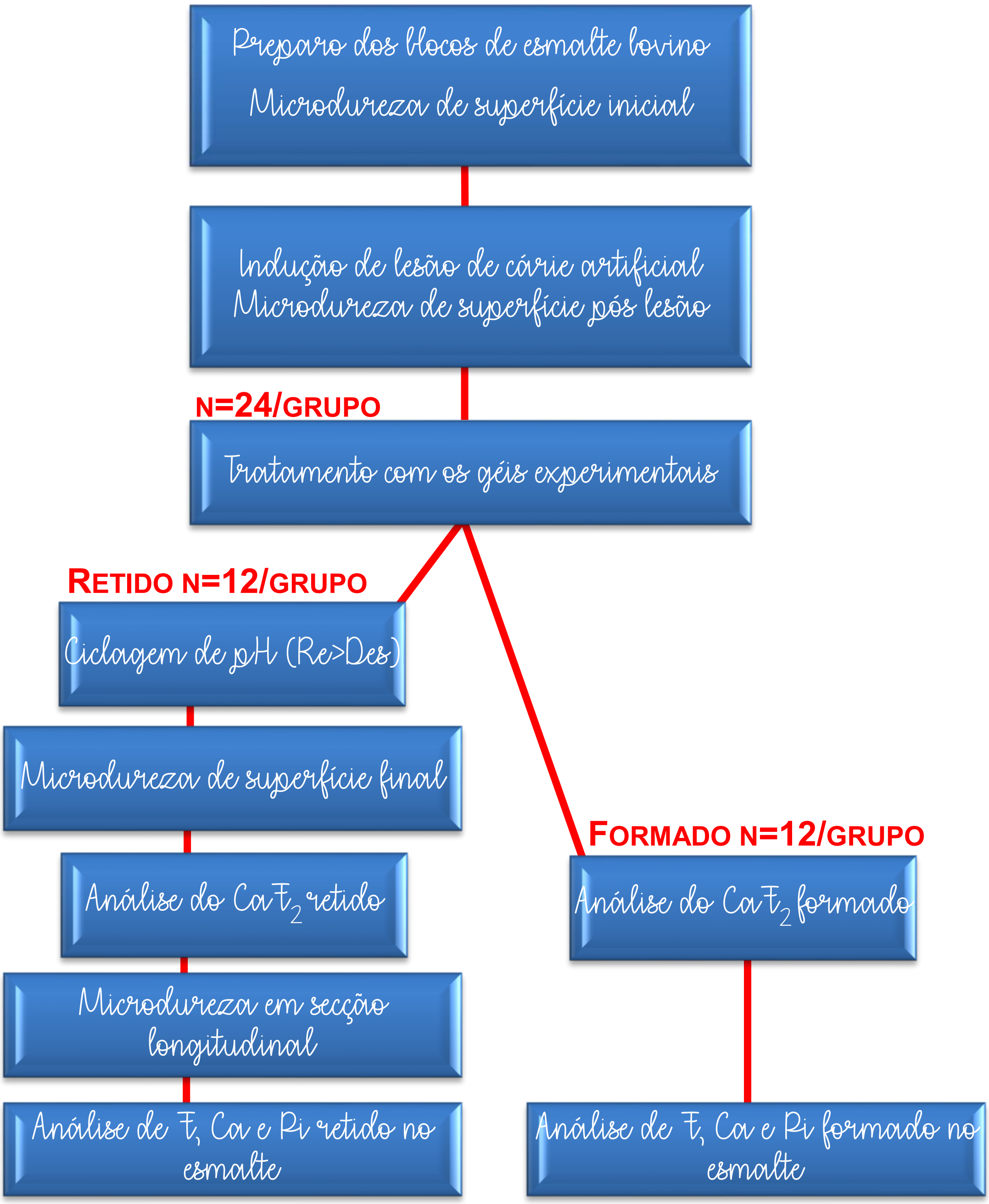
Anexo C

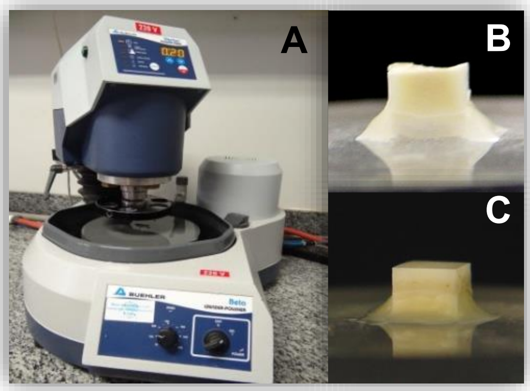
Esquema representativo da metodologia (Capítulo 1)



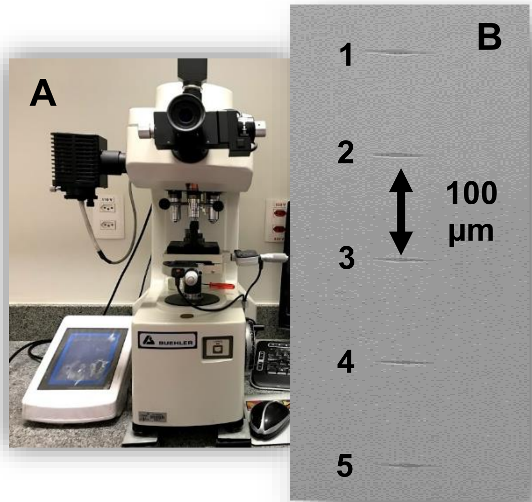
ANEXO D

PREPARO DOS BLOCOS DE ESMALTE

1. *Bloco de esmalte bovino de 4X4 mm, obtido após secção transversal e longitudinal em cortadeira*



2. *Blocos de esmalte fixados em base acrílica com cera pegajosa para polimento da dentina e esmalte. Politriz (A), dentina (B), esmalte (C)*

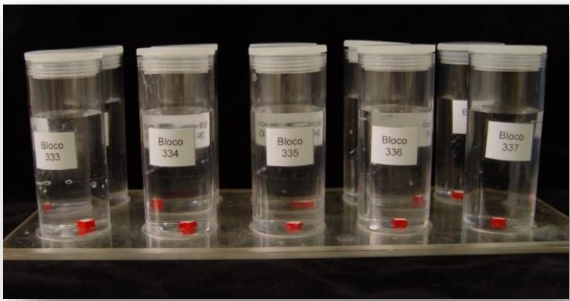


3. *Seleção inicial dos blocos por meio de microdureza de superfície (DS), 320 – 380 KHN, carga de 25 gramas por 10 segundos. (A) Microdurômetro, (B) Fotomicrografia das 5 impressões*

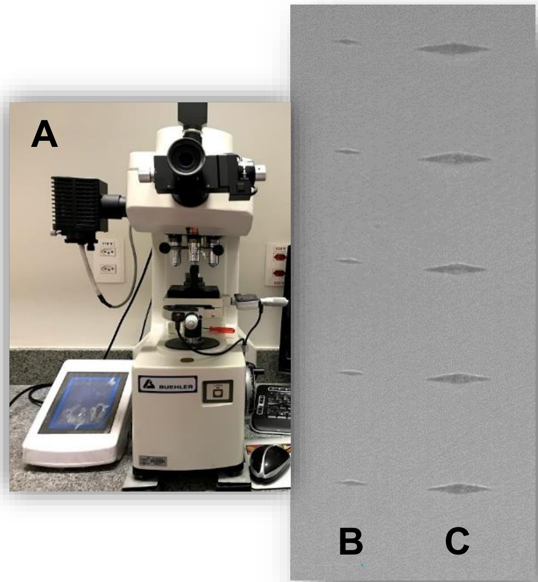
Anexo E

Indução de lesões de cárie artificial

4. Bloco de esmalte bovino protegido com esmalte cosmético



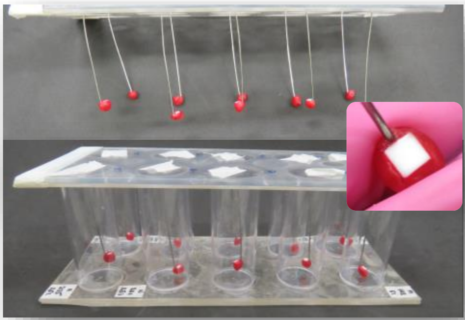
5. Blocos imersos em solução desmineralizadora por 16 horas, em estufa a 37 ºC



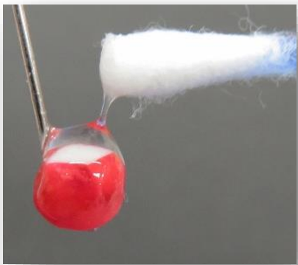
6. (A) Microdurômetro; (B) Fotomicrografia das 5 impressões iniciais e (C) Pós lesão de cárie

ANEXO F

CICLAGEM DE PH RE>DES



7. Blocos foram protegidos com cera rosa e fixados em hastes metálicas

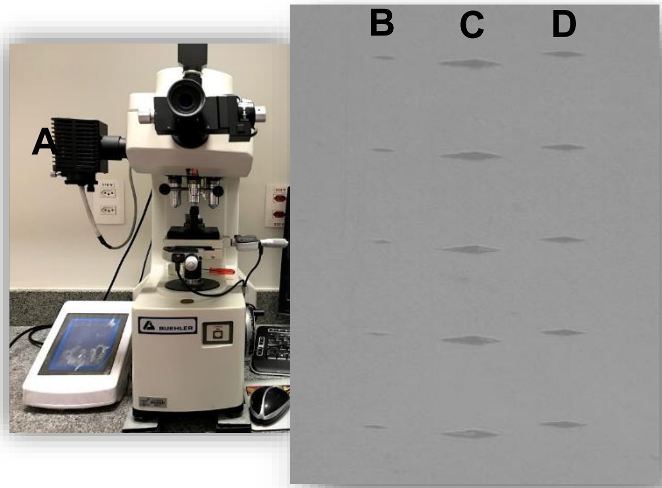


8. Tratamento com os géis experimentais por 1 minuto antes da ciclagem de pH

9. Esquema representativo da ciclagem de pH (Re>Des)

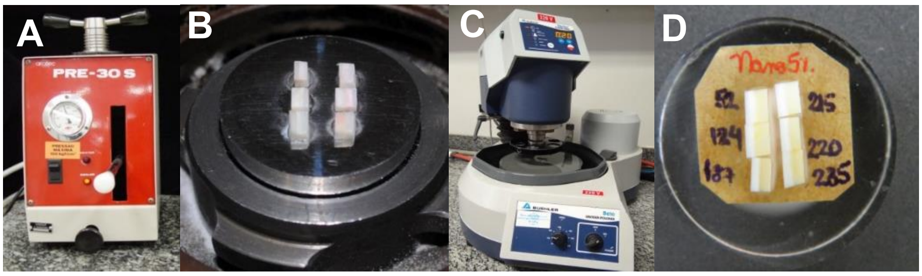
Anexo G

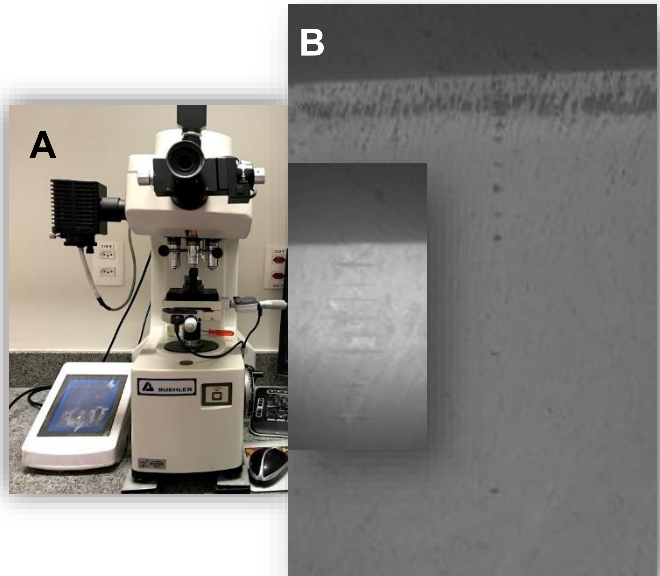
Microdureza Final Pós Ciclagem (Superfície/Secção Longitudinal)



10. (A) Microdurômetro; (B) Fotomicrografia das 5 impressões iniciais; (C) Pós lesão de cárie (D) Pós ciclagem de pH (Re>Des)

11. (A) Embutidora metalográfica; (B) Metade do bloco posicionado para inclusão em resina acrílica; (C) Polimento das bases na politriz; (D) Base acrílica polida para leitura em secção longitudinal

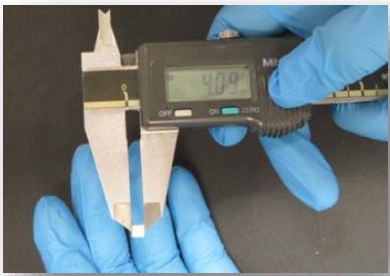




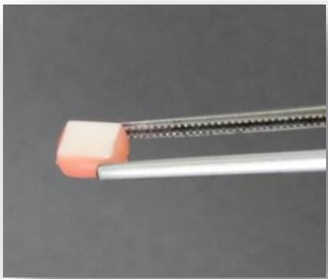
12. (A) Microdurômetro; (B) Fotomicrografia das 14 impressões em secção longitudinal (5 gramas por 10 s)

ANEXO H

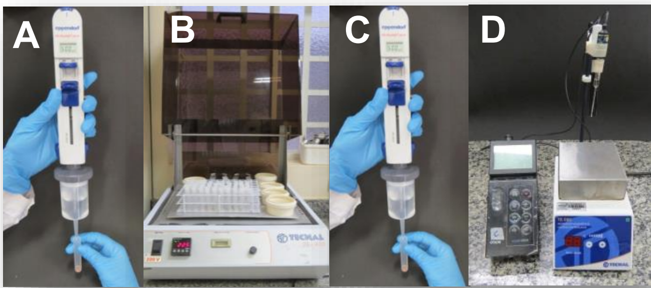
ANÁLISE DA CONCENTRAÇÃO DE CaF_2 NO ESMALTE (FLÚOR FRACAMENTE LIGADO)



13. Blocos foram medidos para determinação da área superficial do esmalte



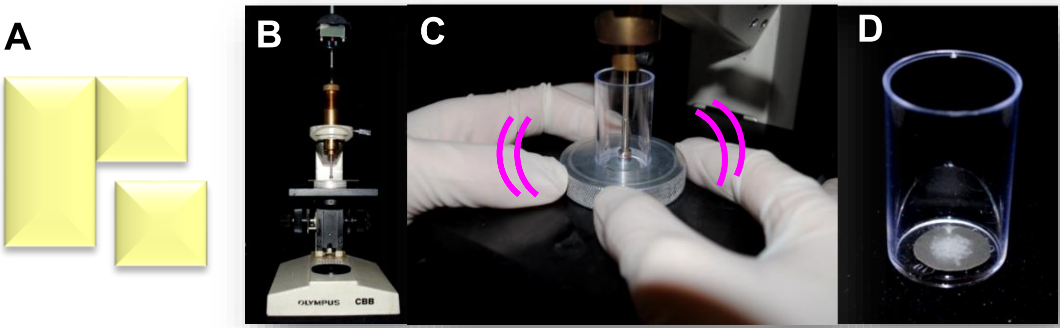
14. Proteção com cera rosa de todas as superfícies exceto da superfície de esmalte



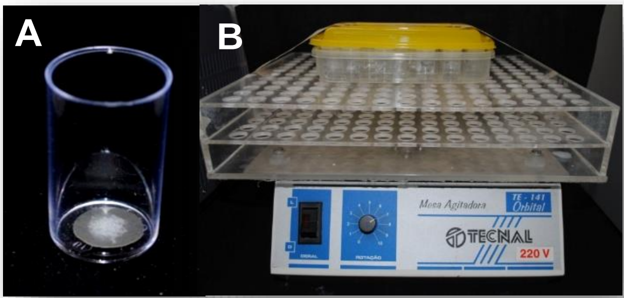
15. (A) Adição de 0,5 mL de HCl, agitação por 1h
(B) Agitação por 1 h
(C) Adição de 0,5 mL de TISAB II
(D) Leitura das amostras com analisador de íons e eletrodo íon específico

ANEXO I

DETERMINAÇÃO DO F (FLÚOR FORTEMENTE LIGADO), CA E PI NO ESMALTE

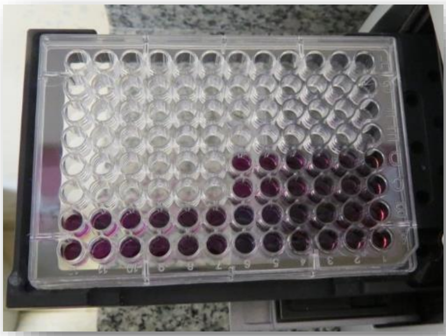


16. (A) Secção do bloco para obter amostra de 2x2 mm
(B) Mandril para peça reta montado em um microscópio modificado com um micrometro
(C) Desgaste de 50 µm da superfície de esmalte para análise de F, Ca e Pi
(D) Após desgaste, pó de esmalte presente na lixa adaptada em frascos de poliestireno cristal

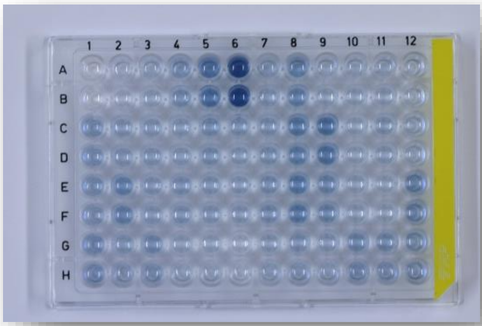


17. (A) Adição de 0,5 mL de HCl
(B) Agitação por 1h

18. Para leitura de F pipetar 0,25 mL da amostra + 0,25 mL de TISAB II modrificado por NaOH. Leitura das amostras com analisador de íons e eletrodo íon específico



19. A concentração de Ca no esmalte foi determinada através de método do Arsenazo III

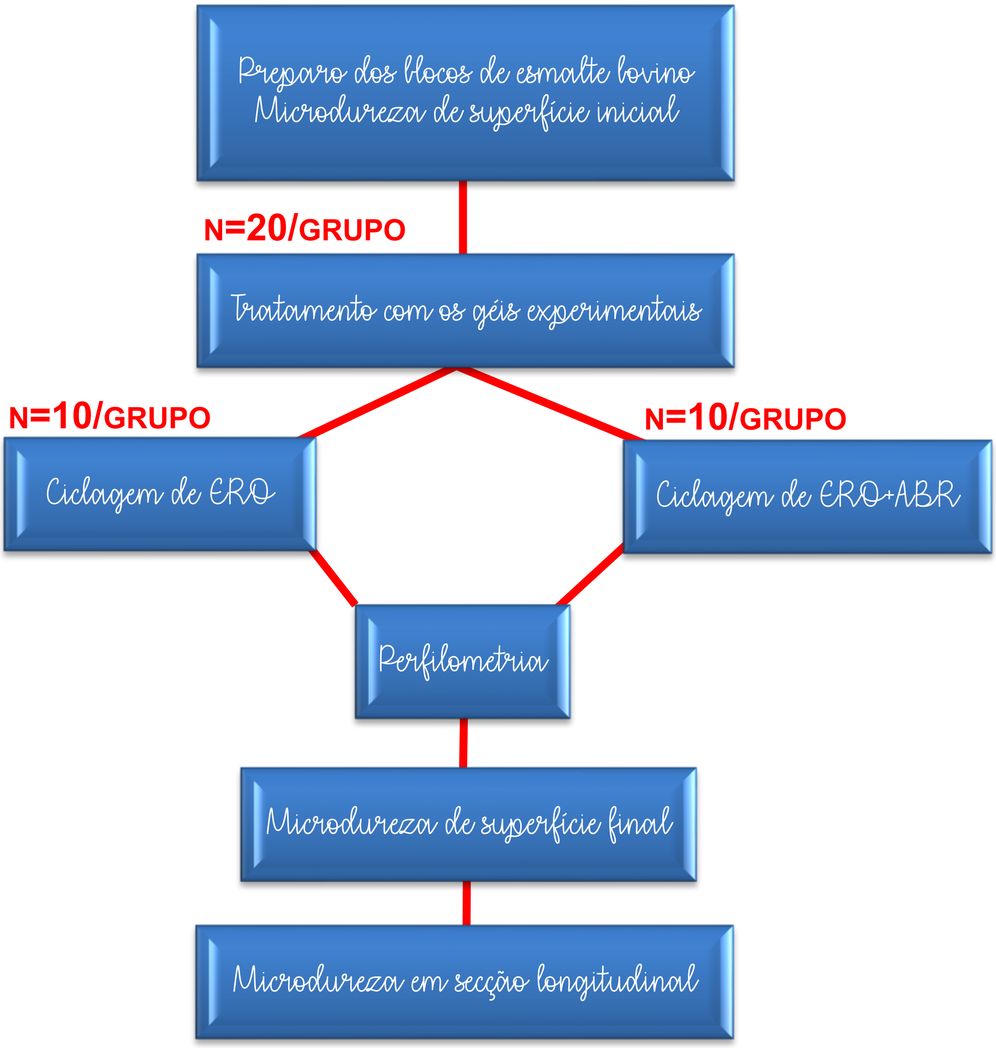


20. A concentração de Pi no esmalte foi determinada através de método colorimétrico

21. As leituras de absorbância na análise da concentração de Ca e Pi no esmalte foram realizadas em leitor de placas

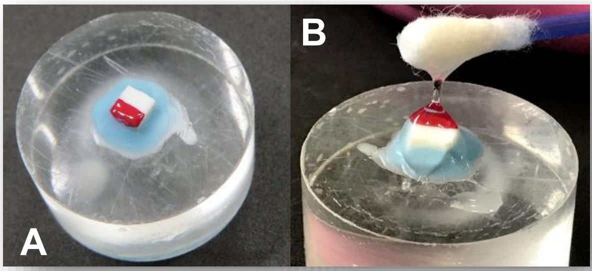
ANEXO J

ESQUEMA REPRESENTATIVO DA METODOLOGIA (CAPÍTULO 2)

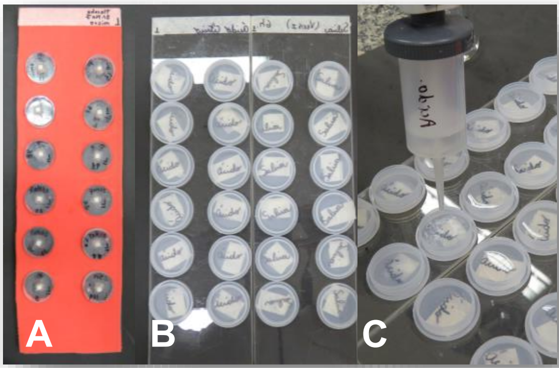


ANEXO K

CICLAGEM DE EROSÃO EROSÃO/ABRASÃO

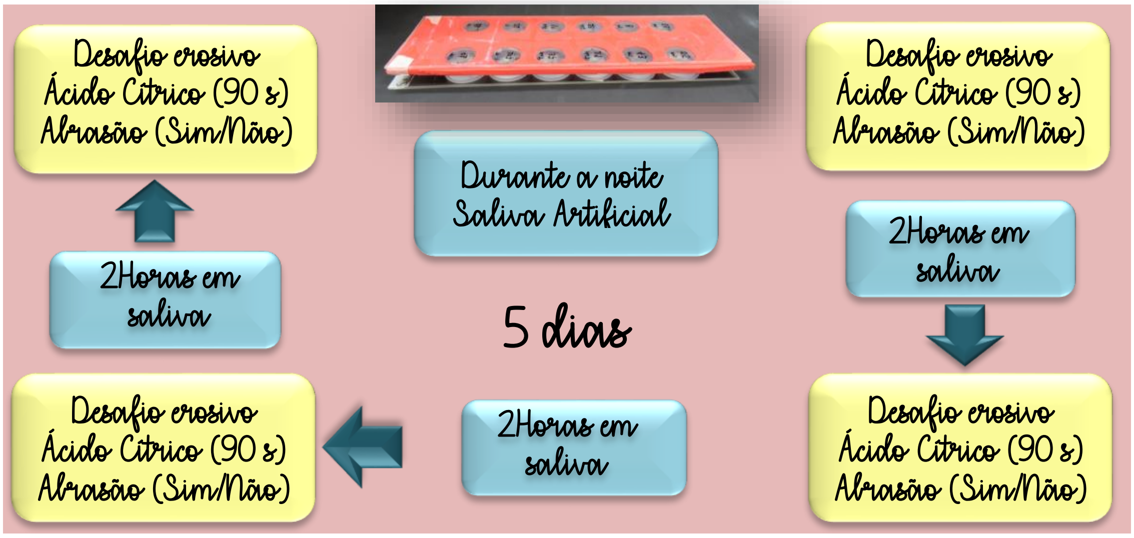


22. (A) Bloco de esmalte fixado em base acrílica. Metade da superfície do bloco protegida com esmalte ácido-resistente (área controle); (B) Aplicação dos géis de tratamento



23. (A) Dispositivo para fixar as bases acrílicas; (B) Dispositivo com recipientes para o desafio erosivo com ácido cítrico e manutenção em saliva artificial; (C) Pipetagem de 4 mL de ácido e saliva

24. Esquema representativo da ciclagem de erosão, erosão/abrasão



ANEXO L

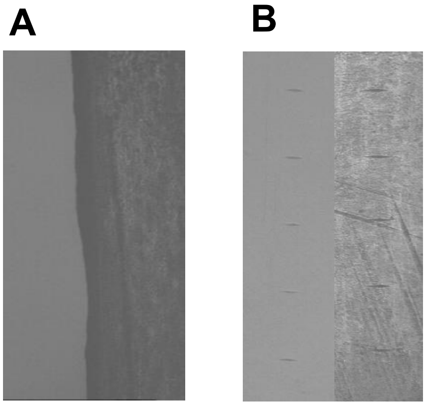
PERFILOMETRIA

25. Rugosímetro adaptado para perfilometria (Programa Surfpak – Surftest Mitutoyo)

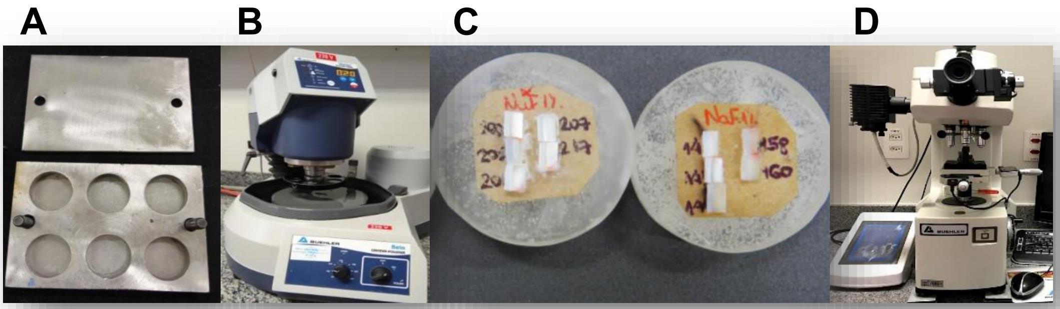
26. Cinco varreduras são feitas da área hígida do esmalte para área erodida

ANEXO M

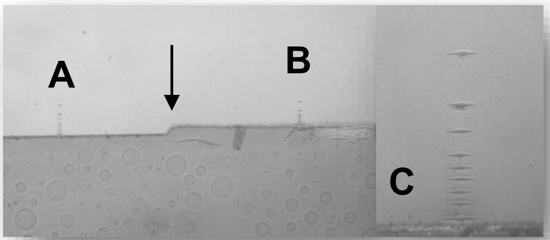
MICRODUREZA FINAL PÓS CICLAGEM EROSÃO EROSÃO/ABRASÃO (SUPERFÍCIE/SECÇÃO LONGITUDINAL)



27. (A) Fotomicrografia do esmalte hígido (lado esquerdo) e erodido (lado direito); (B) Fotomicrografia das impressões iniciais (lado esquerdo) e finais (lado direito)



28. (A) Matriz de metal para inclusão em resina acrília à frio (B) Polimento das bases acrílicas (C) Dentes inclusos em base acrílica (D) Microdurômetro



29. Fotomicrografia das (A) impressões em área hígida e (B) no esmalte erodido; (Seta) Degrau formado entre área hígida e erodida (C) Fotomicrografia das nove impressões