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Efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio sobre desgaste erosivo da dentina e energia livre de superfície do esmalte

Araçatuba
2024

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Tese apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista (Unesp), como parte dos requisitos para a obtenção do título de Doutor em Ciências, área de concentração Saúde Bucal da Criança.

Orientador: Prof. Assoc. Juliano Pelim Pessan
Coorientadora: Profa. Dra. Liliana Carolina Báez-Quintero

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“Bom mesmo é ir **à luta com determinação,** ***abraçar a vida com paixão, perder com classe e vencer com ousadia, pois o triunfo pertence a quem se atreve...”***

Charles Chaplin

RESUMO

FABREGAT, B. D. **Efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio sobre desgaste erosivo da dentina e energia livre de superfície do esmalte.** 2024. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

O presente estudo *in vitro* avaliou o efeito de géis fluoretados suplementados com nanopartículas de trimetafosfato de sódio (TMP) sobre a energia livre de superfície (ELS) do esmalte e o desgaste erosivo da dentina (DED) da dentina. Dentes bovinos foram seccionados em coroa e raiz, e armazenados até o início do experimento em soluções de formol 2% e timol 0,1% respectivamente. Discos de esmalte (*n*=10/8 grupos) e blocos de dentina (*n*=22/grupo, 7 grupos) foram preparados, selecionados por microdureza de superfície e aleatoriamente distribuídos entre os grupos de estudo. Os géis avaliados no estudo foram: Placebo (“PLA”, sem F ou TMP – controle negativo), 4,500 ppm F (“4500F”), 9,000 ppm F (“9000F”, controle positivo), Flúor fosfato acidulado 1,23% (“APF”, controle positivo comercial), 4500F + 5% TMP microparticulado (TMPmicro5), 4500F + 2,5% TMP nanoparticulado (TMPnano2.5) e 4500F + 5% TMP nanoparticulado (TMPnano5), os quais foram aplicados uma única vez (1 min) sobre os discos/blocos. No Capítulo 1, três líquidos de sondagem foram utilizados para determinação de oito parâmetros relacionados a ELS e hidrofobicidade/hidrofilia do esmalte em quatro momentos: no início do estudo, após a exposição por 2 h a saliva humana, após tratamento com os géis, e após 1 min de desafio erosivo com ácido cítrico. Os dados foram submetidos ao teste de Mann-Whitney e a ANOVA de medidas repetidas, dois fatores, seguida do teste de Tukey (p<0,05). No Capítulo 2, após tratamento com os géis, todos os blocos de dentina foram submetidos a desafios erosivos (ERO, ácido cítrico, 90 s), sendo que metade dos espécimes de cada grupo foram adicionalmente abrasionados (ERO+ABR, escovação mecânica, 15 s), totalizando 4 ciclos diários, durante 5 dias. O DED (perfilometria) e a perda de dureza integrada em profundidade (ΔKHN) foram avaliados. Os dados foram submetidos a ANOVA dois fatores, seguida do teste de Tukey, e teste de correlação de Spearman (p<0,05). No Capítulo 1, a ELS foi significativamente alterada após exposição à saliva, passando de hidrofóbica para levemente hidrofílica; o tratamento com gel tornou o esmalte ainda mais a hidrofílico. Após o desafio erosivo, a superfície do esmalte foi significativamente menos hidrofílica, sendo que os géis contendo TMP nanoparticulado exibiram os valores mais altos (hidrofílico) dentre todos os grupos. No Capítulo 2, para ERO, todos os géis suplementados com TMP reduziram significativamente o DED em comparação ao gel 4500F sem TMP, atingindo valores semelhantes ao grupo 9000F. Para ERO+ABR, o gel contendo 5% de TMP nanométrico levou a um DED significativamente menor do que todos os géis contendo 4500F, atingindo valores semelhantes aos dos grupos 9000F e APF. Quanto a ΔKHN, todos os géis contendo TMP promoveram menor perda mineral em profundidade em comparação ao grupo 4500F, atingindo valores semelhantes aos grupos 9000F e APF em ambos os desafios. Foi verificada uma correlação positiva entre DED e ΔKHN. Concluiu-se que géis contendo TMP, especialmente sob a forma de nanopartículas, promoveram maior resistência a alterações na hidrofilia do esmalte e contra o DED da dentina frente a desafios erosivos, trazendo novos dados acerca dos mecanismos de ação do TMP e F em coadministração sobre o desgaste erosivo do esmalte e da dentina.

Palavras-chave: erosão dentária; abrasão dentária; dentina; esmalte dentário; fluoretos; polifosfatos; nanopartículas.

ABSTRACT

FABREGAT, B. D. **Effect of fluoride gels supplemented with nanosized Sodium Trimetaphosphate on dentin erosive wear and enamel surface free energy.** 2024. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

The present *in vitro* study evaluated the effect of fluoride gels supplemented with sodium trimetaphosphate (TMP) nanoparticles on enamel surface free energy (SFE) and dentin erosive wear (DEW). Bovine teeth were sectioned into crown and root, and stored until the beginning of the experiment in solutions of 2% formaldehyde and 0.1% thymol, respectively. Enamel discs (n=10/8 groups) and dentin blocks (n=22/group, 7 groups) were prepared, selected by surface microhardness and randomly distributed among the study groups. The gels evaluated in the study were: Placebo ("PLA", without F or TMP – negative control), 4,500 ppm F ("4500F"), 9,000 ppm F ("9000F", positive control), 1.23% acidulated phosphate fluoride ("APF", commercial positive control), 4500F + 5% microparticulate TMP (TMPmicro5), 4500F + 2.5% nanoparticulate TMP (TMPnano2.5) and 4500F + 5% nanoparticulate TMP (TMPnano5), which were applied only once (1 min) on the discs/blocks. In Chapter 1, three probing liquids were used to determine eight parameters related to SFE and enamel hydrophobicity/hydrophilicity at four moments: at the beginning of the study, after exposure for 2 h to human saliva, after treatment with the gels, and after 1 min of erosive challenge with citric acid. Data were subjected to two-way, repeated-measures ANOVA, followed by Tukey's test, and by the Mann-Whitney's test ($p < 0.05$). In Chapter 2, after treatment with the gels, all dentin blocks were subjected to erosive challenges (ERO, citric acid, 90 s), with half of the specimens in each group being additionally abraded (ERO+ABR, mechanical brushing, 15 s), totaling 4 cycles/day, for 5 days. DEW (profilelometry) and depth-integrated hardness loss (ΔKHN) were evaluated. Data were subjected to two-way ANOVA, followed by Tukey's test, and by Spearman's correlation test ($p<0.05$). In Chapter 1, SFE was significantly altered after exposure to saliva, shifting from hydrophobic to slightly hydrophilic; gel treatment made the enamel even more hydrophilic. After the erosive challenge, the enamel surface was significantly less hydrophilic, with gels containing nanoparticulate TMP exhibiting the highest values (hydrophilic) among all groups. In Chapter 2, for ERO, all TMP-containing gels significantly reduced DEW compared to their TMP-free counterpart (4500F), reaching values similar to the 9000F group. For ERO+ABR, the TMPnano5 gel led to significantly lower DEW than all gels containing 4500F, reaching values similar to 9000F and APF groups. As for ΔKHN, gels containing TMP promoted significantly lower mineral loss in depth compared to the 4500F group, reaching values similar to the 9000F and APF groups under both challenges. A positive correlation was found between DEW and ΔKHN. It was concluded that gels containing TMP, especially as nanoparticles, promoted greater resistance to changes in enamel hydrophilicity and greater protection against dentin DED under erosive challenges, bringing new data about the mechanisms of action of TMP and F in co-administration on enamel and dentin erosive wear.

Keywords: tooth erosion; tooth abrasion; dentin; dental enamel; fluorides; polyphosphates; nanoparticles.

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

LISTA DE SIGLAS

2.5TMPn/ TMPnano2.5	TMP nanoparticulate at 2.5% + 4,500 ppm F *(TMP nanoparticulado a 2,5% + 4.500 ppm F)*
4500F	4,500 ppm F
5TMPm/ TMPmicro5	TMP microparticulate at 5% + 4,500 ppm F *(TMP microparticulado a 5% + 4.500 ppm F)*
5TMPn/ TMPnano5	TMP nanoparticulate at 5% + 4,500 ppm F *(TMP nanoparticulado a 5% + 4.500 ppm F)*
9000F	9,000 ppm F
APF	Acidulated phosphate fluoride *(flúor fosfato acidulado)*
baseline	Aferição ou análise nos espécimes previamente ao início do protocolo experimental
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
ERO	Desafio erosive
ERO+ABR	Desafio erosivo seguido de abrasão por escovação
ETW	Erosive tooth wear (*desgaste dental erosivo)*
F	Fluoride *(fluoreto)*
INPI	National Institute of Industrial Property *(Instituto Nacional de Propriedade intelectual)*
KHN	Surface microhardness *(microdureza de superfície)*
MMP	Matrix metalloproteinases *(metaloproteinases da matriz)*
NaF	Sodium fluoride *(Fluoreto de sódio)*
PLA	Placebo
SFE/ γS	Surface free energy *(energia livre de superfície)*
TMP	Sodium trimetaphosphate *(trimetafosfato de sódio)*
TMPm	TMP microparticulate *(TMP microparticulado)*
TMPn	TMP nanoparticulate *(TMP nanoparticulado)*
UNESP	Sao Paulo State University *(Universidade Estadual Paulista)*
$\gamma-$	Donor component (base)
$\gamma+$	Receptor component (acid)
γ^{AB}	Acid base interaction, polar component
γ^{LW}	Surface tension Lifshiz van der Waals, nonpolar component

$\Delta Gsws$	Free energy of interaction between the surface (s) and the water (w)
$\Delta Gsws^{AB}$	Free energy of interaction between the surface (s) and the water (w) for polar component.
$\Delta Gsws^{LW}$	Free energy of interaction between the surface (s) and the water (w) for nonpolar component
ΔKHN	Integrated hardness loss in depth *(perda integrada de dureza em profundidade)*

SUMÁRIO

1 INTRODUÇÃO GERAL*

Erosão dentária é definida como um processo químico que leva à perda de minerais da estrutura dentária, ocasionada pela exposição a ácidos não relacionados com o biofilme dental (Schlueter et al., 2020). Clinicamente, a erosão raramente atua como um processo isolado (Wiegand & Attin, 2011), uma vez que a abrasão dentária, processo físico causado quando friccionamos dentes, alimentos ou objetos na superfície da estrutural dentária, é frequentemente associada à erosão (Schlueter et al., 2020). Em termos gerais, a interação erosão-abrasão é considerada de substancial importância, dado que esta interação leva à uma condição de perda cumulativa de minerais da estrutura dentária, a qual é nomeada como desgaste dentário erosivo (DDE) (Wiegand & Attin, 2011; Schlueter et al., 2020).

Como principal fator associado ao DDE, destaca-se a exposição a ambientes com pH ácido provenientes dos meios extrínsecos (*i.e.*, alimentos e bebidas ácidas) ou intrínsecos (*i.e.*, refluxo gastroesofágico e distúrbios alimentares associados a vômitos). Tendo em vista modificações no estilo de vida e hábitos cotidianos na população em geral (Azzola, Fankhauser, & Srinivasan, 2023; Chatzidimitriou et al., 2023), tem-se observado uma crescente prevalência e incidência desta condição globalmente na população adulta, sendo que, com o passar da idade, padrões de aumento destes índices podem ser notados (Van't Spijker et al., 2009).

Embora a maioria dos estudos epidemiológicos sobre DDE envolva a população adulta, a crescente prevalência desta condição em crianças tem chamado a atenção de clínicos e pesquisadores, dado que a dentição decídua se mostra menos resistente que os dentes permanentes, possivelmente devido a características histológicas (*i.e.*, esmalte mais poroso e menos mineralizado que os permanentes), o que pode acarretar em uma maior suscetibilidade ao amolecimento (Carvalho et al., 2014). O DDE inicia-se no esmalte dentário, podendo progredir rapidamente para o tecido dentinário dependendo da intensidade do desafio erosivo e forças mecânicas associadas. Com essa progressão, a exposição da dentina a ácidos leva à dissolução mineral mais intensa e rápida em comparação ao esmalte, situação na qual tanto a dentina peritubular quanto a intertubular é inicialmente desmineralizada a taxas semelhantes, havendo maior dissolução da dentina peritubular com a continuidade da exposição a ácidos (Lussi et al., 2011). Com a exposição ácida contínua, o pH baixo leva à ativação de enzimas proteolíticas derivadas do hospedeiro, denominadas metaloproteinases de matriz (MMPs) (Buzalaf, Kato, & Hannas, 2012; Kato et al., 2014). Após a neutralização do microambiente

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* Lista de Referências – Apêndice A

devido à capacidade tampão do colágeno da matriz, além de tampões salivares (Lussi et al., 2011; Buzalaf, Kato, & Hannas, 2012), ocorre a degradação da matriz, consequentemente aumentando a taxa de dissolução da dentina (Lussi et al., 2011). Os dados supracitados evidenciam que, apesar do esmalte apresentar semelhanças químicas à dentina, a progressão do DDE nestes tecidos ocorre de maneiras distintas, de forma que resultados obtidos em estudos utilizando esmalte dentário não podem ser diretamente extrapolados para o tecido dentinário, e vice-versa.

Considerando as evidências sobre os efeitos preventivos e terapêuticos dos fluoretos (F) no controle da cárie dentária, e partindo-se do princípio de que tanto a cárie dentária e DDE resultarem de um desequilíbrio na dinâmica mineral dos tecidos dentários, inicialmente acreditou-se que a utilização de terapias com F fossem também efetivas na prevenção e controle do DDE. Entretanto, as evidências científicas disponíveis para atestar a eficácia do F na proteção contra o DDE são limitadas (Zanatta et al., 2020). De fato, resultados de uma metanálise em rede demonstraram que o fluoreto de sódio não é capaz de proteger o esmalte contra o desgaste dentário erosivo comparado a produtos sem fluoreto (Silva et al., 2022). A principal razão para esses achados é que a camada de fluoreto de cálcio (CaF_2) formada sobre os tecidos dentários após exposição ao F é susceptível a rápida dissolução após severos desafios ácidos, proporcionando, assim, proteção limitada contra a erosão (Lussi & Carvalho, 2015). Nesse sentido, alternativas vêm sendo estudadas no intuito de suprir o efeito limitado do F sobre essa condição, as quais envolvem a utilização de fluoretos metálicos (*e.g.*, fluoreto estanhoso e tetrafluoreto de titânio), biovidros, produtos à base de cálcio e/ou fosfatos e ciclofosfatos).

Dentre as estratégias investigadas, a coadministração de F e ciclofosfatos cíclicos tem ganhado destaque, considerando a extensa evidência sobre sua ação sinérgica sobre os tecidos dentários, em modelos *in vitro*, *in situ* e *in vivo* para o estudo da cárie dentária, erosão dentária e hipersensibilidade dentinária (Moretto et al., 2013; Manarelli et al., 2014; Freire et al., 2016; Akabane et al., 2018; Favretto et al., 2018, 2021; Oliveira et al., 2023; Paiva et al., 2023). Especificamente sobre erosão dentária e DDE, a suplementação de veículos fluoretados de uso profissional (*e.g.*, vernizes e géis) ou autoaplicação (*e.g.*, dentifrícios e colutórios) com TMP tem se mostrado efetiva na potencialização dos efeitos do F no controle do DDE (Manarelli et al., 2011; Pancote et al., 2014; Danelon et al., 2018; Capalbo et al., 2020; Paiva et al., 2023).

Considerando a grande aplicabilidade de géis dentais na prática clínica, em função de seu baixo custo e aceitabilidade por parte dos pacientes, formulações de géis contendo TMP e F em concentrações mais baixas (*i.e.*, 4.500 ppm F) em relação a produtos convencionais (*i.e.*, 9.000 ppm F em géis neutros e 12.300 ppm F em formulações aciduladas) têm sido estudas, tanto em modelos de cárie como de erosão. A redução na concentração de F visa à redução do potencial de toxicidade aguda (dose potencialmente tóxica: 5 mg/Kg de peso corporal), o qual tem sido reportado especialmente em crianças de pequena idade em função de sua massa corporal reduzida (Shulman & Wells, 1997; Whitford, 2011). De forma geral, os estudos desenvolvidos envolvendo modelos de cárie dentária atestam efeitos preventivos e terapêuticos superiores de géis contendo 4.500 ppm F contendo TMP em comparação a géis de mesma concentração de F sem o polifosfato, atingindo efeitos semelhantes ou até mesmo superiores aos de formulações convencionais (Danelon et al., 2013, 2014; Nagata et al., 2023). Um padrão muito semelhante foi observado em estudos avaliando o efeito dos géis supracitados sobre o DDE, ou seja, o uso de gel contendo 4.500 ppm F e TMP superou os efeitos de géis convencionais (Pancote et al., 2014; Capalbo et al., 2020). Em acréscimo, o uso de nanopartículas de TMP evidenciou um potencial protetor ainda mais evidente, superando os efeitos do gel suplementado com TMP microparticulado (Capalbo et al., 2020), o que possivelmente se deve à maior reatividade de nanopartículas em comparação a partículas convencionais (micrométricas) (Jandt & Watts, 2020).

Embora o mecanismo de ação do TMP sobre a proteção do esmalte contra desafios cariogênicos ou erosivos ainda não seja completamente conhecido, estudo utilizando hidroxiapatita carbonatada (semelhante à encontrada nos tecidos dentários duros) demonstrou que a coadminstração de F e TMP interfere positivamente na incorporação de F sobre a mesma, o que reduz sua solubilidade, levando a um padrão de cristalinidade semelhante ao de hidroxiapatita pura (Amaral et al., 2018). Em acréscimo, estudo com biofilmes cariogênicos evidenciou que o tratamento com TMP e F em associação levou a aumentos significativos na concentração de F no fluido do biofilme, promovendo maior efeito tamponante em comparação ao F administrado sozinho, após exposição a sacarose (Cavazana et al., 2020).

Mais recentemente, a avaliação da energia livre de superfície (ELS) tem sido utilizada como ferramenta para fornecer dados sobre as interações físico-químicas entre superfícies e íons ou moléculas de uma determinada terapia (Neves et al., 2018; Nalin et al., 2021; Oliveira et al., 2022). A reatividade superficial é avaliada por SFE a partir de ângulos de contato formados por diferentes líquidos de sondagem em uma superfície sólida. Tal propriedade mostrou ter estreita relação com os processos de mineralização da estrutura dentária, em um estudo que avaliou os efeitos do tratamento com F e hexametafosfato de sódio, seguido da exposição do esmalte a soluções contendo cálcio e/ou fosfato (Neves et al., 2018). Quanto ao TMP, dois estudos avaliaram as alterações do ELS do esmalte (Nalin et al., 2021) e da dentina (Oliveira et al., 2022) após a administração deste polifosfato, isoladamente ou em associação com F, demonstrando que o TMP altera significativamente as propriedades da superfície do esmalte e sua capacidade de doar elétrons, o que favorece a atração de cátions para a superfície do esmalte.

Apesar das evidências promissoras de géis contendo F e TMP sobre o DDE citadas acima, ainda existem importantes lacunas acerca dos mecanismos de ação desta associação, bem como do efeito desta sobre diferentes tecidos. Dentre as ressalvas encontradas, destacam-se:

1. Ausência de estudos avaliando o efeito de géis contendo F e TMP sobre o DDE da dentina: as diferenças estruturais entre os tecidos dentários mineralizados não possibilita a extrapolação direta dos achados obtidos em esmalte para a dentina;
2. Ausência de evidência acerca da superioridade de nanopartículas em comparação a partículas convencionais (micropartículas) de TMP sobre o DDE em dentina: considerando a maior porosidade e presença de túbulos no tecido dentinário, é possível que o uso de micropartículas promovam efeito protetor máximo (*plateau*), sem efeito adicional do emprego de nanopartículas;
3. Necessidade de estudos adicionais sobre os mecanismos de ação do TMP associado ao F sobre o DDE no esmalte: As evidências reportadas no estudo de Nalin et al. (2021) utilizando ELS foram obtidas a partir da administração dos TMP em soluções aquosas e na ausência de F. Considerando a maior complexidade de formulações de géis em comparação a soluções aquosas, não é possível descartar possíveis interações dos componentes não ativos dos géis com os tecidos dentários mineralizados, o que poderia afetar os efeitos do TMP sobre a ELS. Em acréscimo, a coadministração de TMP e F, extensivamente reportada em estudos prévios, ainda não foi investigada sobre a ELS do esmalte;
4. Ausência de película adquirida do esmalte nos estudos de Nalin et al. (2021) e Oliveira et al. (2023): há evidência de que a presença de película salivar altera a ELS (Gironda et al., 2022). Tal alteração, por sua vez, pode afetar a interação de íons e moléculas com o tecido dentário e, consequentemente, a ELS resultante; e

5. Ausência de evidência sobre a ELS do esmalte tratado com F e TMP frente a um desafio erosivo: Até a presente data, os estudos avaliando alterações na ELS do esmalte ou da dentina em função do tratamento com F e TMP não incluíram desafios simulando alterações de pH decorrentes de desafios erosivos ou cariogênicos. Tais informações poderiam contribuir para um melhor entendimento não somente dos efeitos imediatos decorrentes da administração dos ativos, mas também de seus efeitos ao longo do tempo.

Com base no exposto, o presente estudo teve por objetivo responder as quatro questões (lacunas) supracitadas, por meio da avaliação do efeito de géis fluoretados suplementados com TMP microparticulado ou nanoparticulado, (1) sobre a ELS do esmalte na presença de película salivar; e (2) sobre o DDE da dentina. Para tanto, a presente tese foi dividida em dois capítulos distintos, conforme descrito abaixo:

1 Nanoparticulate sodium trimetaphosphate and fluoride in gels affect enamel surface free energy after erosive challenge *in vitro* (redigido de acordo com as normas do periódico *Caries Research*); e

2 Low-fluoride gels supplemented with nano-sized sodium trimetaphosphate reduce dentin erosive wear *in vitro* (redigido de acordo com as normas do periódico *Archives of Oral Biology*).

2 CAPÍTULO 1 - NANOPARTICULATE SODIUM TRIMETAPHOSPHATE AND FLUORIDE IN GELS AFFECT ENAMEL SURFACE FREE ENERGY AFTER EROSIVE CHALLENGE *IN VITRO*[†]

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2.1 Abstract

Aim: To evaluate the effects of sodium trimetaphosphate (TMP) and fluoride (F), alone or in association, on the surface free energy (SFE) of enamel coated with human salivary pellicle *in vitro*.

Methods: Bovine enamel discs (n=10/ group) were randomly allocated into seven treatment groups (gels): Placebo (without any actives), low-fluoride gels (4,500 ppm F – "4500F") supplemented or not with microparticulate TMP (5%) or nanoparticulate (2.5 or 5%) TMP, 9,000 ppm F (positive control), and 12,300 ppm F (acid gel, commercial control); a negative control group (*i.e.*, untreated enamel) was included. Discs were exposed to human saliva (2 h), treated with the gels (1 min) and subjected to a 1-min acid challenge. Three probing liquids were used to assess enamel SFE. Data were submitted to two-way, repeated-measures ANOVA followed by Tukey's test, and by Mann-Whitney's test ($p<0.05$).

Results: SFE was significantly altered after exposure to saliva, changing from hydrophobic to slightly hydrophilic; gel treatment further increased enamel hydrophilicity (*i.e.*, with electron-donor properties), without significant differences among the gels. After the erosive challenge, enamel surface became significantly less hydrophilic for all groups; the highest values were observed for both gels containing nanoparticulate TMP. As for the overall SFE, the best performance was achieved by the gel containing 5% nanometric TMP.

Conclusion: SFE of salivary-coated enamel was significantly influenced by the treatment gels, which promoted increases in hydrophilicity. Gels containing TMP, especially at nanoscale, promoted higher resistance to changes in hydrophilicity after an erosive challenge.

Keywords: Enamel; Nanoparticles; Polyphosphates; Sodium Fluoride; Tooth erosion.

2.2 Introduction

Despite the effects of fluoride (F) on dental caries control are supported by a large number of well-designed clinical trials [Agouropoulos et al., 2014; Yadav et al., 2019; Turska-Szybka et al., 2021], limited scientific evidence is available to attest its efficacy on the protection against erosive tooth wear (ETW) [Zanatta et al., 2020]. In fact, calcium fluoride (CaF_2) precipitates resulting from the application of F products are prone to dissolution after severe acid challenges, thus providing limited protection against erosion [Lussi and Carvalho, 2015]. This scenario has stimulated the search for alternative therapies to boost the effects of F against ETW.

In this context, the evidence attests the synergistic action of sodium trimetaphosphate (TMP) and F in erosion models, when applied in vehicles for professional use or self-application [Manarelli et al., 2011; Pancote et al., 2014; Amaral et al., 2018; Danelon et al., 2018; Capalbo et al., 2020; Paiva et al., 2023]. Furthermore, such effects were shown to be further enhanced by the use of nanoparticles, due to their greater reactivity compared with conventional (*i.e.*, micrometric) particles [Jandt and Watts, 2020]. Considering F gels, the addition of TMP to low-F formulations (1% sodium fluoride – NaF, 4,500 ppm F), either as microparticles or nanoparticles, resulted in a significantly higher protective effect against ETW compared with their TMP-free counterpart, but only the gel supplemented with nano-sized TMP promoted significantly lower ETW compared with a commercial acidic formulation (12,300 ppm F) [Capalbo et al., 2020].

Laboratory experiments with hydroxyapatite [Amaral et al., 2018] and biofilms [Cavazana et al., 2020] provided some evidence on the mechanisms by which TMP and F act on the tooth structures (*i.e.*, adsorption) and in the mineral dynamics (*i.e.*, absorption). More recently, the evaluation of surface free energy (SFE) has been used as a tool to provide data on the physicochemical interactions between surfaces and ions or molecules from a given therapy [Neves et al., 2018; Nalin et al., 2021; Oliveira et al., 2022]. Surface reactivity is evaluated by SFE from the contact angles formed by different probing liquids on a solid surface, and this property was shown to be closely linked to mineralization processes of the tooth structure in a study assessing the effects of F and sodium hexametaphosphate (HMP) [Neves et al., 2018]. As for TMP, two studies assessed the SFE changes of enamel [Nalin et al., 2021] and dentin [Oliveira et al., 2022] after the administration of this polyphosphate, alone or in association with F, demonstrating that TMP significantly alters enamel surface properties and its ability to donate electrons, which favors the attraction of cations.

It is noteworthy, however, that the analysis of SFE is still not widely used in erosion models, so that further studies on the topic could provide additional data on its usefulness to better understand surface changes both after the application of a therapeutic agent and after an erosive challenge. Furthermore, one important limitation of two aforementioned studies [Nalin et al., 2021; Oliveira et al., 2022] is the absence of the acquired enamel pellicle, which is known to greatly affect the interactions between tooth surfaces and ions/molecules [Gironda et al., 2022]. Also, both studies assessed the effects of TMP and F in aqueous solutions, which might differ from the effects of these actives when applied in more complex formulations, such as gels for professional applications. Finally, both studies assessed TMP administered as microparticles, so that the effects of nanometric TMP on enamel SFE remain unknown.

Based on the above, this study aimed to assess the effects of gels containing TMP and F, alone or in association, on the SFE of enamel coated with acquired salivary pellicle *in vitro*, both after treatment with the gels and after an erosive challenge. The null hypothesis tested was SFE would not be affected by TMP, regardless of the particle size, both after gel treatment and erosive challenge.

2.3 Methods

2.3.1 Experimental design, ethical aspects and sample size calculation

Bovine enamel discs were selected by surface hardness, and randomly allocated into were randomly allocated into seven treatment groups (gels): Placebo (without any actives), low-fluoride gels (4,500 ppm F – “4500F”) supplemented or not with microparticulate TMP (5%) or nanoparticulate TMP (2.5 or 5%), 9,000 ppm F (neutral pH), and 12,300 ppm F (acid gel); a negative control group (*i.e.*, untreated enamel) was included. Discs were exposed to human saliva (2 h) to form acquired enamel *pellicle*, treated with the gels (1 min) and subjected to a 1-min acid challenge. Three probing liquids were used to assess enamel SFE using an automatic goniometer at four different moments: prior to the beginning of the experiments (all 8 groups), after exposure to human saliva (negative control only), after treatment with the gels (all 7 test groups), and after an erosive challenge (all 7 test groups) (Figure 1). This *in vitro* study was approved by the IRB from *São Paulo* State University (Unesp), School of Dentistry, *Araçatuba*, Brazil, for experiments involving animals (Protocol 0486-2020[‡]) and humans (Protocol 67881223.5.0000.5420[§]). A pilot study (n = 4/group) was carried out to determine sample size, considering the minimum detectable mean difference between Group 3 and 4. Mean difference (4.7 mN/m), standard deviation (2.6 mN/m), alpha (0.05), power (0.80), and the number of

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[‡] Anexo A - Comissão de Ética no uso de Animais

[§] Anexo B - Parecer consubstanciado do CEP study groups (n=7) were informed, resulting in a sample size of ten discs per study group. The discs were included in the final study.

2.3.2 Preparation of Enamel Discs[**]

Bovine permanent incisors were collected and stored in formaldehyde solution at 2 % (pH 7.0) for 30 days, within room temperature [Delbem and Cury, 2002]. The flattest portion of the vestibular surface of the crowns were used for to obtain the enamel discs (Dinser, *São Paulo*, Brazil). The surfaces of the discs (area = 25.42 mm^2) were flattened using a Beta Grinder-Polisher (Buehler, Lake Bluff, Illinois, USA), silicon carbide grinding discs (30-5108-400, -600, -800, and -012, Buehler), and felt paper (Polishing Cloth, 40-7618, Buehler) with diamond suspension (Extec Corp., Enfield, CT, USA) [Paiva et al., 2023]. The discs were kept in an environment moistened with 2% formaldehyde solution at pH 7.0 [Vieira et al., 2005].

2.3.3 Gel formulation[††]

All gels were manufactured (Department of Preventive and Restorative Dentistry, School of Dentistry, Araçatuba, Sao Paulo State University, Brazil.) according as described by Nagata et al. [2023] using the same concentrations tested in enamel erosion study [Capalbo et al., 2020], and contained: carboxymethylcellulose (Sigma-Aldrich Co., St Louis, MO, USA), sodium saccharin (Vetec, *Duque de Caxias*, *Rio de Janeiro,* Brazil), glycerol (Merck, Darmstadt, Germany), mint oil flavoring (Synth, Brazil) and deionized water. The placebo gel ("PLA") was produced without the addition of F or TMP. Sodium fluoride (NaF, Merck, Germany) was used to prepare all gels containing 4,500 or 9,000 ppm F (positive control) (hereafter abbreviated as "4500F" and "9000F"). A gel containing 12,300 ppm F ("APF", acid gel, DFL Dental Products, Rio de Janeiro, Brazil) was used as a commercial control. TMP microparticulate (average size of 450 ± 250 nm, 70 g, $Na_3O_9P_3$, Aldrich, purity ≥95% CAS 7785- 84-4, Sigma-Aldrich Co., St. Louis, MO, USA) at 5% and TMP nanoparticulate (approximately 22.7 nm, Chemistry Institute of the Federal University of São Carlos) at 2.5 or 5% were added to a 4500F gel and comprised the three test gels (hereafter abbreviated as "TMPmicro5", "TMPnano2.5", and "TMPnano5", respectively)[‡‡]. The nanoparticles synthesis

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[**] Apêndice B - Preparação dos discos de esmalte

[††] Apêndice C - Preparação dos géis

[‡‡] Apêndice D - Síntese e Caracterização de nanopartículas de TMP

and characterization were described in a previous study [Emerenciano et al., 2018]. F concentrations and pH values were determined using the ion analyzer (Orion 720 A+; Orion Research Inc.). Approximately 100 mg of each gel was dissolved in 100 ml of deionized water, in triplicate. Each solution was analyzed in duplicate to assess F concentrations, after buffering with TISAB II added at 1:1 proportion [Delbem et al., 2003]. The pH was checked electrometrically for all gels[§§] [Nagata et al., 2023]. Mean fluoride concentrations (Standard Deviation) in the PLA, 4500F, 9000F, TMPmicro5, TMPnano5, TMPnano2.5, and APF gels averaged 29.6 (1.2); 4,215.8 (111.4); 8,721.6 (141.6); 4,736.1 (90.6); 4,568.3 (97.3); 4,071.6 (78.7); and 12,131.9 (126.3) ppm F, respectively. A neutral pH was determinated for all gels (6.4 ranging from 6.3 to 6.7), except for the APF (pH = 3.6).

2.3.4 Stimulated saliva collection[***]

Stimulated saliva samples were collected from healthy and non-smoking volunteers (n = 4). They were instructed to chew on a piece of PARAFILM® M (Sigma-Aldrich Co., St. Louis, MO, USA) and spit out all saliva formed into ice-chilled vials. Collections were performed in the morning, at least 2 h after eating or drinking anything (except water) [Baumann et al., 2016], and after thoroughly rinsing their mouth with water [Schipper et al., 2007]. The stimulated saliva pooled, centrifuged for 20 min, 4 °C and 4000 *g* [Baumann et al., 2016]. The supernatants were collected and divided into 8 aliquots of 5 ml and stored at -72 °C.

2.3.5 Surface free energy analysis

Enamel SFE (γS, mN/m) was analyzed by an automatic goniometer (DSA 100S, Krüss, Hamburg, Germany), using three probing liquids: di-iodomethane, water and ethylene glycol. The enamel surface was divided into three parts, each liquid (0.5 μl) was automatically dispensed on a different part of surface using a glass syringe and needle with 0.5 mm gauge. Each drop was measured 5 times during 5 s at 23 °C and relative humidity of 44% ± 6 [Nalin et al., 2021; Van der Mei et al., 2002; Harnett, Alderman and Wood, 2007]. The contact angles were measured using the images captured by a CCD camera and the tangent method (Drop Shape Analysis DSA4 Software, version 2.0–01, Krüss). The secondary parameters assessed of

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[§§] Apêndice E - Análise de flúor

[***] Apêndice F - Características dos voluntários e da saliva coletada

γS were acid base interaction (γ^{AB}; acid γ^{+}, receptor component, and base γ^{-}, donor component), and surface tension Lifshiz van der Waals (γ^{LW}, nonpolar component) [Van Oss, 1995; Chaudhury, 1996; Van der Mei et al., 2002; Knorr et al., 2005; Harnett, Alderman and Wood, 2007). In addition, free energy of interaction (ΔGsws, mJ m^{-2}) between the surface (s) and the water (w) was also calculated to determine the hydrophobicity (ΔGsws < 0) or hydrophilicity (ΔGsws > 0) properties of the enamel surface [Van Oss, 1995; Harnett, Alderman and Wood, 2007]. The $\Delta Gsws^{LW}$ and $\Delta Gsws^{AB}$ components were assessed as secondary parameters of hydrophobicity properties. All parameters were assessed initially (*baseline*), after human's saliva exposure (*pellicle*), after gel treatment exposure (*gel*), and after citric acid exposure (*erosion*).

2.3.6 Surface treatment[†††]

The enamel surface was rinsed with deionized water for 20 seconds and was air-dried for 45 min for obtain stable, water contact angles [Van der Mei et al., 2002]. The discs were initially evaluated (γS-*baseline*; ΔGsws-*baseline*) without any exposed treatment. Then, all discs were immersed in 0.4 mL of human saliva in individual containers at 37 °C, unstirred [Danelon et al., 2018], during 2 h, allowing the formation of the acquired enamel pellicle [Taira et al., 2018]. Following, only discs from the negative control group (*i.e.*, untreated) were air-dried for 45 min and evaluated for γS-*pellicle* and ΔGsws-*pellicle.* All remaining blocks (7 test groups) were gently dried with a paper towel (to remove excess of saliva), and received a single application of the gels (1 min), which were removed with deionized water for 20 s. Discs were then air-dried for 45 min prior to the determination of γS-*gel* and ΔGsws-*gel*. Finally, discs were subjected to an erosive challenge for 1 min [Baumann et al., 2017], which consisted of individual immersion in 1% citric acid (0.4 ml/disc), pH=3.6 (Synth, Brazil), under agitation at 70 rpm for 1 min at room temperature, followed by washing with deionized water for 20 s [Baumann et al., 2016]. Discs were again air-dried for 45 min, and γS-*erosion* and ΔGsws-*erosion* were assessed to complete the study protocol.

2.3.7 Statistical analysis

[†††] Apêndice G - Sequência do ensaio de energia livre de superfície

SFE (γS) and hydrophobicity/hydrophilicity properties (ΔGsws) were considered as the response variables, while treatment gels and condition of enamel surface (*i.e.*, *baseline*, *gel* and *erosion*) were considered as the variation factors. To assess the effects of gels and conditions of enamel surface, the data were submitted to two-way, repeated-measures ANOVA followed by Tukey's test. Mann-Whitney's test was used for the comparison between γS and ΔGsws from the negative control group (γS-*baseline* and γS-*pellicle*). Analyzes were performed using SigmaPlot software, version 12.0, assuming a 5% significance level.

2.4 Results

SFE (γS) was significantly influenced by the treatment gels ($F = 19.9$; $p < 0.001$) and condition of enamel surface ($F = 511.1$; $p < 0.001$), with significant interaction between the two factors ($F = 29.2$; $p < 0.001$). γS-*baseline* was lower than 30 mN/m for all groups, characteristic of hydrophobic surfaces. After treatment with the gels, γS-*gel* was significantly higher than γS-*baseline* ($p < 0.05$). Finally, after the erosive challenge (γS-*erosion*), groups treated with PLA, 4500F, TMPmicro5, and APF were significantly higher than the corresponding values f ($p < 0.05$) (Figure 2). As for the negative control group (*i.e.*, untreated), γS-*pellicle* (38.3 mN/m) was significantly higher than γS-*baseline* (26.2 mN/m) ($p<0.05$), so that the surface changed from hydrophobic to slightly hydrophilic (ΔGsws-*baseline* = - 3.6 mJ m^{-2}, and ΔGsws-*pellicle* = 1.2 mJ m^{-2}; $p = 0.910$) (Supplementary Material, Table 1).

In line with the SFE data, surface ΔGsws was significantly influenced by the treatment gels ($F = 85.1$; $p < 0.001$), and condition of enamel surface ($F = 4,594.5$; $p < 0.001$), with significant interactions between the two factors ($F = 100.2$; $p < 0.001$). Baseline enamel surface (*i.e.*, prior to exposure to saliva) had hydrophobic properties (ΔGsws < 0), in contrast with the values obtained after exposure to saliva and treatment with the gels (ΔGsws > 0), resulting in surfaces with hydrophilic characteristics ($p < 0.05$) (Figure 3). After the erosive challenge (ΔGsws-*erosion*), the surface became less hydrophilic than ΔGsws-*gel* for all groups ($p < 0.05$). Enamel treated with both gels containing nanometric TMP (TMPNano2.5 and TMPnano5) exhibited the highest ΔGsws-*erosion* values, followed by 9000F, TMP5micro, and APF, while the lowest values were observed for PLA and 4500F ($p < 0.05$) (Figure 3). The results of secondary parameters that contributed to the calculation of γS and ΔGsws are presented in the Supplementary Material[‡‡‡].

2.5 Discussion

The increased incidence and prevalence of ETW in the primary dentition [Gatt and Attard, 2019], along with the limited effects of conventional F therapies against tooth erosion, has encouraged the development of more effective and safe formulations for use in children. In this context, the addition of TMP to F vehicles intended for professional application has been shown to boost the effects of F against ETW, both under *in vitro* and *in situ* conditions [Pancote et al., 2014; Capalbo et al., 2020; Paiva et al., 2023]. In the present study, SFE was assessed to provide additional knowledge on the mechanisms by which TMP and F act synergistically when administered as low-F gels (*i.e.*, 4500F). Our data showed that SFE was significantly changed after the formation of the acquired enamel pellicle, both after gel treatment and after an erosive challenge, with significant differences among the gels after acid exposure, thus leading to the partial rejection of the study's null hypothesis.

Baseline SFE and a predominantly hydrophobic characteristic observed for the polished specimens prior to exposure to human saliva showed that enamel surface was not prone to ion absorption, in line with data from previous studies employing the same methodology [Neves et al., 2018; Nalin et al., 2021]. In those studies, exposure to aqueous solutions containing cyclophosphates (TMP or HMP) in absence of F led to hydrophilic surfaces which, in turn, greatly increased adsorption of Ca^{2+} and PO_4^{3-} ions from the subsequent exposure to Ca^{+2}/PO_4^{-3} solutions. While this evidence provided valuable information on the mechanisms by which TMP affects tooth mineral dynamics, it only refers to the direct interaction of TMP alone (*i.e.*, without F) and tooth enamel under *in vitro* conditions, which does not represent intraoral exposure.

In contrast, in the present study acquired enamel pellicle was allowed to form on the specimens prior to any treatment, given that salivary proteins are known promote changes in the enamel surface, which enables the attraction of ions and molecules from the oral environment [Buzalaf, Hannas, and Kato, 2012; Gironda et al., 2022]. Our data demonstrated that exposure to saliva significantly increased enamel SFE (from ~26 to ~38 mN/m), shifting

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[‡‡‡] Apêndice H - Material suplementar

the surface from hydrophobic to slightly hydrophilic, the latter being associated with greater mineral deposition compared with hydrophobic surfaces [Neves et al., 2018; Nalin et al., 2021; Oliveira et al., 2022). Interestingly, treatment with all gels promoted further increases in SFE, but to a smaller extent (average increment ~4 mN/m), clearly indicating that the pellicle exerted the most influence on enamel SFE. These results reinforce the importance of including salivary pellicle in studies assessing the effect of current or novel therapies intended to influence tooth mineral dynamics, to better represent their clinical application. Contrarily to data on SFE, the effects of salivary pellicle on hydrophilicity were small (average increase of 4.8 mJ m^{-2}) and not statistically significant, while treatment with the gels massively impacted enamel hydrophilicity (average increase of ~60 mJ m^{-2}). This suggests that enamel SFE and ΔGsws must be interpreted together for a better understanding of the changes in enamel surface. Finally, no significant differences were observed in SFE and ΔGsws after gel application on the saliva-coated enamel specimens, which was somehow unexpected based on previous studies involving the same gels used in the present investigation.

The implications of such findings need to be carefully addressed. Firstly, the lack of significant differences in SFE and ΔGsws after gel treatment might suggest that one or more components of the gels played the main role on both outcomes, so that any differences that might result from the actives could not be observed. Considering all ingredients of the basic gel formulation, the high content of negatively charged components (*e.g.*, carboxymethylcellulose and glycerol) seem to be the main responsible for the high hydrophilicity, as the surface became richer in electron-donor sites after gel application. In fact, it has been shown that treatment of enamel with gels [Kato et al., 2010] or solutions [Magalhães et al., 2009] resulted in different protein profile of salivary enamel pellicle, reinforcing the concept that non-active ingredients may also influence changes in enamel SFE and ΔGsws. In this sense, it could be expected that the influence of non-active ingredients would be slowly washed out from tooth surfaces due to salivary clearance, so that any influence of the actives on enamel SFE and ΔGsws might have been detected if time and clearance had been considered as variables in the present study.

The above-mentioned assumption is supported by the final set of results, related to SFE and ΔGsws after the erosive challenges. Overall, SFE significantly decreased for all groups treated with gels containing actives after the erosive challenges, except for 4500F, which behaved similarly to PLA, resulting in high enamel SFE (slightly higher than their counterparts after gel application). An opposite trend was observed for ΔGsws, which plummeted after PLA and 4500F application, while decreases for the other gels were less pronounced. From the trend above, it is likely that exposure to the acidic solution under agitation promoted intense clearance of the enamel surfaces, removing possible remnants of the negatively charged non-active compounds, making it possible to detect differences in SFE and ΔGsws due to the actives.

An important aspect deserves comment. The acid-base theory adopted in our study allows for the decomposition of SFE into polar and non-polar components [Van Oss, 1995; Harnett, Alderman and Wood, 2007], which help to explain the overall effect on SFE. For instance, the high SFE values observed for PLA and 4500F groups after erosive challenges were shown to be greatly influenced by a non-polar component, which is related to aspects not involving electron-donor (γ^-) or electron-receptor (γ^+) properties (Supplementary Material). The lesser influence of the polar component resulted in a surface with low ΔGsws values, characteristic of low electron-donor potential, which does not facilitate the deposition of positively charged ions or molecules. Conversely, the remaining groups were mostly influenced by the polar (negative) component (γ^-) [Van Oss, 1995; Harnett, Alderman and Wood, 2007]. As γ^- is characteristic of high electron-donor potential, it could be expected that, in the oral environment, treatment with the TMP-containing gels would significantly enhance deposition of salivary minerals compared with, similarly with *in vitro* data on enamel Ca^{2+}, PO_4^{-3}, F^- uptake after treatment with the same gels of the current study [Nagata et al., 2023].

As for the additional benefit of TMP nanoparticles over micrometric ones when administered in F gels on ETW [Capalbo et al., 2020], the present study provides further insights into the mechanisms involved. After the erosive challenge, both gels containing nanoparticulate TMP had significantly higher ΔGsws values compared to all the other gels, indicating that the products led to higher resistance to reductions in γ^-, thus maintaining its high ability to retain positively charged ions or molecules. Reasons for the superior effect of nanoparticles over micrometric ones have been extensively reported, being mainly associated to their higher surface-to-volume ratio, what provides more electrons for heat transfer, making them more reactive than micrometric particles [Joudeh and Linke, 2022], It may be hypothesized that the higher availability of electrons and consequent higher reactivity of nanoparticles were responsible for the higher resistance to reductions in enamel γ^- after the erosive challenge, justifying the higher protective effect of low-fluoride gels containing nanometric TMP against ETW, even after intensive and cumulative erosive/abrasive challenges [Capalbo et al., 2020].

As for the APF, its higher protective effect against ETW [Capalbo et al., 2020] was not associated with high ΔGsws values after the erosive challenge in the present study. In fact, ΔGsws values were the lowest among all gels containing any active, except for 4500F. Considering that application of APF promotes the deposition of large amount of CaF_2, it would be possible that Ca^{2+} ions released during the erosive challenge could retain into enamel electron-donor sites resulting from the gel application, thus justifying the final ΔGsws for the group treated with APF. Also, the enhanced enamel F uptake and CaF_2 deposition promoted by high F levels under low pH may be regarded as the main reasons for the higher performance of APF compared with all other gels [Nagata et al., 2023].

The above-mentioned considerations on the mineral dynamics could be supported by data on enamel F, Ca and P, as well as the ions released into the acid solution, which was not assessed in the present investigation, thus being a study limitation. Also, it should be pointed out that the assessment of enamel SFE demands a dry surface, which may have changed some properties of the acquired pellicle resulted from the dehydration and possible changes in protein quaternary structure and functions. Thus, the results should be interpreted with caution, in light with this methodological aspect. Within the limitations of this short-term, *in vitro* protocol, it can be concluded that enamel SFE is greatly influenced by salivary enamel pellicle, so that protocols assessing the effect of different formulations on enamel mineral dynamics should consider this factor to better mimic clinical application. Also, the superior effect of TMP-containing formulations against ETW reported in the literature is associated with changes in enamel SFE, especially ΔGsws, by promoting a surface with electron-donor properties. The higher reactivity of nanometric TMP was shown to enhance the resistance in ΔGsws changes (γ^- reduction). This provides further data on the mechanisms by which TMP, especially as nanoparticles, boost the effect of low-F gels against ETW.

Declaration of Competing Interest

The second and the last author have a patent for a product used in the study, by the National Institute of Industrial Property – INPI/SP, on April 11, 2017, under number C1 0801811-1.

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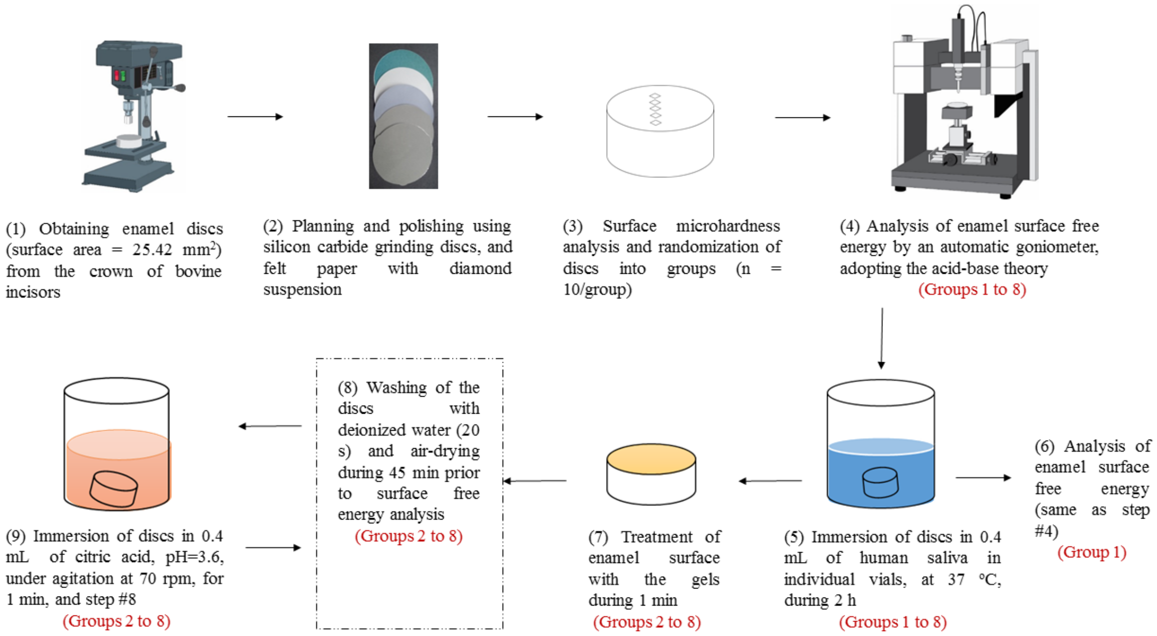

Fig. 1. Schematic flowchart summarizing the experimental design of the study. Group 1: Negative control; Group 2: Placebo (without any actives), Group 3: low-fluoride gel (4,500 ppm F, 4500F); Group 4: conventional neutral gel (9,000 ppm F); Group 5: 4500F + 5% microparticulate TMP; Group 6: 4500F + 2.5% nanoparticulate TMP; Group 7: 4500F + 5% nanoparticulate TMP; and Group 8: 12,300 ppm F (acid gel).

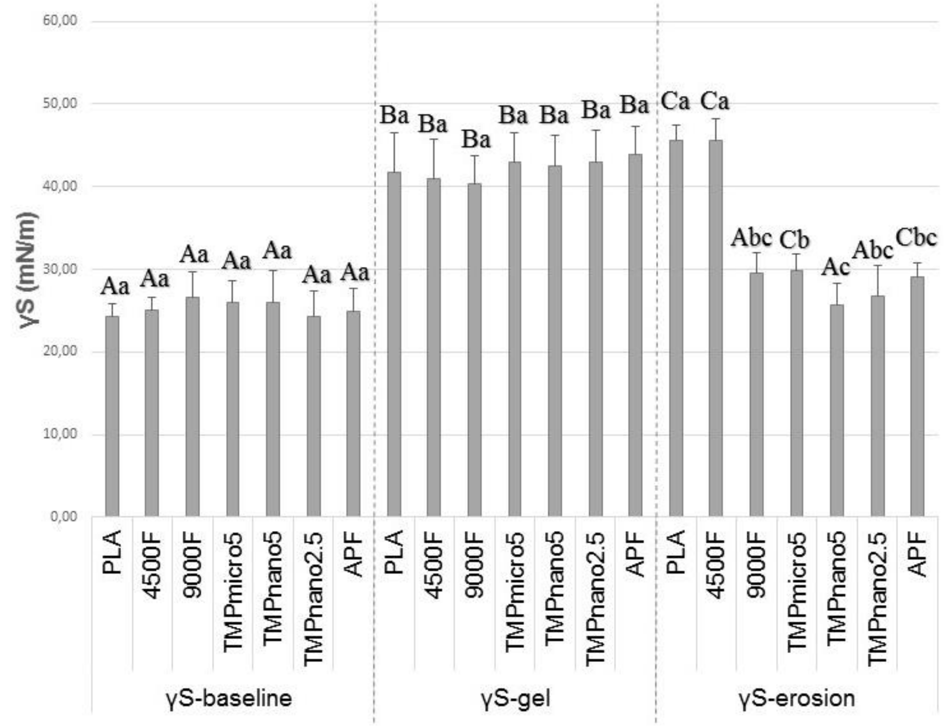

Fig. 2. Mean surface free energy (γS, mN/m) according to the test groups, prior to any exposure (γS-*baseline*), after salivary exposure and treatment with the gels (γS-*gel*), and after citric acid exposure (γS-*erosion*). Bars denote standard deviations. Different letters indicate significant differences among the conditions of enamel surface within each group (upper-case) and among the groups within each condition of enamel surface (lower-case). Two-way ANOVA and Tukey's test ($p < 0.05$, n = 10/group). Captions: PLA = placebo (with no actives); 4500F = 4,500 ppm F; 9000F = 9,000 ppm F; APF = 12,300 ppm F (acidulated); TMPmicro5 = 4500F + 5% micrometric TMP; TMPnano2.5 = 4500F + 2.5%TMP nanosized; TMPnano5 = 4500F + 5%TMP nanosized). TMP = sodium trimetaphosphate.

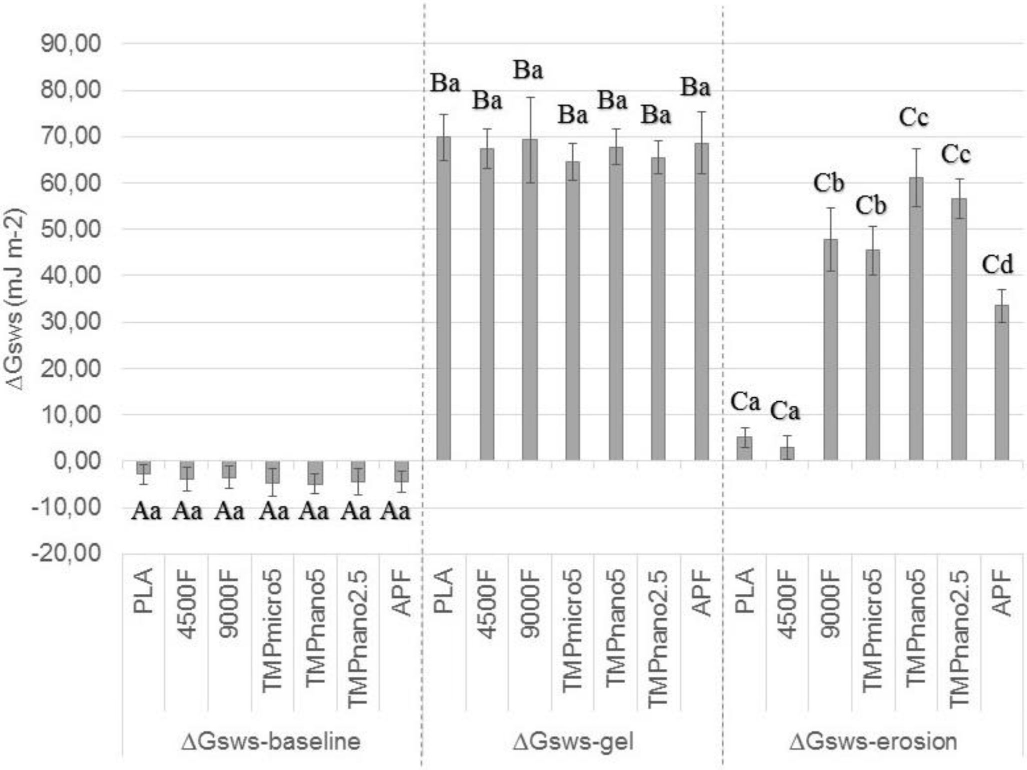

Fig. 3. Mean of the hydrophobicity properties (ΔGsws, mJ m^{-2}), according to the test groups, prior to any exposure (ΔGsws-*baseline*), after salivary exposure and treatment with the gels (ΔGsws-*gel*), and after citric acid exposure (ΔGsws-*erosion*). Bars denote standard deviations. Different letters indicate significant differences among the conditions of enamel surface within each group (upper-case) and among the groups within each condition of enamel surface (lower-case). Two-way ANOVA and Tukey's test ($p < 0.05$, n = 10/group). Two-way ANOVA and Tukey's test ($p < 0.05$, n = 10/group).
Captions: PLA = placebo (with no actives); 4500F = 4,500 ppm F; 9000F = 9,000 ppm F; APF = 12,300 ppm F (acidulated); TMPmicro5 = 4500F + 5% micrometric TMP; TMPnano2.5 = 4500F + 2.5%TMP nanosized; TMPnano5 = 4500F + 5%TMP nanosized). TMP = sodium trimetaphosphate.

Supplementary Material[§§§]

Table 1. Mean (Standard Deviation) of all parameters adopted to calculate enamel surface free energy (γS, mN/m) and hydrophobicity/hydrophilicity. Mean of secondary parameters assessed according to the test groups, prior to any exposure (*baseline*), after salivary exposure (*pellicle*) and treatment with the gels (*ge*l), and after citric acid exposure (*erosion*). Bars denote standard deviations. Different letters indicate significant differences among the conditions of enamel surface within each group (upper-case) and among the groups within each condition of enamel surface (lower-case). *Different between *baseline* and *pellicle* in untreated group. Two-way ANOVA and Tukey's test, and by Mann-Whitney's test ($p < 0.05$, n = 10/group).

Captions: Untreated = negative control group; PLA = placebo (with no actives); 4500F = 4,500 ppm F; 9000F = 9,000 ppm F; APF = 12,300 ppm F (acidulated); TMPmicro5 = 4500F + 5% micrometric TMP; TMPnano2.5 = 4500F + 2.5%TMP nanosized; TMPnano5 = 4500F + 5%TMP nanosized). TMP = sodium trimetaphosphate. γ^{LW}: surface tension Lifshiz van der Waals, nonpolar component; γ^{AB}: acid base interaction, polar component; γ+: receptor component (acid); γ−: donor component (base); $\Delta Gsws^{LW}$: free energy of interaction between the surface (s) and the water (w) for nonpolar component; and $\Delta Gsws^{AB}$: free energy of interaction between the surface (s) and the water (w) for polar component.

[§§§] Apêndice H - Material suplementar

3 CAPÍTULO 2 - LOW-FLUORIDE GELS SUPPLEMENTED WITH NANO-SIZED SODIUM TRIMETAPHOSPHATE REDUCE DENTIN EROSIVE WEAR *IN VITRO*[****]

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3.1 Abstract

Objective. This study assessed the effect of low-fluoride gels supplemented with micrometric (TMPm) or nano-sized (TMPn) sodium trimetaphosphate (TMP) on dentin erosive wear *in vitro*.

Design. Bovine dentin blocks (n=154) were selected by surface microhardness and randomly allocated into seven groups (n=22/group), according to the gels: Placebo (without TMP or F); 4,500 ppm F (4500F); 9,000 ppm F (9000F); 5% TMP microparticulate plus 4500F (5TMPm+4500F); 2.5% TMP nanoparticulate plus 4500F (2.5TMPn+4500F); 5% TMP nanoparticulate plus 4500F (5TMPn+4500F); and 12,300 ppm F acid gel (APF). All blocks were treated only once for 60 s and cyclically eroded (ERO, citric acid, 4×90 s/day) or eroded and brushed (4×15 s/day, five strokes/s, ERO+ABR) over five days (each subgroup n=11). Dentin wear and integrated hardness loss in depth (ΔKHN) were determined, and the data were submitted to two-way ANOVA, followed by Tukey's test, and Spearman's correlation ($p<0.05$).

Results. For ERO, all gels containing 4500F supplemented with TMP significantly reduced dentin wear compared with their counterpart without TMP, reaching values similar to 9000F. For ERO+ABR, 5TMPn+4500F gel led to significantly lower wear than all its counterparts, reaching values similar to 9000F and APF. As for ΔKHN, all gels containing TMP promoted superior protective effects compared with 4500F, reaching values similar to 9000F and APF under both challenges. A positive correlation between dentin wear and mineral content in depth was verified.

Conclusions. Gels containing 4500F supplemented with TMP significantly reduced dentin erosive wear compared with pure 4500F, with additional benefit from the use of nanoparticles.

Keywords: Dentin; Nanoparticles; Polyphosphates; Sodium Fluoride; Tooth erosion.

3.2 Introduction

Dental erosion is a process of chemical loss of mineralized tooth substance caused by the exposure to acids not derived from oral bacteria, while abrasion is a physical loss by objects other than teeth (Schlueter et al., 2020). These processes lead to a condition called erosive tooth wear, and dental erosion is the primary etiological factor (Schlueter et al., 2020). Current studies confirm the increase in the prevalence of erosive tooth wear affecting the child population (Kitasako et al., 2024; Pereira Cenci et al., 2023; Tschammler et al., 2016; Wiegand et al., 2006). One-third of preschool children suffer from this contidition, and digestive disorders and dietary factors are the main potential contributing factors (Yip, Lam, & Yiu, 2022).

The limited effect of conventional fluoridated formulations on preventing erosive tooth wear (Silva et al., 2022) has stimulated the search for alternatives to enhance the protective effects of fluoride products have been proposed, including the addition of phosphates, such as sodium trimetaphosphate (TMP). Previous studies demonstrated a synergistic effect of fluoride (F) and TMP on enamel erosive wear, in which low-F formulations containing TMP were shown to promote similar or superior protective effects compared with their counterparts containing twice as much F (Danelon et al., 2018; Manarelli et al., 2011; Moretto et al., 2013; Pancote et al., 2014).

Considering the widespread use of F gels in clinical practice, as well as their potential for acute toxicity, especially when applied to young children without appropriate safety measures (Whitford, 2011), emphasizes the need for formulations with lower F content while maintaining its preventive and therapeutic effects. In this sense, low-F gels containing TMP were shown to be more effective on enamel erosive wear than its TMP-free counterpart (Pancote et al., 2014), and such effects were further enhanced by the use of nanoparticles (Capalbo et al., 2020). Despite promising, these results cannot be directly extrapolated to dentin erosive wear, due to its lower mineral content, structural arrangement, and the existence of an organic matrix (Lussi & Carvalho, 2015), both of which could interfere with the mechanisms by which TMP acts in acid resistance. Thus, this study assessed the effects of low-fluoride gels supplemented with micrometric or nano-sized TMP on dentin erosive wear *in vitro*. The study's null hypotheses were that (1) dentin erosive wear and (2) dentin mineral content would not be affected by the addition of TMP, regardless of the particle size.

3.3 Methods

3.3.1 Experimental design and sample size calculation

The study was approved by the Ethics Committee for Animal Use (Protocol No. 0486-2020[††††]). Dentin blocks were randomly allocated into 7 groups (n = 22/group): Placebo (without TMP or F); 4,500 ppm F (4500F); 9,000 ppm F (9000F); 5% TMP microparticulate plus 4500F (5TMPm+4500F); 2.5% TMP nanoparticulate plus 4500F (2.5TMPn+4500F); 5% TMP nanoparticulate plus 4500F (5TMPn+4500F); and 12,300 ppm F acid gel (APF) (Capalbo et al., 2020). All blocks were treated only once for 60 s, and cyclically eroded (ERO, citric acid, 4×90 s/day) or eroded and brushed (4×15 s/day, 5 strokes/s, ERO+ABR) over 5 days (each subgroup n=11). Dentin erosive wear (profilometry analysis) and integrated hardness loss in depth (ΔKHN) were determined (Danelon et al., 2020; Capalbo et al., 2020; Pancote et al., 2014) (Figure 1).

A pilot study (n = 4/group) to determine sample size was performed, considering the minimum detectable mean difference between 4500F and 5TMPm+4500F gels on dentin erosive wear. Mean difference (1.6 µm), standard deviation (0.8 µm), alpha 0.05, beta 0.20, and a potential loss of 20% were parameters used, resulting in 11 blocks per treatment group for each condition (ERO or ERO+ABR).

3.3.2 Synthesis and characterization of nano-sized TMP particles[‡‡‡‡]

The nanoparticles were provided from a previous project in which the synthesis and characterization were described (Emerenciano et al., 2018). In brief, nano-sized TMP was obtained from microparticulate TMP (average size of 450 ± 250 nm, 70 g, $Na_3O_9P_3$, Aldrich, purity ≥95% CAS 7785- 84-4) by ball milling (500 g of zirconia spheres in 1 L of isopropanol) for 48 h. The material was filtered and sealed with aluminum foil, and the vials were dried at 75°C to evaporate isopropanol. The powder crystallinity was characterized by X-ray diffraction (the crystalline structure was maintained). Scanning electron microscopy (Philips XL-30 FEG) was used to determine the size of the nanoparticles (approximately 22.7 nm) (Emerenciano et al., 2018).

[††††] Anexo A - Comissão de Ética no uso de Animais

[‡‡‡‡] Apêndice D - Síntese e Caracterização de nanopartículas de TMP

3.3.3 Gel formulation and determination of fluoride and pH in products[§§§§]

Gels were performed in Department of Preventive and Restorative Dentistry, School of Dentistry, Araçatuba, Sao Paulo State University (UNESP), SP, Brazil. The following compounds were used as base formula for each gel (50 g): carboxymethylcellulose (4.00 g, Sigma-Aldrich Co., St. Louis, MO, USA), sodium saccharin (0.05 g, Vetec, Duque de Caxias, Rio de Janeiro, Brazil), glycerol (14.00 g, Merck, Darmstadt, Germany), and mint flavoring oil (0.25 g, Synth, Brazil), completing with deionized water. Formulations containing F in their form sodium fluoride (NaF, Merck, Germany) at concentrations of 4,500 (4500F) and 9,000 ppm F (9000F) were obtained by adding 0.4974 g and 0.9948 g NaF to base formula, respectively. Micrometric TMP (Sigma-Aldrich Co., St. Louis, MO, USA) was added at a concentration of 5% (2.5 g), and nano-sized TMP (synthesized and characterized at the Chemistry Institute of the Federal University of São Carlos, Brazil) was added at concentrations of 2.5% (1.25 g) or 5% (2.5 g) to the 4500F gel for obtaining to 5TMPm+4500F, 2.5TMPn+4500F or 5TMPn+4500F gels, respectively. A placebo gel was produced without NaF or TMP. In addition, an acidulated phosphate fluoride gel (APF, 12,300 ppm F, pH=3.6, *DFL Indústria e Comércio* S.A., *Rio de Janeiro*, RJ, Brazil) was used as a commercial positive control (Capalbo et al., 2020). F concentrations in the gels were determined using a specific electrode for ion F (9609 BN; Orion Research Inc., Beverly, Mass., USA), attached to an ion analyzer (Orion 720 A plus; Orion Research Inc.), previously calibrated[*****]. The pH was checked electrometrically for all gels using a pH analyzer (Orion 720 A plus; Orion Research Inc.) calibrated with standard solutions of pH 4.0 and 7.0 (Danelon et al., 2014).

3.3.4 Dentin specimens[†††††]

From bovine teeth, the crowns were separated from the roots, and the blocks were obtained from the root portion closest to the cervical region (approximately 1 to 2 mm up to the cementoenamel junction). Then, they were flattened and serially polished (Beta Grinder-Polisher, Buehler, Lake Bluff, Illinois, USA) using 400, 600, 800, and 1200-grade silicon

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[§§§§] Apêndice C - Preparação dos géis

[*****] Apêndice E - Análise de flúor

[†††††] Apêndice I - Preparação dos blocos de dentina

carbide paper discs (Buehler, 36-08-0400) under constant irrigation with water. Blocks were subsequently polished using a felt disk (Buehler Polishing Cloth 40–7618) and 1/4-µm diamond polishing suspension (Extec Corp., Enfield, CT) (Capalbo et al., 2022).

Dentin blocks were selected and randomly allocated by surface microhardness (as described below) into the 14 treatment subgroups. Half of the surface of each block was protected with acid-resistant nail varnish (control area) (Capalbo et al., 2022).

3.3.5 Erosive and abrasive cycling [‡‡‡‡‡]

A thin layer of each gel was applied using a cotton swab for 1 min, only once on all blocks (n = 22/group) on the first experimental day, subsequently removed with deionized water (20 s), and dried with absorbent paper. All blocks were treated only once for 60 s and cyclically eroded (ERO, citric acid, pH 3.2, 4×90 s/day, Synth, Brazil), for five consecutive days (Capalbo et al., 2022). After each erosive challenge, half of the blocks (n = 11) were immersed in a placebo dentifrice slurry (1 g of toothpaste: 3 mL of deionized water) for 15 s under agitation at 35 rpm at room temperature. For ERO+ABR, the other half of the blocks (n = 11) were subjected to brushing abrasion using a mechanical brushing machine (15s, 250 g axial load, five strokes/s; *Elquip Maq Escovação, São Carlos*, Brazil) and a placebo dentifrice (Danelon et al., 2020; Pancote et al., 2014). The composition of the placebo dentifrice[§§§§§] used has been previously described (Paiva et al., 2017). Blocks remained in artificial saliva (pH 7.0; without agitation at 37 ºC) for 2 h between erosive challenges, as well as overnight (Danelon et al., 2018). The artificial saliva was performed without F, containing 1.5 mmol/L $Ca(NO_3)_2 \cdot 4H_2O$, 0.9 mmol/L $NaH_2PO_4 \cdot 2H_2O$, 150 mmol/L KCl, and 0.1 mol/L Tris buffer (Danelon et al., 2018).

3.3.6 Profilometry analysis

Dentin wear was determined in relation to the reference surfaces by profilometry (Surftest SJ 401, Mitutoyo American Corporation). The tip of the profilometer was moved from the sound surface (control area) of the specimens over the treated area, and five readings were used to calculate the average wear for each dentin block (Capalbo et al., 2022; Manarelli et al., 2013).

[‡‡‡‡‡] Apêndice J - Sequência do ensaio de ciclagem

[§§§§§] Apêndice K - Componentes do dentifrício placebo

3.3.7 Analysis of surface microhardness and integrated hardness loss

Surface microhardness (KHN) was used for the selection and randomization of dentin blocks (Shimadzu HMV- 2000 microhardness tester, Shimadzu Corp., Kyoto, Japan) with a Knoop diamond indenter under a 10 g load for 10 s (Capalbo et al., 2022). After the experiment, the blocks were longitudinally sectioned for the analysis of cross-sectional hardness. The microhardness tester (magnification ×500, 0.1648 µm/pixel) was used for cross-sectional hardness, under a 2 g load, for 10 s. A sequence of 9 prints, at distances of 5, 10, 15, 20, 25, 30, 40, 50, and 70 µm from the external dentin surface, was performed at the center of both the control and test areas (Capalbo et al., 2022). The integrated hardness area (KHN × µm) of the lesion up to the intact dentin was measured by the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated area of intact dentin hardness, allowing the calculation of the integrated hardness loss in depth (ΔKHN) (Pancote et al., 2014).

3.3.8 Statistical analysis

Dentin wear (µm) and integrated hardness loss in depth (ΔKHN) were considered as the response variables, with two variation factors: gels (with seven levels) and type of challenges (with two levels). The data did not pass normality and homogeneity tests (Shapiro-Wilk) and were submitted to two-way ANOVA. The relationship between dentin wear and integrated hardness loss in depth was verified by Spearman's correlation coefficient. Analyses were performed using SigmaPlot software, version 12.0, assuming a 5% significance level.

3.4 Results

Mean fluoride concentrations (Standard Deviation) in the Placebo, 4500F, 9000F, 5TMPm+4500F, 2.5TMPn+4500F, 5TMPn+4500F, and APF groups averaged 29.6 (1.2); 4,215.8 (111.4); 8,721.6 (141.6); 4,736.1 (90.6); 4,568.3 (97.3); 4,071.6 (78.7); and 12,131.9 (126.3) ppm F, respectively. A neutral pH was determinated for all gels (6.4 ranging from 6.3 to 6.7), except for the APF (pH = 3.6).

Dentin erosive wear was significantly affected by the type of challenge ($F = 1,871.0$, $p < 0.001$) and treatments ($F = 449.3$, $p < 0.001$), with significant interactions between challenges and treatments ($F = 57.7$, $p < 0.001$). An inverse dose-response relationship was observed between F concentrations in the gels without TMP and dentin wear, both for ERO and ERO+ABR ($p < 0.05$) (Figure 1). For ERO, all gels containing 4500F supplemented with TMP significantly reduced dentin wear compared with their counterpart without TMP, reaching values similar to the positive controls (9000F and/or APF). For ERO+ABR, 5TMPn+4500F gel led to significantly lower wear than all its counterparts, reaching values similar to both 9000F and APF (Figure 2).

Dentin integrated hardness in depth (ΔKHN) was significantly affected by the type of challenge ($F = 151{,}490$, $p < 0.001$) and treatments ($F = 36{,}750$, $p < 0.001$), with significant interactions between challenges and treatments ($F = 2{,}325$, $p = 0.042$). Similarly to dentin wear, an inverse dose-response relationship was observed between F concentrations in the gels without TMP and ΔKHN, both for ERO and ERO+ABR (Figure 3). All gels supplemented with TMP promoted superior protective effects compared with 4500F, reaching values similar to both positive controls (9000F and APF) under both challenges (ERO and ERO+ABR).

A positive correlation was detected between dentin erosive wear and integrated hardness loss in depth (ERO, $r = 0.775$, $p < 0.0001$; ERO+ABR, $r = 0.685$, $p < 0.0001$).

3.5 Discussion

Despite the effect of low-fluoride gels supplemented with TMP on erosive enamel wear has been previously explored by our research group (Capalbo et al., 2020; Pancote et al., 2014), such effects had not yet been investigated on dentin erosive wear. The results of our study demonstrated that the addition of 5% TMP (nanoparticles or microparticles) or 2.5% TMP nanoparticles to low-fluoride gels significantly reduced dentin erosive wear under *in vitro* both under ERO and ERO+ABR conditions, with superior protective effects on dentin mineral content of the remaining dentin than Placebo and 4500F groups. Additionally, a better performance was associated with nanoparticulate gels, specifically 5TMPn+4500F. Therefore, both null hypotheses were rejected.

The reasons for such effects can be attributed to the higher proportion of atoms on the surface area concerning the volume of nanoparticles compared with microparticles, which leads to a greater capacity for interaction with the surrounding environment (Capalbo et al., 2020; Jandt & Watts, 2020). Despite their reduced size, nanoparticles were shown to maintain their crystalline structure, while reducing particle agglomeration, which may contribute to their superior effect (Emerenciano et al., 2018). The only study assessing the influence of TMP's particle size added to F gels on enamel erosive wear (Capalbo et al., 2020) attested the superior effects of nanoparticles over microparticles when added to low-fluoride gels (4500F). As a similar trend was observed in the present study, such an effect may be mainly attributed to the structural characteristics of the tested particles concerning their size, form, and agglomeration (Jandt & Watts, 2020).

Integrated hardness loss in depth is used to assess the resistance capacity of a given material and is often used as a surrogate method to determine enamel and dentin mineral content in caries models (Capalbo et al., 2020, 2022; Danelon et al., 2014; Gonçalves et al., 2018; Kielbassa et al., 1999). In erosive models, this method is useful to estimate the mineral content of the remaining tissue after the removal of the outmost layers by erosive/abrasive challenges, both on enamel (Manarelli et al., 2011; Moretto et al., 2013) and dentin (Capalbo et al., 2022). In the present study, the test gels promoted significant protective effects against mineral loss, given the higher mineral content (ΔKHN) observed for gels containing 4500F supplemented with TMP compared with their TMP-free counterpart (Figure 3). Also, the dose-response relationship between F concentrations in the neutral gels and the mineral content in depth strengthens the suitability of cross-sectional hardness to indicate mineral changes on the dentin tissue left after erosive wear, which is in line with a recent study assessing the effects of fluoride solutions dentin erosive wear (Capalbo et al., 2022). In fact, dentin erosive wear and ΔKHN values were positively correlated in this work and the above-mentioned study, while data obtained by microtomography (Nunes et al., 2022) were negatively correlated with ΔKHN data (unpublished data) for dentin remineralization of subsurface lesions. These data analyzed collectively indicate that ΔKHN values in erosion models may be relevant under clinical conditions when considering the long-term effects of the treatment since the protective effect on wear was accompanied by effects on the rehardening of the remaining tissue, and/or on the protection of the remaining tissue against mineral loss.

It must also be emphasized that the abrasion after exposure to erosive solutions is remarkably intensive for the dentin tissue (Danelon et al., 2020), totaling 30 minutes of exposure in only five days. Despite this, the residual effect of the gels at the end of the study was verified, both for dentin loss and mineral content of the remaining structure. Another relevant aspect is that the effects of nanoparticles under more aggressive challenges (ERO+ABR) were shown to be influenced by TMP's concentration, with a higher protective effect observed for TMPnano at 5%, which justifies the use of 5% TMPnano for enhanced results. It is worth mentioning that TMPmicro at 2.5% was not included in this study due to the lack of significant enhancement promoted by a low-fluoride gel (4500 ppm F) containing TMPmicro at 3% compared with its counterpart without TMP (Danelon et al., 2014).

Dentin is a tissue that structurally presents greater solubility and lower hardness than enamel due to dentin's lower mineral content and its organic composition (Lussi et al., 2011). While the dentin mineral content undergoes faster mineral loss (compared with enamel) under continuous exposure to acids, the remaining organic matrix has a crucial role in protecting the remaining inorganic tissues, being one of the main defense mechanisms against erosion. However, salivary matrix metalloproteinase enzymes (MMP) subsequently degrades the exposed organic matrix, and the successive cycles of acid exposure results in dentin loss (Capalbo et al., 2022; Delbem et al., 2022; Lussi et al., 2011). In this sense, the association of TMP and F was shown to promote a high inhibitory effect on the gelatinolytic activity of MMP-2 and MMP-9 (Gonçalves et al., 2018; Nunes et al., 2022) in caries models *in vitro*, what might help to explain the promising protective effects of the TMP-containing gels on dentin erosive wear.

At the initial stages of dentin erosion, both peritubular and intertubular dentin are demineralized at a similar rate but, as the process evolves, the peritubular dentin undergoes faster mineral loss, leaving spaces for the interaction with both erosive acids and other molecules (*e.g.*, TMP and F) (Lussi et al., 2011). In this sense, it was recently demonstrated that TMP is absorbed on the dentin surface and obliterates the dentinal tubules (Favretto et al., 2021). The effects of solutions containing F and TMP were also assessed on the remineralization and antiproteolytic activity of the dentin tissue, demonstrating that this association led to a significantly lower mineral loss at the deeper layers of the lesion, in addition to reducing lesion depth compared to its TMP-free counterpart (Nunes et al., 2022). Based on the aforementioned studies, it may be hypothesized that both effects of TMP (*i.e.*, tubule obliteration and enhanced remineralization) are possible mechanisms by which the TMP-containing gels led to significantly lower dentin loss and mineral content of the remaining dentin tissue in the current study, despite the 2- and ~3-fold lower fluoride content compared to the positive and commercial controls, respectively.

A few considerations on the study protocol must be pointed out. Firstly, despite artificial saliva being used instead of human saliva, a previous study from our group demonstrated that the type of saliva (*i.e.*, artificial or human) did not influence enamel erosion (Danelon et al., 2018). However, salivary MMPs play an important role in dentin mineral loss due to the degradation of dentin collagen fibers (Buzalaf, Kato, & Hannas, 2012), so the present results must not be extrapolated to *in vivo* conditions. Also, despite intense erosive and abrasive challenges are necessary in short-term *in vitro* studies, these do not fully resemble ordinary conditions of acid consumption and toothbrushing, which may have important implications when assessing the long-term effects of anti-erosive therapies. In addition, the treatments might have induced macroscopic and histological variations, so that microscopic and histological analyses could have provided important data for a more comprehensive discussion of the mechanisms of action involved (Kierdorf, Kierdorf, & Fejerskov, 1993). Finally, given that the study was carried out with bovine teeth and under *in vitro* conditions, the extrapolation of the effects observed here to *in vivo* conditions cannot be done. This emphasizes the need for future studies addressing the present limitations, and with protocols that better resemble the high complexity of the oral environment under *in vivo* conditions. Nonetheless, despite the aspects listed above, the results presented are unquestionably promising, considering both their enhanced anti-erosive effects and the lower possibility of acute side effects, especially when used by young children.

In conclusion, gels containing 4500F supplemented with TMP were shown to significantly reduce dentin erosive wear compared with pure 4500F, with superior protective effects on the mineral content of the remaining dentin tissue. Also, the use of nanoparticles further increased such effects, with an additional benefit of TMP at 5%.

Declaration of Competing Interest

The second and the last author have a patent for a product used in the study, by the National Institute of Industrial Property – INPI/SP, on April 11, 2017, under number C1 0801811-1.

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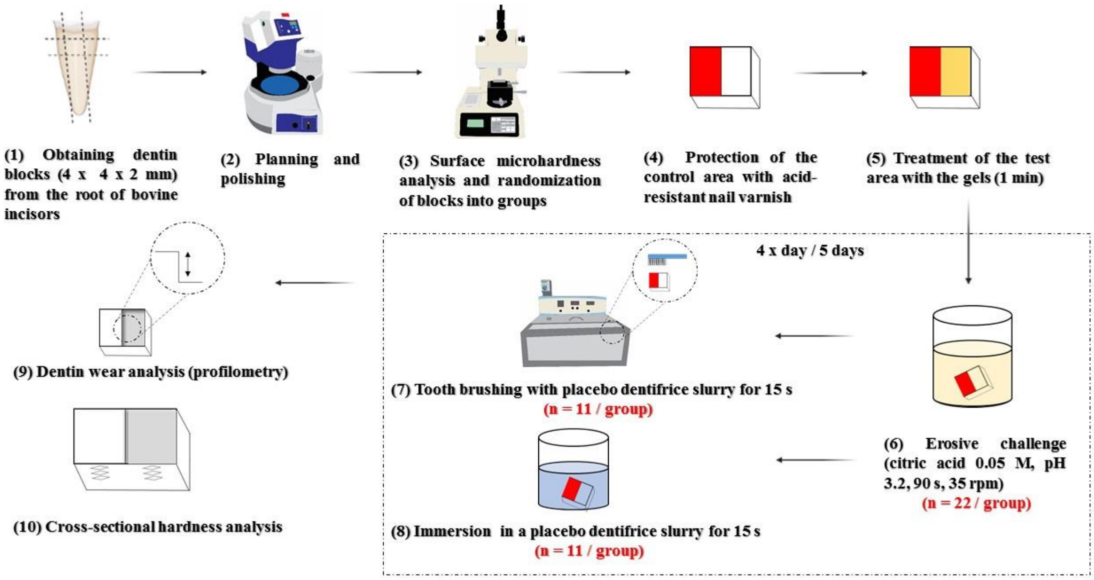

Fig. 1. Schematic flowchart summarizing the experimental design of the study.

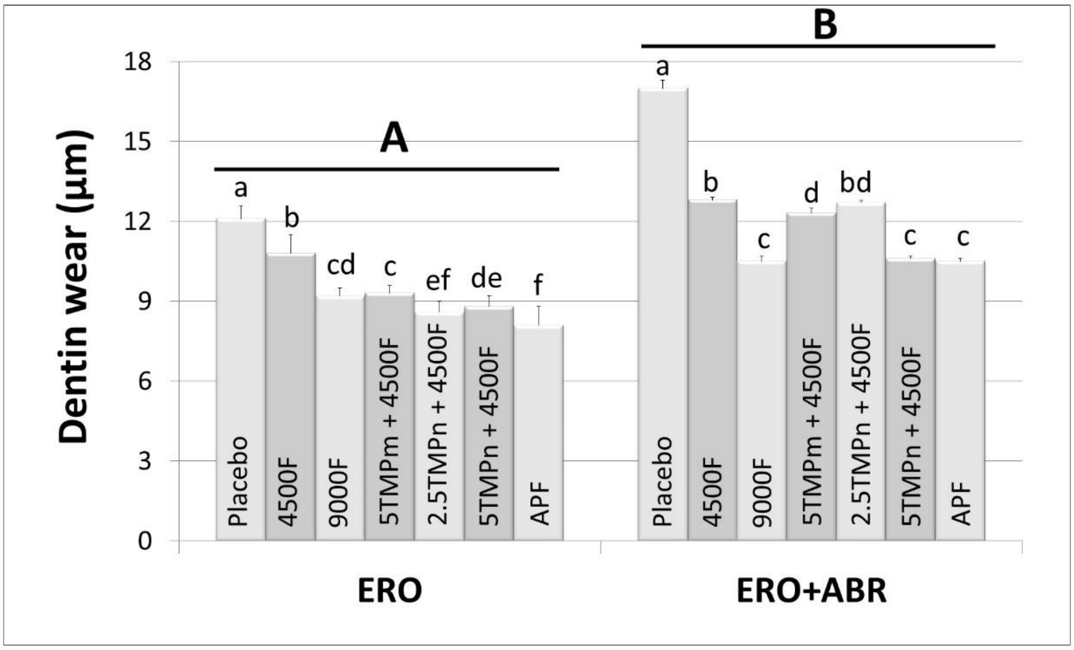

Fig. 2. Mean dentin erosive wear (µm) according to challenges (ERO or ERO+ABR) and treatment groups. Bars denote SD. Different uppercase and lowercase letters indicate significant differences between challenges within each treatment gels, and among the gels within each challenges, respectively. Two-way ANOVA and Tukey's test ($p < 0.05$, n = 11/group). ERO: erosion challenges. ERO+ABR: erosion+abrasion challenges.

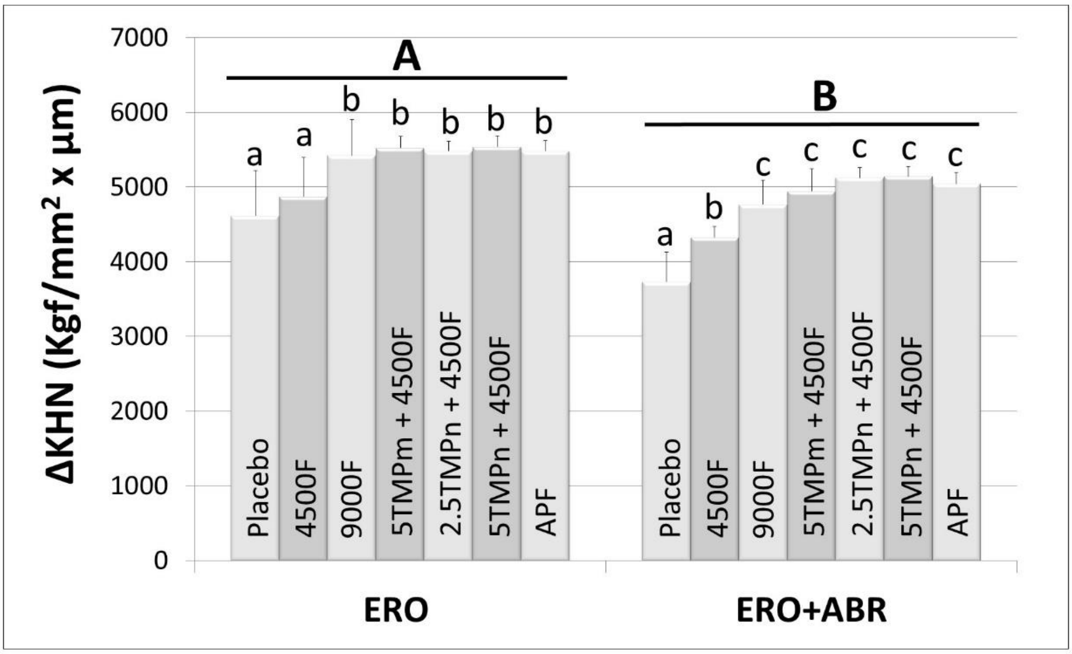

Fig. 3. Mean integrated hardness in depth (ΔKHN) according to challenges (ERO or ERO+ABR) and treatment groups. Bars denote SD. Different uppercase and lowercase letters indicate significant differences between challenges within each treatment gels, and among the gels within each challenges, respectively. Two-way ANOVA and Tukey's test ($p < 0.05$, n = 11/group). ERO: erosion challenges. ERO+ABR: erosion+abrasion challenges.

3 CONSIDERAÇÕES GERAIS

Os resultados do estudo apresentado no Capitulo 1 permitem concluir que a energia livre de superfície foi significativamente alterada após exposição à saliva, passando de hidrofóbica para levemente hidrofílica. Em acréscimo, o tratamento com os géis tornou o esmalte ainda mais a hidrofílico, independentemente da presença ou ausência de agentes ativos nos mesmos. Após o desafio erosivo, a hidrofilia do esmalte foi significativamente reduzida para todos os grupos, sendo que os géis contendo trimetafosfato de sódio (TMP) nanoparticulado apresentaram maior resistência a esta redução, visto que exibiram os valores mais altos (hidrofílico) dentre todos os grupos. Cabe ressaltar, no entanto, que o estudo foi desenvolvido sob um modelo de erosão *in vitro* de curta duração (1 min), e que a metodologia de análise da energia livre de superfície demanda, necessariamente, a secagem da superfície para aplicação dos líquidos de sondagem, o que pode ter afetado as propriedades da película adquirida observadas *in natura* no ambiente bucal. 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 intraorais. De igual forma, a análise do conteúdo mineral do esmalte após a exposição a ácidos poderia trazer informações relevantes para uma melhor compreensão dos efeitos dos géis analisados, considerando, simultaneamente, as alterações na energia livre de superfície e dinâmica mineral frente a desafios erosivos.

Quanto aos resultados apresentados no Capítulo 2, estes permitem concluir que todos os géis suplementados com TMP reduziram significativamente o desgaste erosivo da dentina em comparação ao gel contendo concentração reduzida de fluoreto (4.500 ppm F) sem TMP, atingindo valores semelhantes ao grupo de gel contendo 9.000 ppm F. Já para desafios erosivos seguido de abrasão, o gel contendo 5% de TMP nanométrico levou a um desgaste erosivo da dentina significativamente menor do que todos os géis contendo 4.500 ppm F, atingindo valores semelhantes aos dos grupos tratados com géis contendo 9.000 ppm F (neutro) e 12.300 ppm F (acidulado). Quanto ao conteúdo mineral em profundidade (avaliado por dureza em secção longitudinal), todos os géis contendo TMP promoveram menor perda mineral em comparação ao grupo tratado com 4.500 ppm F, atingindo valores semelhantes aos grupos tratados com géis contendo 9.000 ppm F e 12.300 ppm F, sob ambos os desafios. Além disso, destacamos a verificação de uma correlação positiva entre o desgaste erosivo da dentina e o conteúdo mineral em profundidade.

Diante dos resultados obtidos nos dois capítulos, considera-se que a suplementação de géis com concentração reduzida de F com TMP promoveu resultados promissores no desgaste erosivo em ambos tecidos, visto que a redução na concentração de F no produto reduz o risco de toxicidade aguda, aumentando a efetividade dos mesmos comparando-se a produtos convencionais neutros e/ou acidulados. Especialmente sob a forma de nanopartículas, estes promoveram maior resistência a alterações na hidrofilia frente a desafios erosivos no esmalte e contra o desgaste erosivo da dentina, trazendo novos dados acerca dos mecanismos de ação do TMP e F em coadministração.

APÊNDICES

APÊNDICE A - Referências da Introdução Geral

Akabane, S., Delbem, A. C., Pessan, J., Garcia, L., Emerenciano, N., Gonçalves, D. F., & Danelon, M. (2018). In situ effect of the combination of fluoridated toothpaste and fluoridated gel containing sodium trimetaphosphate on enamel demineralization. *Journal of dentistry, 68,* 59–65. https://doi.org/10.1016/j.jdent.2017.10.013.

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APÊNDICE B - Preparação dos discos de esmalte

	Preparo dos discos de esmalte
1	Separação das coroas e raízes.
2	Preservação das coroas em formol 2% até processamento.
3	Corte dos discos de esmalte (área superficial de 25,42 mm^2).
4	Planificação e polimento dos discos (Beta Grinder-Polisher, Buehler, Lake Bluff, Illinois, USA) com lixas de granulação (Buehler, 36-08-0400) conforme sequência abaixo: *i.* Fixação com cera pegajosa (Kota, São Paulo, Brasil) em base de acrílico o disco com a dentina voltado para cima; *ii.* Desgaste com lixa de granulação 400, durante 60 segundos, para planificar a dentina; *iii.* Descolamento do disco da sua base; *iv.* Colagem do disco com o esmalte voltado para cima; *v.* Polimento dos discos com lixa de granulação 600, durante 60 segundos, para planificar o esmalte, sem desgastar a dentina excessivamente; *vi.* Polimento com lixa de granulação 800 (120 segundos), seguida por lixa de granulação 1 200 (120 segundos); *vii.* Polimento com dico de feltro (40-7618), por 60 segundos, com 1/4 µm de solução diamantada (Extec Corp., Enfield, CT).
5	Lavagem dos discos em cuba de ultrassom (Elmasonic P, Germany), durante 20 min.
6	Análise de microdureza de superfície (Knoop) (Shimadzu Corp., Kyoto, Japan), com 25 g de carga por 10 s, realizando-se 5 indentações equidistantes a 100 µm. No estudo, discos com valores médios de dureza fora do intervalo entre 320 e 380 ΔKHN foram descartados.
7	Aleatorização dos discos de esmalte utilizando os valores de microdureza de superfície.

Em todas as fases, manter disco em água deionizada.

APÊNDICE C - Preparação dos géis

Quantidades (gramas) e componentes das preparações dos géis para 50 gramas

Componentes	**Grupos de Géis** **Placebo**	**4500F**	**9000F**	**5TMPm+4500F**	**2.5TMPn+4500F**	**5TMPn+4500F**
Carboximetilcelulose[1]	4	4	4	4	4	5
Sacarina sódica[2]	0,05	0,05	0,05	0,05	0,05	0,05
Glicerol[3]	14	14	14	14	14	14
Óleo de menta[4]	0,25	0,25	0,25	0,25	0,25	0,25
NaF[3]		0,4974	0,9948	0,4974	0,4974	0,4974
TMPm[1]				2,5		
TMPn					1,25	2,5
Água deionizada (mL)	31,7	31,2026	30,7052	28,7026	29,9526	28,7026

Fluoreto de sódio (NaF); Trimetafosfato de sódio (TMP); TMP microparticulado (TMPm); TMP nanoparticulado (TMPn); 4 500 ppm F (4500F); 9 000 ppm F (9000F); 5% de TMPm mais 4500F (5TMPm+4500F); 2,5% de TMPn mais 4500F (2,5TMPn+4500F); 5% de TMPn mais 4500F (5TMPn+4500F). Fabricantes: [1]Sigma-Aldrich Co., St. Louis, MO, USA; [2]Vetec, Duque de Caxias, Rio de Janeiro, Brasil; [3]Merck, Darmstadt, Germany; e [4]Synth, Brasil.

Passo a passo do preparo dos géis

Dia anterior	Separar todos os materiais e componentes a serem usados. Leitura do protocolo.
Passo 1	Ligar e calibrar a balança 30 min antes.
Passo 2	Pesar as quantidades correspondentes dos componentes da fórmula, exceto o óleo de menta, o qual será pesado no final para evitar evaporação.
Passo 3	Adicionar carboximetilcelulose e glicerol a um béquer de vidro e homogeneizar com espátula de teflon, evitando-se a formação de grumos.
Passo 4	Separar a água deionizada em alíquotas suficientes para diluir os componentes sacarina sódica, NaF e TMP, respeitando-se a composição específica de cada gel.
Passo 5	Agregar a diluição de sacarina sódica à mistura do Passo 3 e homogeneizar com espátula de teflon.
Passo 6	Agregar a diluição de *NaF* à mistura do Passo 5 e homogeneizar.
Passo 7	Sonicar a diluição de *TMP* antes de agregar à mistura do Passo 6. Homogenizar.
Passo 8	Pesar o óleo de menta e agregar à mistura do Passo 7. Homogenizar.

Nota: Caso grumos sejam identificados, verter a mistura em placa de PVC e espatular até obter uma mistura homogênea livre de grumos.

APÊNDICE D - Síntese e Caracterização de nanopartículas de TMP

Para preparar TMP nanométrico, 70 g de TMP puro (micrométrico) ($Na_3O_9P_3$, Aldrich, pureza $\geq$ 95% CAS 7785-84-4, tamanho médio de 450 ± 250 nm) foram moídos com 500 g de esferas de zircônio em 1 L de isopropanol. Após 48 h, o material foi filtrado e selado com papel alumínio, e os frascos foram secos a 75 °C para evaporação do isopropanol. A cristalinidade do pó foi caracterizada por difração de raios-x (DRX) utilizando um difratômetro Rigaku Dmax 2500 PC. Os domínios cristalinos coerentes (tamanho do cristal) foram estimados usando a equação de Scherrer: $L = \frac{K\lambda}{B\cos{}^{\theta}B}$. Onde L é a dimensão linear de uma nanopartícula monocristalina, λ é o comprimento de onda do raio-x incidente, B é a largura da linha de difração do pico de difração, θ_B é o ângulo de Bragg obtido a partir do padrão XRD e K é uma constante cujo valor é 0,9. A morfologia das partículas de TMP micro e nanométrico foram analisadas por meio de Microscopia Eletrônica de Varredura (SEM, Philips XL-30 FEG). O processamento de moagem reduziu o tamanho das partículas de TMP sem afetar sua estrutura cristalina. O padrão de difração de raios-x (XRD) do TMP nanométrico após 48 h de moagem mostra picos mais amplos devido aos cristalitos menores, que foram usados para estimar um tamanho médio de partícula de 22,7 nm. Após a moagem foi possível visualizar as partículas nanométricas sem aglomeração.

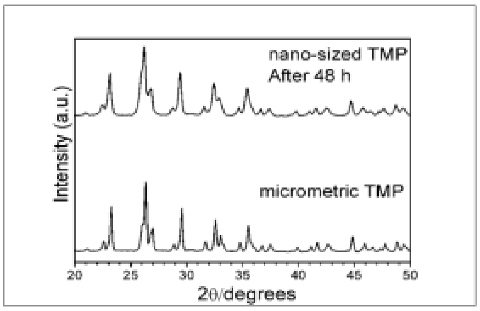

Figure 2. X-ray patterns of the micrometric TMP and of the nano-sized TMP after milling for 48 h.

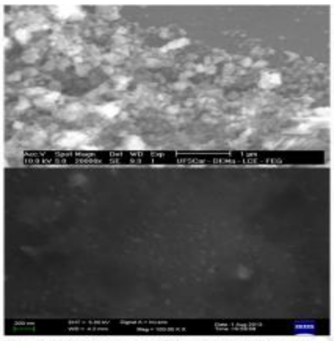

Figure 3. SEM images of the TMP powders (a) before milling and (b) after milling for 48 h.

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APÊNDICE E - Análise de flúor

Preparação das amostras para análise de F

Dia anterior	Separar todos os materiais e componentes a serem usados. Leitura do protocolo.
Passo 1	Ligar e calibrar a balança 30 min antes.
Passo 2	Pesar 100 ± 0,5 mg de cada gel (incluindo Placebo e APF)
Passo 3	Diluir em 20 mL de água deionizada e agitar durante 10 min
Passo 4	Colocar em balão volumétrico plástico de 100 mL e completar até alcançar essa capacidade. Repetir o processo totalizando três vezes (triplicata).

Nota: Assim teremos no final três amostras de cada gel para avaliar a concentração de F (o resultado de ppm F pode ter uma variação máxima de ± 3%). Estas amostras serão tamponadas em TISAB II respeitando-se a proporção 0,5 mL amostra: 0,5 mL TISAB II. Todas as leituras são realizadas em duplicata.

Preparação dos padrões de F

Fórmula

$C_1V_1=C_2V_2$

C_1 : padrão 100 ppm F

V_1 : desconhecido

C_2 : padrão que quero fazer

V_2 : 10 mL

Exemplo

Sendo C_1 : 100 ppm F, V_1 : desconhecido, C_2 : 16 ppm F, e V_2 : 10 mL

$C_1V_1=C_2V_2$

100 ppm F * V_1= 16 ppm F * 10 mL

V_1=1,6 mL

	Amostra do padrão 100 ppm F (mL)	Amostra de água deionizada (mL)
Padrão 1 ppm F	0,1	9,9
Padrão 2 ppm F	0,2	9,8
Padrão 4 ppm F	0,4	9,6
Padrão 8 ppm F	0,8	9,2
Padrão 16 ppm F	1,6	8,4

Nota: Estes padrões serão tamponados em TISAB II,
respeitando-se a proporção 0,5 mL padrão: 0,5 mL TISAB II.

APÊNDICE F - Características dos voluntários e da saliva coletada

Total de voluntários	4 voluntários
Sexo	2 masculinos, 2 femininos
Idade	25 a 33 anos
Fluxo salival	3,03 mL/min
Concentração de fósforo da saliva	94,8 ± 0,8 µg/mL
Concentração de cálcio da saliva	27,9 ± 0,3 µg/mL
Concentração de flúor[21] da saliva	1,1 ± 0,3 µM (0,02 ± 0,01 ppm F)

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[21] Foram utilizados os padrões de 1 µM, 2,5 µM, 5 µM, 10 µM, 25 µM, 50 µM e 100 µM.

APÊNDICE G - Sequência do ensaio de energia livre de superfície

1	Lavar com água deionizada a superfície dos discos por 20 segundos, posteriormente secar ao ar livre por 45 min a temperatura ambiente.
2	Avaliar energia livre de superfície (*baseline*) para todos os grupos de estudo.
3	Imergir todos os discos em saliva humana (0,4 mL) por 2 horas (37 ºC).
4	Para os discos do controle negativo (não tratados), deixar secar ao ar livre por 45 min. Avaliar energia livre de superfície (*pellicle*).
5	Para os demais discos, secar delicadamente a superfície com papel absorvente. Aplicar os géis sobre a superfície dos discos por 1 min, com ajuda de um bastão com ponta de algodão (aplicação única durante todo o experimento). Repetir o passo 1.
6	Avaliar energia livre de superfície (*gel*) para todos os grupos de géis.
7	Imergir os discos tratados com os géis em 0,4 mL de 1% de ácido cítrico pH 3,6, durante 60 segundos, sob agitação a 70 rpm. Repetir o passo 1.
8	Avaliar energia livre de superfície (*erosion*) para todos os grupos de géis.

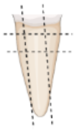
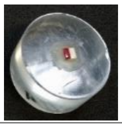
APÊNDICE H - Material suplementar

Mean (Standard Deviation) of all parameters adopted to calculate enamel surface free energy (γS, mN/m) and hydrophobicity/hydrophilicity

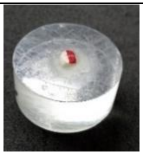
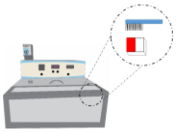
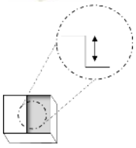
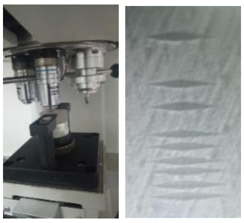
		γ_S (mN/m)	γ_S^{LW} (mN/m)	γ_S^{AB} (mN/m)	γ_S+ (mN/m)	γ_S- (mN/m)	ΔG_{sws}^{LW}	ΔG_{sws}^{AB}	ΔG_{sws}^{TOTAL}
baseline	**Untreated**	26.2 (1.8) ^{Aa}	29.0 (1.7) ^{Aa}	-2.8 (1.6) ^{Aa}	0.1 (0.2) ^{Aa}	24.2 (2.0) ^{Aa}	-1.1 (0.4) ^{Aa}	-2.6 (4.0) ^{Aa}	-3.6 (3.9) ^{Aa}
	PLA	24.3 (1.6) ^{Aa}	27.4 (1.5) ^{Aa}	-3.3 (1.2) ^{Aa}	0.3 (0.3) ^{Aa}	24.3 (1.1) ^{Aa}	-0.7 (0.3) ^{Aa}	-2.2 (2.0) ^{Aa}	-2.9 (2.1) ^{Aa}
	4500F	25.1 (1.6) ^{Aa}	28.9 (1.5) ^{Aa}	-3.8 (1.4) ^{Aa}	0.3 (0.4) ^{Aa}	24.0 (1.3) ^{Aa}	-1.0 (0.4) ^{Aa}	-2.8 (2.7) ^{Aa}	-3.9 (2.6) ^{Aa}
	9000F	26.6 (3.1) ^{Aa}	29.2 (2.2) ^{Aa}	-3.2 (1.5) ^{Aa}	0.2 (0.1) ^{Aa}	24.3 (1.2) ^{Aa}	-1.2 (0.6) ^{Aa}	-2.3 (2.3) ^{Aa}	-3.4 (2.4) ^{Aa}
	TMPmicro5	26.0 (2.7) ^{Aa}	29.7 (2.1) ^{Aa}	-4.0 (2.8) ^{Aa}	0.2 (0.2) ^{Aa}	23.7 (1.3) ^{Aa}	-1.3 (0.6) ^{Aa}	-3.3 (2.6) ^{Aa}	-4.6 (2.9) ^{Aa}
	TMPnano5	26.0 (3.8) ^{Aa}	29.2 (2.7) ^{Aa}	-4.0 (1.9) ^{Aa}	0.3 (0.3) ^{Aa}	23.5 (1.1) ^{Aa}	-1.2 (0.8) ^{Aa}	-3.7 (2.1) ^{Aa}	-4.9 (2.2) ^{Aa}
	TMPnano2.5	24.4 (3.0) ^{Aa}	28.8 (2.1) ^{Aa}	-4.5 (1.9) ^{Aa}	0.3 (0.2) ^{Aa}	23.7 (1.4) ^{Aa}	-1.0 (0.6) ^{Aa}	-3.4 (2.6) ^{Aa}	-4.4 (2.8) ^{Aa}
	APF	25.0 (2.7) ^{Aa}	28.4 (2.0) ^{Aa}	-3.4 (2.0) ^{Aa}	0.4 (0.4) ^{Aa}	23.5 (1.3) ^{Aa}	-0.9 (0.5) ^{Aa}	-3.6 (2.3) ^{Aa}	-4.5 (2.3) ^{Aa}
pellicle	**Untreated**	38.3 (3.3)*	27.9 (4.3)	10.7 (2.4)*	2.3 (1.7)	27.8 (18.8)	-1.0 (1.2)	2.2 (27.0)	1.2 (26.3)
gel	**PLA**	41.8 (4.7) ^{Ba}	14.5 (2.7) ^{Bab}	28.3 (4.5) ^{Bab}	0.1 (0.1) ^{Aa}	76.0 (3.9) ^{Babc}	-1.7 (1.0) ^{Aab}	71.5 (4.2) ^{Ba}	69.8 (5.0) ^{Ba}
	4500F	40.9 (4.9) ^{Ba}	14.6 (4.2) ^{Bab}	26.3 (5.6) ^{Bab}	0.1 (0.1) ^{Aa}	76.2 (4.5) ^{Babc}	-2.1 (1.6) ^{Aab}	69.6 (4.5) ^{Ba}	67.5 (4.2) ^{Ba}
	9000F	40.3 (3.4) ^{Ba}	14.3 (2.6) ^{Bab}	27.4 (4.5) ^{Bab}	0.3 (0.4) ^{ABa}	79.7 (7.2) ^{Ba}	-1.8 (1.1) ^{Aab}	71.1 (8.7) ^{Ba}	69.3 (9.2) ^{Ba}
	TMPmicro5	43.0 (3.5) ^{Ba}	15.8 (4.6) ^{Babc}	28.2 (2.7) ^{Bab}	0.1 (0.1) ^{Aa}	73.6 (2.6) ^{Bbc}	-1.7 (1.5) ^{Aab}	66.3 (3.9) ^{Ba}	64.6 (4.0) ^{Ba}
	TMPnano5	42.5 (3.7) ^{Ba}	12.8 (2.8) ^{Ba}	30.4 (3.2) ^{Ba}	0.1 (0.1) ^{Aa}	75.8 (3.1) ^{Babc}	-2.7 (1.6) ^{Bb}	70.5 (3.2) ^{Ba}	67.7 (3.8) ^{Ba}
	TMPnano2.5	43.0 (3.8) ^{Ba}	16.9 (3.4) ^{Bbc}	26.1 (4.1) ^{Bb}	0.2 (0.1) ^{Aa}	74.5 (3.3) ^{Bbc}	-1.0 (1.2) ^{Aa}	66.5 (3.6) ^{Ba}	65.5 (3.5) ^{Ba}
	APF	44.0 (3.3) ^{Ba}	18.4 (3.3) ^{Bc}	26.0 (4.1) ^{Bb}	0.3 (0.3) ^{ABa}	77.2 (4.2) ^{Babc}	-0.6 (0.6) ^{Aa}	69.2 (6.7) ^{Ba}	68.6 (6.6) ^{Ba}
erosion	**PLA**	45.5 (1.9) ^{Ca}	48.4 (1.5) ^{Ca}	-2.8 (1.8) ^{Aa}	0.1 (0.1) ^{Aa}	34.4 (1.3) ^{Ca}	-10.5 (1.0) ^{Ba}	15.6 (2.0) ^{Ca}	5.1 (2.2) ^{Ca}
	4500F	45.7 (2.6) ^{Ca}	48.7 (1.4) ^{Ca}	-3.1 (2.5) ^{Aa}	0.1 (0.1) ^{Aa}	33.2 (2.0) ^{Ca}	-10.7 (0.9) ^{Ba}	13.5 (3.1) ^{Ca}	2.9 (2.5) ^{Ca}
	9000F	29.6 (2.3) ^{Abc}	39.7 (4.0) ^{Cbcd}	-10.1 (3.1) ^{Cb}	0.4 (0.2) ^{Bb}	65.4 (7.8) ^{Cbc}	-5.4 (2.0) ^{Bbc}	53.2 (7.8) ^{Cb}	47.7 (6.8) ^{Cb}
	TMPmicro5	29.9 (2.0) ^{Cb}	41.0 (4.1) ^{Cde}	-12.1 (3.9) ^{Cb}	0.2 (0.3) ^{Aab}	61.0 (5.5) ^{Cc}	-6.1 (2.1) ^{Bb}	51.5 (4.9) ^{Cb}	45.4 (5.3) ^{Cb}
	TMPnano5	25.7 (2.7) ^{Ac}	36.9 (2.8) ^{Ccfg}	-12.2 (4.7) ^{Cb}	0.1 (0.2) ^{Aa}	71.7 (4.6) ^{Cd}	-4.0 (1.3) ^{Ccd}	65.2 (6.5) ^{Cc}	61.2 (6.2) ^{Cc}
	TMPnano2.5	26.8 (3.6) ^{Abc}	37.8 (2.4) ^{Cbeg}	-10.9 (4.1) ^{Cb}	0.1 (0.1) ^{Aa}	67.8 (4.2) ^{Cbd}	-4.4 (1.2) ^{Bc}	61.0 (4.1) ^{Cc}	56.6 (4.2) ^{Cc}
	APF	29.0 (1.8) ^{Cbc}	34.0 (1.3) ^{Cf}	-5.0 (2.0) ^{Aa}	0.1 (0.1) ^{Ba}	48.5 (3.7) ^{Ce}	-2.7 (0.5) ^{Bd}	36.2 (3.6) ^{Cd}	33.5 (3.6) ^{Cd}

Mean of secondary parameters assessed according to the test groups, prior to any exposure (*baseline*), after salivary exposure (*pellicle*) and treatment with the gels (*ge*l), and after citric acid exposure (*erosion*). Bars denote standard deviations. Different letters indicate significant differences among the conditions of enamel surface within each group (upper-case) and among the groups within each condition of enamel surface (lower-case). *Different between *baseline* and *pellicle* in untreated group. Two-way ANOVA and Tukey's test, and by Mann-Whitney's test ($p < 0.05$, n = 10/group). Captions: Untreated = negative control group; PLA = placebo (with no actives); 4500F = 4,500 ppm F; 9000F = 9,000 ppm F; APF = 12,300 ppm F (acidulated); TMPmicro5 = 4500F + 5% micrometric TMP; TMPnano2.5 = 4500F + 2.5%TMP nanosized; TMPnano5 = 4500F + 5%TMP nanosized). TMP = sodium trimetaphosphate. γ^{LW}: surface tension Lifshiz van der Waals, nonpolar component; γ^{AB}: acid base interaction, polar component; γ+: receptor component (acid); γ–: donor component (base); $\Delta Gsws^{LW}$: free energy of interaction between the surface (s) and the water (w) for nonpolar component; and $\Delta Gsws^{AB}$: free energy of interaction between the surface (s) and the water (w) for polar component.

APÊNDICE I - Preparação dos blocos de dentina

	Preparo dos blocos de dentina	
1	Após 30 dias de armazenamento dos dentes bovinos em solução de timol a 0,1%, separar as coroas das raízes. Realizar a limpeza do tecido externo com bisturi nº 20, cabo nº 4. Remover o tecido pulpar com lima nº 80.	
2	Preservar as raízes em timol a 0,1% até processamento.	
3	Corte dos blocos de dentina radicular 4 x 4 x 2 mm, a partir do terço cervical das raízes.	
4	Realizar a planificação e polimento dos blocos (Beta Grinder-Polisher, Buehler, Lake Bluff, Illinois, USA) com lixas de granulação (Buehler, 36-08-0400) conforme sequência abaixo: *i*. Colar com cera pegajosa (Kota, São Paulo, Brasil) em base de acrílico o bloco com a dentina interna (pulpar) voltado para cima; *ii*. Desgastar com lixa de granulação 400, durante 5 segundos, para planificar a dentina; *iii*. Descolar o bloco; *iv*. Colar o bloco com o cemento voltado para cima; *v*. Polir os blocos com lixa de granulação 600, durante 2 segundos, para remover a camada de cemento sem desgastar a dentina excessivamente; *vi*. Polir com lixa de granulação 800 (5 segundos), seguida por lixa de granulação 1 200 (20 segundos); *vii*. Polir com dico de feltro (40-7618), por 30 segundos, com 1/4 µm de solução diamantada (Extec Corp., Enfield, CT).	  
5	Lavar os blocos em cuba de ultrassom (Elmasonic P, Germany), durante 20 min.	
6	Realizar a análise de microdureza de superfície (Knoop)(Shimadzu Corp., Kyoto, Japan), com 10 g de carga por 10 s, realizando-se 5 indentações equidistantes a 100 µm. No estudo, blocos com valores médios de dureza fora do intervalo entre 60 e 85 ΔKHN foram descartados.	
7	Aleatorizar os blocos de dentina utilizando os valores de microdureza de superfície.	
8	Proteger a metade da superfície de cada bloco com verniz resistente a ácido na cor vermelha (Gabriele, Colorama).	
Em todas as fases, manter a dentina umedecida com água deionizada para preservar as características do tecido.		

APÊNDICE J - Sequência do ensaio de ciclagem

	Sequência	
1	Aplicar os géis sobre a superfície dos blocos por 1 min, com ajuda de um bastão com ponta de algodão (aplicação única durante todo o experimento). Remover os géis com água deionizada (20 s) e secar os blocos delicadamente com papel absorvente, sem abrasionar.	
2	Realizar o desafio erosivo em todos os blocos com ácido cítrico 0,05 M, pH 3,2 durante 90 s, sob agitação a 35 rpm.	
3	Imergir metade dos blocos em suspensão de dentifrício placebo (1 g do dentifrício para 3 mL de água deionizada) durante 15 segundos, sob agitação 35 rpm. Em seguida, lavar os blocos em jatos de água deionizada durante 20 s. Secar com papel absorvente a superfície dos blocos, sem abrasionar.	
4	Submeter a outra metade dos blocos a desafios abrasivos em uma máquina de escovação automática (250 g de carga axial, 5 movimentos de vai-e-vem/s) utilizando a mesma suspensão de dentifrício placebo descrita no item 3, durante 15 segundos. Em seguida, lavar os blocos em jatos de água deionizada durante 20 s. Secar com papel absorvente a superfície dos blocos, sem abrasionar.	
5	Imergir os blocos em solução de saliva artificial (pH 7,0, sem agitação) e armazenamento em estufa (37 ºC) por 2 h entre os desafios ácidos e *overnight* (entre o último desafio do dia e o primeiro desafio do dia seguinte).	
6	Realizar 4 ciclagens erosivas ou erosivas+abrasivas por dia, durante 5 dias.	
7	Remover o verniz ácido-resistente com o auxílio de uma espátula metálica, iniciando pelas bordas dos blocos para não tocar a região central dos mesmos. Analisar o desgaste dentinário dos blocos por perfilometria de contato (Surftest SJ 401, Mitutoyo American Corporation). A ponta do perfilômetro percorreu a área hígida (controle) até a área exposta, totalizando 5 leituras por bloco.	
8	Realizar uma secção longitudinal nos blocos, de forma que os mesmos tenham uma área controle (hígida) e uma área exposta (erodida).	
9	Embutir os blocos foram em resina acrílica a frio para posterior polimento, usando a mesma sequência de polimento de superfície inicial descrita no Apêndice I.	
10	Para avaliar a microdureza (magnificação *x* 500; 0,1648 µm/pixel, carga de 2 g, durante 10 s) (Buehler, Lake Bluff, USA and Mitutoyo Corporation, Kanagawa, Japan), realizar uma sequência de 9 indentações a 5, 10, 15, 20, 25, 30, 40, 50 e 70 µm de distância da superfície externa, sendo uma sequência na área controle e outra na área exposta.	

Após a quarta ciclagem do dia 5, os blocos de dentina foram mantidos em saliva artificial por 2 horas em estufa (pH 7,0, sem agitação, a 37 ºC). Após o final da ciclagem manter a dentina umedecida em água deionizada para preservar as características do tecido.

APÊNDICE K - Componentes do dentifrício placebo

Dentifrício placebo (500 gramas)
Carboximetilcelulose[1] (7 g)
Glicerina[2] (133 g)
Água deionizada (298,1 mL)
Metil p-hidroxibenzoato de sódio[1] (0,4 g)
Sacarina[3] (0,5 g)
Dióxido de titânio[1] (2,5 g)
Sílica abrasiva[1] (50 g)
Lauril sulfato de sódio[1] (8,5 g)

Fabricantes: [1]Sigma-Aldrich Co., St. Louis, MO, USA; [2]Merck, Darmstadt, Germany; e [3]Vetec, Duque de Caxias, Rio de Janeiro, Brasil.

ANEXOS

ANEXO A - Comissão de Ética no uso de Animais

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

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

CERTIFICADO

Certificamos que o Relatório Final do trabalho intitulado **"Efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio sobre desgaste erosivo da dentina e energia livre de superfície do esmalte"**, Processo FOA nº 2020-486, sob responsabilidade de Juliano Pelim Pessan e colaboração de Beatriz Diaz-Fabregat foi aprovado pela CEUA em 22 de Agosto de 2023.

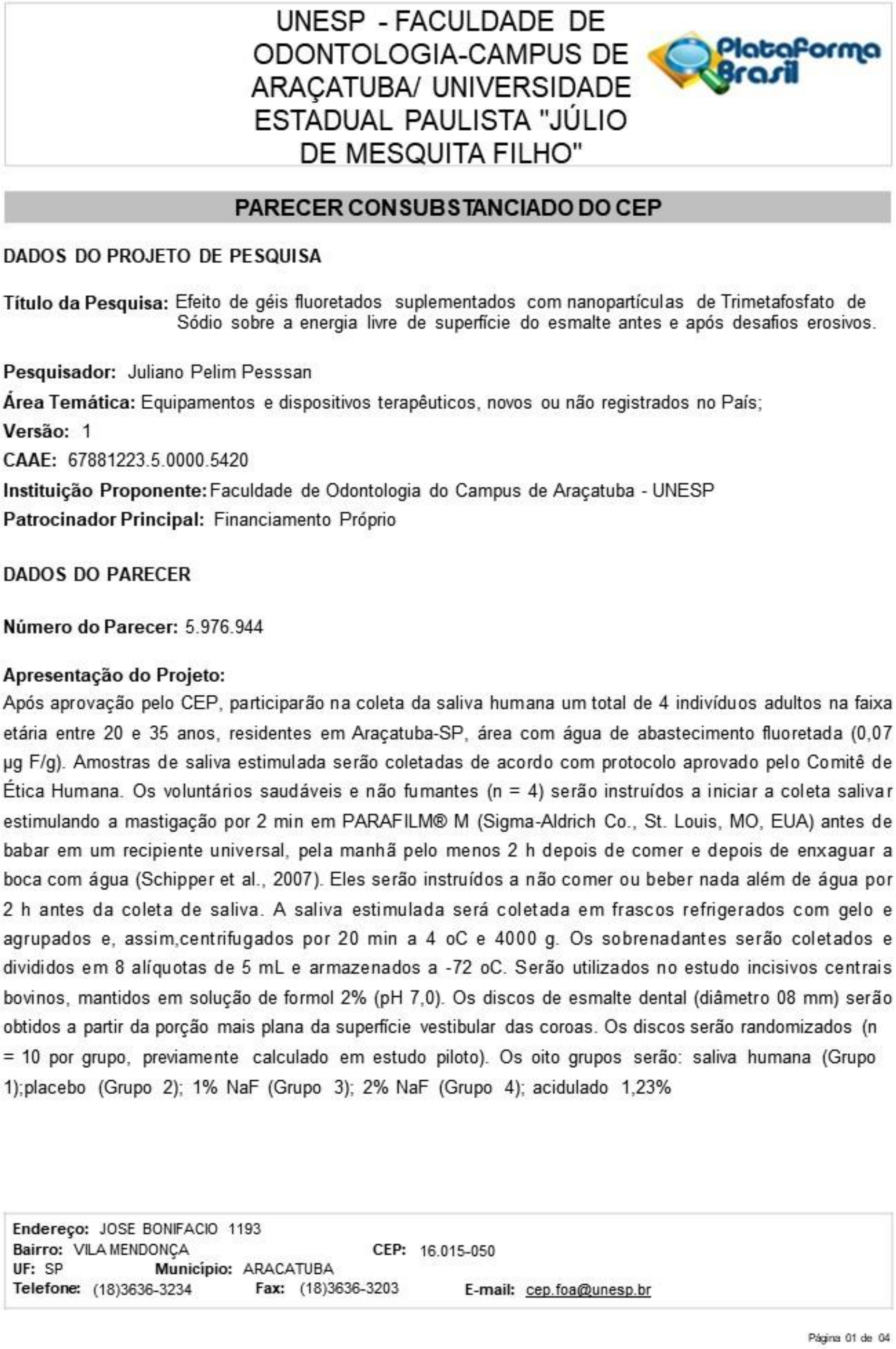
CERTIFICATE

We certify that the study entitled **"Effect of fluoride gels supplemented with nanosized Sodium Trimetaphosphate on dentin erosive wear and enamel free surface energy"**, Process FOA nº 2020-486, under the supervision of Juliano Pelim Pessan and collaboration of Beatriz Diaz-Fabregat had its the Final Report approved by the CEUA on August 22, 2023.

Prof. Assoc. Marcos Franke Pinto
Vice-Coordenador da CEUA
CEUA Vice-Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
Faculdade de Medicina Veterinária de Araçatuba
Rua José Bonifácio, 1193 – Vila Mendonça - CEP: 16015-050 – ARAÇATUBA – SP
Fone (18) 3636-3234 Email CEUA: ceua.foa@unesp.br

ANEXO B - Parecer consubstanciado do CEP



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ESTADUAL PAULISTA "JÚLIO
DE MESQUITA FILHO"

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio sobre a energia livre de superfície do esmalte antes e após desafios erosivos.

Pesquisador: Juliano Pelim Pesssan
Área Temática: Equipamentos e dispositivos terapêuticos, novos ou não registrados no País;
Versão: 1
CAAE: 67881223.5.0000.5420
Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.976.944

Apresentação do Projeto:
Após aprovação pelo CEP, participarão na coleta da saliva humana um total de 4 indivíduos adultos na faixa etária entre 20 e 35 anos, residentes em Araçatuba-SP, área com água de abastecimento fluoretada (0,07 µg F/g). Amostras de saliva estimulada serão coletadas de acordo com protocolo aprovado pelo Comitê de Ética Humana. Os voluntários saudáveis e não fumantes (n = 4) serão instruídos a iniciar a coleta salivar estimulando a mastigação por 2 min em PARAFILM® M (Sigma-Aldrich Co., St. Louis, MO, EUA) antes de babar em um recipiente universal, pela manhã pelo menos 2 h depois de comer e depois de enxaguar a boca com água (Schipper et al., 2007). Eles serão instruídos a não comer ou beber nada além de água por 2 h antes da coleta de saliva. A saliva estimulada será coletada em frascos refrigerados com gelo e agrupados e, assim,centrifugados por 20 min a 4 oC e 4000 g. Os sobrenadantes serão coletados e divididos em 8 alíquotas de 5 mL e armazenados a -72 oC. Serão utilizados no estudo incisivos centrais bovinos, mantidos em solução de formol 2% (pH 7,0). Os discos de esmalte dental (diâmetro 08 mm) serão obtidos a partir da porção mais plana da superfície vestibular das coroas. Os discos serão randomizados (n = 10 por grupo, previamente calculado em estudo piloto). Os oito grupos serão: saliva humana (Grupo 1);placebo (Grupo 2); 1% NaF (Grupo 3); 2% NaF (Grupo 4); acidulado 1,23%

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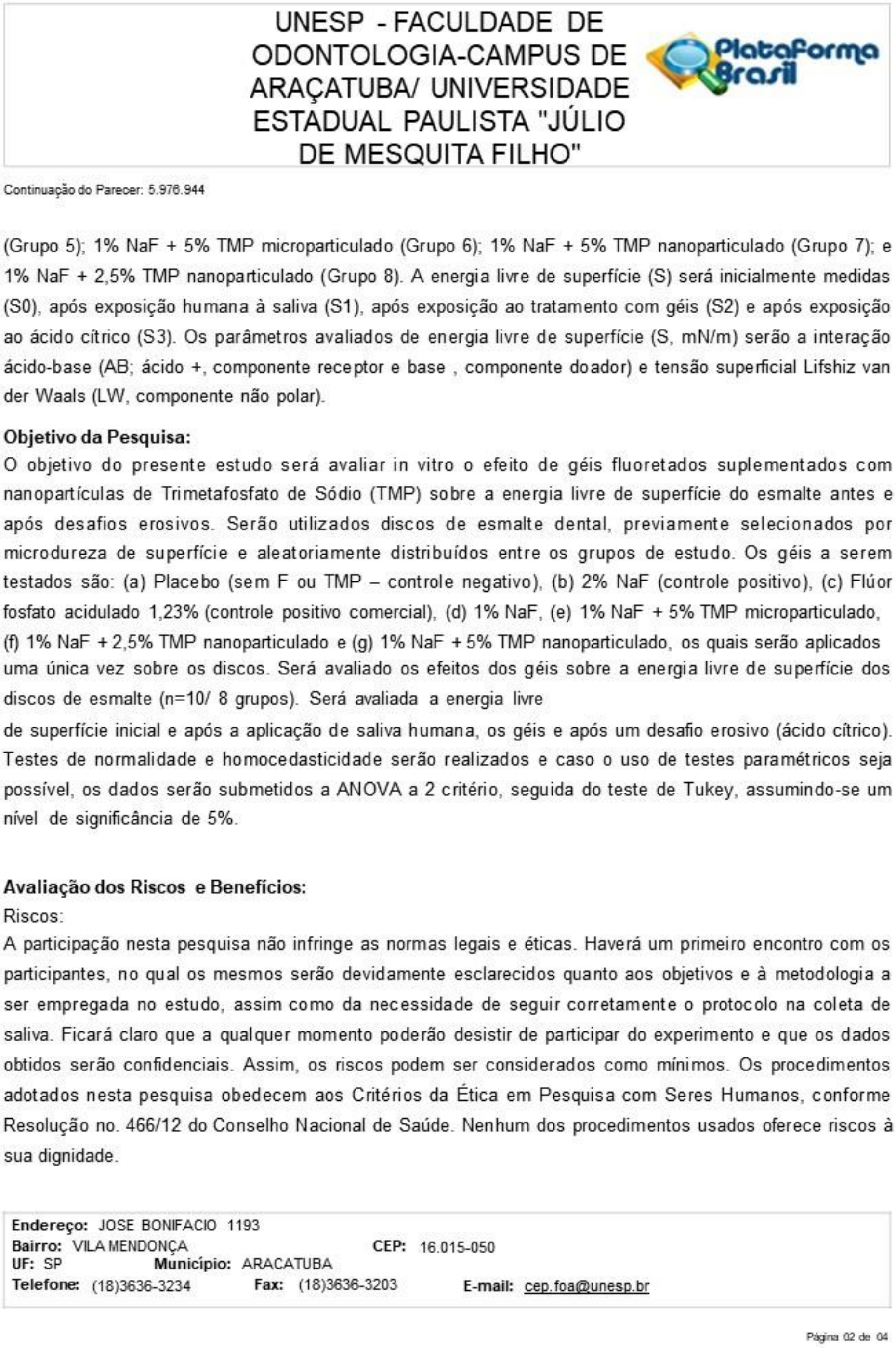
Página 01 de 04



Continuação do Parecer: 5.976.944

(Grupo 5); 1% NaF + 5% TMP microparticulado (Grupo 6); 1% NaF + 5% TMP nanoparticulado (Grupo 7); e 1% NaF + 2,5% TMP nanoparticulado (Grupo 8). A energia livre de superfície (S) será inicialmente medidas (S0), após exposição humana à saliva (S1), após exposição ao tratamento com géis (S2) e após exposição ao ácido cítrico (S3). Os parâmetros avaliados de energia livre de superfície (S, mN/m) serão a interação ácido-base (AB; ácido +, componente receptor e base , componente doador) e tensão superficial Lifshiz van der Waals (LW, componente não polar).

Objetivo da Pesquisa:

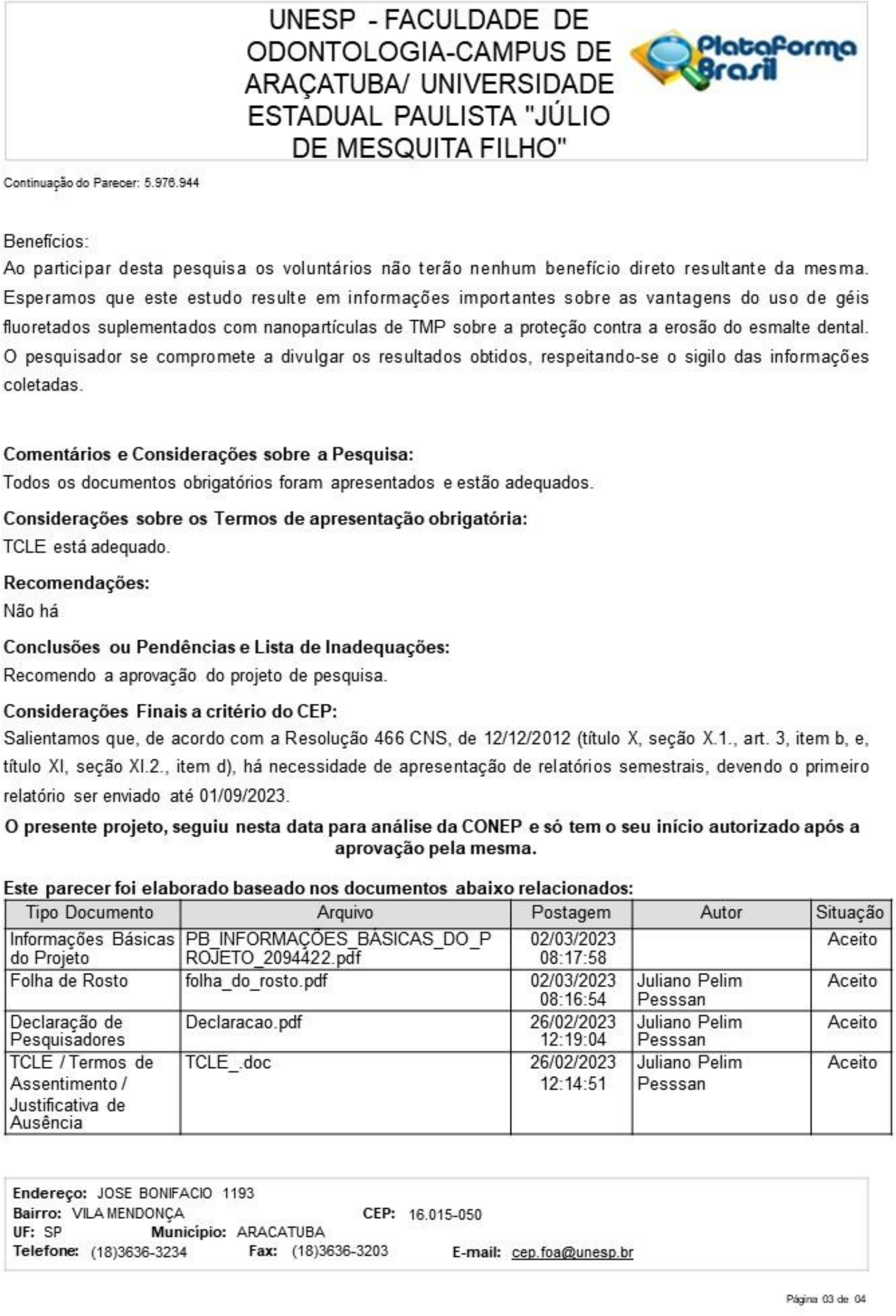
O objetivo do presente estudo será avaliar in vitro o efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio (TMP) sobre a energia livre de superfície do esmalte antes e após desafios erosivos. Serão utilizados discos de esmalte dental, previamente selecionados por microdureza de superfície e aleatoriamente distribuídos entre os grupos de estudo. Os géis a serem testados são: (a) Placebo (sem F ou TMP – controle negativo), (b) 2% NaF (controle positivo), (c) Flúor fosfato acidulado 1,23% (controle positivo comercial), (d) 1% NaF, (e) 1% NaF + 5% TMP microparticulado, (f) 1% NaF + 2,5% TMP nanoparticulado e (g) 1% NaF + 5% TMP nanoparticulado, os quais serão aplicados uma única vez sobre os discos. Será avaliado os efeitos dos géis sobre a energia livre de superfície dos discos de esmalte (n=10/ 8 grupos). Será avaliada a energia livre

de superfície inicial e após a aplicação de saliva humana, os géis e após um desafio erosivo (ácido cítrico). Testes de normalidade e homocedasticidade serão realizados e caso o uso de testes paramétricos seja possível, os dados serão submetidos a ANOVA a 2 critério, seguida do teste de Tukey, assumindo-se um nível de significância de 5%.

Avaliação dos Riscos e Benefícios:

Riscos:

A participação nesta pesquisa não infringe as normas legais e éticas. Haverá um primeiro encontro com os participantes, no qual os mesmos serão devidamente esclarecidos quanto aos objetivos e à metodologia a ser empregada no estudo, assim como da necessidade de seguir corretamente o protocolo na coleta de saliva. Ficará claro que a qualquer momento poderão desistir de participar do experimento e que os dados obtidos serão confidenciais. Assim, os riscos podem ser considerados como mínimos. Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos, conforme Resolução no. 466/12 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos à sua dignidade.



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Benefícios:
Ao participar desta pesquisa os voluntários não terão nenhum benefício direto resultante da mesma. Esperamos que este estudo resulte em informações importantes sobre as vantagens do uso de géis fluoretados suplementados com nanopartículas de TMP sobre a proteção contra a erosão do esmalte dental. O pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o sigilo das informações coletadas.

Comentários e Considerações sobre a Pesquisa:
Todos os documentos obrigatórios foram apresentados e estão adequados.

Considerações sobre os Termos de apresentação obrigatória:
TCLE está adequado.

Recomendações:
Não há

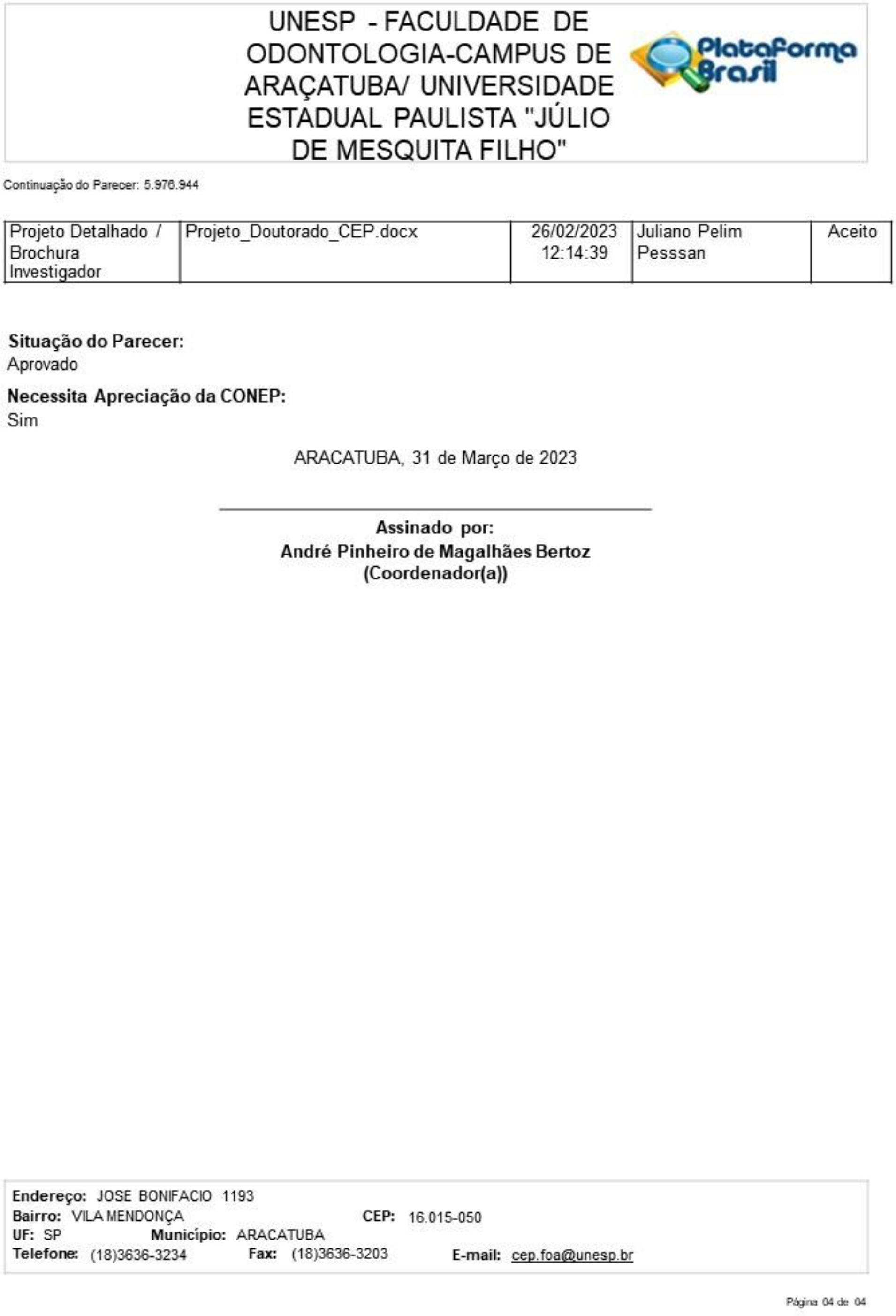
Conclusões ou Pendências e Lista de Inadequações:
Recomendo a aprovação do projeto de pesquisa.

Considerações Finais a critério do CEP:
Salientamos que, de acordo com a Resolução 466 CNS, de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/09/2023.

O presente projeto, seguiu nesta data para análise da CONEP e só tem o seu início autorizado após a aprovação pela mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2094422.pdf	02/03/2023 08:17:58		Aceito
Folha de Rosto	folha_do_rosto.pdf	02/03/2023 08:16:54	Juliano Pelim Pesssan	Aceito
Declaração de Pesquisadores	Declaracao.pdf	26/02/2023 12:19:04	Juliano Pelim Pesssan	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_.doc	26/02/2023 12:14:51	Juliano Pelim Pesssan	Aceito



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Continuação do Parecer: 5.976.944

Projeto Detalhado / Brochura Investigador	Projeto_Doutorado_CEP.docx	26/02/2023 12:14:39	Juliano Pelim Pesssan	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Sim

ARACATUBA, 31 de Março de 2023

Assinado por:
André Pinheiro de Magalhães Bertoz
(Coordenador(a))