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EFICÁCIA DE DENTIFRÍCIO COM TECNOLOGIA INOVADORA PARA REMINERALIZAÇÃO DE LESÕES INICIAIS DE CÁRIE E EROSÃO: ABORDAGEM *IN VITRO* E *IN VIVO*

Nayanna Lana Soares Fernandes

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Orientador: Prof. Dr. Fábio Correia Sampaio
Coorientador: Prof. Dr. Anderson Takeo Hara

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NAYANNA LANA SOARES FERNANDES

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Banca Examinadora

Prof. Dr. Fábio Correia Sampaio

Orientador - UFPB

Prof. Dr. Franklin Delano Soares Forte

Examinador - UFPB

Profa. Dr.ª Edja Maria Melo de Brito Costa

Examinador – UEPB

ATA DA DEFESA PÚBLICA DE DISSERTAÇÃO DE MESTRADO

DEFESA DE Nº: ____

Aos três dias do mês de fevereiro do ano de 2021, às 14:00 horas, a partir de utilização do modelo remoto, reuniram-se os membros da banca examinadora composta pelos professores(as) doutores(as): Fábio Correia Sampaio (Orientador e Presidente), Franklin Delano Soares Forte (membro vinculado ao Programa de Pós-graduação em Odontologia – UFPB) e Edja Maria Melo de Brito Costa (membro externo) a fim de arguirem a mestranda Nayanna Lana Soares Fernandes, com relação ao seu trabalho final de curso de mestrado (dissertação), sob o título "Eficácia de dentifrício com tecnologia inovadora para remineralização de lesões iniciais de cárie e erosão: abordagem *in vitro* e *in vivo*". Aberta a sessão pelo presidente da mesma, coube a candidata, na forma regimental, expor o tema de sua dissertação, dentro do tempo regulamentar. Em seguida, foi questionada pelos membros da banca examinadora, sendo as explicações necessárias fornecidas e as modificações solicitadas registradas. Logo após, os membros da banca examinadora reuniram-se em sessão secreta, tendo chegado ao seguinte julgamento, que, de público, foi anunciado: 1º Examinadora (membro externo): Conceito "Aprovado"; 2º Examinador (membro vinculado ao PPGO): Conceito "Aprovado, 3º Examinador (Orientador e Presidente): Conceito "Aprovado". O que resultou em conceito final igual: **"APROVADO"**, o que permite a candidata fazer jus ao título de Mestre em Odontologia. Os documentos utilizados para avaliação da candidata durante o processo aqui descrito apresentam-se como prova documental do mesmo e, como tal, serão anexadas a esta ata para arquivamento. Nada mais havendo a tratar, foi lavrada a presente ata, que será por mim assinada, Ricardo Dias de Castro, coordenador do Programa de Pós-Graduação em Odontologia da UFPB, pelo presidente, pelos demais membros da banca, e pela candidata.

Coordenador do PPGO

1º Examinadora – Membro Externo

2º Examinador – Membro do PPGO

3º Examinador – Presidente

Candidata

DEDICATÓRIA

À Deus, minha família, meu noivo e amigos pelo apoio e incentivo em todos os momentos.

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À Deus, meu guia e protetor, que despertou em mim desde o início da graduação o desejo de seguir a docência, e me encorajou para superar todos os obstáculos durante esse árduo e gratificante caminho que venho percorrendo.

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Ao meu grupo de pesquisae todos os seus participantes, em especial minha grande parceira Ingrid Meira.Os amantes da cárie e erosão, que se empenharam diariamente junto comigo em busca de adquirir conhecimento científico e disseminá-lo através das publicações.

À todos os voluntários que aceitaram participarda fase clínica desta pesquisa, mesmo com vários percalços não desistiram e me ajudaram a concretizar esse sonho.

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"É justo que muito custe o que muito vale".
Santa Teresa D'Ávila

RESUMO

Objetivo: Avaliar a eficácia de dentifrícios com tecnologias inovadoras para remineralização de lesões iniciais de cárie e erosão *in vitro,* e quanto à capacidade de retenção de flúor *in vivo* em biomarcadores de exposição. **Materiais e métodos:** Para o modelo *in vitro*, 60 blocos de esmalte bovino foram cariados artificialmente por ciclagem de pH durante 6 dias, intercalada com exposição aos dentifrícios: G1- Controle negativo (NC); G2- Controle positivo (PC); G3- Regenerador diário Dentalclean (RDC); G4- Regenerate Enamel Science (RES) e G5- Sensodyne Repair & Protect (SRP). Os espécimes foram submetidos à análise de microdureza superficial, análise quantitativa de fluorescência induzida por luz (QLF), para calcular o percentual de remineralização da superfície (%SMH_R) e o percentual de recuperação mineral (ΔF_{RE}). A morfologia da superfície das amostras foi avaliada através de microscopia eletrônica de varredura (MEV) e espectroscopia por energia dispersiva (EDS). Os dados foram analisados por meio de teste ANOVA seguido de Tukey, ANOVA de medidas de repetidas e Correlação de Pearson (α= 5%). Logo em seguida, alguns desses blocos de esmalte (n=5), foram submetidos a um desafio erosivo, sendo imersos em 50% de ácido cítrico por 2 minutos e avaliados na MEV e EDS. No ensaio clínico cruzado randomizado, 15 indivíduos usaram os dentifrícios por uma semana: G1- Regenerador Diário Dentalclean Neutro (RDCN); G2- Sensodyne Repair & Protect (SRP); G3- Regenerador Diário Dentalclean Ácido (RDCA); G4 Colgate Total Reparação Diária (CTDR). No sétimo dia de uso do dentifrício, o biofilme foi coletado em 1 e 12 h, e a saliva foi coletada até 60 min e 12 h após a última escovação. As concentrações de F foram determinadas usando usando a técnica de difusão facilitada. Resultados de saliva foram analisados pelo teste de ANOVA de medidas repetidas seguido de Bonferroni (α= 5%). Área sob a curva (AUC) após 1h de uso dos dentifrícios foi calculado para os dados de saliva. Para biofilme, foram aplicados teste de Wilcoxon, teste de Friedman e Pós-teste de Bonferroni (α= 5%). **Resultados:** Para cárie dentária (*in vitro*), os valores de %SMH_R variaram de: 1,8 a 22,8. Já em ΔF_{RE}, ficaram na faixa de -6,6 a 11,9. O RDC obteve os maiores valores em ambas as aferições (p<0,05). A análise morfológica demonstrou a formação de uma espessa camada mineral na superfície das amostras tratadas com esse grupo. Após a realização do desafio erosivo, o RDC apresentou a superfície mais lisa. No estudo *in vivo*, os maiores valores de AUC em saliva foram para G3, G4, G1 e G2, respectivamente. Para o biofilme, na avaliação entre os grupos e no mesmo tempo de coleta, o grupo G3 (RDCA) foi o que apresentou maiores valores de mediana em 1 e 12 horas: 452,90 e 373,60 ppm, respectivamente; e diferentes do placebo ($p<0,05$). **Conclusão:** Conclui-se que o Regenerador Diário Dentalclean foi o mais eficaz (*in vitro*) para remineralização da lesão cariosa e proteção contra desafios erosivos. Os resultados do modelo *in vivo,* corroboram esses achados para as lesões iniciais de cárie dentária.

Palavras-chave: Remineralização dentária, cárie dentária, erosão dentária, dentifrícios, dureza, microscopia e biomimética.

ABSTRACT

Objective: To evaluate the effectiveness of dentifrices with innovative technology for remineralization of initial carious lesions and erosion *in vitro*, and for the ability to retain fluoride *in vivo* in exposure biomarkers. **Materials and methods**: For the *in vitro* model, 60 blocks of bovine enamel were artificially decayed by pH cycling for 6 days, interspersed with exposure to dentifrices: G1- Negative control (NC); G2- Positive control (PC); G3- Daily Regenerator Dentalclean (RDC); G4- Regenerate Enamel Science (RES) and G5- Sensodyne Repair & Protect (SRP). The specimens were subjected to surface microhardness analysis, quantitative analysis of light-induced fluorescence (QLF), to calculate the percentage of surface remineralization (% SMH_R) and the percentage of mineral recovery (ΔF_{RE}). The surface morphology of the samples was evaluated using scanning electron microscopy (SEM) and dispersive energy spectroscopy (EDS). The data were analyzed using ANOVA test followed by Tukey, ANOVA of repeated measures and Pearson's correlation (α = 5%). Soon after, some of these enamel blocks (n = 5), were subjected to an erosive challenge, being immersed in 50% citric acid for 2 minutes and evaluated in SEM and EDS. In the randomized crossover clinical trial, 15 subjects used the following toothpaste for one week: G1- Daily Regenerator Dentalclean Neutral (RDCN); G2- Sensodyne Repair & Protect (SRP); G3- Daily Regenerator Dentalclean Acid (RDCA); G4 Colgate Total Daily Repair (CTDR). On the seventh day of use of the toothpaste, the biofilm was collected at 1 and 12 h, and saliva was collected up to 60 min and 12 h after the last brushing. F concentrations were determined using using the facilitated diffusion technique. Saliva results were analyzed by the repeated measures ANOVA test followed by Bonferroni (α= 5%). Area under the curve (AUC) after 1 hour of using the toothpaste was calculated for the saliva data. For biofilm, Wilcoxon test, Friedman test and Bonferroni post-test (α = 5%) were applied. **Results:** For dental caries (in vitro), the% SMH_R values ranged from: 1.8 to 22.8. In ΔF_{RE}, they were in the range of -6.6 to 11.9. The RDC obtained the highest values in both measurements ($p<0,05$). The morphological analysis showed the formation of a thick mineral layer on the surface of the samples treated with this group. After the erosion challenge, the RDC presented the smoothest surface. In the *in vivo* study, the highest values of AUC in saliva were for G3, G4, G1 and G2, respectively. For biofilm, in the evaluation between the groups and at the same collection time, the G3 group (RDCA) was the one with the highest median values in 1 and 12 hours: 452.90 and 373.60 ppm, respectively; and different from placebo ($p < 0.05$). **Conclusion**: It is concluded that the Daily Regenerator Dentalclean was the most effective (*in vitro*) for for remineralization of the carious lesion and protection against erosive challenges. The results of the *in vivo* model corroborate these findings for the initial lesions of dental caries.

Keywords: Tooth Remineralization, Dental caries, Dental erosion, Dentifrices, Hardness, Microscopy and Biomimetics.

SUMÁRIO

1. INTRODUÇÃO

A cárie e a erosão dentária são doenças freqüentes da cavidade bucal, sendo consideradas como importantes problemas de saúde pública (Pitts *et al*., 2017; Carvalho, Lussi, 2019). Apesar do declínio na prevalência da cárie nos últimos anos, pesquisas realizadas recentemente demonstram um aumento mundial dessa patologia (Nozari *et al.,* 2017; Pitts *et al*., 2017). No Brasil, segundo o último levantamento epidemiológico nacional, as taxas de prevalência variam entre 37,3 a 78,2% (Firmino *et al*., 2018). O perfil epidemiológico da cárie é heterogêneo, apresentando uma distribuição desigual, dependendo principalmente do nível socioeconômico da região, com os maiores índices de prevalência em populações desfavorecidas economicamente (Engelmann *et al*.,2016). No que diz respeito à erosão dentária, ela se encontra cada vez mais presente na população, especialmente nos jovens, devido a mudanças nos hábitos alimentares, com um aumento no consumo de alimentos e bebidas ácidas (Colombo *et al*., 2017; Carvalho, Lussi, 2019) .

A cárie possui uma etiologia multifatorial, com vários os fatores de risco envolvidos no seu desenvolvimento, a exemplo da alta ingestão de carboidratos fermentáveis, composição microbiana (Llena*et al*., 2015), condições do fluido salivar (Farooq, Bugshan, 2020), assim como fatores comportamentais (Pitts*et al*., 2017). Em contrapartida, a erosão dental é uma condição clínica sem envolvimento microbiano, decorrente do ataque ácido as superfícies dentárias, causando dissolução mineral e perda da integridade estrutural (Moazzez, Austin, 2018). A etiologia dessa patologia envolve a interação entre fatores extrínsecos e intrínsecos. Os fatores extrínsecos são os ácidos advindos da dieta, da ingestão de medicamentos e/ou das doenças ocupacionais. Enquanto que os fatores intrínsecos estão relacionados com o próprio individuo, como a presença de refluxo gastro-esofágico e/ou a bulimia (Passos, Rodrigues, Santiago,2018).

Os elementos dentais encontram-se em equilíbrio na cavidade oral, com a saliva e o biofilme que os rodeia, mantendo sua estrutura integra e inalterada (Magalhães*et al*., 2017). O aumento na acidez do ambiente bucal, seja provocado pelo metabolismo microbiano ou pelo ataque ácido advindo de outras fontes, altera esse equilíbrio, deixando a saliva com pH ácido e insaturado em relação aos íons que compõem os cristais minerais do dente (Magalhães *et al*., 2017). A partir daí, a reação de desmineralização se sobressai, e ocorre aperda mineral com a formação das lesões de cárieou erosão (Magalhães *et al.,* 2017). Esses processos são distintos em alguns aspectos, tendo em vista que a cárie é uma lesão que se inicia na subsuperfície, enquanto que a erosão começa diretamente na superfície dentária, levando ao amolecimento desta, seguido pela dissolução contínua de camada por camada dos cristais de hidroxiapatita do esmalte (Fita; Kaczmarek, 2016; Magalhães*et al.,* 2017). No entanto, uma característica em comum é que ambos podem ser minimizados e o equilíbrio fisiológico pode ser restabelecido, através especialmente de íons flúor, cálcio e fosfato, que promovem a remineralização das superfícies de esmalte e inibem a perda mineral (Ten Cate, Buzalaf, 2019; Fita; Kaczmarek, 2016).

Dessa forma, é essencial reduzir os fatores nocivos que aumentam a probabilidade da cárie e/ou erosão dentária (Bossú*et al*., 2019; Lussi, Carvalho, 2015). O flúor surge nesse contexto, pois é reconhecido como principal agente de higiene oral, responsável pelo declínio da cárie em todo o mundo (Amaechi, Loveren, 2013). Para a erosão dentária os seus benefícios ainda não são totalmente esclarecidos quanto para a cárie (Magalhães *et al*., 2017). Porém, sabe-se que a formação de uma camada de fluoreto de cálcio na superfície dentária, ajuda a proteger os elementos durante uma queda de pH, fornecendo íons flúor e cálcio para o meio circundante, aumentando a saturação, inibindo a desmineralização e favorecendo a redeposição mineral (Carvalho *et al.,* 2016). Além disso, o fluoreto consegue penetrar na estrutura dentária, substituindo os grupos hidroxila e resultando na formação da fluorapatita, uma fase mineral mais resistente ao ataque ácido (Tahmasbi*et al*., 2019). Portanto, tendo em vista todos os benefícios do flúor para a manutenção da integridade dos elementos dentais, a constância dos níveis intrabucais desse íon é muito relevante do ponto de vista clínico (Ten Cate, Buzalaf, 2019).

Das mais variadas formas de fluoretos disponíveis, o dentifrício fluoretado é uma das alternativas mais acessíveis e eficazes, por seu uso tópico e diário, além da associação com a limpeza mecânica dos dentes (Walsh *et al.*, 2019; Carvalho *et al.,* 2016; Pitts*et al.,* 2017). A escovação com creme dental fluoretado se enquadra no controle dessas patologias, especialmente da cárie, pois combina a desorganização e/ou remoção do biofilme, diminuindo seu potencial patogênico, com a manutenção de concentrações intraorais satisfatórias de flúor para auxiliar no equilíbrio mineral dos dentes (Esteves-oliveira *et al.*, 2016; Nassar*et al.,* 2018).

A cárie é uma doença que só ocorre na presença de bactérias específicas, que se unem formando um biofilme cariogênico, capaz de permanecer íntegro por longos períodos de tempo e destruir os sítios dentários onde se acumula (Pitts*et al.*, 2017). Portanto, é de extrema importância a remoção e/ou modificação desse biofilme, para tentar minimizar a dissolução dos cristais de hidroxiapatita (Pitts *et al.*, 2017). Além disso, a quantidade de fluoreto retida nesse compartimento têm grande impacto clinicamente. Durante uma queda de pH provocada pelo metabolismo microbiano da sacarose, íons flúor são liberados para o fluído do biofilme e em seguida para o esmalte dentário, agindo contra a desmineralização (Buzalaf *et al.,* 2011).

Os níveis intrabucais de flúor são resultado da interação complexa entre dois sistemas: de eliminação (“clearance”) e de retenção (“F-uptake”). O clearance salivar representa o processo de remoção do flúor da cavidade bucal, seja através da ingestão ou pela lavagem do fluxo salivar. Já o sistema de retenção ou “F-uptake”, são os sítios onde o íon flúor fica aderido, na maioria das vezes associado ao cálcio. Os sítios de ligação do íon flúorsão representados pela matriz intercelular e o fluído do biofilme, além das células da mucosa bucal e o cálcio salivar. Esses dois sistemas são opostos, porém agem simultaneamente, sendo influenciados por diversos fatores, como: fluxo salivar, freqüência e duração da escovação, além da concentração de flúor no dentifrício utilizado (Duckworth, 2013; Duckworth, Jones, 2015; Alves *et al.*, 2018).

Estudos demonstram que quando é feito o uso de produtos fluoretados, como os dentifrícios, a saliva transporta esse íon livre para os sítios de retenção e ajuda no processo de remineralização pelo contato direto com os elementos dentais (Souza *et al.,* 2015; Kondo *et al*., 2016). Rapidamente a concentração desse íon cai, devido à deglutição e lavagem do fluxo salivar (Souza *et al.,* 2015; Kondo *et al*., 2016). No biofilme, a concentração de flúor é bem maior e vai sendo liberada gradualmente, funcionando como um local para troca de íons com o esmalte dental (Larsen *et al*., 2018; Alves *et al*., 2018). Sendo assim, a saliva e o biofilme funcionam como biomarcadores orais de exposição, ou seja, são parâmetros biológicos mensuráveis experimentalmente, que vão indicar uma resposta ao flúor. Estudos com esses biomarcadores demonstram que os níveis intrabucais do flúor estão em constante flutuação ao longo do dia (Larsen *et al*., 2018; Alves *et al*., 2018).

Diante desse contexto, o aumento do tempo de atuação do fluoreto na cavidade bucal após a escovação, ainda é um desafio para a odontologia nos dias atuais. Além disso, a eficácia do flúor, representada pela capacidade desse composto de atingir o seu objetivo, é limitada para erosão dentária. Em relação à cárie, observamos o seu crescimento mesmo diante do uso de produtos fluoretados pela população, evidenciando a dificuldade desse elemento em controlar a doença em determinados indivíduos. Por isso, esforços estão sendo realizados para desenvolver novos compostos. Ingredientes ativos que consigam estabelecer uma relação de sinergismo com o flúor, ajudando a manter o elemento dental em equilíbrio químico com o meio circundante (Magalhães *et al*., 2017; Philip *et al*., 2018). Assim, surgem as tecnologias biomiméticas, que estão sendo promissoras para prevenir e tratar a cárie e a erosão dentária. Esses produtos têm o objetivo de simular a remineralização natural, promovendo a formação de cristais de hidroxiapatita menos solúveis e porosos através da associação do fluoreto com diversos ingredientes ativos (Philip, 2019; Delbem *et al.,* 2019; Vilhena *et al.,* 2020).

Portanto, a hipótese desse estudo, é que um sistema odontológico multifuncional à base de gel (tecnologia Refix), com propriedade para formar complexos estáveis em meio ácido, ricos em fosfato e silício, seja capaz de aumentar os níveis intrabucais de flúor ao longo do tempo e atuar na remineralização e/ou prevenção de lesões cariosas e erosivas. Resultados satisfatórios já foram encontrados em alguns estudos (Tomaz *et al*., 2020;

Vilhena *et al*., 2020), demonstrando a formação de uma camada chamada de "enamel like" na superfície dentária após utilização desse creme dental. Entretanto, este estudo é pioneiro na avaliação quantitativa e qualitativa do conteúdo mineral formado por esses dentifrícios com novas tecnologias de remineralização. Assim, o objetivo desse trabalho é avaliar *in vitro* a remineralização de lesões iniciais de cárie e a proteção contra erosão em esmalte bovino, além da retenção *in vivo* de flúor em biomarcadores intraorais de exposição, após a utilização de dentifrícios com tecnologia Refix.

2. CAPÍTULO 1

O manuscrito a seguir será submetido para publicação no periódico Caries Research. (Qualis: A1, IF 2019-2020 = 2.8)

Effectiveness of fluoride-containing toothpastes associated with different technologies to remineralize enamel after pH cycling

Running title: Effectiveness of fluoride toothpastes to remineralize enamel

Nayanna Lana Soares Fernandes[a], José Gabriel Victor Costa Silva[b], Elizabeth Barreto Galvão de Sousa[b], Paulo Henrique Perlatti D'Alpino[c], Andressa Feitosa Bezerra de Oliveira[d], Elbert de Josselin de Jong[e], Anderson Takeo Hara[g], Fábio Correia Sampaio[f]

[a]Department of Clinical and Community Dentistry, Health Science Center, University of Paraíba, João Pessoa, PB, Brazil;
[b]Dentistry undergraduate course, Federal University of Paraíba, João Pessoa, PB, Brazil;
[c]Secretaria de Estado da Saúde de São Paulo, São Paulo, SP.
[d]Department of Morphology, Health Science Center, University of Paraíba, João Pessoa, PB, Brazil,
[e]Research & Development, Inspektor Research Systems BV, Amsterdam, Netherlands,
[f]Oral Health Research Institute, Indiana University School of Dentistry, Indianapolis, IN, USA.

***Corresponding Author:**
Prof. Dr. Fábio Correia Sampaio
Department of Clinical and Community Dentistry, Health Science Center, University of Paraíba, João Pessoa, PB, Brazil;
Street Name & Number: Street Mario Batista Júnior, number 75, Miramar. João Pessoa, Paraíba, Brazil.
Postal code: 58043-130
Tel: + 55 (83) 3216 - 7795
E-mail: fcsampa@gmail.com

Abstract

Objectives: The present *in vitro* study evaluated the effectiveness of fluoride-containing toothpastes containing different technologies to remineralize artificial caries lesions in enamel. **Materials and Methods**: Enamel blocks were prepared from extracted bovine teeth and randomly distributed into five groups according to the treatment (n=12): Fluoride-free toothpaste Colgate Oral Cares (NC); Colgate Total Daily Repair (PC); Regenerador Diário DentalClean (RDC),Regenerate Enamel Science (RES), and Sensodyne Repair & Protect (SRP). For the microhardness test, the enamel surfaceswere divided into three parts: sound, demineralized, and treated areas. One-third of the specimens surfaces were coated twice with nail varnish to create control areas (sound and demineralized). The specimens were submitted to a pH cycling model for 6 days. Briefly, the enamel samples were exposed to toothpaste slurry (3:1 weight ratio of deionized water to toothpaste; 2 mL/block) for 1 minute, twice a day, before and after immersion into to demineralization solution (for 2 h), and subsequently the specimens were immersed into a remineralization solution for 22 h. The effectiveness of the toothpastes to remineralize the enamel was evaluation with surface microhardness (%SMH_R) and Quantitative Light-induced Fluorescence (QLF, ΔF_{RE}). The surface and cross-sectional micromorphology was assessed using scanning electron microscopy (SEM). The elemental analyses (weight%) were determined with energy-dispersive X-ray spectrometer (EDS). Data was statistically analyzed (α= 5%). **Results:** The highest mean of remineralization (%SMH_R and ΔF_{RE}) was found for RDC, 22.8 and 11.9 respectively. A strong positive correlation was observed between these variables measuring remineralization (r^2=0.9371). In the morphology analysis, the same group provided the formation of a mineral layer on the surface enriched with silica. **Conclusions**: It can be concluded that the RDC, silicate-based fluoride toothpaste containing proprietary REFIX technology, demonstrated higher effectiveness for remineralizing artificial caries when compared to products.

Keywords: Tooth Remineralization, Toothpaste, Dental caries, Hardness, Microscopy, Biomimetics.

Introduction

For a long period of time it was believed that the fluoride effect was only systemic. The more exposed the individual was to any method of fluoride ingestion, the more of that ion would be incorporated into the forming dental elements and the more resistant the enamel would be, with less susceptibility to dental caries [Fejerskov, 2004]. However, with advances in dental researches, it was discovered that the mechanism of action of fluoride is topical, through the reduction of enamel dissolution and favoring remineralization, being considered the main agent in the fight against caries worldwide [Hellwig and Lennon, 2004; Scholz et al., 2019; Tahmasbi et al., 2019; Ten Cate and Buzalaf, 2019]. Several fluoridated products are available on the market, with different compositions, with toothpastes being the most used, especially for their easy access and low cost [Cruz and Narvai, 2018; Vilhena et al., 2020]. Thus, brushing with fluoridated toothpastes is the most effective non-professional intervention to prevent tooth decay, especially in places where there is no presence of fluoridated water [Ganavadiya et al., 2014; Tomaz et al., 2020].

Currently, research has been carried out in order to find active principles with more sophisticated action mechanisms, which can be added in new products to increase fluoride efficiency and improve its performance [Cardoso et al., 2015; Delbem and Pessan, 2019; Farooq et al., 2019]. The innovative aspects are not limited to trying to reduce enamel demineralization, but also to increase the mineral precipitation capacity of these formulations [de Camargo Smolarek et al., 2015; Delbem and Pessan, 2019; Farooq et al., 2019]. Thus, several compounds are being introduced in fluoridated toothpastes, which may alter their pH and abrasiveness [Tomaz et al., 2020]. In this context, biomimetic agents appear which function as fluoride and calcium carriers for tooth enamel [Delbem and Pessan, 2019; Philip, 2019]. This is an alternative mechanism with the objective of mimicking natural remineralization, promoting the formation of less soluble and porous hydroxyapatite crystals [Volponi et al., 2018; Xiao et al., 2017].

Silica, a component of so-called bio-glass, has been used in toothpaste formulations, acting as a site for the precipitation of calcium and phosphate ions, leading to the nucleation of hydroxyapatite and mineral formation [Carvalho et al., 2019]. Thus, this compound intensifies the remineralization process, and can be associated with calcium in the dental structure, forming calcium silicate [Parker et al., 2014]. Calcium silicate is responsible for a protective effect on the surface, stimulating the deposition of other minerals and reducing the effects of demineralization [Parker et al., 2014].

Recently, a technology that combines low pH fluoride toothpaste with phosphate and silica was developed. This proprietary technology REFIX, it is a multifunctional dental compound, based on gel, which promotes the formation of a new mineral phase on the dental tissue, called fluoride apatite rich in silicate. According to the manufacturer, this layer or "protective shield" promotes an increase in the enamel's mechanical properties and reduces its solubility, being able to remineralize the subsurface and protect against new acid attacks. In addition, this same mineral layer can precipitate on the dentin surface, when it is exposed, occluding the tubules and reducing dentin hypersensitivity. Recently published studies confirm this mineral deposition [Vilhena et al., in press] and the beneficial effects of this toothpaste, either *in vitro* [Tomaz et al., 2020] or *in vivo* [Vilhena et al., 2020].

Therefore, in view of the current efforts in search of effective components against caries, the purpose of this *in vitro* study was to analyze the mineral gain and changes in the surface of artificial caries lesions in enamel, treated with different toothpastes containing silica and other bioactive components.

Materials and methods

Study design

Enamel blocks (4x4x2 mm) were prepared from extracted bovine incisor teeth and stored in 0.08% Thymol solution. The specimens were embedded in self-cured acrylic resin circular using molds of 16 mm diameter and 3 mm deep. The outer enamel surface was ground flat with grit papers (600–1500 grades) under water refrigeration and polished with 1 µm diamond paste (Extec Corporation, Enfield, CT) in a rotating polishing Machine PSK-2V (Skill-tec Comércio e Manutenção Ltda, São Paulo, SP, Brazil). Baseline enamel surface microhardness (SH_0) analysis was performed with a microhardness tester (Shimadzu HMV - AD Easy Test Version 3.0). Five indentations spaced 100 µm from each other were made at the center of the enamel surface (50 g, 10 s). Enamel blocks between 360 and 400 VHN surface microhardness were selected for the study. Sixty enamel specimens were randomly distributed into five groups (n = 12) according to their surface microhardness (Table 1).

Lesion formation

Subsurface enamel demineralization was carried out using a modified model [Queiroz et al., 2008]. Following 5 min sonication in water using an ultrasonic device, one third of the exposed enamel surface were covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) as a reference sound area.The enamel blocks were immersed individually in 32 mL of a demineralizing solution containing 1.3 mM/L $Ca(NO_3)_2.4H_2O$, 0.78 mM/L NaH_2PO_4 H_2O in 0.05 M/L acetate buffer, 0.03 µgF/mL (NaF), pH 5.0, 32 mL/specimen, during 16 h at 37ºC. After that, the blocks were submitted to a post-demineralization surface hardness (SH_1) with the same parameters described previously. The percentage of surface hardness change was calculated to randomize the enamel blocks in the treatment groups, as follows (Equation 1):

$$\%SMHC = \frac{(\mathrm{SH1} - \mathrm{SH0})}{\mathrm{SH0}} \text{ x } 100 \qquad (1)$$

Remineralizing pH-cycling

Before the remineralization pH cycling model,[Vieira et al., 2005] the enamel specimens had another one third of its surface covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) as a reference for caries lesion area. The specimens were individually submitted to a pH cycling model at 37º C during 6 days. The blocks were immersed individually in a remineralization solution (1.5 $mM.L^{-1}$ calcium, 0.9 $mM.L^{-}1$ phosphate, 150 $mM.L^{-1}$ potassium chloride in 0.02 $mM.L^{-1}$cacodylic buffer, pH 7.0; 0.02 µgF/mL, $1mL/mm^2$), for 22 h. The cariogenic challenge was performed by a demineralization solution (2.0 mM.L-1 calcium and phosphate in 75 $mM.L^{-1}$ acetate buffer, pH 4.7; 0.03 µgF/mL, 3 mL/mm^2) during 2 h per day (12 pm – 2 pm). Twice a day, 10 am and 2 pm, enamel samples were exposed to toothpaste slurries (toothpaste: deionized water, 1:3 w/w; 2 mL/enamel specimen) for 1 minute, under agitation. Deionized water rinses were performed between each step. In between treatments, each enamel block was individually immersed in remineralization solution at 37ºC. The de- and remineralizing solutions were freshly changed every day. After the remineralizing pH-cycling and treatments, enamel SMH was then determined (SH_2), as previously described. The percentage surface microhardness recovery was then calculated, as follows (Equation 2):

$$\%SMHR = \frac{(\mathrm{SH2} - \mathrm{SH1})}{(\mathrm{SH1} - \mathrm{SH0})} \text{ x } 100 \qquad (2)$$

Quantitative Light-induced Fluorescence (QLF) analysis

The bovine enamel blocks were evaluated for fluorescence loss in caries lesions and treated areas, using the Qraycam pro device. The nail varnish in each window was carefully removed with surgical blade associated with cotton swabs soaked in diluted acetone. Then, the specimens were water rinsed with deionized water and dried with a cotton roll. To standardize the QLF measurements, a camera was attached to a stand in the same position for all the images. The images were taken in a dark room, with an exposure of 0, a contrast of 0, and a distance between the device and a sample of 8 cm, according to the manufacturer's instructions [Diniz et al., 2019; Park et al., 2019]. A software (Q-ray version 1.38, Inspektor Research Systems) analyzed the changes in the amount of mineral in the enamel based on the ΔF value.

ΔF represents the percentage decrease in the autofluorescence intensity in a carious lesion and remineralized areas when compared that in sound enamel, and it reflects changes in the mineral contents of enamel [Park et al., 2019]. For the calculation of the Percentage fluorescence recovery (ΔF_{RE}), the measurements were made in two stages: ΔF_0, which represented a loss of initial fluorescence, passing through the difference between the sound and decayed enamel, and ΔF_1, which represents a difference in final fluorescence, using the difference between the sound enamel and the treatment area with toothpaste [Gokce et al., 2017; Kim et al., 2018]. Then, the percentage of fluorescence recovery was calculated as follows [Kim et al., 2018] (Equation 3):

$$\Delta FRE = \frac{(\Delta F1 - \Delta F0)}{\Delta F0} \times 100 \qquad (3)$$

Scanning Electronic Microscopy (SEM) plus Energy-dispersive X-ray spectroscopy (EDS)

The morphological analysis of the specimens was performed in a scanning electron microscope (EGA 3, TESCAN, LMU, Kohoutovice, Czech Republic), operating at 15 kV. For the morphological analysis of the specimens, the blocks were previously sputter-coated with gold in a vacuum evaporator (MED 010; Balzers, Balzer, Liechtenstein), and then microscopically analyzed to obtain photomicrographs of the superficial morphology of the treated specimens (1,000× magnification). Representative images of selected regions of the sputter-coated specimens were taken in order to characterize the morphological aspect of the surface. The EDS point analysis (80 mm^2, SDD Detector, Oxford Instruments, Concord, MA, USA)was performed to determine a qualitative elemental analysis of specimens, operating in high vacuum mode and an accelerating voltage of 15 kV. For each sample, five points were randomly selected (300 μm^2 for each point), and the mean values were calculated.

For the analysis at the subsurface, cross-sections of the bovine blocks were obtained by longitudinally sectioning the specimens under water-cooling. Both half-blocks were used for the SEM and the elemental analyses. The halves were dehydrated in silica gel for 3 h. The specimens were then gold-sputtered and evaluated using a SEM coupled with an EDS.

Statistical analysis

Data wasanalyzed statistically using the SPSS package for Windows, version 21.0 (SPSS, Inc., Chicago, IL, USA). The Shapiro-Wilk test and Levene's test were used to determine normality and homogeneity of variances, respectively. As the data demonstrated equal variances and Gaussian distribution, no data transformation was needed. The following tests were performed: 1) ANOVA followed Tukey for the analysis of differences between groups regarding SH_0, SH_1, SH_2, $\%SMH_R$ and ΔF_{RE}; 2) ANOVA Repeated Measures, followed by Bonferroni, for the analysis of the variables SH_0, SH_1, SH_2 into the same group at the different analysis times; 3) Pearson's correlation between variables. The level of significance considered was 5 %.

Results

The average values of surface microhardness and standard deviation for the variables SH_0, SH_1 and SH_2 are described in Table 2. For SH_0, the average surface microhardness ranged from 373.5 to 384.4 and for SH_1 it was 31.4 to 32.8. For the variable SH_2, the highest mean found was 112.5 and the lowest 38.1. Comparing each group individually, there was a statistically significant difference between the times SH_0 and SH_1 and between SH_1 and SH_2, for all groups (p <0. 05).

Comparing the groups by the variables SH_0 and SH_1, no significant differences were observed between the groups ($p > 0.05$). However, for SH_2 a significant lower mean was observed when the enamel blocks were treated with the fluoride-free toothpaste Colgate Oral Care. There were statistically significant differences between dentifrices for this variable ($p < 0.05$). It is noticed that RES and SRP were similar to each other, and that PC showed similar behavior to them and to RDC ($p > 0.05$).

When the% SMH_R results were compared, RDC and PC exhibited significantly higher means, followed by RES and SRP (Figure 1). After treatment with fluoride-free dentifrice, the lowest recovery value of surface hardness was observed ($p < 0.05$). Conversely, the ΔF_{RE} was calculated, RDC exhibited significantly higher mean, when compared to other treatments, followed by PC and RES, which were statistically similar ($p > 0.05$). A strong positive correlation was found when variables ΔF_{RE} and $\%SMH_R$ were plotted ($r^2 = 0.9371$).

In the Figure 2, representative scanning electron micrographs of the enamel cross-sections are displayed. It is clearly demonstrated the differences among the experimental groups. The product containing REFIX technology, induced the formation of a mineralized layer onto the enamel surface when associated with pH cycling. This mineralized surface layer was not observed when the enamel blocks were treated with the other products. In Table 3, the elemental mapping analysis was displayed, also demonstrating differences among the groups. RDC exhibited the highest silicon content.

Discussion

Biomimetic systems represents an alternative mechanism that reproduces the natural process of dental tissue mineralization [Elgamily et al., 2019; Vilhena et al., in press]. Currently, new commercially available oral care products, containing different active ingredients associated with fluoride, claim to boost the remineralization and regeneration potential of these formulations [Cardoso Cde et al., 2015; Kraivaphan and Amornchat, 2017].The quantitative analysis showed that the most satisfactory behaviors were from the RDC and PC. The high fluorescence recovery value presented by the RDC demonstrated the greatest mineral gain in the samples after treatment, as previously reported [Tomaz et al., 2020]. The recovery of surface hardness followed the same trend, with the highest values presented by RDC followed by PC. Satisfactory remineralization for the Positive Control (PC) group validated the model *in vitro*, and furthermore, corroborated by previous findings [Oliveira et al., 2019; Rana et al., 2007]. For these results, it can be assumed that the increase in enamel mineral content after treatment with RDC and PC, had a direct effect on increasing the surface hardness of the samples. Besides that, the MEV qualitative analysis was able to ratify the hardness and fluorescence values. The thick mineral layer in the surface and subsurface images showed that the RDC had an excellent remineralizing capacity, promoting the recovery of the enamel's fluorescence properties, in addition to making the surface harder and more resistant. These findings were corroborate in another previous study [Vilhena et al., in press], which also demonstrated the formation of a new mineral layer rich in calcium and silicon in the samples treated with this same toothpaste.

Two of the fluoride toothpastes tested contain tetrasodium pyrophosphate in association with sodium fluoride (CTDR and RDC). It has been advocated that the phosphate contained in the composition of these toothpastes helps to oversaturate the tooth tissues with calcium and phosphate, which contributes with the decrease of ions on the previously demineralized enamel surface, consequently increasing the resistance to the cariogenic process in the presence of fluoride ions [Dai et al., 2019]. RDC also contains proprietary Refix technology, which is a complex of salts, organic components, silicon and phosphates, according to the manufacturer. This complex is acidified, which further contributes to its effectiveness. In addition, it is established in saliva and when it comes into contact with the dental structure, it favors the formation of new mineral phases. The presence of a large amount of silicon in its composition seems to guarantee greater bioactivity, facilitating the formation of a modified hydroxyapatite on the surface and subsurface of tooth enamel [Vilhena et al., 2020].

The other product evaluated in the present study contains Novamin technology (Sensodyne Repair & Protect). It has sodium and calcium phosphosilicate (Bioglass) in the form of an amorphous inorganic compound [Joshi et al., 2013]. Despite presenting statistical similarity with the PC, it was the one that obtained the lowest absolute values of mineral gain and hardness recovery. According to the manufacturer, a series of chemical reactions occurs when Bioglass comes into contact with an aqueous solution. This can lead to the formation of a carbonated hydroxyapatite in the dentin, functioning as an insoluble mineralized layer on the surface. This technology seems to favor another mechanism of action in the enamel, which can alter the structure of hydroxyapatite in this tissue and reinforce it, without actually forming a mineralized layer on the surface. In the present study, no deposition of minerals was observed in the enamel (Figure 2), although the formation of a less soluble surface hydroxyapatite that is resistant to acid challenges may occur [Burwell et al., 2009]. Parkinson et al. [Parkinson et al., 2017], claim that the bioglass failed to improve the remineralizing performance of SRP toothpaste. However, satisfactory values of fluorescence and microhardness were observed by Gocke et al. [Gocke et al., 2017] and Jagga et al. [Jagga et al., 2018].

In an *in vitro* study, the protective effect of four commercial toothpastes was investigated [Wang et al., 2011]. It was found that the toothpaste containing proprietary technology Novamin was not effective in preventing acid challenges when applied before or after in specimens [Wang et al., 2011]. In a systematic review, the effectiveness of Novamin was evaluated based in publications evaluating its action as a remineralizing agent [Khijmatgar et al., 2020]. The authors concluded that Novamin had significantly less clinical evidence to demonstrate its effectiveness as a remineralization agent in treating both carious and non-carious lesions. Clinical trials better designed to recommend this technology have been suggested [Khijmatgar et al., 2020].

RES toothpaste has patented NR-5 technology. This technology claims to combine calcium silicate, sodium phosphate salts and fluoride, in order to increase the saliva mineralization processes by hydroxyapatite nucleation and the formation of dental enamel minerals [Gorrepati et al., 2010]. Despite the inferior performance of this technology in relation to the RDC, mineral gain and increased surface hardness were also observed. These findings corroborate those of Joiner et al. [Joiner et al., 2014], which found that this technology was able to repair the carious lesion. However, disagreeing with this remineralizing capacity, a previous study [Chandru et al., 2020] found a poor performance of this toothpaste, with lower hardness values and without evidence of remineralization. Following SEM analysis with PC and RES toothpaste, the formation of a new, less thick mineral phase on the surface and subsurface was observed. Therefore, these findings confirm the remineralizing tendency found by the quantitative analysis reported above. Sun et al. [Sun et al., 2014] ratify the result of this study, as they also found the formation of a new hydroxyapatite derived from the calcium silicate contained in the RES.

Finally, based on the findings of this research, it is important to highlight that the *in vitro* methodology has limitations, such as the impossibility of simulating all the variables existing in the oral environment. Despite these limitations, efforts were made to get as close as possible to the demineralization and remineralization processes, especially through pH cycling [Amaechi, 2019]. These *in vitro* analyzes allowed greater control of conditions with reduced costs, mainly to test the effectiveness of new products designed to remineralize the enamel tissue. In addition, in the present study, two analyzes were performed on the same specimen (surface hardness and mineral gain). In a complementary way, seeking a qualitative analysis of the specimens, a morphological analysis was also performed. These methods are validated in the literature to assess *in vitro* remineralization of tooth enamel [Gokce et al., 2017; Gomez et al., 2014; Krishnan et al., 2017; Vilhena et al., in press].

Conclusion

The toothpaste containing REFIX technology provided remineralization of superficial hardness and recovery of light-induced fluorescence in carious lesions. In addition, it was possible to verify the formation of a new layer of minerals on the surface of the samples. Its performance is even better than that found for positive control. Therefore, this toothpaste can be an alternative to prevent and treat patients with dental caries.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author contributions

Fernandes, Silva, Sousa, and D'Alpino contributed to the planning and execution of the research, through the preparation of samples, the performance of artificial caries lesions, the execution of pH cycling, in addition to measures of surface microhardness, fluorescence induced by light and scanning electron microscopy. Sampaio, Oliveira, Jong, and Hara designated the research methodology, the planning, coordinated each stage of the study, when necessary, made the guidelines during data collection and preparation of the manuscript. Therefore, all participated together in the acquisition, analysis and interpretation of the data. In addition to elaborating the writing of this study and reviewing it critically.

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Table 01: Dentifrices used in the study according to their active ingredients and manufacturer.*

Product	Active Agents	Manufacturer
Fluoride-free toothpaste Colgate Oral Care- Negative Control (NC)	No active ingredients.	Colgate-Palmolive Manufacturing, São Bernardo do Campo, SP, Brazil.
Colgate Total Daily Repair- Positive Control (PC)	1450 ppm F- of as sodium fluoride, 0.30% triclosan, arginine, tetrasodium pyrophosphate.	Colgate-Palmolive Manufacturing, São Bernardo do Campo, SP, Brazil.
Daily Regenerator Dentalclean (RDC)	1450 ppm F- of sodium fluoride; tetrasodium pyrophosphate and silicon (Refix technology).	Rabbit Corp. Londrina/PR, Brazil.
Regenerate Enamel Science (RES)	1450 ppm F- of sodium fluoride and sodium monofluorophosphate, calcium silicate and sodium phosphate (NR-5 technology).	Unilever UK Limited, Leatherhead, Surrey, UK.
Sensodyne Repair & Protect (SRP)	1426 ppm of sodium fluoride and calcium sodium phosphosilicate 5% (NOVAMIN technology).	GSK Consumer Healthcare, Norreys Drive, Maidenhead, Berkshire, SL6 4BL, UK.

*Manufactures' information.

Table 2. Mean and standard deviation of surface microhardness values (SH_0, SH_1 e SH_2) of the analyzed samples.*

Product	SH_0	SH_1	SH_2
NC	373.5 (9.4)^{a,A}	31.9(5.4)^{b,B}	38.1 (3.7)^{c,A}
PC	376.6 (15.6)^{a,A}	31.5(4.0)^{b,B}	96.5 (11.9)^{c,B}
RDC	384.4 (14.3)^{a,A}	32.8(4.4)^{b,B}	112.5 (27.3)^{c,B}
RES	382.2 (16.5)^{a,A}	31.4(2.6)^{b,B}	89.5 (11.7)^{c,B}
SRP	377.0 (13.8)^{a,A}	32.6 (4.1)^{b,B}	81.8 (4.3)^{c,B}

*Means preceded by distinct lower case letters differ statistically within the same line for each group, $p < 0.05$, ANOVA Repeated Measures, followed by the Bonferroni test.

**Different capital letters differ statistically between groups for each variable, in the same column, $p < .05$, ANOVA followed by the Tukey test.

Table 3- Elemental mapping according to the experimental groups.

Element	NC	PC	RDC	RES	SRP
C	49.51	8.8	54.83	28.71	16.78
O	38.79	27.54	32.36	36.22	35.45
F	0.00	0.41	0.05	0.05	1.13
Na	0.20	0.69	0.10	0.32	0.41
Mg	0.00	0.51	0.15	0.29	0.34
Al	0.00	0.00	0.25	0.00	0.00
Si	0.29	0.00	7.14	0.07	0.00
P	4.02	23.82	1.73	11.45	15.63
K	0.00	0.00	0.19	0.00	0.00
Cl	0.15	0.53	0.00	0.41	0.00
Ca	7.05	37.7	3.21	22.48	30.26

Abbreviations: NC: Fluoride-free toothpaste Colgate; PC: Colgate Total 12; RDC: Regenerador Diário DentalClean; RES: Regenerate Enamel Science; SRP: Sensodyne Repair & Protect.

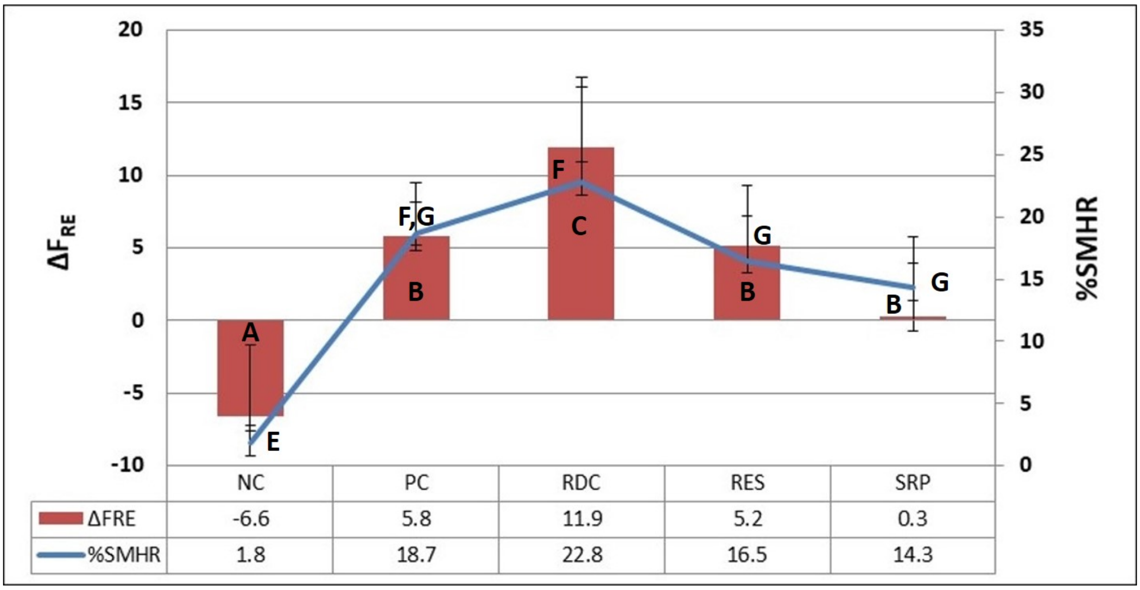
Figure legends

Figure 1- Means and standard deviation of remineralization evaluated with fluorescence (ΔF_{RE}) and microhardness measurements ($\%SMH_R$) according to the experimental groups.
Different capital letters, for each column: significant ($p < 0.05$).
Vertical bar= ± 1 standard deviation

Figure 2- Representative scanning electronmicrographs of the enamel cross-sections. A: NC; B: PC; C: RDC; D: RES; E: SRP.

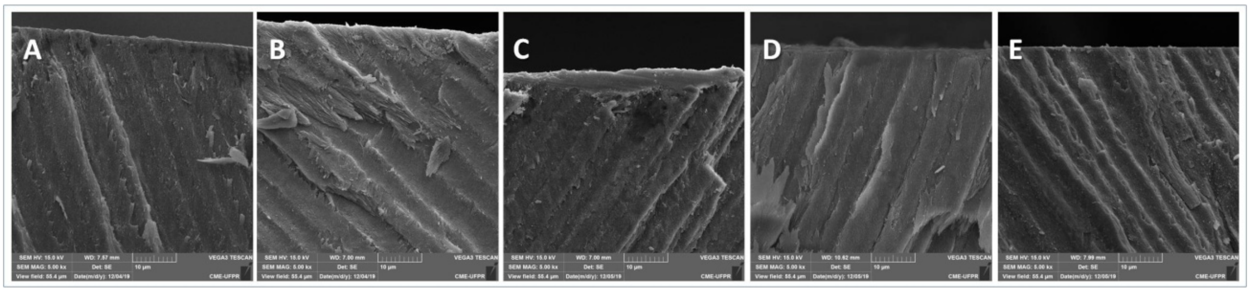
Figures

Fig.1



	NC	PC	RDC	RES	SRP
ΔFRE	-6.6	5.8	11.9	5.2	0.3
%SMHR	1.8	18.7	22.8	16.5	14.3

Fig.2



1. CAPÍTULO 2

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Resistance against erosive challenge of dental enamel treated with 1450-ppm fluoride toothpastes containing different biomimetics compounds

Nayanna Lana Soares Fernandes[1], e-mail: fernandesnayanna58@gmail.com
Pedro Luiz Santos Tomaz[2], e-mail: pedrolstomaz@gmail.com
Artur José Carreira[2], e-mail: dr.arturjcarreira@gmail.com
Juliellen Luiz da Cunha[3], e-mail: juliellenluizcunha@gmail.com
Andressa Feitosa Bezerra de Oliveira[3], e-mail: andressafeitosaboliveira@gmail.com
Paulo Henrique Perlatti D'Alpino[2,5], e-mail: paulodalpino@gmail.com
Fábio Correia Sampaio[1,4], e-mail: fcsampa@gmail.com

[1]Programa de Pós-Graduação Stricto sensu em Odontologia, Universidade Federal da Paraíba (UFPB), PB, Brazil.
[2]Programa de Pós-Graduação Stricto sensu em Biotecnologia e Inovação em Saúde, Universidade Anhanguera de São Paulo (UNIAN-SP), SP, Brazil;
[3]Universidade Federal da Paraíba (UFPB), Centro de Ciências da Saúde Departamento de Morfologia – DM
[4]Universidade Federal da Paraíba (UFPB), Centro de Ciências da Saúde Departamento de Clínica e Odontologia Social – DCOS
[5]Programa de Pós-Graduação Stricto sensu em Ensino de Ciências e Saúde, Universidade Anhanguera de São Paulo (UNIAN-SP), SP, Brazil.

Corresponding author*:
Prof. Dr. Paulo H. P. D'Alpino
Anhanguera University of São Paulo – UNIAN SP
Graduate Program in Biotechnology and Health Innovation
Av. Raimundo Pereira de Magalhães, 3.305
São Paulo – SP, Brazil
CEP: 05145-200
Email: paulodalpino@gmail.

Original Article

Resistance against erosive challenge of dental enamel treated with 1450 ppm fluoride toothpastes containing different biomimetic compounds

Running title: Effectiveness of fluoride toothpastes containing different technologies

Abstract

Objectives: This *in vitro* study aimed to characterize the superficial and subsurface morphology of dental enamel treated with fluoridated gels containing different biomimetic compounds after erosive challenge. **Materials and Methods:** Bovine incisor teeth were sectioned to obtain enamel blocks (4 × 4 × 6 mm; N = 5) that were demineralized to create an artificial caries lesion and treated by pH cycling interspersed with exposure to fluoridated toothpaste slurries under agitation. During pH cycling (demineralization and remineralization for 2 and 22 hours, respectively) for 6 days, the enamel blocks were exposed to toothpaste slurries under agitation with one of the dental gels: Regenerate Enamel Science (NR-5 technology), Daily Regenerator Dental Clean (REFIX technology), and Sensodyne Repair & Protect (Novamin technology). The enamel blocks were subjected to an erosive challenge, immersed in 50% citric acid for 2 min, and then washed with plenty of distilled water. The surface and cross-sectional micromorphology were assessed using scanning electron microscopy (SEM). The elemental analyses (weight%) were determined with an energy-dispersive X-ray spectroscopy (EDS). **Results:** Enamel treated with the product containing REFIX technology presented a smoother surface morphology compared to the other treatments. The higher resistance to the erosive challenge can be attributed to a silicon-enriched mineral layer formed on the enamel induced by the REFIX-based toothpaste. This was not observed in the specimens treated with the other technology-containing toothpastes. **Conclusions:** The REFIX technology seemed to be the most promising compared to the Novamin and NR-5 technologies. In addition to forming a superficially mineralized layer on the enamel, the enamel treated with

REFIX technology associated with the pH cycling resisted a subsequent erosive challenge.

Keywords: Enamel; Dentin; Tooth Remineralization; Toothpaste; Microscopy, Electron, Scanning

Introduction

Dental erosion, defined as the irreversible chemical wear of the dental hard tissue without the involvement of bacteria, represents a tooth pathology that causes patient discomfort. [1] Enamel remineralized by natural saliva is not able to withstand the recurrent erosive attack to the tooth structure.[2] Therefore, preventive measures are indicated for preventing progressive, erosive tooth wear.[3] Fluoride-containing oral care products used against enamel and dentin erosion might promote remineralization through apatite crystallization or replacement of the lost mineral.[4] In fact, most of these products only reduce the hydroxyapatite dissolution to some extent. [4] Recently, a new generation of biomimetic oral care products using advanced technologies with stronger surface bioactivity has been developed to optimize the interaction with the dental tissues. [5] Different components or supplements associated with fluoride were added as ingredients in these products aiming to reproduce the natural process of dental tissue mineralization[5- 6] and boost the remineralization and regeneration potential of the hydroxyapatite. [7-8]

Several promising biomimetic approaches to prevent tooth erosion have been investigated. One of the approaches comprises altering the dissolution properties of the hydroxyapatite with different foreign ions as substituents in the different sites of the hydroxyapatite molecule. [9-10] Each ionic grouping of the hydroxyapatite molecule can be replaced by another of the same or different valence, either anionic or cationic.[9, 11]Changes in the solubility of hydroxyapatite may occur depending on the substitution at the calcium, phosphate, and/or hydroxyl sites. [11] The degree of substitution by foreign ions can vary from low substitution (such as fluoride, magnesium, and potassium) to a complete substitution (three sites). [12] The properties of the ion-substituted hydroxyapatite may vary according to crystallite morphology, crystallinity, particle size, and foreign ion substitute.[9]

A proprietary technology named REFIX was recently developed. It comprises a fluoride-containing toothpaste in association with phosphates and silica. According to the manufacturer, this association favors the formation of a fluoridated apatite and the deposition of silicon, which was also incorporated deep into the hydroxyapatite and the open dentinal tubules. [13] A recent *in vitro* study[14] demonstrated that brushing teeth with the REFIX-containing toothpaste induced the formation of a silicon-enriched mineral layer on the enamel surface, proving the biomimetic mechanism of action of this fluoridated oral care product. To date, the effect of REFIX technology to prevent tooth erosive wear is unclear. This *in vitro* study aimed to characterize the mineral content and surface and cross-sectional morphology of enamel treated with fluoridated toothpaste containing REFIX technology after an erosive challenge.

Materials and methods

Specimen preparation of dental enamel

Enamel blocks (4 × 4 × 2 mm) were prepared from extracted bovine incisor teeth and stored in 0.08% Thymol solution. The specimens (N = 5) were embedded in self-cured acrylic resin circular molds 16 mm diameter and 3 mm deep. The outer enamel surface was ground flat with grit papers (600–1500 grades) under water cooling and polished with 1 µm diamond paste (Extec Corporation, Enfield, CT) in a rotating polishing machine PSK-2V (Skill-tec Comércio e Manutenção Ltda, São Paulo, SP, Brazil).

Caries-Like Lesion Formation

Following 5 min sonication in water using an ultrasonic device, one third of the exposed enamel surface was covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) as a reference sound area. Then, the specimens were demineralized to form an artificial caries lesion. Subsurface enamel demineralization was carried out using a modified model. [15] Following 5 min sonication in water using an ultrasonic device, the enamel blocks were immersed individually in 32 mL of a demineralizing solution containing 1.3 mM/L $Ca(NO_3)_2.4H_2O$, 0.78 mM/L NaH_2PO_4 H_2O in 0.05 M/L acetate buffer, 0.03 µgF/mL (NaF), pH 5.0, 32 mL/specimen, during 16 h at 37 °C.

pH Cycling

Before the remineralization pH cycling model, [16] the enamel specimens had another one third of its surface covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) as a reference for caries lesion area. The specimens were submitted to a pH cycling model at 37 °C for 6 days. The blocks were immersed individually in a remineralization solution (1.5 mM.L-1 calcium, 0.9 mM.L-1 phosphate, 150 mM.L-1 potassium chloride in 0.02 mM.L-1 cacodylic buffer, pH 7.0; 0.02 µgF/mL, 1mL/mm2), for 22 h. The cariogenic challenge was performed by immersing the enamel blocks in a demineralization solution (2.0 mM.L-1 calcium and phosphate in 75 mM.L-1 acetate buffer, pH 4.7; 0.03 µgF/mL, 3mL/mm2) for 2 h per day (12 pm–2 pm). Twice a day, at 10 am and 2 pm, the enamel blocks were exposed to toothpaste slurries (toothpaste: deionized water, 1:3 w/w; 2 mL/enamel specimen) for 1 minute, under agitation. Enamel blocks were then rinsed with deionized water between each step. Then, the enamel blocks were individually immersed in a remineralization solution at 37ºC. The de- and remineralizing solutions were changed daily. Between the steps, the specimens were water rinsed with deionized water for 5 s. Deionized water rinses were performed between each step. In between treatments, each enamel block was individually immersed in remineralization solution at 37ºC. The de- and remineralizing solutions were freshly changed every day. The toothpastes selected for the present study are described in Table 1.

Erosive challenge

After the caries pH cycling, the blocks were then subjected to an erosive challenge. Enamel blocks were immersed in 50% citric acid for 2 min, and subsequently washed in abundant distilled water for at least 5 min. [17] The results were compared to untreated control half blocks.

Characterization of the enamel surfaces by scanning electron microscopy (SEM) imaging observation and energy-dispersive X-ray spectroscopy (EDS)

The morphological analysis of the specimens was performed using a scanning electron microscope (TESCAN VEGA3, LMU, Kohoutovice, the Czech Republic) operating at 15 kV. The blocks were first sputter-coated with gold in a vacuum evaporator (MED 010; Balzers, Balzers, Liechtenstein), and then microscopically analyzed to obtain photomicrographs of the surface morphology of the treated specimens (×1,000 magnification). Representative images of selected regions of the specimens were obtained to characterize the morphological aspect of the surface. The EDS point analysis (80 mm^2, SDD [silicon drift detector], Oxford Instruments, Concord, MA, USA) was performed to determine a qualitative elemental analysis of the specimens, operating in high vacuum mode with an accelerating voltage of 15 kV. Five points per sample were randomly selected (300 μm^2 per point), and the mean values were calculated.

Characterization of the cross-sections by scanning electron microscopy (SEM) imaging observation

For the subsurface analysis, cross-sections of the bovine blocks were obtained by longitudinally sectioning the specimens under water cooling. Both half-blocks were used for the SEM analysis. The halves were dehydrated in silica gel for 3 h. The specimens were then gold-sputtered and evaluated using SEM.

Results

Figure 1 shows the representative scanning electron micrographs of the enamel surfaces treated with the different toothpastes after the pH cycling (images above) and after the erosive challenge (images below). After the erosive challenge, the extent to which the enamel surface presented a characteristic morphological aspect of eroded mineral tissues depended on the previous treatments (Figure 1B). The morphological aspect of the eroded enamel surfaces in the specimens treated with the toothpaste containing NR-5 technology (Regenerate Enamel Science) resembled the control group (Figures 1D and 1B, respectively). Similarly eroded morphology was observed for the specimens treated with the product containing Novamin (Sensodyne Repair & Protect) (Figure 1H), which also resembled the control group. On the other hand, the specimens treated with the product containing REFIX technology presented a smoother enamel surface morphology compared to the other treatments (Figure 1F).

Table 2 shows the elemental mapping of the enamel treated with the different toothpastes and after the erosive challenge. EDS detected different amounts of carbon, oxygen, silicon, phosphorus, and calcium before and after the erosive challenge. In the specimens treated with the REFIX technology, no changes in the chemical elements were observed after the erosive challenge, which agrees with the morphological analysis. Conversely, a reduction in the percentage weight of calcium was observed in the specimens treated with Novamin after the erosive challenge (from 34.11 to 29.33%). The percentage weight of calcium increased in the eroded specimens treated with NR-5 (from 28.07 to 30.39%). However, this increase may be not a real increase, but instead, the resulting of measuring the calcium content of the enamel layer exposed after the erosive challenge. This can be confirmed by the Ca/P ratio after the erosive challenge (2.11), which is similar to that found in untreated bovine hydroxyapatite (2.08).[18] The percentage weight of silicon was similar after the erosive challenge for the specimens treated with the REFIX and Novamin technologies. Conversely, when the enamel was treated with the NR5-containing toothpaste, a decrease in the percentage weight was observed (from 0.33 to 0.27% weight). The highest percentage weight of silicon was found in the specimens treated with REFIX technology, before and after the erosive challenge (0.42 and 0.41%, respectively).

Figure 2 shows micrographs of cross-sectional areas of the enamel treated with the different toothpastes. A mineralized layer formed on the enamel surface after treatment with NR-5 (Figure 2A) and REFIX technologies (Figure 2B). A thicker mineral layer was observed for REFIX in all the specimens evaluated. Conversely, no mineralized surface layer was observed in the specimens treated with Novamin technology (Figure 2C). This toothpaste is known to induce the formation of a mineralized layer on the dentin and inside the dentinal tubules.[19]

Figure 3 shows micrographs of cross-sectional areas of the enamel treated with REFIX technology, in which a mineralized layer formed on the enamel surface is observed (Figure 3B), in comparison with the untreated enamel (Figure 3A). The product containing REFIX technology induced the formation of a mineralized layer when associated with pH cycling. This layer was approximately 6 μm thick (Figure 3B).

Figure 4 shows photomicrographs of the morphology of cross-sectional areas of the enamel treated with the REFIX-containing toothpaste comparing the effect of the erosive challenge with an intact, untreated enamel area. The eroded area was around 15 μm deep in the intact enamel (Figure 4A). Conversely, in the specimens treated with the REFIX technology, virtually no erosion was observed and the mineralized layer after the erosion challenge (Figure 4B).

Discussion

As dental erosion may lead to irreversible loss of hydroxyapatite, it is of paramount importance to prescribe products with remineralizing potential that can assist in the mineral gain of the demineralized surface and reduce the solubility of the dental structure in recurrent acidic challenges. [9] As previously pointed out, fluoride-containing products may have anti-erosive properties, but they are only able to repair smaller enamel lesions. [20-21] Promising biomimetic approaches to erosion prevention have been developed. [4, 22]

The toothpaste with the proprietary technology called NR-5 (Regenerate Enamel Science) contains, according to the manufacturer, sodium phosphate associated with calcium silicate. Also, according to the manufacturer, this technology was developed by combining calcium silicate, sodium phosphate salts and fluoride. This technology was proposed to accelerate the mineralization processes provided by saliva, assisting the nucleation of hydroxyapatite and in the formation of minerals in the enamel, thereby remineralizing, protecting and repairing the enamel. A previous *in vitro* study[23] investigated the repair and protective properties after the deposition of calcium silicate on acid-eroded enamel surfaces. That study demonstrated that calcium silicate could transform into hydroxyapatite and be deposited on both intact and eroded enamel surfaces, providing significant protection against erosive challenges. This technology seems to induce the formation of a mineralized layer on the enamel surface after treatment with NR5, as demonstrated in the present study (Figure 2A). In another *in vitro* study[13] the toothpaste with NR-5 technology favored the recovery of superficial enamel hardness more than 100% compared to the untreated control. Conversely, this effect was not observed at the enamel subsurface, demonstrating that this technology was less effective at remineralizing the enamel in depth. This helps to explain the reasons for not promoting an effective protection against the erosive challenge.

The other product containing Novamin technology (Sensodyne Repair & Protect) uses sodium and calcium phosphosilicate (Bioglass) in the form of an amorphous inorganic compound. [24] According to the manufacturer, a series of chemical reactions occurs when Bioglass is in contact with an aqueous solution, leading to the formation of a layer of carbonated hydroxyapatite on the dentin that forms an insoluble mineralized layer on the surface. This technology may favor another mechanism of action in enamel, possibly altering the structure of the enamel hydroxyapatite and reinforcing it, without actually forming a superficial mineralized layer on the enamel.In the present study, it was not observed the formation of a mineralized layer on the enamel surface (Figure 2C), although the formation of a less-soluble surface hydroxyapatite, which is resistant to acid challenges, may occur.[25]

In an *in vitro* study, [26] the protective effect of four commercial toothpastes containing anti-erosion agents was investigated. The authors found that the toothpaste containing Novamin was not effective in preventing the erosion effect caused by orange juice when applied either before or after the erosive challenge. The authors of a recent systematic review [27] searched for clinical evidence of the effectiveness of Novamin in publications evaluating its action as a remineralizing agent. The analysis of the different studies led to the conclusion that Novamin had significantly less clinical evidence to demonstrate its effectiveness as a remineralization agent in treating both carious and noncarious lesions. The authors recommended better-designed clinical trials to make definitive recommendations about this technology. [27]

The toothpaste containing proprietary REFIX technology, according to the manufacturer, represents a novel, multifunctional phosphate-based dental gel technology in an acidified stabilized phosphate/fluoride complex, which is established especially in saliva. [13] The combination of toothpaste, saliva, and dental tooth structures favors the generation of new minerals containing calcium/phosphate/fluoride, promoting the enamel surface and remineralizing within the subsurface carious lesion. [13] This product presents an acid pH that may be the main reason for its effectiveness due to the formation of calcium phosphate crystals in an acidic environment.[13, 28]

In the present study, it was demonstrated that a mineralized layer was formed on the enamel surface after treatment with REFIX technology (Figures 2B and 3B), in comparison with the untreated enamel (Figure 3A). A previous study demonstrated the formation of a silicon-enriched mineral layer on the enamel surface induced by the REFIX-based toothpaste was favored by the formation of complexes of the bioactive particles of calcium, phosphorus, and sodium.[14] Substituting PO4 with SiO4 is believed to affect the mechanical properties of the silicon-enriched hydroxyapatite in a dosedependent manner, decreasing hardness and the elastic modulus.[29] Conversely, the silicon content in the toothpaste formulation in association with fluoride and phosphate groups induces increased bioactivity and apatite-forming ability of hydroxyapatite, which is enhanced by the substitution of silicon, or silicate, into the remineralizing hydroxyapatite.[30-31] In this manner, a protective effect is provided by inducing the formation of hydroxyapatite after its deposition onto the eroded surfaces.[23]

The results of the present study can also be explained by the pH at which the remineralization processes occur (Table 1). The biomimetic effect of the technologycontaining fluoride toothpastes may induce the nucleation and growth of new enamel crystals by incorporation into the porous spaces of the lesion, and at later stages by means of the growth and fusion with the preexisting crystals. In this manner, a faster remineralization process may be expected when treating the enamel with these multifunctional toothpastes compared with conventional fluoride toothpastes. Fluoride ions, known to reduce hydroxyapatite solubility, can replace hydroxyl ions. [32] The smallsized fluoride anions are able to diffuse throughout the enamel matrix in either acidic or basic pH, inducing the remineralization process using a nucleophilic attack on silicon, coordinating to it, and promoting subsequent reactions. [33] In an acidic pH, such as the REFIX-containing toothpaste, the remineralization process in the presence of silicon leads to the formation of a less porous hydroxyapatite structure (less than 2 nm). [34] In addition, the REFIX product contains 30% more silicon than the other products (Table 2). Conversely, in a basic medium, such as the NR-5- and Novamin-containing toothpastes, there is a tendency to form a mesoporous enamel structure, [34] with porosity varying from 2 to 50 nm.[35-36] This also helps to explain the differences in the resistance to the erosive challenge after treatment among the technology-containing fluoride toothpastes.

Considering the limitations of the present *in vitro* study, the protocol used to evaluate the protective effectiveness of the selected fluoride toothpastes can be explained considering that both cariogenic and erosive challenges might simultaneously occur in the oral cavity, depending on different etiological factors.[37-38] By treating the enamel with the fluoride toothpastes allowed changes in the hydroxyapatite structure, which ends up forming fluoridated apatite and the deposition and/or replacement of hydroxyapatite sites with other substitutes. This also seems to occur deeper into the hydroxyapatite. [39] Without this protocol it would not be possible to evaluate the effectiveness of the toothpastes to promote a protective effect after exposure to the erosive challenges. This protective effect of the toothpastes containing different technologies may not only be restricted to the enamel surface but also to the enamel subsurface. It is true that the erosive challenge used in the present study has also limitations, but it is a valid and well-established method.[17] Another limitation relies on the fact that this morphologic evaluation is qualitative, and one may argue that the results are quite subjective. In spite of this fact, the images are clear to demonstrate the results when the treatments were compared.

The present study found that the protective effect against the erosive challenge was material dependent. The toothpaste containing 1450 ppm of sodium fluoride with REFIX technology enabled the formation of a mineralized surface layer less affected by the erosive challenge. This outcome appears to be due to the formation of an acid-resistance silicon-enriched mineral surface layer on the enamel surface. Although the treatment with the toothpaste containing NR-5 technology also enabled the formation of a mineralized surface layer, an eroded enamel surface morphology was observed after the erosive challenge, similar to the untreated enamel control. Conversely, no mineralized surface layer was observed in the specimens treated with the toothpaste containing Novamin technology, and the enamel surface morphology was significantly affected by the erosive challenge.

Conclusion

The present study characterized the enamel surface and subsurface morphology of specimens treated with different 1450-ppm fluoride toothpastes containing different biomimetic technologies. These technologies were developed to accelerate the remineralization process or to minimize the demineralization process of dental tissues, especially in the event of repetitive erosive challenges. Despite the limitations of the present *in vitro* study, the preferred REFIX technology was the most promising compared to other Novamin and NR-5 technologies. In addition to forming a mineralized layer superficially on the enamel, the most important result was the ability to resist acid dissolution by the erosive challenge. Further studies are needed to investigate the performance of the dental gel containing the REFIX technology using in situ and in vivo studies on the effectiveness of the dental gel on dental substrates

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Figure captions

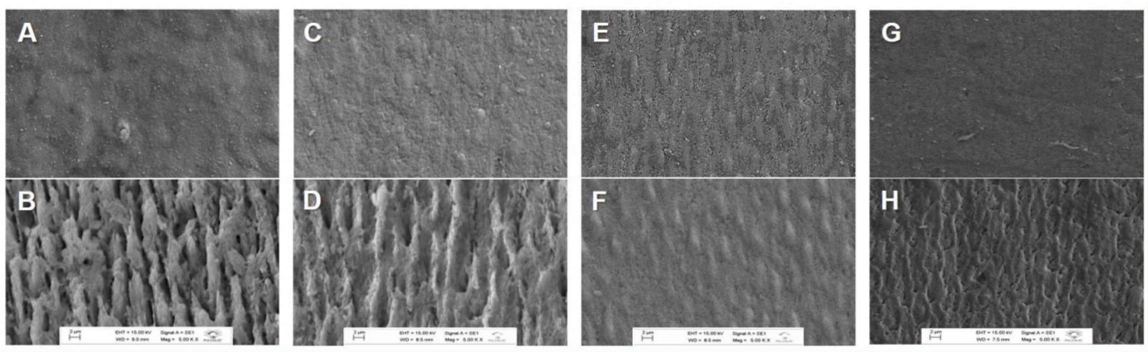

Figure 1: Figure 1 shows a representative scanning electron micrograph of the enamel surfaces treated with the different toothpastes during the pH cycling (images above) and after the erosive challenge (images below). Figure 1A and 1B: the morphological aspect of the control (untreated area) before and after the erosive challenge of the control, untreated group; Figure 1C and 1D: morphology of the enamel surface treated with toothpaste containing NR-5 technology (Regenerate Enamel Science), before and after the erosive challenge. Figure 1E and 1F: representative image of the morphology of the enamel surface treated with the product containing REFIX technology before and after the erosive challenge. Figure 1G and 1H: representative micrograph of the morphology of the enamel surface treated with a product containing Novamin (Sensodyne Repair & Protect), before and after the erosive challenge.

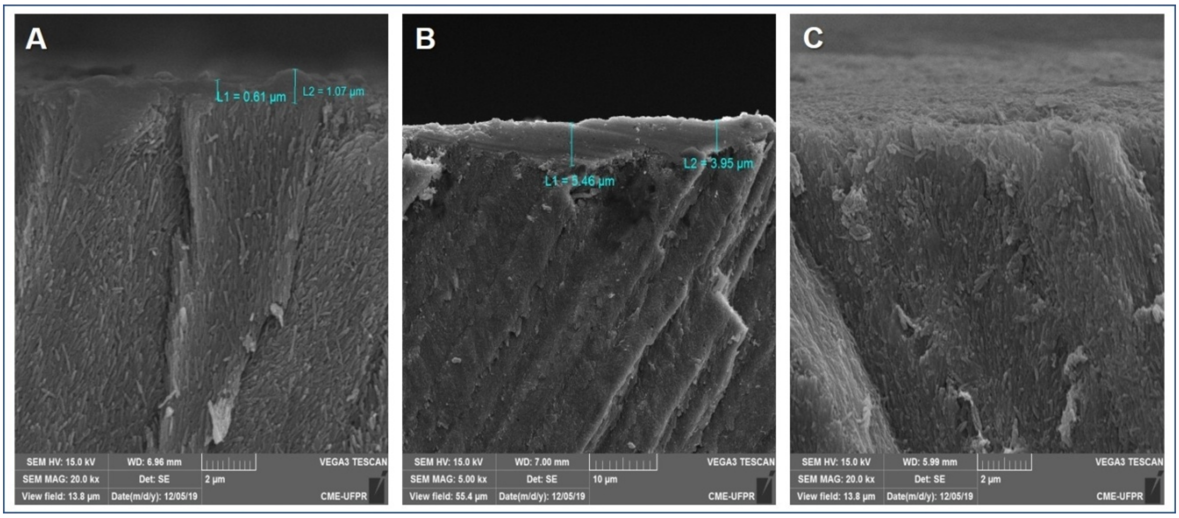

Figure 2: Representative scanning electron micrographs of the enamel cross sections of the enamel treated with the different technology-containing fluoride toothpastes. A: NR-5 technology (Regenerate Enamel Science); B: REFIX

technology (Regenerador + Sensitive DentalClean); C: Novamin technology (Sensodyne Repair & Protect).

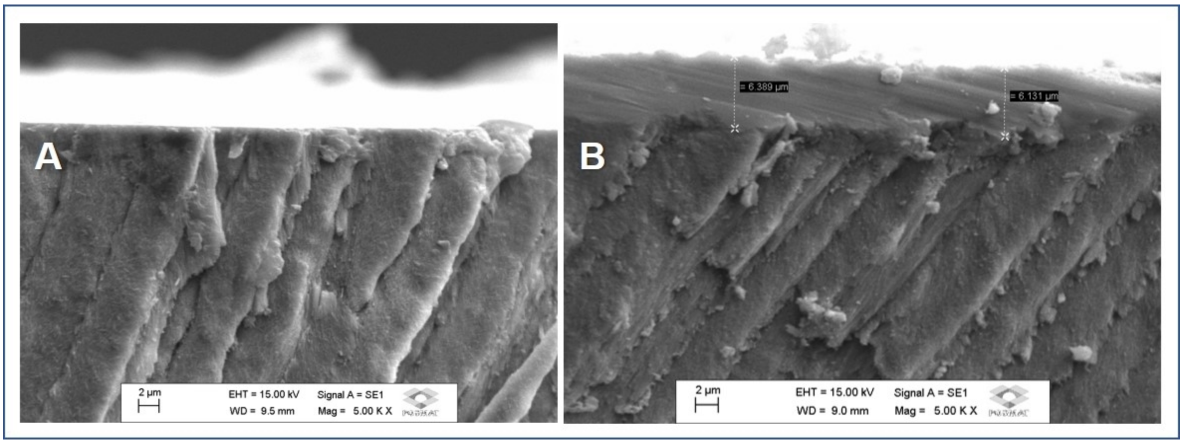

Figure 3: Scanning electron micrographs of the morphological analysis of the cross-sectional areas of the enamel showing the formation of a mineralized surface layer after treatment with the REFIX-containing fluoride toothpaste (B), in comparison to the untreated specimen (A).

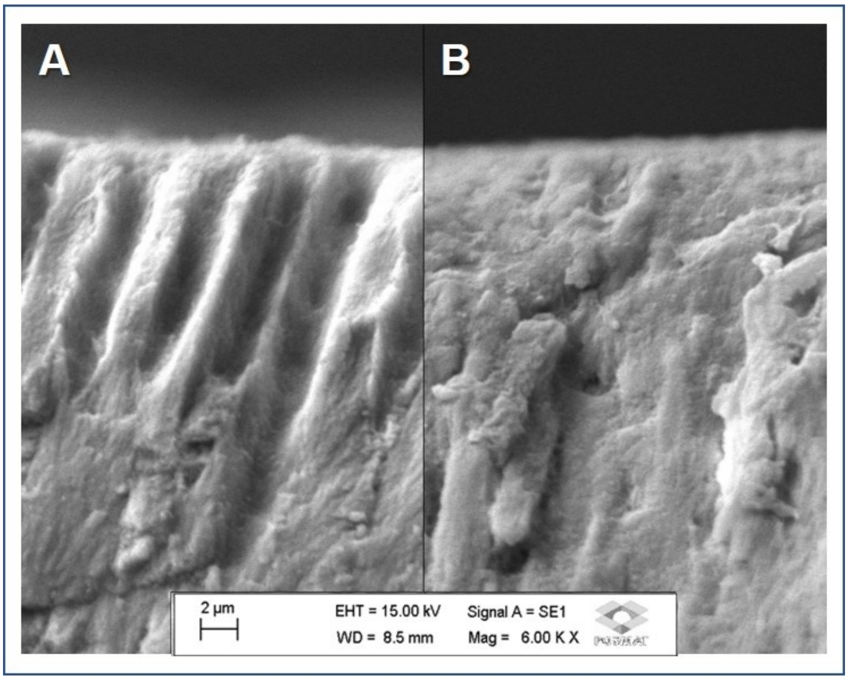

Figure 4: Scanning electron micrographs of the morphological analysis of the cross-sectional areas of the enamel comparing the untreated control area (A) and the treated area (B) with the REFIX-containing fluoride

Table 1 – Composition of the toothpastes selected for the study*.

Product	Ingredients	Active Agents	Lot# Exp. Date
Regenerate Enamel Science [a]	Glycerin, Calcium Silicate, PEG-8, Hydrated Silica, Trisodium Phosphate, Sodium Phosphate, Aqua, PE-60, Sodium Lauryl Sulfate, Aroma, Flavor, Synthetic Fluorphlogopite, Sodium Saccharin, Polyacrylic Acid, Tin Oxide, Limonene, CI 77891. pH: 8.92 (Tomaz et al., 2020)	1450 ppm F^- of sodium fluoride and sodium monofluorophosphate; calcium silicate and sodium phosphate (NR-5 technology)	L72878CC 04/2020
Regenerator + Sensitive DentalClean [b]	Glycerin, Silica, Sorbitol, Sodium Lauryl Sulfate, Aqua, Aroma, PEG-12, Cellulose Grum, O-Phosphoric acid, Xylitol, sodium Sacharin, Triclosan, Menthol, Mica, Sodium Benzoate. pH: 4.73 (Tomaz et al., 2020)	1450 ppm F^- of sodium fluoride and Tetrasodium Pyrophosphate (REFIX technology)	41531 05/2021
Sensodyne Repair & Protect [c]	Glycerin, PEG-8, hydrated silica, pentasodium triphosphate, sodium lauryl sulfate, flavor, titanium dioxide, polyacrylic acid, cocamidopropyl betaine, sodium saccharin. pH: 8.63 (João-Souza et al., 2017)	1450 ppm F^- of sodium fluoride and Calcium Sodium Phosphosilicate 5% (NOVAMIN technology)	BN 028E 12/2019

*Manufacturers' information. [a] Rabbit Corp, Londrina, PR, Brazil; [b] Unilever UK Limited, Leatherhead, Surrey, UK, [c]GlaxoSmithKline, Philadelphia, PA, USA.

Table 2 –Elemental mapping of the enamel treated with the different dentifrices and after erosive challenge.

	C weight %		O weight %		Ca weight %		P weight %		Si weight %		Na weight %		Ca/P ratio	
Dentifrice	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Regenerate Enamel Science	7.36	6.48	32.09	30.90	28.07	30.39	14.17	14.39	0.33	0.27	0.42	0.53	1.98	2.11
Regenerator Diário DentalClean	6.92	7.14	31.97	32.35	31.61	30.72	15.31	15.11	0.42	0.41	0.43	0.31	2.06	2.03
Sensodyne Repair & Protect	4.95	7.60	18.97	25.14	34.11	29.33	16.74	14.27	0.32	0.33	0.29	0.36	2.03	2.05

2. CAPÍTULO 3

O manuscrito a seguir será submetido para publicação no periódico *Clinical Oral Investigations* (Qualis: A1, IF 2019-2020 = 2.7)

Evaluation of dentifrice with innovative remineralizing technology: A randomized clinical study

Nayanna Lana Soares Fernandes[1]
Helene Soares Moura[2]
Marcel Alves Avelino de Paiva[1]
Jéssica Bonifácio de Carvalho[3]
Fábio Correia Sampaio[1]

[1]Department of Clinical and Community Dentistry, Health Science Center, University of Paraiba, João Pessoa, PB, Brazil.
[2]Departament of Dentistry, State University of Paraiba, Campus VIII, Araruna, PB, Brazil.
[3]Dentistry Graduation, Federal University of Paraiba, João Pessoa, PB, Brazil.

Corresponding Author: Fábio Correia Sampaio
Address: DCOS, Health Science Center, Federal University of Paraíba, Castelo Branco, João Pessoa, PB, 58051-900, Brazil.
Telephone: + 55 83 3216 7795
Fax: + 55 83 3216 7409
E-mail: fcsampa@gmail.com

Abstract

Objective: The aim of this study is to evaluate *in vivo* dentifrices containing different active ingredients in fluoride (F) retention in saliva and dental biofilm. **Material and methods:** In double-blind, randomized crossover with washout, 15 individuals without a water fluoridation program, used the following different dentifrices for a week: G1- Daily Regenerator Dentalclean Neutral (RDCN); G2- Sensodyne Repair & Protect (SRP); G3- Daily Regenerator Dentalclean Acid (RDCA), G4-Colgate Total Daily Repairc (CTDR). On the seventh day of dentifrice use, biofilm was collected at 1 and 12 h, and saliva was collected up to 60 min and 12 h after the last toothbrushing. The concentrations of F in saliva and biofilm were analyzed by specific electrode using the hexamethyldisiloxane facilitated diffusion technique (HMDS). Data of saliva were analyzed by repeated measures ANOVA followed by the Bonferroni test (α= 5%). For biofilm, the results were evaluated by the Wilcoxon test, Friedman test and Bonferroni post-test (α= 5%). Area under the curve (AUC) was calculated for saliva data. **Result:** The highest values of AUC in saliva were found in groups G3 and G4. For biofilm, G3 had the highest medians at 1h and 12h collection times, with a statistically significant difference from placebo (p<0,05). **Conclusion:** The toothpaste RDCA, containing Refix technology, presented the most promising results for fluoride retention in oral exposure biomarkers. **Clinical Relevance:** Daily Regenerator Dentalclean, with innovative technology, can contribute to a greater anti-caries effect, especially in groups at risk.

Keywords: Dental caries. Dentifrice. Fluoride. Biomimetics.

Introduction

Dental caries is a multifactorial disease, which involves the interaction between: dental structure, presence of bacterial biofilm on the dental surface, sugar from the diet, saliva and biological factors [1]. This pathology causes a slow and progressive demineralization of the dental tissue, starting with changes in the optical properties of the enamel until the formation of cavities that can reach the dentin and the dental pulp [2]. Even today, caries is considered an important public health problem, being the most prevalent oral disease in the population, whose prevention is done through the association of adequate eating habits, with regular oral hygiene and control of microbial biofilm [2,3] . Therefore, it is necessary to use fluoridated products to keep the intraoral levels of this ion high, as it acts by inhibiting demineralization and favoring mineral precipitation on the tooth surface, being the main agent used to combat caries in the world [4, 5].

Considering that this is a biofilm-dependent disease [1, 6], the amount of fluoride retained in this compartment plays a relevant role from a clinical point of view [7]. In addition, saliva is also a crucial factor in the biochemical process of incidence and establishment of caries, presenting a protective characteristic and diverse physical-chemical properties [8]. Thus, there is an inversely proportional relationship between fluoride concentrations in dental biofilm and saliva, and the development of this disease [8-10].

Of the most varied forms of fluoride availability, fluoride toothpaste is the one that best fits the control of caries, for its topical and daily use. It is possible to associate the disorganization of the bacterial biofilm through brushing, with the release of the soluble fluoride ion to interfere with the mineral dynamics [1, 11, 12]. Although conventional dentifrices have managed to reduce the prevalence of caries in the majority of the population in recent years [13, 14], some groups are at greater risk for the development of this pathology. Thus, it is necessary to use toothpastes with other active ingredients associated with fluoride, to intensify its protective and therapeutic potential [1, 15, 16]. Studies have been done testing the efficacy of fluoridated toothpastes with the addition of other remineralizing components [17-21]. These demonstrate that the composition of the products directly influences their ability to promote the absorption of fluoride by the enamel and contribute to the remineralization of carious lesions [10, 15].

The increase in the amount of bioavailable fluoride in the oral cavity after brushing is still a challenge for dentistry today. High concentrations of this ion for a longer period of time, especially in dental biofilm and saliva, favor its release during a new cariogenic challenge and guarantee the protection and / or remineralization of already existing carious lesions [12, 21, 22]. Therefore, efforts are being made to develop products that can increase the substantivity of these ions and help to maintain the chemical balance of the dental element with the surrounding environment.

In this context, innovative technologies appear, such as the Refix that claims to increase the anti-caries efficiency of dentifrices, forming a layer of fluoridated apatite rich in silicon on the dental surface, called “enamel like”, ensuring the remineralization of the lesions [23]. In addition, it also claims to have desensitizing and anti-erosive properties [24]. Therefore, the hypothesis of this study is that a gel based on this technology, can form stable mineral complexes in an acidic environment, increasing the amount of fluoride retained in oral biomarkers to act during further pH drops. Thus, the aim of this study was to evaluate the fluoride retention in saliva and biofilm, after using toothpastes with Refix technology and other bioactive components.

Materials and Methods

Ethical considerations

This study was approved by the Research Ethics Committee of the Health Sciences Center of the Federal University of Paraíba (UFPB), in compliance with the rules of resolutions for research on human beings of the National Health Council (CNS 466/2012) with the numbers CAAE:20079319.9.0000.5188. All participants received the necessary information and signed the Free and Informed Consent Form before the beginning of the study. This study followed CONSORT statement and it was registered in ClinicalTrials.gov in January 2021(NCT03761485).

Study Design

A randomized, duple-blind crossover clinical trial study was performed to evaluate the bioavailability of intraoral fluoride in biomarkers of exposure (biofilm and saliva) after the use of dentifrice with experimental fluoride for one week (7 days), with wash-out periods between them. The biofilm was collected 1 hour (h) and 12h after tooth brushing, and saliva was collected 1h (T1, T15, T30, T45, T60 min) and 12h. The data was obtained by potentiometry (ion selective electrode) for fluoride in these samples.

Study population

The sample size was determined by sample calculation [25], according to previous studies [26], α = 5%, β = 10%, and a dropout ratio of 10%. Fifteen individuals with an average of 26 years old, including both males and females, participated in this study. The eligibility criteria were people over 18 years old, without systemic commitment and living in the city of João Pessoa-Paraíba, without water fluoridation (<0.1 µg/mL F). Individuals were excluded if they used drugs that interfere with biofilm formation and salivary flow or fluoride products (> 5000 ppm) in the last 4 weeks, used orthodontic appliances, or had cavity lesions, periodontal disease, and/or tooth sensitivity.

Experimental Dentifrices

The participants were allocated randomly to use the following experimental dentifrices according to Table 1. They were coded by an independent investigator not involved in the trial. A simple randomization was performed taking into account a cross design. The unit of randomization was "dentifrice", so all dentifrices had the same chance of being selected. The design of the study was created for each participant and for each new one another draw was made.

Clinical Stage

First, the participants were submitted to dental prophylaxis and scraping to remove all biofilm and dental calculus. An oral hygiene kit and instructions were provided, explaining the correct brushing technique and that no other fluoride product could be used besides the toothpaste provided. At first they used a placebo, dentifrice without fluoride, for 7 days. Then, the Whitford protocol was followed [16]. Briefly, the subjects were instructed to brush their teeth three times/day for 1 min (transversal technique) and also to rinse their mouths after brushing with 10 mL of water. On the seventh day, the subjects were instructed to brush only the occlusal surfaces and do not use dental floss, to allow biofilm accumulation. After going to bed, they abstained from eating or drinking anything except water and did not brush their teeth. The next morning, after 12 hours of last brushing, and fasting, the first samples of saliva and biofilm (upper and lower right hemi-arch) were collected. Then, the volunteers brushed the occlusal surfaces for 1 min. Soon after, saliva samples were collected in the following times: t1, t15, t30, t45, and t60 minutes. Biofilme samples (left side) were collected later (1 hour after brushing). Resting saliva was collected for 5 minutes. The biofilm was collected from all tooth surfaces, in both buccal and lingual areas, using a 3S hollenback spatula, from which the samples were immediately transferred to an eppendorff tube, centrifuged and subsequently dried at 90°C for two hours and then weighed. The saliva samples were centrifuged at 6,000 rpm for 10 minutes in order to separate the saliva debris.

Determination of the concentration of fluoride

The samples were analyzed by the hexamethyldisiloxane (HMDS) facilitated diffusion method of Taves [20] modified by Whitford [21]. The analyzes were performed with a fluoride specific electrode (model 9409; Orion Research) and a potentiometer (model EA 940; Orion Research, Cambridge, MA, USA) (Model 720 A Orion). Fluoride standards (1-100 nM) were used to prepare calibration curves.

Statistical analysis

The data were analyzed using descriptive and inferential statistics, using the statistical program SPSS - v. 21.0 and software Graphpad. The Shapiro-Wilk test was applied to test the normality of the *in vivo* study data ($n < 50$). Nonparametric and parametric tests were performed. The area under the curve (AUC) was calculated by taking baseline values (12 h after the last brush) up to 60 min after the last brush to indicate the effectiveness of F retention in saliva over time. For the purpose of comparing the concentration of F in saliva between the groups, the ANOVA test of repeated measures was performed followed by the Bonferroni post-test. For biofilm, the results were evaluated by the Wilcoxon test, Friedman test and Bonferroni post test. This study assumed a significance level of 5% ($p < 0.05$).

Results

According to the data of this study, a dose-response relationship could be observed between the use of fluoride dentifrices and the levels of fluoride concentration in saliva and biofilm. Fig. 1 shows the fluoride kinetics in saliva for all toothpastes, with all experimental groups showing a peak value in 1 minute and then a rapid drop in fluoride concentration. As shown, a second mild decrease in the fluoride concentration in saliva can also be observed after 15 min, until almost complete depletion 1 hour after the last brushing, which reaches the baseline line. The cumulative effect of fluoride retention in baseline saliva up to 60 minutes after the last brushing can be seen in the AUC values in Fig. 2. The highest values were associated with G3: Daily Regenerator Dentalclean Acid (RDCA) and G4: Colgate Total Daily Repair (CTDR).

In Table 2, we can see the mean(±SD) fluoride concentrations in saliva at different time intervals. There were statistically significant differences between the toothpaste after 1, 15 and 30 minutes of use (ANOVA, $p < 0.05$). The placebo was different from G3 and G4 for the first moment (T1), and different only from G3 for T15 and T30 (Anova followed by Tukey, $p < 0.05$). For repeated measures ANOVA, each group was evaluated individually, and similarity was found only at 12 hours (T12) and 60 minutes (T60) after brushing (ANOVA of repeated measures followed by Bonferroni, $p < 0.05$). For the differences found, the placebo was different from G3 and G4, and G3 had a statistically different behavior from G1 and G2, being similar only to G4 (ANOVA of repeated measures followed by Bonferroni, $p < 0.05$).

For biofilm, Table 3 shows the results at 1 and 12 hours. Only G3 was different from placebo in 1 hour. In the second moment of collection, 12 hours after the last brushing, differences were found between placebo, G2 and G3 (Friedman followed by Bonferroni test $p < 0.05$). A relationship between fluoride in saliva and biofilm from different groups (Figs. 3, 4, 5, 6 and 7) demonstrated a contribution between these sites to the rate of intraoral fluoride retention, 1 and 12 hours after brushing. The placebo groups, G1 and G4, showed similar trends, with a decrease in the concentration of F in the biofilm over time. On the other hand, G2 dentifrices and especially G3, managed to keep the oral fluoride levels more stable.

Discussion

Fluoride has a recognized protective and remineralizing action on dental caries, in its free form and in appropriate concentrations [12]. Therefore, the evaluation of the concentration of bioavailable fluoride in the oral cavity is used in a recurrent way in the literature, being considered one of the best strategies to verify the efficacy of fluoridated products [14, 27-31]. Through these studies, much has been discovered about the mechanism of action of fluoride and its relationship with other ingredients present in toothpastes. However, increasing the substantivity of this ion still represents a major challenge for dentistry today [32].

In the present study he was assess *in vivo* fluoride retention in the oral cavity (biofilm and saliva) after the use of silica-based toothpaste with refix technology. For this, a cross design was used, where each participant functions as their own control. The intention of this model was to reduce the bias and control the individual variables. In addition, according to Hall et al. [30] and Sampaio et al. [33], the amount of fluoride used and the technique used in brushing directly influence the fluoride retention in oral exposure biomarkers. Therefore, in this study, the use of the transversal technique was indicated for the participants, with the appropriate amount of toothpaste on the toothbrush. This guidance aimed to positively impact the performance of all tested toothpastes.

Furthermore, ensuring that the main source of fluoride should come from the toothpastes used, the work was done in a city without the water fluoridation program. However, this methodology also has some limitations, such as the existence of large variations in fluoride levels, which is a challenging aspect for many clinical trials [14, 26, 28, 31, 34].

The hexamethyldisiloxane (HMDS) facilitated diffusion method was used to provide the total fluoride concentration present in the biofilm and saliva, including the insoluble one. However, it is important to note that fluoride ions strongly adhered to the biofilm and those in residual concentrations have no significant clinical implications. The reason for this lesser impact is the absence of the water fluoridation program in that location [14, 35, 36].

As this element is electronegative and, consequently, very reactive, pH, ionic concentration and vehicle composition can modulate its action [37]. In view of this, active ingredients can be added to establish a synergistic relationship with fluoride, helping to keep the tooth in chemical balance with the surrounding environment [22, 37]. The multifunctional dental system (Refix technology) is based on this principle, having the property of forming stable complexes in acidic medium, rich in phosphate and silicon. This technology, according to the manufacturer, is able to increase intraoral fluoride levels over time and act in remineralization and / or prevention of carious lesions. This work is a pioneer in evaluating, *in vivo*, the performance of this toothpaste in oral exposure biomarkers.

Although the effect of remineralization is not measured in this study, the high levels of fluoride in saliva and dental biofilm, provide a favorable environment and a good indication of the therapeutic potential of the DentalClean Daily Regenerator [38]. For the periods of 1 and 12 hours after brushing, no differences were found between groups in saliva. However, the kinetics of fluoride in this fluid had a behavior similar to that previously discovered by other authors [14, 30, 39, 40], with a sharp drop in concentration in the first minutes after brushing.

In 1 minute, G3 (RDCA) and G4 (CTDR) were different from placebo, and in T15 and T30 only G3 (RDCA) stood out with significant differences. In addition, the AUC measurements show that G3 (RDCA) and G4 (CTDR) dentifrices obtained significantly higher values than the others, indicating a potential for fluoride retention in saliva. These findings corroborate Tomaz et al. [23] and Vilhena et al. [24], who found in their *in vitro* studies, a promising remineralizing ability for Refix technology (RDCA), with the formation of a layer called “enamel like” on the tooth surface after using this toothpaste. Vilhena et al. [41], also conducted clinical research evaluating dentin hypersensitivity, and the RDCA stood out in reducing patients' painful perception. This confirms the results found here, indicating that this toothpaste can really help in the bioavailability of fluoride and mineral deposition on the tooth surface. In addition, CTDR has in its composition some ingredients similar to RDCA, such as sodium fluoride and pyrophosphate teterasodium. Thus, the good performance found also for this group, seems to be related to the amount of calcium, fluoride and phosphate present in this formulation. These ions leave the oral environment supersaturated to favor remineralization and minimize mineral loss [23].

In this present research, to test the influence of acidity on the performance of the Refix technology, two toothpastes with the same formulation were used, varying only the pH. One was acid (RDCA), as it is sold commercially, and the other neutral (RDCN). Superior results were found for RDCA, both in saliva and in biofilm. This finding makes it clear that toothpastes with acidic pH are able to mobilize more fluoride in the oral cavity and perform better against tooth decay. Studies in the literature validate this statement [31, 43].

It is important to highlight that technologies withtenders to increase the substantivity of fluoride, that is, to prolong its effect with the use of small amounts of product, have been developed [43]. Alves et al. [28] found a fluoride retention in saliva up to 12 hours after brushing, promoted by a hydrocolloid-based toothpaste. These results differ from those found in this study, demonstrating that the Refix technology does not work as a slow-release system of fluoride in saliva. However, there are some factors related to salivary clearance, a physiological process by which substances are removed from the oral cavity. One of the main factors is the adhesion of fluoride to other compounds present in toothpaste, such as abrasives. With that, part of that ion can be expectorated or even swallowed, decreasing its concentration over time [12, 29].

Regarding dental biofilm, the RDCA was the group that managed to maintain the highest concentration levels 1 and 12 hours after brushing. Right after it, Sensodyne Repair and Protect also performed well 12 hours after use. The findings of Naumova et al. [27] corroborate this study, showing that the bioavailability of fluoride lasted longer after the use of bioglass-containing toothpaste.

In view of the results, it is noticeable that the RDCA managed to transform the biofilm into a deposit of fluoride, and this was being systematically released into saliva, with a similarity in the drop in ion levels in these two compartments. According to Pessan et al. [14] and Kondo et al. [31], this finding is satisfactory, since biofilm can be considered the main compartment for fluoride retention, and the one of greatest clinical relevance. This can be explained by the proximity to the caries lesion and by functioning as a reservoir of this ion. High concentrations of fluoride in the biofilm are gradually released into the saliva, which has a more labile fluoride [29].

In conclusion, the Daily Regenerator Dentaclean showed the potential for greater fluoride bioavailability in the oral cavity when compared to the other tested toothpaste. Other clinical studies evaluating the remineralization effects of this technology on dental caries are necessary to better understand and validate its mechanism of action.

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This study was developed with the authors' own funding.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures in this study were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Table 1. Dentifrices used in the study according to their active ingredients and manufacturer.

Group	Active ingredients	Manufacturer
Placebo: Fluoride-free toothpaste Colgate Oral Care	No active ingredients.	Colgate-Palmolive Manufacturing, São Bernardo do Campo, SP, Brazil.
G1: Daily Regenerator Dentalclean Neutro (RDCN)	1450 ppm F- of sodium fluoride and tetrasodium pyrophosphate (Refix technology).	Rabbit Corp. Londrina/PR, Brazil.
G2: Sensodyne Repair & Protect (SRP)	1426 ppm of sodium fluoride and calcium sodium phosphosilicate 5% (NOVAMIN technology).	GSK Consumer Healthcare, Norreys Drive, Maidenhead, Berkshire, SL6 4BL, UK.
G3: Daily Regenerator Dentalclean Acid(RDCA)	1450 ppm F- of sodium fluoride and tetrasodium pyrophosphate (Refix technology).	Rabbit Corp. Londrina/PR, Brazil.
G4: Colgate Total Daily Repair (CTDR)	1450 ppm F- of as sodium fluoride, 0.30% triclosan, arginine, tetrasodium pyrophosphate.	Colgate-Palmolive Manufacturing, São Bernardo do Campo, SP, Brazil.

Table 2. Mean and standard deviation of fluoride concentration in saliva (ppm) at different collection times.

	Time (min)					
Groups	T720	T1	T15	TT30	TT45	T60
Placebo	0,30 (0,25)^{a,A}	8,50 (6,08)^{b,B}	0,35 (0,27)^{c,D}	0,35 (0,24)^{d,F}	0,36 (0,27)^{e,H}	0,31 (0,27)^{a,I}
G1 RDCN	0,42 (0,25)^{a,A}	18,32 (11,81)^{b,B}	0,70 (1,43)^{c,D}	0,76 (0,41)^{d,F}	0,51 (0,26)^{e,H}	0,43 (0,27)^{a,I}
G2 SRP	0,37 (0,27)^{a,A}	18,84 (11,14)^{b,B}	0,42 (1,33)^{c,D}	0,67 (0,40)^{d,F}	0,49 (0,31)^{e,H}	0,41 (0,27)^{a,I}
G3 RDCA	0,48 (0,27)^{a,A}	34,59 (31,35)^{b,C}	0,23 (2,19)^{c,E}	0,98 (0,84)^{d,G}	0,58 (0,33)^{e,H}	0,48 (0,28)^{a,I}
G4 CTDR	0,38 (0,28)^{a,A}	32,87 (28,14)^{b,C}	0,75 (1,60)^{c,D}	0,83 (0,49)^{d,F}	0,59 (0,33)^{e,H}	0,45 (0,27)^{a,I}

*Means preceded by distinct lower case letters differ statistically within the same line for each group, $p < .05$, ANOVA Repeated Measures, followed by the Bonferroni test.

**Different capital letters differ statistically between groups for each variable, in the same column, $p < .05$, ANOVA followed by the Tukey test.

Table 3. Median of fluoride concentration in biofilme (mg/Kg) at different collection times.

Time (h)	Groups				
	Placebo	**G1 (RDCN)**	**G2 (SRP)**	**G3 (RDCA)**	**G4 (CTDR)**
1	199,50$^{b, C}$	350,00$^{b\ C,D}$	474,40^{b,C,D}	452,90$^{b, D}$	380,00$^{b,C, D}$
12	92,70$^{a, A}$	297,50$^{a\ AB}$	351,30^{a,B}	373,60$^{a, B}$	252,5$^{a, A, B}$

*Median preceded by distinct lower case letters differ statistically within the same column for each group, p < 0.5, Wilcoxon test.

**Different capital letters differ statistically between groups for each variable, in the same line, p < .05, Friedman's test, followed by the Bonferroni Dunn.

Legend of Figures

Fig. 1 Mean of the F concentration in saliva (ppm) over a 1h period after brushing with experimental dentifrices

Fig. 2. Area under the curve (AUC) of the mean fluoride concentration in saliva

Fig. 3 Relationship between the [F] in the biofilm and saliva after using placebo at 1 and 12 h

Fig. 4 Relationship between the [F] in the biofilm and saliva after using RDCN dentifrice at 1 and 12 h

Fig. 5 Relationship between the [F] in the biofilm and saliva after using SRP dentifrice at 1 and 12 h

Fig. 6 Relationship between the [F] in the biofilm and saliva after using RDCA dentifrice at 1 and 12 h

Fig. 7 Relationship between the [F] in the biofilm and saliva after using CTDR dentifrice at 1 and 12 h

Fig. 1

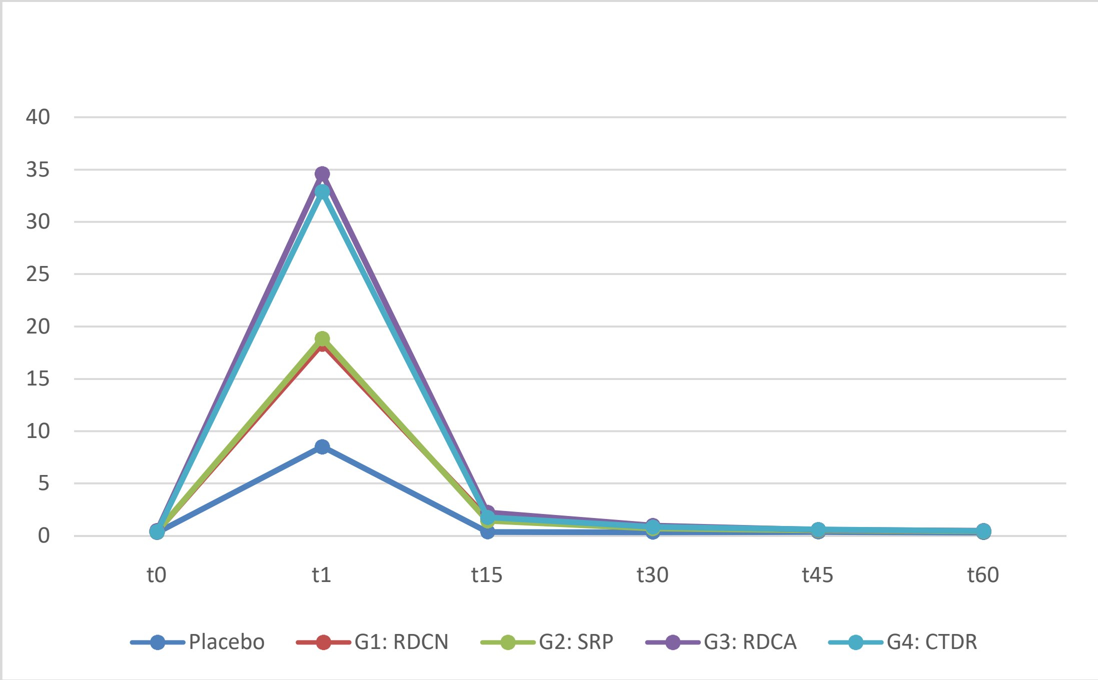

40
35
30
25
20
15
10
5
0
t0
t1
t15
t30
t45
t60
Placebo
G1: RDCN
G2: SRP
G3: RDCA
G4: CTDR

Fig. 2

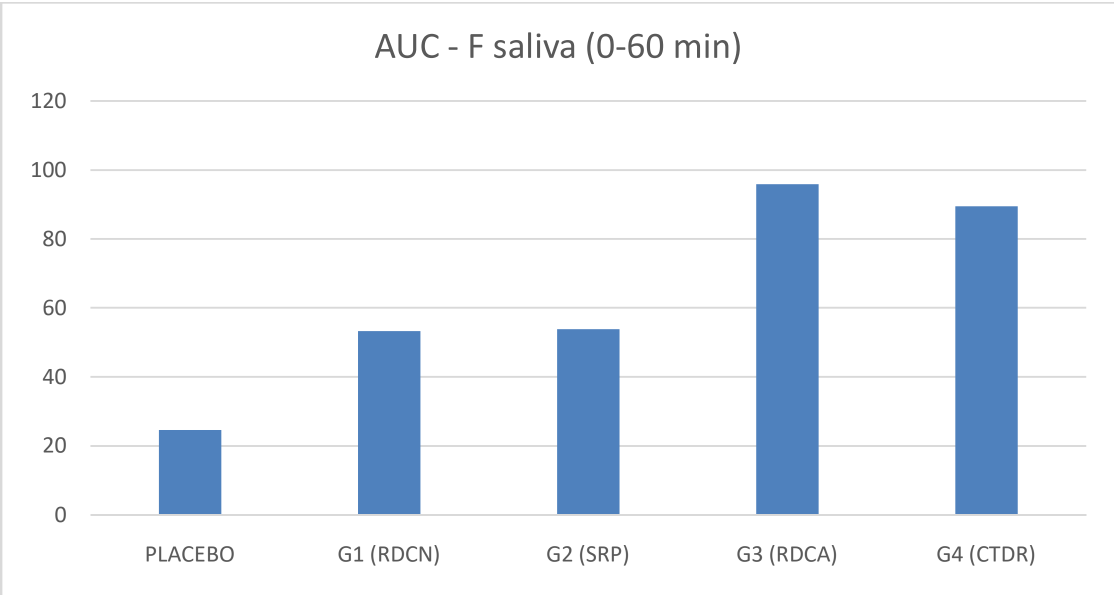

AUC - F saliva (0-60 min)
120
100
80
60
40
20
0
PLACEBO
G1 (RDCN)
G2 (SRP)
G3 (RDCA)
G4 (CTDR)

Fig. 3

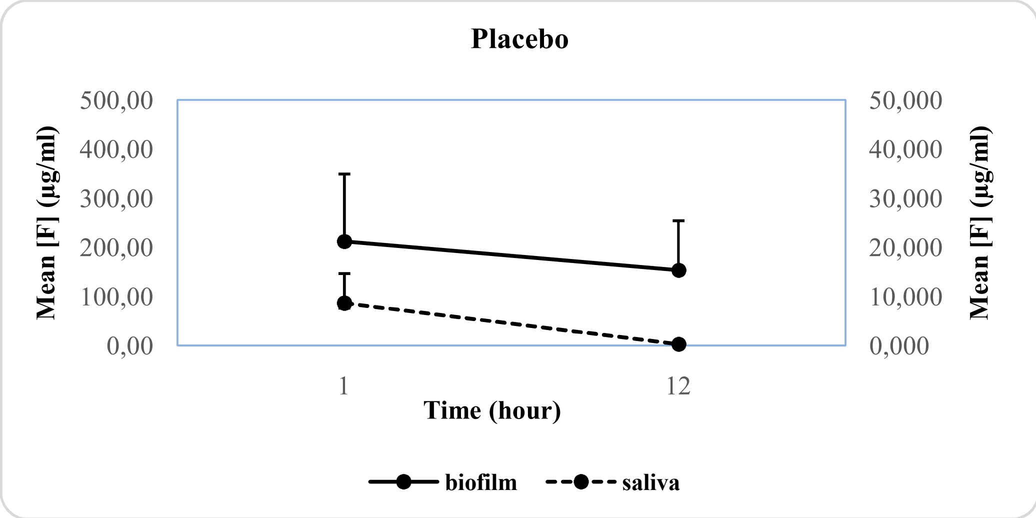


Fig. 4

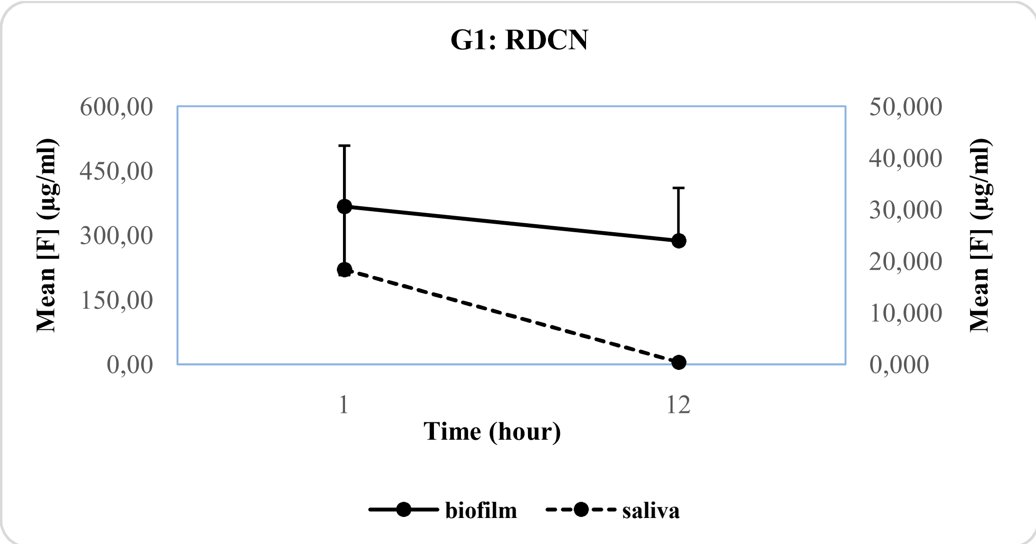


Fig. 5

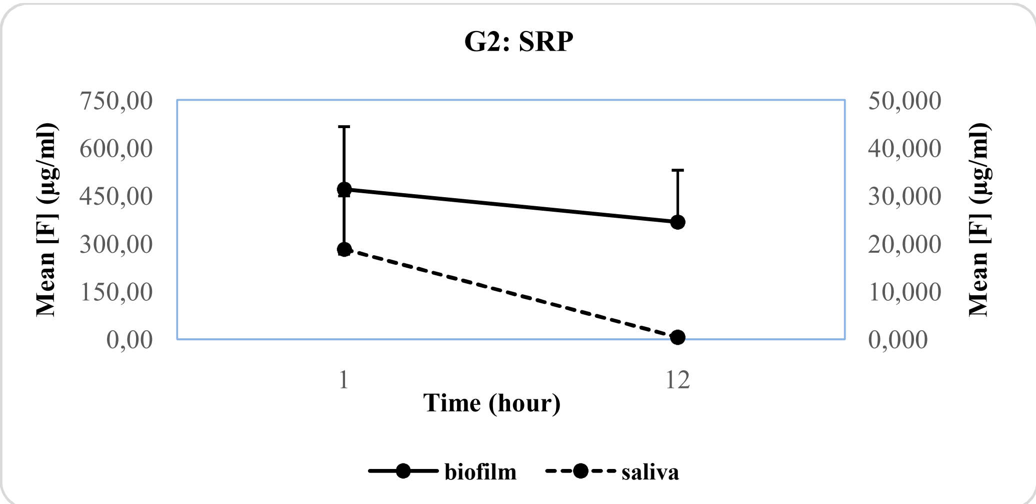


Fig. 6

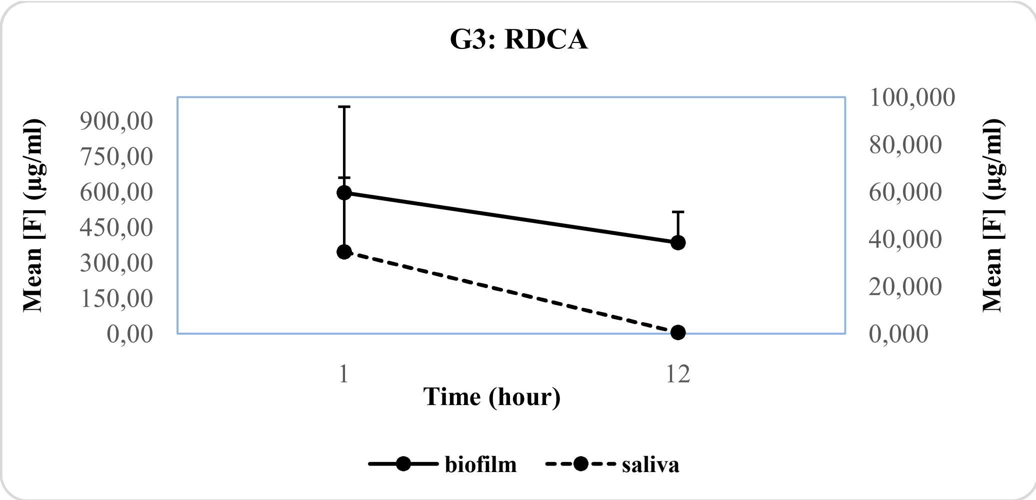
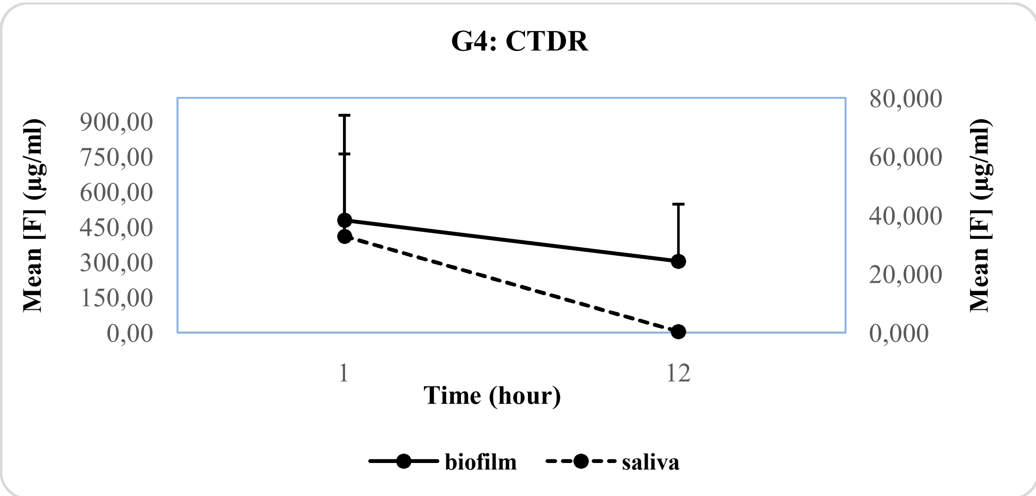

G3: RDCA
Mean [F] (µg/ml)
900,00
750,00
600,00
450,00
300,00
150,00
0,00
100,000
80,000
60,000
40,000
20,000
0,000
Mean [F] (µg/ml)
1
12
Time (hour)
biofilm
saliva

Fig. 7


G4: CTDR
Mean [F] (µg/ml)
900,00
750,00
600,00
450,00
300,00
150,00
0,00
80,000
60,000
40,000
20,000
0,000
Mean [F] (µg/ml)
1
12
Time (hour)
biofilm
saliva

3. CONSIDERAÇÕES GERAIS

Os fluoretos apresentam limitações na remineralização de lesões erosivas e tem sido questionado quanto ao risco de fluorose em crianças e baixa biodisponibilidade intra-bucal em indivíduos com clearance salivar elevada. Diante disso, novas tecnologias estão sendo desenvolvidas para atuar simultaneamente ou melhorando a capacidade remineralizadora dos fluoretos.

Nesse contexto, a tecnologia Refix, com o produto Regenerador Diário Dentalclean (RDC) foi lançada no mercado com o objetivo de atuar como "regenerador" de tecidos mineralizados dos elementos dentais.

Os estudos dessa dissertação demonstraram que o produto com a tecnologia REFIX se apresentou eficaz e promissor na proteção contra erosão e no tratamento à cárie dentária (em modelo *in vitro*). Além disso, a pesquisa *in vivo* indicou que o RDC foi capaz de aumentar significativamente as concentrações de flúor na saliva e no biofilme em comparação com outros produtos já disponíveis no mercado.

Os aspectos de pioneirismo e inovação deste trabalho podem ser comprovados pelo uso de diferentes metodologias e técnicas de análise, a exemplo do QLF para mensuração de perda e ganho mineral. Em adição, o estudo *in vivo* apresenta o primeiro RCT (randomized clinical trial) para avaliação da eficácia de tecnologia inovadora em gel dental no controle cárie e erosão dentária.

Por fim, a avaliação de outros parâmetros (atividade antimicrobiana) e em outras condições (lesões iniciais de cárie e/ou erosão dentária *in vivo*) se faz necessário. Os resultados de estudos de intervenção mais prolongados e em indivíduos com desafios cariogênicos poderá elucidar e mensurar os benefícios do Regenerador Diário DentalClean na cavidade bucal em condições de vida real.

4. CONCLUSÕES

De acordo com os estudos apresentados, pode-se concluir que o dentifrício Gel Regenerador Diário DentalClean, contendo a tecnologia Refix:

- Foi eficaz na remineralização do esmalte previamente cariado, em um modelo de ciclagem de pH remineralizante *in vitro*.
- Foi eficaz na proteção do esmalte dentário contra desafio erosivo, *in vitro*.
- Demonstrou desempenho satisfatório no ensaio clínico randomizado, apresentando retenção do flúor em saliva e biofilme em até 12 horas após a escovação.

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* De acordo com as normas do PPGO/UFPB, baseadas na norma do International Committee of Medical Journal Editors - Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

ANEXO

ANEXO A –**Certidão de Aprovação do Comitê de Ética em Pesquisa**

UFPB - CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Eficácia de dentifrício com tecnologia inovadora para remineralização de lesões iniciais de cárie: estudo clínico randomizado

Pesquisador: Nayanna Lana Soares Fernandes

Área Temática:

Versão: 3

CAAE: 20079319.9.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.933.795

Apresentação do Projeto:

Trata-se de uma pesquisa do programa de pós-graduação em Odontologia da UFPB, onde a pesquisadora responsável é a Mestranda Nayanna Lana Soares Fernandes, sob a orientação do professor Fábio Sampaio. Que tem o objetivo de avaliar in vivo a retenção de flúor na cavidade bucal, após o uso de um dentifrício com tecnologia inovadora (sob patente), e verificar a eficácia desse produto na remineralização de cárie. A amostra será composta por 108 indivíduos com idade de 10 a 16 anos, residentes na Paraíba. O estudo será dividido em duas etapas. Etapa I: será realizado estudo quantitativo do tipo clínico cruzado, triplo-cego randomizado com 18 residentes de um município da Paraíba sem fluoretação de águas. Etapa II: será realizado estudo clínico randomizado duplo-cego com 90 adolescentes(10 a 16 anos) residentes de um município da Paraíba sem fluoretação de águas. Os dentifrícios utilizados serão: Grupo 1 (1450 ppm de NaF); Grupo 2 (Dentifrício regenerador com pH ácido) e Grupo 3 (Dentifrício regenerador com pH neutro). Etapa I: Durante o uso dos dentifrícios, serão coletadas amostras de biofilme dental e saliva estimulada. Ostempos de coleta serão 1 e 12 horas (h) após a última escovação para biofilme e, 1, 5, 10, 15, 20, 30, 45, 60 minutos e 12h para saliva. As concentrações de Flúor [F] em saliva e no biofilme dental serão analisadas por eletrodo específico através da técnica de difusão facilitada por hexametildisiloxano (HMDS).

Etapa II: Serão realizadas fotografias intrabucais em três momentos no decorrer do estudo (Baseline, 45dias e 90dias), a regressão/progressão das lesões serão avaliadas por três examinadores calibrados com auxilio do software ImageJ. Imagens de fluorescência das lesões de cárie incipientes (manchas brancas) serão obtidas através do dispositivo Qraycam pro, e depois analisadas com o programa de (C3 versão 1.24, Inspektor Research Systems), para determinar as alterações na quantidade de mineral nas lesões de mancha branca, com base no valor da perda percentual na intensidade de autofluorescência (F).

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar in vivo a retenção de flúor na cavidade bucal (biofilme e saliva) após o uso de um dentifrício com tecnologia inovadora (sob patente), e verificar a eficácia desse produto na remineralização de lesões de mancha branca de cárie.

Objetivos Secundários:

•Verificar a área de redução de mancha branca ativa após o uso dos dentifrícios testes, utilizando imagens fotografadas padronizadas e inseridas no sofware Image J para gerar dados em pixels (mm2);
•Avaliar a recuperação do conteúdo mineral após o tratamento com os dentifrícios testes, por meio da avaliação da fluorescência quantitativa (QLF);
•Avaliar a prevalência e experiência de cárie por meio dos índices CPO-D e CPO-s;
•Avaliar a atividade de cárie por meio do índice de Nyvad;
• Estimar a biodisponibilidade de flúor em biofilme dental nos períodos de 1 e 12 horas após a escovação;
• Avaliar a cinética do flúor em saliva ao longo de 60 minutos após a escovação com os dentifrícios testes;
• Comparar a concentração de flúor no biofilme dental e saliva após uso dos dentifrícios testes e do controle;
• Verificar se as concentrações de flúor intra-bucais após o uso do dentifrício teste indicam um aumento de substantividade desse elemento a ponto de influenciar condições clínicas de cárie.

Avaliação dos Riscos e Benefícios:
Riscos:

Os riscos mínimos previsíveis são esperados. Uma vez que iremos coletar saliva dos voluntários doadores e pode haver desconforto durante a coleta que dura cerca de 4 a 10 minutos. No entanto, os pesquisadores, estarão orientando todos os pacientes para interromperem a coleta no caso de haver algum desconforto no uso do hiperboloide (que funciona como um chiclete sem sabor para estimular a produção de saliva).

Benefícios:

O maior benefício da pesquisa é a elucidação do mecanismo de ação do sistema REFIX de remineralização, contendo fluoreto de sódio (NaF) com compostos acidulados.

Comentários e Considerações sobre a Pesquisa:
O presente projeto apresenta coerência científica, mostrando relevância para a academia, haja vista a ampliação do conhecimento, onde se busca, principalmente, avaliar in vivo a retenção de flúor na cavidade bucal (biofilme e saliva) após o uso de um dentifrício com tecnologia inovadora (sob patente), e verificar a eficácia desse produto na remineralização de lesões de mancha branca de cárie.

Considerações sobre os Termos de apresentação obrigatória:
Os termos de apresentação obrigatória foram anexados tempestivamente.

Recomendações:
RECOMENDAMOS QUE, CASO OCORRA QUALQUER ALTERAÇÃO NO PROJETO (MUDANÇA NO TÍTULO, NA AMOSTRA OU QUALQUER OUTRA), A PESQUISADORA RESPONSÁVEL DEVERÁ SUBMETER EMENDA SOLICITANDO TAL(IS) ALTERAÇÃO(ÕES), ANEXANDO OS DOCUMENTOS NECESSÁRIOS.

RECOMENDAMOS TAMBÉM QUE AO TÉRMINO DA PESQUISA A PESQUISADORA RESPONSÁVEL ENCAMINHE AO COMITÊ DE ÉTICA PESQUISA DO CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA, RELATÓRIO FINAL E DOCUMENTO DEVOLUTIVO COMPROVANDO QUE OS DADOS FORAM DIVULGADOS JUNTO À INSTITUIÇÃO ONDE OS MESMOS

OBTENÇÃO DA CERTIDÃO DEFINITIVA.

Conclusões ou Pendências e Lista de Inadequações:

A PESQUISADORA RESPONSÁVEL ENCAMINHOU EMENDA APRESENTANDO AS SEGUINTES JUSTIFICATIVAS: "No projeto original, a avaliação dos dentifiricos com flúor será em duas etapas: Etapa I – estudo de curta duração (clínica- laboratorial, n=18) com coleta de saliva e biofilme, apenas pacientes adultos e uso por 7 dias cada produto; Etapa II (ECR duplo cego) – estudo longo - estudo clínico randomizado duplo-cego com 90 adolescentes, uso por 45 e 90 dias. Durante a realização da etapa I os pesquisadores sentiram a necessidade de avaliar a aceitação do sabor do dentifrício para garantir o uso prolongado. Notar que na etapa II o participante fará uso contínuo por 90 dias e precisamos garantir que o mesmo tenha aceitação do produto. Em adição, por questões protocolares de segurança do participante, avaliar se o uso prolongado com determinado sabor pode gerar algum desconforto (exemplo: ardência na mucosa, excesso de salivação). Em tempo: não se observou nenhuma queixa com o uso do produto por 07 dias, mas precisamos registrar oficialmente queixas pelo uso de mais de 14 dias para garantir aceitação e segurança na etapa II do projeto".

O ORA REQUERIDO NÃO COMPROMETE EM NADA A EXECUÇÃO DO PRESENTE PROJETO DE PESQUISA, DESTA FORMA SOMOS DE PARECER FAVORÁVEL A EXECUÇÃO DO MESMO. SALVO MELHOR JUÍZO.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1518876_E1.pdf	01/03/2020 21:26:37		Aceito
Outros	Emendadentifriciosabor.doc	01/03/2020 21:25:16	Fabio Correia Sampaio	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEfaseintermediaria.docx	01/03/2020 21:22:34	Fabio Correia Sampaio	Aceito
TCLE / Termos de	TCLE_ETAPA2.pdf	19/10/2019	Nayanna Lana	Aceito

Assentimento / Justificativa de Ausência	TCLE_ETAPA2.pdf	16:40:33	Soares Fernandes	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ETAPA1.pdf	19/10/2019 16:40:09	Nayanna Lana Soares Fernandes	Aceito
Outros	Resposta_aoparecer.pdf	19/10/2019 16:38:02	Nayanna Lana Soares Fernandes	Aceito
Declaração de Instituição e Infraestrutura	CARTA_ANUENCIA.pdf	19/10/2019 16:36:38	Nayanna Lana Soares Fernandes	Aceito
Declaração de Instituição e Infraestrutura	Certidao_posgraduacao.pdf	19/10/2019 16:34:56	Nayanna Lana Soares Fernandes	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_comite.pdf	19/10/2019 16:33:35	Nayanna Lana Soares Fernandes	Aceito
Cronograma	CRONOGRAMA.pdf	19/10/2019 16:31:34	Nayanna Lana Soares Fernandes	Aceito
Folha de Rosto	FOLHA_DEROSTO.pdf	20/08/2019 22:07:21	Nayanna Lana Soares Fernandes	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMODEASSENTIMENTO_ETAPA2.pdf	20/08/2019 22:04:45	Nayanna Lana Soares Fernandes	Aceito
Orçamento	ORCAMENTO.pdf	02/08/2019 07:50:36	Nayanna Lana Soares Fernandes	Aceito
Declaração de Instituição e Infraestrutura	Carta_anuencianayanna.pdf	01/08/2019 18:13:33	Nayanna Lana Soares Fernandes	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 25 de Março de 2020

Assinado por:
Eliane Marques Duarte de Sousa
(Coordenador(a))

ANEXO B – **Comprovante de aceitação do artigo na revista: European Journal of Dentistry**

European Journal of Dentistry

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Paulo D'Alpino as Author [CHANGE ROLE] (role.php) DASHBOARD PROFILE (redirect.php?option=profile&id=133) [SIGN OUT] (logout.php)

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https://orcid.org/0000-0002-1829-409X (http://orcid.org/0000-0002-1829-409X)

	Submission/Title/Type	Status	Action
Delete [Author files] (authors_file_list.php? &paramID=GQSyAiLwSqzVw5Ke9P3DcamlQUdz+3tkdw6XdayudBU=)	Manuscript ID: **EJD-2020-11-17/R1 RESUBMISSION - (1180)** Resistance against erosive challenge of dental enamel treated with 1450-ppm fluoride toothpastes containing different biomimetic compounds Type: Original Article Authors: Nayanna Lana Fernandes (Co-author), Juliellen Cunha (Co-author), Andressa de Oliveira (Co-author), Paulo Henrique Perlatti D'Alpino (Corresponding Author), Fabio Sampaio (Co-author) Submitted: 2021-01-06	Decision **Accept**	See decision (view_decision.php? &paramID=ooBXobt7MZrcmQS+lV6VrKJlyF0x+JipU

APÊNDICES

APÊNDICE A **– Termo de consentimento livre e esclarecido**

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (ETAPA I)

Prezado(a) Senhor(a),

Esta pesquisa intitulada como **"Eficácia de dentifrício com tecnologia inovadora para remineralização de lesões iniciais de cárie: ensaio clínico randomizado"** está sendo desenvolvida pela aluna da Pós - Graduação em Odontologia, Nayanna Lana Soares Fernandes e seu orientador, Prof. Fabio Correia Sampaio do Curso de Odontologia da Universidade Federal da Paraíba.

A finalidade deste trabalho é avaliar a eficácia de um dentifrício fluoretado com tecnologia inovadora, na remineralização da lesão de cárie inicial em esmalte. Os resultados desta pesquisa fornecerão importantes informações a respeito da eficácia de cremes dentais novos, recém lançados no mercado, dispondo assim de mais um dado clínico para prevenção e tratamento da cárie dentária. O papel do cirurgião-dentista é muito importante nesse processo de informação e conscientização, pois de acordo com a Organização Mundial da Saúde, a cárie ainda é um grande problema de saúde pública que precisa ser enfrentado especialmente com estratégias de promoção e prevenção, que atuem antes que a doença esteja em estágio mais avançado.

Solicitamos a sua colaboração para utilização dos cremes dentais da pesquisa e participação dos exames clínicos, para que sejam coletados biofilme bacteriano dos seus elementos dentários e saliva. Pedimos também sua autorização para apresentar os dados deste estudo em eventos da área de saúde e publicar em revista científica. Por ocasião da publicação dos resultados, seu nome e imagens serão mantidos em sigilo. Informamos que esse procedimento pode gerar um certo desconforto, na medida em que você ficará por cerca de cinco a dez minutos cuspindo em um tubo de vidro. Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano. Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

Assinatura do Participante da Pesquisa
ou Responsável Legal

OBERVAÇÃO: (em caso de analfabeto - acrescentar)

Espaço para impressão
dactiloscópica

Assinatura da Testemunha

Contato do Pesquisador Responsável:

Caso necessite de maiores informações sobre o presente estudo, favor ligar para o pesquisador:
Nayanna Lana Soares Fernandes (tel.: (83) 99638-7160)

Ou

Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba
Campus I - Cidade Universitária - 1º Andar – CEP 58051-900 – João Pessoa/PB
☎ (83) 3216-7791 – E-mail: **comitedeetica@ccs.ufpb.br**

Atenciosamente,

Assinatura do Pesquisador Responsável

Assinatura do Pesquisador Participante

APÊNDICE B **– Ficha de instruções ao Participante da Pesquisa**

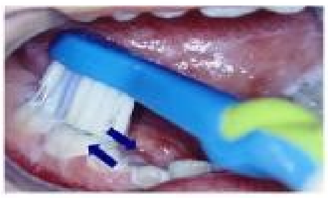
Escovar os dentes com o dentifrício fornecido durante 1 minuto, 3 vezes(manhã, tarde e noite) ao dia.

-Usar somente a pasta e a escova fornecida, utilizando um tanto de pasta como mostra afigura;

-Após a escovação, enxaguar com 10 ml de água.Utilizar o **copinho de plástico** para medir a quantidade de água (**observar a marcação preta, ver figura).**

-Fazer a marcação no lugar de cada escovação através de “X”. Caso esqueça-se da escovação por algum motivo, não marcar com “X”. Deve-se marcar no campo correspondente o motivo da não escovação.

	Dia 1	**Dia 2**	**Dia 3**	**Dia 4**	**Dia 5**	**Dia 6**	**Dia 7**
Escovação da Manhã							
Escovação da tarde							
Escovação da Noite							



No dia 7, somente escovar a parte de cima dos dentes do fundo (molares e pré molares).

Ao ir para cama, não comer e beber mais nada até o dia seguinte, com exceção de beber água. Também não pode escovar mais os dentes.**Tem que estar em um período de 12 horas de jejum e 12 horas da última escovação para fazer a coleta no dia seguinte (8º dia).**

No **oitavo dia**, deverá se dirigir para a Clínica de Odontologia na Universidade Federal da Paraíba no período da manhã no horário combinado. **NÃO TOMAR CAFÉ DA MANHÃ E NEM ESCOVAR OSDENTES**.

Deve levar junto com você o creme dental, escova, copinho medidor e esta ficha.No caso de qualquer dúvida, entrar em contato:Nayanna Lana Soares Fernandes (83) 99638-7160.