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GUILHERME SILVA DOS SANTOS

EFEITOS DE DENTIFRÍCIOS COM POTENCIAL ANTI-EROSIVO NA SUPERFÍCIE DO ESMALTE BOVINO SUBMETIDO À BEBIDA ÁCIDA

EFFECTS OF DENTIFRICES WITH ANTI-EROSIVE POTENTIAL ON THE SURFACE OF BOVINE ENAMEL SUBMITTED TO ACIDIC BEVERAGE

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgião Dentista.

Undergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon.

Orientadora: Profª. Drª. Vanessa Cavalli Gobbo

Coorientador: Dr. Matheus Kury Rodrigues

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RESUMO

Este estudo avaliou o efeito de diferentes dentifrícios contendo fluoreto de sódio (NaF) associado ao Novamin (Sensodyne Repair&Protect – SRP), NaF associado ao fluoreto de estanho (SnF_2, Oral-B Pró-gengiva – OBP) e outro contendo fluoreto de amina (AmF, Colgate Elmex – ELM) no esmalte submetido à ciclagem erosiva. Quarenta blocos de esmalte-dentina bovinos (n=10) foram submetidos à ciclagem erosiva com suco de laranja (pH = 3,29, 5min, 3x/dia), saliva artificial (SA – 2h, 3x/dia e overnight) e tratados com dentifrícios (2min, 2x/dia) ou sem tratamento (CONT). A microdureza de superfície (KNH) foi avaliada inicialmente (T_0), no 1º (T_1) e 5º (T_5) dia de ciclagem. A rugosidade de superfície (Ra, μm) foi determinada em T_0 e em T_5. Os dados foram analisados com ANOVA/Tukey ou Bonferroni (α=5%). A morfologia e conteúdo mineral foram avaliados usando microscopia eletrônica de varredura (MEV) e espectroscopia de raio-X por energia dispersiva (EDS). Não houve diferenças na porcentagem de perda de dureza de superfície (%PDS) entre os grupos em T_1 ($p>0,05$). Em T_5, OBP promoveu %PDS inferior ao CONT, SRP e ELM ($p<0,05$), enquanto ELM e SRP não diferenciaram do CONT ($p>0,05$). O grupo OBP promoveu Ra e ΔRa significativamente inferiores aos demais grupos ($p<0,05$). Todos os grupos apresentaram alterações morfológicas de topografia. Os dentifrícios comerciais testados não foram capaz de reverter a perda de dureza de superfície (PDS) e o aumento da rugosidade promovida pelo desafio ácido. Contudo, o dentifrício contendo SnF_2, atenuou o impacto negativo causado pela exposição ao suco de laranja às propriedades do esmalte dental.

Palavras-chave: Erosão dentária. Remineralização dental. Dentifrícios. Fluoretos de Estanho.

ABSTRACT

This study evaluated the effect of different dentifrice containing sodium fluoride (NaF) combined with Novamin (Sensodyne Repair&Protect – SRP), NaF combined with stannous fluoride (SnF_2, Oral-B Pro-gengiva – OBP), and another containing amine fluoride (AmF, Colgate Elmex – ELM) on enamel submitted to simulated erosive cycling. Forty bovine enamel-dentine blocks (n=10) were submitted to an erosive cycling with orange juice (pH = 3.29, 5 min, 3x/day), artificial saliva (SA – 2h, 3x/day and overnight) and treated with dentifrice (2 min, 2x/day) or without treatment (CONT). The surface microhardness (SMH) was evaluated at baseline (T_0), on the 1st (T_1), and 5th (T_5) days. Surface microhardness loss (%SHL) was calculated. Surface roughness (Ra, μm) was determined at T_0 and T_5. Morphology and mineral content were evaluated under scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), respectively. Data were analyzed using ANOVA/Tukey or Bonferroni (α=5%). No differences in %SHL were detected among groups at T_1 ($p>0.05$). At T_5, the OBP promoted a lower %SHL than CONT, SRP and ELM ($p<0.05$), but there were no differences between ELM and SRP to CONT ($p>0.05$). The OBP group promoted Ra and ΔRa significantly lower than the other groups ($p<0.05$). All groups exhibited morphological changes in topography. None of the commercial dentifrices tested prevented the microhardness loss and increase in roughness promoted by erosion. However, Oral-B Pro-gengiva, which contains SnF_2, minimized the negative effects on the surface microhardness and roughness caused by exposure to orange juice after 5 days of simulated cycling.

Key Words: Tooth erosion. Tooth remineralization. Dentifrices. Tin Fluorides.

SUMÁRIO

1 INTRODUÇÃO

A erosão dental é um processo químico definido pela perda progressiva e irreversível de tecido dental duro por um processo químico que não envolve ação bacteriana (Barac et al., 2023). Pode-se destacar que a etiologia da erosão dental é dependente de uma condição ácida, seja ela intrínseca ou extrínseca (Barac et al., 2023). Os ácidos de origem intrínseca são provenientes do metabolismo, ou seja, hábitos e doenças que promovem o desgaste dental, devido à acidificação do meio (Helle et al., 2023). De acordo com a autora, estes ácidos são oriundos da bulimia e da doença do refluxo gastroesofágico, condições nas quais o paciente está sujeito a exposição ao ácido hidroclorídrico proveniente da mucosa gástrica. Portanto, estas condições favorecem o aparecimento da erosão na face palatina dos dentes anteriores superiores e região vestibular de dentes posteriores superiores. Já os ácidos de origem extrínseca são provenientes do ambiente externo, estando intimamente relacionados ao consumo de alimentos e bebidas ácidas, como suco de frutas cítricas, condimentos, refrigerantes e medicamentos (Barac et al., 2023). Embora a saliva desempenhe um papel importante na neutralização da acidez bucal, por meio de seu sistema tampão constituído de bicarbonato de cálcio e íons, a frequência destes desafios ácidos supera a capacidade regulatória e reparadora da saliva e a perda mineral ocorre na superfície dental (Enax et al., 2023).

Desta forma, a dissolução gradual de tecido dental duro pelos ácidos que não são subprodutos bacterianos promove o amolecimento do esmalte e o aumento da perda de estrutura dentária (Malcangi et al., 2023), contribuindo para alterações nas propriedades físicas como microdureza, rugosidade, morfologia de superfície e conteúdo mineral do esmalte (Loretto et al., 2023). Em razão disso, dentifrícios com potencial anti-erosivo que possuem fluoreto de sódio (NaF) ou de amina (AmF) em sua composição podem produzir uma barreira composta por fluoreto de cácio (CaF_2) na superfície dental comprometida (Cury et al., 2017). Esta barreira protege a estrutura dental contra a exposição aos ácidos e auxilia no tamponamento mediante o desafio.

Além destes compostos, trabalhos realizados por Ganss et al., 2010 e Zanatta et al., 2020 relataram que o mecanismo de ação do fluoreto de estanho (SnF_2) consiste na deposição de material semelhante ao CaF_2, e foi demonstrado que o íon estanhoso pode interagir com as superfícies dentárias formando outros precipitados resistentes a ácidos, como hidróxido fosfato de estanho ($Sn_2(OH)PO_4$) e fosfato de flúor estanhoso (SnF_3PO_4) (Ganss et al., 2010). Também foi postulado que o estanho pode se incorporar ao esmalte, modificando sua camada superior para aumentar sua resistência (Zanatta et al., 2020). Um estudo realizado recentemente demonstrou que o SnF_2 desempenha ação altamente eficaz na proteção do esmalte mediante desafios ácidos comparado com fluoretos convencionais (Gümüştaş et al., 2023).

Além destes, outros componentes têm sido propostos e estudados ao longo dos anos com o intuito de minimizar os processos de perda mineral na superfície dental. Biovidros ativos, desenvolvidos para regenerar tecidos altamente mineralizados como tecido ósseo, foram adaptados para o uso na cavidade oral para serem utilizados no tratamento da hipersensibilidade, gengivite, lesão não cariosa e lesão cariosa (Gaona et al., 2019). Segundo estes autores, o NovaMin é um biovidro ativo biocompatível à base de fosfosilicato de cálcio e sódio. Seu mecanismo de ação baseia-se na formação de uma camada de íons cálcio e fosfato semelhante à hidroxiapatita na superfície dental, que serve como reservatório capaz de liberar íons Ca e P quando em contato com meio aquoso ou saliva, com intuito de proteger o esmalte e dentina e ocluir os túbulos dentinários. Um estudo realizado por Khijmatgar et al., (2020) apontou que o fosfosilicato de cálcio e sódio é capaz de ocluir grande quantidade de túbulos dentinários e reduzir a perda de estrutura da superfície dental devido à alta concentração de cálcio e fosfato.

Uma vez que a erosão dental tem se tornado um problema frequente em pacientes cada vez mais jovens, a análise da eficácia de terapias não invasivas, como o uso de dentifrícios com potencial de minimizar as perdas iniciais do conteúdo mineral do esmalte promovidas pela erosão dental, são necessárias (Kanaan et al., 2022). Não obstante, apesar de diversos autores avaliarem a eficácia de dentifrícios que contêm flúor e seu potencial anti-erosivo, como o biovidro fosfosilicato de cálcio e sódio (Gümüştaş et al., 2023), o fluoreto de estanho (Ramos et al., 2022) e o fluoreto de amina (Reise et al., 2021), em condições que simulam o desafio erosivo de pacientes que consomem continuamente soluções ácidas (pH 3,0 – 5,0), esses dentifrícios anti-erosivos não foram avaliados simultaneamente em um mesmo estudo.

Portanto, o objetivo principal desta investigação foi avaliar a resistência do esmalte contra um desafio erosivo com bebida ácida mediante tratamento um dentifrício contendo biovidro ativo NovaMin (fosfosilicato de cálcio e sódio a 5% + NaF 1426 ppm, Sensodyne Repair and Protect), outro contendo fluoreto de amina (AmF 1250 ppm F, Colgate Elmex) e, finalmente, um dentifrício contendo fluoreto de estanho (SnF_2 1100 ppm F + NaF 350 ppm F, Oral B Pró-gengiva).

2 ARTIGO: EFFECTS OF DENTIFRICES WITH ANTI-EROSIVE POTENTIAL ON THE SURFACE OF BOVINE ENAMEL SUBMITTED TO ACIDIC BEVERAGE

Authors: Guilherme Silva dos Santos, Amanda Ferreira Felix,
Iago César Ribeiro Teles Matos, Matheus Kury, Vanessa Cavalli

ABSTRACT

Objectives: This study evaluated the effect of dentifrices containing sodium fluoride (NaF) combined with Novamin (Sensodyne Repair&Protect – SRP), NaF combined with stannous fluoride (SnF_2, Oral-B Pro-gengiva – OBP) and amine fluoride (AmF, Colgate Elmex – ELM) on enamel submitted to simulated erosive cycling. **Materials and Methods:** Forty bovine enamel-dentine blocks (n=10) were submitted to an erosive cycling with orange juice (pH = 3.29, 5 min, 3x/day), artificial saliva (SA – 2h, 3x/day and overnight) and treated with dentifrice (2 min, 2x/day) or without treatment (CONT). The surface microhardness (SMH) was evaluated at baseline (T_0), on the 1st (T_1) and 5th (T_5) days. Surface microhardness loss (%SHL) was calculated. Surface roughness (Ra, µm) was determined at T_0 and T_5. Morphology and mineral content were evaluated under scanning electron microscopy and energy dispersive X-ray spectroscopy. Data were analyzed using ANOVA/Tukey or Bonferroni (α=5%). **Results:** No differences in %SHL were detected among groups at T_1 ($p>0.05$). At T_5, the OBP promoted lower %SHL than CONT, SRP and ELM ($p<0.05$), but there were no differences among them ($p>0.05$). OBP group promoted Ra and ΔRa significantly lower than all groups ($p<0.05$). All groups exhibited morphological changes in topography. Groups showed similar numeric Ca/P means before and after treatments. **Conclusions:** Oral-B Pro-gengiva, which contains SnF_2, minimized the negative effects on the SMH and Ra caused by exposure to orange juice after 5 days of simulated cycling. **Clinical relevance:** Patients who are more exposed to risk factors for dental erosion could benefit from the use of dentifrice containing SnF_2.

Keywords: Tooth erosion. Tooth remineralization. Dentifrices. Tin Fluorides

INTRODUCTION

Dental erosion is a chemical process characterized by progressive and irreversible loss of dental hard tissue not involving acids from bacterial origin [1]. Erosive demineralization depends on an acidic condition that may be caused by intrinsic and extrinsic factors [1]. Intrinsic erosion is originated from metabolism, habits and conditions that promote dental wear due to acidification of the environment [2]. These clinical oral challenges are originated from endogenous acids such as gastric acid in patients suffering from bulimia and/or gastroesophageal reflux disease (GERD) [2]. On the other hand, exogenous acids are related to the consumption of acidic foods and beverages such as soft drinks, sport drinks and citrus fruit juice with low pH values [1]. Although the saliva plays an important role in neutralizing oral acidity due to its buffer system composed of calcium bicarbonate and ions, the frequency of the acidic challenges surpasses the regulatory and reparative capacity of saliva resulting in mineral loss on the dental surface [3].

As a result, the gradual softening of dental tissue by non-bacterial acid byproducts weakens the enamel and increases the loss of tooth structure [4], thereby negatively influencing enamel physical properties such as microhardness, roughness, surface morphology, and mineral content [5]. To minimize these effects, dentifrices with anti-erosive potential containing sodium fluoride (NaF) or amine fluoride (AmF) may produce a barrier composed of calcium fluoride (CaF_2) on the dental surface, and that barrier may lead to fluorapatite (FA) formation on the enamel surface and promote higher resistance than hydroxyapatite during acid attacks [6]. This barrier protects dental structure from acid exposures and assists the buffering capacity during the acid challenge.

However, a recent study demonstrated that stannous fluoride (SnF_2) plays a highly effective role in protecting enamel against acid challenges compared to conventional fluorides [7]. The mechanism of action of dentifrices containing SnF_2 involves the deposition of a compound similar to CaF_2, and it has been demonstrated that Sn can interact with dental surfaces to form other acid-resistant precipitates [8]. Also, tin can incorporate into enamel, modifying its top layer, increasing the surface resistance [9].

Furthermore, other components have been proposed to suppress mineral loss, such as Novamin, which is a commercial biocompatible bioactive glass that consists of calcium sodium phosposilicate [10]. Its mechanism of action relies on the formation of a calcium (Ca) and phosphate (PO_4^{3-}) ion layer similar to hydroxyapatite on dental surface. This layer provides a reservoir capable of releasing Ca and PO_4^{3-} ions when in contact with aqueous medium or saliva. Khijmatgar et al. [10] indicated that calcium and sodium phosphosilicate reduced dental surface loss due to its high concentration of calcium and phosphate, but evidence is scarce concerning Novamin's action against an erosive challenge.

The prevalence and severity of dental erosion in the adult population are significant and has become an increasingly frequent problem in adolescents and young adults [11]. In this sense, it is paramount to analyze the effectiveness of non-invasive therapies to uphold the enamel mineral content. Although previous studies assessed the efficacy of the aforementioned dentifrices [6,12,13], the results are still controversial and the potential anti-erosive effects of such dentifrices have not been compared simultaneously up to this moment. Therefore, this study evaluated enamel resistance against erosive challenge with an acidic beverage using an adapted erosive cycling model [14,15], and compared the protective ability of dentifrices containing NovaMin bioactive glass (5% calcium and sodium phosphosilicate), amine fluoride (AmF 1250 ppm F) and stannous fluoride (SnF_2 1100 ppm F). The null hypotheses postulated were that the dentifrices would not impact enamel surface i) microhardness and ii) roughness during erosive challenges.

METHODOLOGY

Experimental Design

Forty bovine enamel-dentin blocks were randomized assigned into groups (n = 10/group), according to the dentifrice treatment:

- **SRP:** bioactive glass of calcium sodium phosphosilicate combined with NaF (1426 ppm F) (Sensodyne Repair&Protect, GSK);
- **ELM:** AmF (1250 ppm F, Colgate Elmex);
- **OBP:** SnF_2 combined with sodium fluoride (SnF_2 1100 ppm F + NaF 350 ppm F, Oral-B Pro-gengiva);
- **CONT:** Control (without treatment).

Enamel surface microhardness (SMH), percentage of microhardness loss (%SHL), surface roughness (Ra and ΔRa), morphology and calcium to phosphorous content were evaluated (Ca/P). SMH data were obtained before (baseline, T_0), at the first day of cycling (T_1), and after erosive cycling (5^{th} day of cycling, T_5), Ra data was obtained at T_0 and T_5, while morphology and Ca/P content were determined at T_5.

Specimens Preparation

Forty bovine incisors were collected and cleaned using periodontal scalers and scalpel blades. Enamel-dentin discs (diameter = 5.6mm, thickness = 3.0mm) were obtained from the central third of the buccal face of the incisors using a diamond-coated hole saw drill bit (DiMartino, Campinas, SP, Brazil) attached to a bench drill (F16 – Pratika Schultz) under refrigeration. Subsequently, the dentin was flattened, and the enamel surface was polished in a polishing machine (Arotec, Cotia, São Paulo, Brazil) with #600- and #1200-grit sandpapers (Norton Saint-Gobain, Guarulhos, SP, Brazil). The blocks were ultrasonically cleaned in distilled water for 10 minutes. The discs were fixed into acrylic plates using sticky wax to cover all the exposed dentin surface, and only enamel remained exposed. One half of the exposed enamel surface of the specimen was covered with adhesive tape to create a reference area unaffected by erosion.

Surface Microhardness (SMH)

At the baseline (T_0), three indentations were performed in the central area of each specimen, 100 µm-distant from each other, with a Knoop-type indenter (Future Tech-FM-1e, Tokyo, Japan) under a static load of 50 g/5 s. The mean SMH values of all specimens were obtained (320.0 kg/cm^2) and a 10% (+/-) variation of the mean values was used for specimens' selection. The specimens were randomly distributed into four experimental groups, submitted to one-way ANOVA, and no statistical differences were found among groups ($p>0.05$). Yet, during the simulated cycling on the first and fifth days (T_1 and T_5), three indentations were performed in the exposed area. At T_1 and T_5, the percentage of the surface microhardness loss [%SHL = Initial KNH – Final KNH / Initial KNH * 100] was calculated.

pH Analysis and solution's preparation

The pH of acidic beverage (orange juice, Del Valle) was measured (pH = 3.29) at 0 min, 5 min and 24h using a pHmeter (Equilam, Diadema, SP, Brazil) coupled with a potentiometer (Orion Research Incorporated, Boston, MA), previously calibrated with pH 4.0 and 7.0 standards.

The artificial saliva (SA) was prepared containing 1.5 mM Ca, 0.9 mM PO_4, 150 mM KCl and 20mM Tris, pH 7.0 [16]. Also, the slurries of each dentifrice were diluted in distilled water at 1:3 ratio (dentifrice, g / distilled water, mL), according to the groups previously described. Table 1 displays the complete composition of the dentifrices used.

Table 1. Composition of the anti-erosive dentifrices used.

Dentifrices	Ingredients
Sensodyne Repair & Protect (GlaxoSmithKline, GSK, Brazil)	Calcium sodium phosphosilicate 5% (NovaMin), Sodium fluoride (1426ppm), Glycerin, PEG 8, hydrated silica, cocamidropropyl betaine, sodium methyl cocoyl taurate, aroma, titanium dioxide, carbomer, sodium sacchari, limonene, flavour.
Colgate Elmex (Colgate Palmolive Company, São Paulo, SP, Brazil)	Amine fluoride (1250 ppmF), Water, Hydrated Silica, Sorbitol, Hydroxyethylcellulose, Olaflur, Aroma, Saccharin, CI 77891, Limonene, 3-(N-hexdecyl-N-2-hydroxyethylammonium) propyl bis (2-hydroexyethyl) ammonium dihydrofluoride.
Oral B Pro-Gengiva (Procter & Gamble, P&G, Brazil)	Stannous fluoride (1100 ppmF) + Sodium fluoride (350 ppmF), Glycerin, silica, sodium hexametaphosphate, PEG-6, propylene glycol, water, zinc lactate, aroma, sodium gluconate, titanium dioxide (CI 77891), sodium lauryl sulfate, sodium saccharin, stannous chloride, carrageenan, trisodium phosphate, xanthan gum, cinnamal.

Erosive Cycling Model

This study used a modified version of an *in situ* erosion-remineralization cycling model [14,15]. The specimens were immersed in dentifrices slurries (10 mL, 2 min, 2x/day) under constant agitation. Additionally, specimens were immersed in orange juice (20 mL, 5 min, 3x/day) and the %SHL was measured on the 1st and 5th days of cycling. The specimens were stored in artificial saliva (SA – 20 mL, 120 min) at the intervals among treatments and overnight, after the last slurry treatment. Figure 1 shows a schematic representation of the erosive cycling model, which was repeated for 5 consecutive days at 37ºC, always initiated by with slurry immersion. The control group (CONT) was maintained in SA during the slurry agitation.

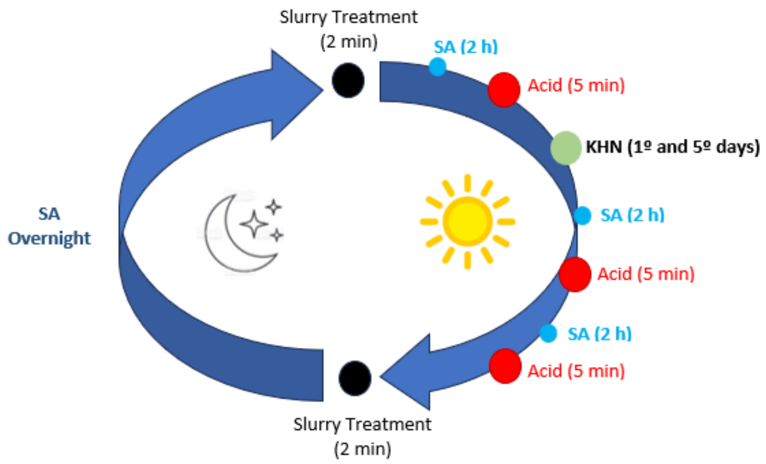

Fig 1.: Schematic representation of the erosive cycling for 5 days.

Surface Roughness (Ra)

The surface roughness (Ra, µm) was determined at the baseline (T_0) and after the treatments (T_5) using a roughness meter (Surfcorder SE 1700, Kosalab), calibrated at a 0.8 mm cut-off. The measuring tip of the equipment remained perpendicular to the surface and three measurements were performed on each side of the sample (untreated at T_0 and treated at T_5), rotating the specimen 45^o, and the mean Ra value was calculated. The surface roughness average variation (ΔRa) was determined (T_5 – T_0).

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

One representative specimen from each group was selected and analyzed for morphology (SEM) and mineral content (EDS) at T_5. After treatments, specimens were washed in an ultrasonic bath (Ultra Cleaner, Unique, Indaiatuba, SP, Brazil) for 10 min and allowed to dry for 24 h in an oven at 37ºC. After drying, the specimens were sputter-coated with a thin carbon layer and observed under SEM (JEOL - JSM, 6460 LV, Tokyo, Japan), operating at 15kV in vacuum mode (45 Pa) [17]. Images were acquired at 1000x magnification. Concurrently to the SEM images acquisition, the software of the energy-dispersive X-ray spectroscopy (EDS, Vantage System – Easymicro Noran Instruments, Middleton, Wisconsin, USA) provided semi-quantitative data on the percentage of chemical elements (atomic percentage) in the selected area of the sample surface.

Statistical analyses

The collected data, except for the EDS analysis, were submitted to exploratory analysis to verify the normal distribution (Shapiro-Wilk and Levene, $p > 0.05$). The values obtained from the analyzed variables met the assumptions of normality and homoscedasticity. The results of %SHL and ΔRa were submitted to one-way ANOVA and Tukey post hoc tests when significance was observed. The values of KNH and Ra assessed overtime were submitted to two-way ANOVA (treatment*time) and Bonferroni post hoc test. A 5% significance level was set for all the analyses.

RESULTS

Surface Microhardness (SMH)

Figure 2A shows that no significant differences in the %SHL were detected among groups ($p > 0.05$) on the first day of cycling (T_1). However, Figure 2B shows that, on the last day of cycling (T_5), OBP exhibited significantly lower %SHL than the CONT, SRP and ELM ($p < 0.05$), and no differences were found among them ($p > 0.05$). Figure 2C depicts a significant decrease in surface microhardness (KNH) for all groups over time, and no significant differences were found among the groups within each time point (T_0, T_1 and T_5).

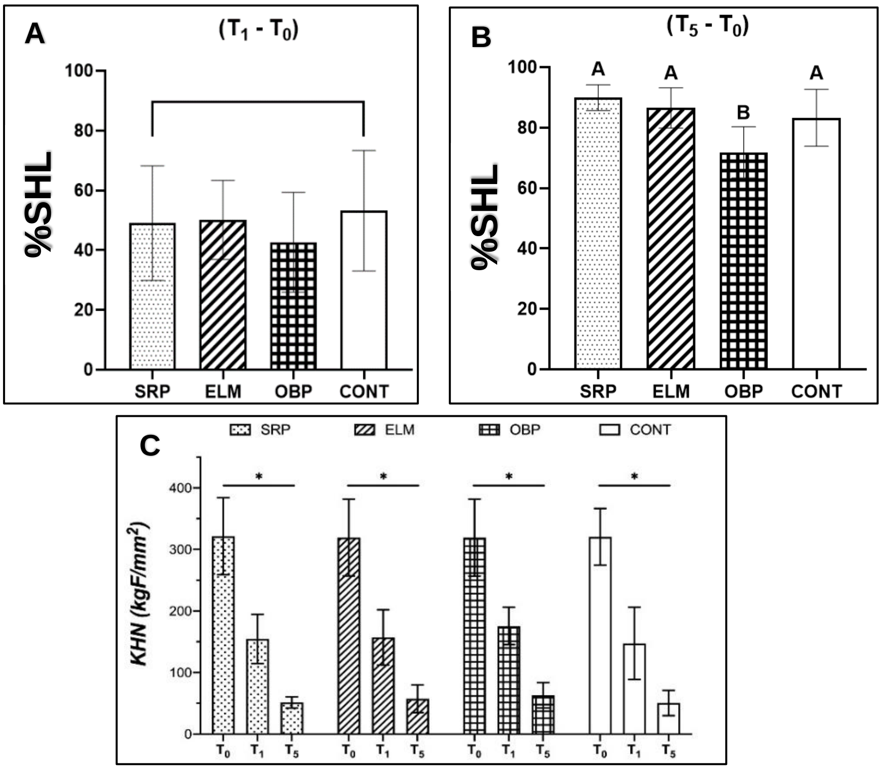

Fig 2A: A graphical representation of the %SHL results on the 1st day of cycling (T_1) compared to the baseline (T_0). Bars connected by bracket indicate that there were no differences between the groups according to the one-way ANOVA; **Fig 2B:** A graphical representation of the %SHL results on the 5th day of cycling (T_5). Different letters indicate differences between the groups according to one-way ANOVA and Tukey post hoc test; **Fig 2C:** A graphical representation of the mean values and standard deviation of KNH over time (T_0, T_1 and T_5). Horizontal lines with asterisks indicate statistical differences within each group among all evaluation times.

Surface Roughness

Figure 3A displays the mean surface roughness results, which increased after 5 days of cycling (T_5), but OBP promoted significantly lower Ra than all other groups.

Although SRP showed significantly lower mean Ra than ELM ($p = 0.044$), both dentifrices exhibited no differences in comparison to CONT at T_5. Figure 3B shows ΔRa values, in which SnF_2 (OBP) treatment showed significantly lower Ra variation than all the other groups. No differences in ΔRa were noted among SRP, ELM and CONT groups ($p > 0.05$).

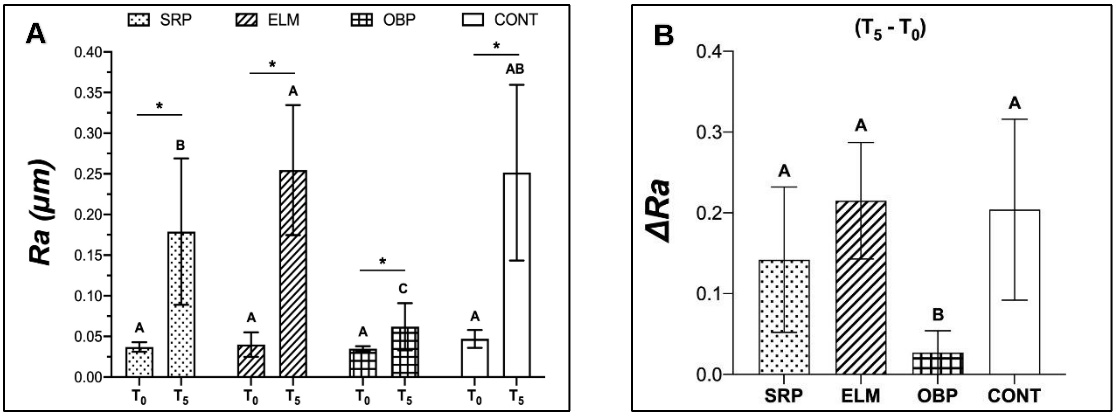

Fig 3A: Mean values and standard deviation of Ra at the baseline (T_0) and after the treatments (T_5). Horizontal lines with asterisks indicate statistical differences within each group between the evaluation times and different letters represent statistical differences among groups within each time point according to one-way ANOVA and Tukey ($\alpha = 5\%$). **Fig 3B:** Graphical representation of the ΔRa results ($T_5 - T_0$), different letters represent statistical differences according to one-way ANOVA and Tukey ($\alpha = 5\%$).

Morphology of enamel surface

Representative images of SEM show that all groups exhibited noticeable enamel surface alterations in the treated areas (T) compared to the non-treated areas (NT). The interface between the areas (IF) clearly demonstrates the transition from a polished enamel surface (NT) to another with different degrees of grooves, valleys, depressions and surface porosity that represents the enamel submitted to acid cycling (T). The eroded area of the CONT and SRP exhibited more irregularities and porosities than OBP and ELM, which showed areas compatible with mineral precipitate formation indicated by the white arrows. The mean percentages (%) of atomic weight for calcium (Ca) and phosphorus (P), as well as the Ca/P, are displayed below each SEM image. The only group showing a mean reduction after treatment was ELM, whereas all the others showed closer Ca/P means before and after treatments.

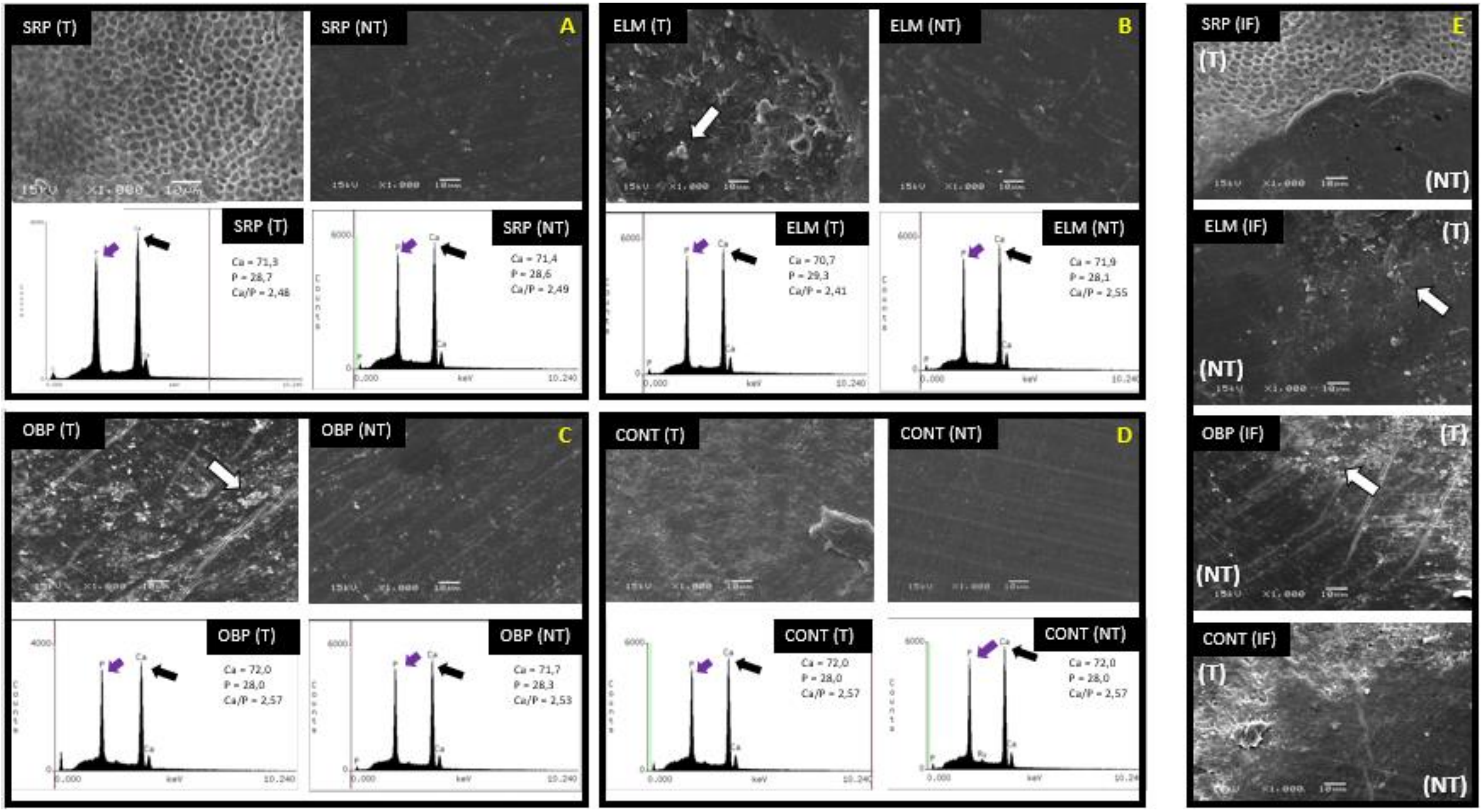

Fig 4: Representative Images collected from SEM and EDS for SRP (A), ELM (B), OBP (C) and CONT (D) groups, showing that the non-treated area (NT) presented a polished, flat and regular surface of enamel in comparison with the treated area one (T), in which enamel exhibited valleys, pits and depressions. ELM and OBP presented depositions that could be result of precipitates from the slurries used specifically in these group's treatments. The interface (IF) for each group is shown (E), indicating a clear transition between the sound and eroded enamel. White arrows represent mineral precipitate formation. In the EDS plots, black arrows represent calcium (Ca) peaks, and purple arrows represent phosphorus (P) peaks.

DISCUSSION

The results of this study showed that orange juice can cause dental enamel erosion after successive immersion cycles. This erosive effect was confirmed by the significant decrease in surface microhardness, regardless of the dentifrice treatment [18]. Even though all groups presented mineral loss above 70%, it is noteworthy that the stannous fluoride-containing dentifrice combined with a low amount of NaF (OBP) promoted a significantly lower microhardness loss. Therefore, the first null hypothesis that no differences in terms of surface microhardness would be detected among treatments was rejected. Previous authors reported that tin-containing salts are able to deposit a more resistant layer than CaF_2 alone on the enamel surface [19], interacting with salivary pellicle [20] or being incorporated onto the demineralized surface [21]. A recent meta-analysis demonstrated that, indeed, none of the potential anti-erosive dentifrices included in that systematic review were capable to avoid dental erosion. However, SnF_2 showed a higher effect against erosion when compared to monovalent dentifrices containing NaF [22]. In this context, the present study corroborates that stannous would be an effective agent to control the microhardness loss promoted by acidic beverages, suggesting lower softening of this eroded surface [15].

On the other hand, the Novamin-containing dentifrice (SRP), which was combined with a high amount of NaF (1426 ppm F), was not capable to reduce the microhardness loss detected in the control group. Although it was expected that the formation of a calcium (Ca) and phosphate (PO_4^{3-}) ion layer similar to hydroxyapatite would turn the enamel surface more resistant against the erosive cycling, the short slurry application time might have hampered this layer formation or did not allow enough formation to combat the acidic attack [15]. It is important to bear in mind that since this dentifrice is effective in occluding dentin tubules, it would be more strategic to use it when the patient already presents exposed dentin and dentin hypersensitivity should be avoided/treated [23]. Therefore, the use of dentifrices with Novamin technology for preventing or minimizing the enamel microhardness loss due to erosion should be discouraged.

Besides, the amine fluoride-containing dentifrice (AmF) was herein evaluated because there is evidence that it is more effective in preventing carious demineralization among the monovalent fluoride dentifrices (containing only either sodium fluoride, amine fluoride, or sodium monofluorophosphate) [24]. Nevertheless, the present findings clearly demonstrated that such monovalent fluoride compound into the dentifrice was not effective in preserving the enamel surface microhardness facing an erosive challenge. On the contrary, researchers pointed out that its combination with stannous fluoride or chloride was more effective to prevent dental erosion [7,19]. In such direction, further studies could investigate whether amine fluoride could synergically decrease the mineral loss found for enamel treated with SnF_2+NaF.

Despite the fact that artificial saliva, containing calcium, potassium and phosphate, was used to mimic as close as possible the remineralizing effect played by human saliva, the acidic challenge likely overcame the buffering capacity provided by saliva in this *in vitro* model. It is important to bear in mind that factors such as pH, abrasivity [25], and mechanical force, may modulate the effect of dentifrice on dental erosion and abrasion. Due to the contact with acid, it is expected that toothbrushing would produce greater tissue loss as eroded enamel becomes softened and, consequently, more vulnerable when submitted to abrasive forces [19]. Therefore, aiming to evaluate the dentifrice's effect on surface microhardness, the slurry agitation was chosen, based on previously conducted studies [15,19], to analyze more effectively the dentifrice's ability to protect the enamel surface without the influence of other factors, such as dental abrasion.

On this note, it is important to emphasize that Silva et al. [26] and Zanatta et al. [15] assessed surface loss through microhardness and optical profilometry methods. Optical profilometry analysis is essential in erosion studies and its absence may be a limitation of the current study. However, microhardness evaluation is considered an indirect method for assessing surface loss [27]. As mentioned before, the findings from this research might indicate that the softening of the eroded enamel treated with SnF_2 was lower than the other dentifrices, possibly inferring that the progression of enamel loss for this group was slower. Further studies could indicate if there is a correlation between the enamel surface microhardness and volume loss under the present treatments and orange juice challenge.

The enamel surface roughness increased over time following exposure to the orange juice. This event was in accordance with the study conducted by Rajeev et al. [28], which reported that the immersion in acidic beverages itself increases the surface roughness. Under clinical examination, at the end of the erosive cycling the enamel showed an opaque surface similar to the study reported by Fuji et al. [29], wherein the enamel surfaces placed in orange juice were visibly roughened and exhibited gloss loss. However, enamel treated with dentifrice containing SnF_2 showed more brightness than the others, and such group significant lower Ra increase than all the other groups. Therefore, the second null hypothesis that no differences in terms of surface roughness would be detected among treatments was rejected. A recent network meta-analysis [30] demonstrated that the enamel wear is lower following the use of polyvalent fluoride group (SnF_2) compared with the monovalent fluoride, because stannous fluoride solutions assist the formation of different precipitates on the enamel surface [31]. Furthermore, enamel volume loss due to erosion may be associated with surface roughness, as indicated by a study conducted by Mullan et al. [32], in which polished enamel exhibited higher surface roughness and loss after 45-minute immersion in orange juice compared to 15- and 30-minute immersion times. Therefore, considering the ability of the stannous fluoride to minimize the enamel surface roughness increase, as suggested by the current study, it can be inferred that there is a relationship of this fluoride in maintaining the surface volume.

Although quantitative analyses of hard dental tissues altered by erosion offer more objective results, scanning electron microscopy (SEM) can be used for the qualitative assessment of changes in the tissue surface morphology [33,34]. Images obtained by SEM depicted that treated area (T) of the OBP group, containing stannous fluoride (SnF_2), presented a uniform surface with precipitate formation. A study conducted by Ganss et al. [8] obtained similar results with the formation of mineral precipitates on the enamel surface such as calcium stannous fluoride, stannous hydroxyphosphate and stannous fluorophosphate which are acid-resistant layers. Furthermore, the ELM group also showed uniform surface with precipitate formation, which could be explained through its mechanism of action of CaF_2 precipitation on the enamel surface [24]. However, according to the authors, these surface precipitates were not sufficiently acid-resistant to effectively prevent demineralization, confirming the findings of the current study. On the other hand, the SRP and CONT images showed changes in the interprismatic space on the enamel surface, which are characteristic of enamel submitted to acid attack [5]. Such evaluation is complementary to surface microhardness and surface roughness measurement [35] confirming the surface roughness results, in which OBP group produced a more uniform surface and significantly lower Ra than all other groups.

Even though the specimens treated with stannous fluoride (SnF_2) demonstrated a positive effect in minimizing damage caused by acid exposure, Jiemkim et al. [36] detected a rare absence of stannous ion release in specimens treated with dentifrice containing SnF_2. According to the authors, this could be attributed to the complex compositions of excipients in dentifrice such as detergents and abrasives like silica, by which the stannous ion may be absorbed, reducing its availability, corroborating the absence of this ion in the EDS analysis, as shown in Fig 4.

Furthermore, the present outcomes should be interpreted with caution because surface dissolution behavior can be influenced by the presence of acquired pellicle and saliva under in situ/in vivo conditions, as these are essential factors in protection against erosion [37]. The clinical relevance of this study is associated with the increased excessive consumption of citrus beverages and food directly influencing the dental erosion process, which has become a global issue in terms of hard dental tissue wear. Thus, patients who are more exposed to these risk factors for dental erosion development could benefit from the use of dentifrice containing stannous fluoride (SnF_2). Nevertheless, while this approach may not offer a comprehensive solution to completely forestall erosion, it underscores the necessity for rigorous clinical investigations. Such studies are imperative to evaluate the plausible anti-erosive efficacy intrinsic to these dentifrices. This evaluation extends beyond merely mitigating the adverse effects of erosion, encompassing the prevention of microhardness reduction and the escalation of surface roughness.

CONCLUSION

None of the commercial dentifrice tested was able to reverse or prevent the microhardness loss and the increase in surface roughness promoted by the erosive challenge. However, the OBP dentifrice, which contains SnF_2, minimized the average of surface microhardness loss and the roughness increase caused by exposure to orange juice after 5 days of simulated cycling.

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3 CONCLUSÃO

Nenhum dos dentifrícios comerciais testados foi capaz de reverter a perda de microdureza de superfície e o aumento da rugosidade promovida pelo desafio ácido. Entre os dentifrícios testados, Oral B Pró-gengiva, que contém SnF_2, promoveu menor perda de microdureza de superfície e menor aumento de rugosidade de superfície causada pela exposição ao suco de laranja após 5 dias de ciclagem simulada.

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ANEXOS

Anexo 1 - Verificação de originalidade e prevenção de plágio

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https://www.slideshare.net/AlyaaAsaad1/non-carious-lesions-operetive-research-project

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https://rjme.ro/RJME/resources/files/620121255262.pdf

<1% match (Adelisa Rodolfo Ferreira Tiveron, Alberto Carlos Botazzo Delbem, Gabriel Gaban, Kikue Takebayashi Sassaki, Denise Pedrini. "In vitro enamel remineralization capacity of composite resins containing sodium trimetaphosphate and fluoride", Clinical Oral Investigations, 2015)
Adelisa Rodolfo Ferreira Tiveron, Alberto Carlos Botazzo Delbem, Gabriel Gaban, Kikue Takebayashi Sassaki, Denise Pedrini. "In vitro enamel remineralization capacity of composite resins containing sodium trimetaphosphate and fluoride", Clinical Oral Investigations, 2015

<1% match (Ahmed Mohsen Foad, Amina Hamdy, Ghada Abd el Fatah, Ahmad Aboelfadl. "Influence of CAD/CAM Material and Preparation Design on the Long-term Fracture Resistance of Endocrowns Restoring Maxillary Premolars", Brazilian Dental Science, 2020)
Ahmed Mohsen Foad, Amina Hamdy, Ghada Abd el Fatah, Ahmad Aboelfadl. "Influence of CAD/CAM Material and Preparation Design on the Long-term Fracture Resistance of Endocrowns Restoring Maxillary Premolars", Brazilian Dental Science, 2020

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Daniel Mendes Filho. "Poluição sonora e perda auditiva podem levar a prejuízo cognitivo e quadros de demência?", Universidade de Sao Paulo, Agencia USP de Gestao da Informacao Academica (AGUIA), 2022

<1% match (documentos dos alunos a partir de 23-fev-2022)
Submitted to Ivy Tech Community College Central Office on 2022-02-23

<1% match (Valdir Gouveia GARCIA, Tiago Esgalha da ROCHA, Natália Amanda GOMES, Daniela Maria Janjácomo MIESSI et al. "Adjuvant effects of Saccharomyces cerevisiae in the treatment of experimental periodontitis in rats undergoing chemotherapy", Journal of Applied Oral Science, 2023)
Valdir Gouveia GARCIA, Tiago Esgalha da ROCHA, Natália Amanda GOMES, Daniela Maria Janjácomo MIESSI et al. "Adjuvant effects of Saccharomyces cerevisiae in the treatment of experimental periodontitis in rats undergoing chemotherapy", Journal of Applied Oral Science, 2023

<1% match (Yuhao Yang, Qiaoqiao Xue, Yubao Zhang, Xingdao He, Zekun Li, Jian Yang. "Quantitative analysis of the degree of demineralization for bleached enamel by optical coherence tomography", Photodiagnosis and Photodynamic Therapy, 2023)
Yuhao Yang, Qiaoqiao Xue, Yubao Zhang, Xingdao He, Zekun Li, Jian Yang. "Quantitative analysis of the degree of demineralization for bleached enamel by optical coherence tomography", Photodiagnosis and Photodynamic Therapy, 2023

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https://www.depositonce.tu-berlin.de/bitstream/11303/11932/1/water-12-02997-v2.pdf

<1% match (documentos dos alunos a partir de 24-out-2023)
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<1% match (Internet a partir de 30-jun-2023)
https://www.science.gov/topicpages/m/magnetically+responsive+alginate

ANEXO 2 – Comprovante de Submissão de Artigo

Gmail **Guilherme Santos <gui.santos272001@gmail.com>**

Clinical Oral Investigations - Receipt of Manuscript 'EFFECTS OF DENTIFRICES...'
1 mensagem

Clinical Oral Investigations <boygeorge.miranda@springernature.com> 22 de janeiro de 2024 às 21:20
Para: gui.santos272001@gmail.com

Ref: Submission ID 762f7448-50a1-4bfa-970a-f717aeaa2761

Dear Dr Santos,

Please note that you are listed as a co-author on the manuscript "EFFECTS OF DENTIFRICES WITH ANTI-EROSIVE POTENTIAL ON THE SURFACE OF BOVINE ENAMEL SUBMITTED TO ACIDIC BEVERAGE", which was submitted to Clinical Oral Investigations on 23 January 2024 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Editorial Assistant
Clinical Oral Investigations

ANEXO 3 – Iniciação Científica

Programa Institucional de Bolsas de Iniciação Científica e Tecnológica da UNICAMP pibic pibiti

Relatório Final

Resistência do esmalte dental tratado com dentifrício contendo biovidro ativo e submetido ao consumo de bebida ácida

Versão enviada em 10/09/2023 20:49:39

ver relatório (../arquivos/rel_final/AlunoCod_29676_1-RelFinal_2022.pdf)

— Parecer do(a) orientador(a) emitido em 14/09/2023 10:38:56

Desempenho do(a) aluno(a) no projeto: Aluno com excelente desempenho no projeto: comprometido, responsável e atuante. Demonstra postura, educação e atenção no desenvolvimento e cuidado na metodologia. Participativo e interessado, discute fases, resultados e métodos para aprimorar as próximas fases da pesquisa.

Desempenho acadêmico do(a) aluno(a): Aluno muito bom nas atividades clínicas: responsável, educado, comprometido e humano com os pacientes. As mesmas qualidades que demonstrou durante a realização do projeto, foram observadas na atuação nas atividades clínica. Apresenta qualidades que o destacam entre os pares e certamente, está entre meus melhores alunos de IC e na graduação.

— Parecer do(a) Assessor(a) dado em 18/12/2023 17:36:19

O relatório científico final descreve objetivamente, com o apoio da literatura, a justificativa e o problema focalizado, sua relevância no contexto da área de dentística e sua importância específica para o avanço do conhecimento quanto a resistencia do esmalte mediante o desafio erosivo com bebida ácida e comparar a capacidade de remineralização e proteção de um dentifrÌcio contendo NaF associado ao biovidro NovaMin (fosfosilicato de cálcio e sódio a 5% - Sensodyne Repair and Protect), um dentifrício contendo fluoreto de amina (AmF 1250 ppm F, Colgate Elmex Anticarie) e outro dentifrício contendo SnF_2 (SnF_2 1100 ppm F + NaF 350 ppm F, Oral B Pró-Saúde). Objetivos e descrição da metodologia empregada bem definidos. Resultados e discussão claros e satisfatoriamente ilustrados por meio de imagens, gráficos e diagrama. O estudo concluiu que nenhum dos dentifrícios comerciais testados foi capaz de reverter a perda de microdureza e o aumento da rugosidade promovida pelo desafio ácido. Entre os dentifrícios testados, Oral B Pró-gengiva, com SnF_2, promoveu menores médias de perda de dureza de superfÌcie e menores médias de aumento de rugosidade de superfície causada pela exposição ao suco de laranja após 5 dias de ciclagem simulada. O bolsista demonstrou competência na realização do estudo proposto, com destaque para a apresentação do trabalho científico no congresso do PIBIC e outros congressos científicos. Além disso, o artigo proveniente do presente estudo está sendo estruturado e encaminhado para futura publicação. Considero o relatório final aprovado pelo ótimo desempenho do aluno, assim como do grupo de pesquisa.

● Aprovado