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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA – MESTRADO ACADÊMICO
ANA PAULA LIMA DA SILVA

# AVALIAÇÃO DA EFICÁCIA DE TRATAMENTOS PREVENTIVOS NO CONTROLE DA CÁRIE DENTAL

UBERABA-MG
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Dissertação apresentada ao Programa de Pós-graduação em Odontologia – Mestrado Acadêmico da Universidade de Uberaba, como requisito para obtenção do título de Mestre em Odontologia, área de concentração: Clínica Odontológica Integrada.

Orientador: Prof. Dr. Vinícius Rangel Geraldo Martins

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**RESUMO**

A cárie dentária em seu estágio inicial é vista como uma região opaca e esbranquiçada sem cavitação visível. A prevenção é geralmente feita com a aplicação de fluoretos, mas para garantir esse efeito, os níveis de $F^-$ sempre precisam estar elevados. O objetivo deste estudo foi avaliar a resistência ácida de lesões de mancha branca do esmalte dental tratadas por diferentes métodos de prevenção de cárie. Foram obtidos 50 incisivos bovinos e preparadas amostras padronizadas (5x5x3). Em seguida, realizou-se a leitura da microdureza do esmalte dental antes de qualquer intervenção, seguido pelo primeiro desafio cariogênico, onde os espécimes permaneceram imersos por 6 horas em solução desmineralizante e 18 horas em solução remineralizante por 14 dias. Após, foi conduzida uma nova avaliação da microdureza do esmalte, seguida da aplicação de cinco tratamentos não invasivos: gel fluoretado (G1), verniz fluoretado (G2), dentifrício com CPP-ACP (G3), resina infiltrante ICON (G4) e laser de Er,Cr:YSGG (G5). Posteriormente, todas as amostras foram submetidas a uma nova avaliação da microdureza e ao último desafio cariogênico, com subsequente e última análise da microdureza. Os dados foram submetidos ao teste de normalidade D'Agostino. ANOVA unidirecional e teste de Tukey foram utilizados para analisar as diferenças de microdureza de todos os grupos. O nível de significância estatística foi 5%. Os resultados revelaram que o valor da microdureza do esmalte dental do G1 (GF) permaneceu estável após o tratamento ($453 \pm 137^a$), comparável aos valores iniciais ($457 \pm 150^a$), e após o 2º desafio cariogênico, houve um aumento da resistência deste grupo ($428 \pm 79^a$). Já o G2 (VF), apresentou microdureza pós-tratamento ($472 \pm 38^{ab}$) semelhante aos valores iniciais ($498 \pm 37^a$), mas após segunda acidez registrou uma diminuição ($420 \pm 63^b$). O G3 (CPP-ACP) também demonstrou um aumento na microdureza pós-escovação ($448 \pm 107^{ab}$) seguida por uma redução frente a um novo desafio acidogênico ($439 \pm 111^b$). O G4 (ICON) recuperou a microdureza do esmalte desmineralizado após a infiltração ($480 \pm 40^a$), além de resistir a nova desmineralização ($431 \pm 66^a$). Resultados semelhantes foram observados para o G5 (L), onde os valores de microdureza após a irradiação ($445 \pm 47^a$) mantiveram-se similares aos do momento inicial ($507 \pm 85^a$), assim como após o 2º desafio cariogênico ($443 \pm 40^a$). Todos os métodos avaliados conseguiram restaurar a dureza do esmalte dentário, contudo, o verniz fluoretado e a escovação com dentifrício CPP-ACP demonstraram uma eficácia relativamente menor em comparação com as outras abordagens. Por outro lado, o gel fluoretado, o laser Er,Cr:YSGG e o infiltrante resinoso conseguiram manter a dureza do esmalte dentário em níveis semelhantes aos valores iniciais. O desempenho destes métodos pode aumentar se os tratamentos forem realizados em conjunto, portanto, novos estudos são necessários para avaliar a sinergia dessas técnicas, a fim de promover maiores resultados na prevenção da cárie dentária.

Palavras-chave: Cárie dental; Dentifrícios; Desmineralização do dente; Flúor; Testes de dureza.

**ABSTRACT**

Dental caries are its early stage is seen as an opaque, whitish region without visible cavitation. Prevention is typically done through fluoride application, but to ensure this effect, fluoride levels must always be elevated. The aim of this study was to assess the acid resistance of white spot lesions on dental enamel treated by different caries prevention methods. Fifty bovine incisors were obtained and standardized samples (5x5x3) were prepared. Subsequently, the enamel microhardness was measured before any intervention, followed by the initial cariogenic challenge, where specimens were immersed for 6 hours in demineralizing solution and 18 hours in remineralizing solution for 14 days. Afterward, a new assessment of enamel microhardness was conducted, followed by the application of five non-invasive treatments: fluoride gel (G1), fluoride varnish (G2), CPP-ACP-containing toothpaste (G3), ICON infiltrating resin (G4), and Er,Cr:YSGG laser (G5). Later, all samples underwent another microhardness assessment and the final cariogenic challenge, with subsequent and final microhardness analysis. The data were subjected to D'Agostino normality test. One-way ANOVA and Tukey's test were employed to analyze the differences in microhardness among all groups. The statistical significance level was set at 5%. The results revealed that the enamel microhardness value of G1 (GF) remained stable after treatment ($453 \pm 137^{a}$), comparable to the initial values ($457 \pm 150^{a}$), and following the 2nd cariogenic challenge, there was an increase in the resistance of this group ($428 \pm 79^{a}$). On the other hand, G2 (VF) showed post-treatment microhardness ($472 \pm 38^{ab}$) similar to the initial values ($498 \pm 37^{a}$), but after the second acidity challenge, a decrease was recorded ($420 \pm 63^{b}$). G3 (CPP-ACP) also demonstrated an increase in microhardness post-brushing ($448 \pm 107^{ab}$) followed by a reduction in the face of a new acidogenic challenge ($439 \pm 111^{b}$). G4 (ICON) regained the microhardness of demineralized enamel after infiltration ($480 \pm 40^{a}$), as well as resisting new demineralization ($431 \pm 66^{a}$). Similar results were observed for G5 (L), where microhardness values after irradiation ($445 \pm 47^{a}$) remained similar to those at the initial moment ($507 \pm 85^{a}$), as well as after the 2nd cariogenic challenge ($443 \pm 40^{a}$). All evaluated methods were able to restore dental enamel hardness; however, fluoride varnish and brushing with CPP-ACP toothpaste showed relatively lower efficacy compared to other approaches. On the other hand, fluoride gel, Er,Cr:YSGG laser, and resin infiltrant were able to maintain dental enamel hardness at levels similar to initial values. The performance of these methods may increase if treatments are performed in conjunction; therefore, further studies are needed to evaluate the synergy of these techniques in order to promote better outcomes in dental caries prevention.

Keywords: Dental caries; Dentifrices; Fluoride; Hardness tests; Tooth demineralization.

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**SUMÁRIO**

# 1. INTRODUÇÃO

A cárie dental é uma doença dinâmica, multifatorial, mediada por biofilme bacteriano, que afeta grande parte da população e promove a destruição localizada dos tecidos dentários (Cheng *et al.,* 2022). Esse distúrbio pode ocorrer ao longo da vida, tanto na dentição decídua quanto na permanente, com potencial de danificar a coroa do dente e, posteriormente, as superfícies radiculares expostas. Sua origem depende da correlação entre fatores determinantes primários, representados pelo biofilme dental, a microbiota cariogênica, o hospedeiro (dieta) e o tempo, e pelo fator determinante secundário, representado pela saliva (Pitts *et al.*, 2017). Somado a eles estão os fatores modificadores ou confundidores, caracterizados pela renda, pelo conhecimento sobre higiene oral, pelo comportamento do indivíduo e pela escolaridade (Tinanoff *et al.*, 2019). Na infância a cárie é vista como uma doença crônica, sendo uma das patologias evitáveis mais comuns, tornando-se um problema de saúde pública (Seow, 2018). Conhecida como uma "epidemia silenciosa", a cárie afeta desproporcionalmente as populações pobres, em especial crianças e jovens (Frencken *et al.*, 2020).

As lesões iniciais de cárie são caracterizadas pela presença de áreas opacas no esmalte, criadas pela perda mineral da camada subsuperficial do tecido duro, sem que haja cavitação clinicamente visível (Ebrahimi *et al.*, 2017). Nota-se ainda uma coloração mais esbranquiçada em comparação ao esmalte sadio, devido à diferença dos índices de refração (IR) entre a região desmineralizada (IR= 1,33 em meio aquoso e 1 em meio seco) e a área de esmalte íntegro (IR=1,65) (Oguro *et al.*, 2016).

O diagnóstico precoce da desmineralização do esmalte dental permite que seja empregada a terapia de remineralização do tecido duro, interrompendo a progressão da lesão incipiente com a menor perda tecidual possível, utilizando diferentes abordagens minimamente invasivas como, por exemplo, a educação/motivação do paciente para uma correta higiene oral, o controle da dieta e a aplicação de compostos à base de flúor no dente e cavidade oral (Chan *et al.*, 2022).

Os fluoretos, forma iônica do elemento químico flúor, são os principais responsáveis pelo declínio da cárie dentária em países desenvolvidos e também no Brasil. Além da redução da prevalência da cárie, o F- age reduzindo a velocidade de progressão de novas lesões (Clarck *et al.*, 2020). O efeito preventivo do flúor tem sido investigado extensivamente nos últimos anos. Várias modalidades e métodos de aplicação dos compostos fluoretados foram estudados, cada um com sua própria concentração recomendada, frequência de uso e esquema de dosagem (O'Mullane *et al.*, 2016).

A aplicação profissional de flúor é realizada nos consultórios odontológicos e é frequentemente recomendada para indivíduos de alto risco/atividade de cárie. Essa aplicação envolve uso de géis fluoretados, vernizes, espumas e pastas que podem, em alguns casos, serem implementados em escolas ou outras instituições como parte de programas de prevenção de cárie (Soares *et al.*, 2021).

Apesar da American Dental Association sugerir que indivíduos que apresentam alto risco à cárie deveriam receber verniz fluoretado duas vezes por ano, a literatura não é conclusiva sobre esse assunto, principalmente devido ao risco da fluorose dentária, que é o resultado da ingestão crônica de flúor durante o desenvolvimento dental, que se manifesta como mudanças visíveis de opacidade do esmalte devido a alterações no processo de mineralização do dente (Grohe e Mittler, 2021). Outro fator que pode ser considerado uma desvantagem é a necessidade da utilização constante de compostos fluoretados para que os níveis de F- na saliva se mantenham elevados e possam atuar na prevenção ou na progressão das lesões cariosas (Benedito *et al.*, 2023). Por esse motivo, outras abordagens não invasivas como, infiltrantes resinosos, dentifrícios contendo caseína (CPP-ACP) e o laser estão sendo estudados para diminuir a utilização ou mesmo substituir o flúor no processo de higienização bucal (Doméjean *et al.*, 2014; Gulec e Goymen, 2019).

O infiltrante resinoso ICON (DMG, Chemisch – Pharmazeutische Fabrik GmbH, Hamburgo, Alemanha) é um método promissor de tratamento minimamente invasivo, uma vez que sua baixa viscosidade e seu poder de penetração permitem que ele infiltre no corpo da lesão de esmalte e preencha as porosidades evitando a difusão de ácidos e sais minerais dissolvidos que levariam a uma maior desmineralização e progressão da lesão (Askar *et al.*, 2015). Além de impedir a progressão da lesão, o infiltrante resinoso (Icon®) possui um índice de refração muito semelhante ao do esmalte dental saudável (1,46-1,52) o que confere a esse material, a capacidade de mascarar o aspecto esbranquiçado que a lesão incipiente apresenta devido ao diâmetro aumentado de seus poros (Andrade *et al.*, 2020; Farias *et al.*, 2022).

Na última década uma nova perspectiva de tratamento não invasivo de lesões iniciais de cárie foi adotada. A caseína, uma proteína do leite, demonstrou atividade anticariogênica através da capacidade de estabilizar cálcio e fosfato na superfície dentária, aumentando o gradiente destes íons e, consequentemente, promovendo a remineralização (Nakamura *et al.*, 2021). O fosfopeptídeo caseína se liga ao fosfato de cálcio amorfo, constituindo o complexo Fosfopeptídeo Caseína - Fosfato de Cálcio Amorfo (CPP ACP). O fosfato de cálcio amorfo é capaz de liberar íons de cálcio e fosfato, mantendo um estado supersaturado, otimizando o processo de remineralização. O complexo CPP-ACP possui formulações em produtos como enxaguante bucal, dentifrício, mousse e goma de mascar (Tao *et al.*, 2018).

Outro tratamento alternativo que tem sido bastante discutido na literatura é a utilização de lasers em alta intensidade para aumentar a resistência ácida do esmalte dental. Os relatos da literatura têm demonstrado que a irradiação do esmalte dental com lasers de alta intensidade tem promovido redução significativa da perda mineral (Al-Maliky *et al.*, 2020). Diferentes hipóteses têm sido propostas para explicar as alterações do esmalte que resultam no efeito preventivo e todas elas se relacionam com o aumento de temperatura no tecido irradiado, diminuindo a solubilidade e permeabilidade dele (Al-Maliky *et al.*, 2020; Luk *et al.*, 2020).

Como discutido, vários são os métodos que se propõem a remineralizar o esmalte dental. Em grande parte das vezes os estudos sobre prevenção de cárie são realizados em esmalte sadio (Taher *et al.*, 2012; Abufarwa *et al.*, 2022). No entanto, seria interessante compreender a eficácia dessas abordagens na prevenção da progressão das lesões de cárie, ou seja, avaliar a efetividade de diferentes tratamentos no controle de lesões iniciais da cárie dental. Dessa maneira, o objetivo do presente estudo é avaliar a microdureza de lesões de mancha branca tratadas com diferentes métodos de prevenção de cárie. A hipótese nula é de que os tratamentos não são capazes de impedir a desmineralização das lesões de mancha branca do esmalte dental.

## 2. OBJETIVOS

O objetivo do presente estudo foi avaliar a resistência ácida de lesões de mancha branca do esmalte dental tratadas por diferentes métodos de prevenção de cárie.

## 3. CAPÍTULO 1

**Evaluation of preventive methods used to interrupt the progression of early caries lesions.**

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## 4. ABSTRACT

The objective was to evaluate the surface hardness of white spot lesions (WSL) treated with different caries prevention methods. The Knoop microhardness (µKHN) of 50 bovine dental enamel fragments was evaluated. WSL were induced (pH cycling) in these fragments and, after µKHN analysis, the specimens received the following treatments (n=10): fluoride gel (NaF 2% - G1), fluoride varnish (NaF 5% - G2), brushing with CPP-ACP dentifrice (G3), infiltrant resin (Icon-DMG) (G4) and Er,Cr:YSGG laser (8.92 J/cm2, 0.5 W) (G5). A second cariogenic challenge was done after treatments, and surface microhardness was measured before and after those treatments. The Knoop hardness (KHN) values obtained were analyzed by one-way ANOVA followed by Tukey´s test (α=5%). After treatments, the KHN was similar to the baseline in all groups. After the second cariogenic challenge, it was observed that G1, G4 and G5 presented KHN similar to their baseline numbers, and in G2 and G3 the KHN was similar to the post-treatment numbers. In general, the recovery of enamel hardness after treatment was above 86%, where fluoride gel, varnish and icon achieving slightly better results than the CPP-ACP and Er,Cr:YSGG laser. The analysis of the acid resistance of WSL showed that in groups 1, 3 and 5 the loss of hardness was lower than that observed in groups 2 and 4. It was concluded that the treatments recovered the hardness of the demineralized enamel and that, despite the methods tested did not prevent demineralization of WSL, their effectiveness was greater than 85%.

**KEYWORDS:** Dental caries; Dentifrices; Fluoride; Hardness tests; Tooth demineralization.

## 5. INTRODUCTION

Dental caries is a dynamic, multifactorial disease, mediated by bacterial biofilm, affecting a large part of the population and promoting localized destruction of dental hard tissues [1]. Caries can occur throughout life, both in primary and permanent dentition, and can damage the tooth crown and, subsequently, the exposed root surfaces. Its origin depends on the correlation between primary determining factors, represented by dental biofilm, cariogenic microbiota, sugar-based diet and time. In addition, salivary flow, saliva components and socioeconomic factors contribute to the occurrence of the disease [2]. Initial caries lesions, known as white spot lesions (WSL), are characterized by the presence of opaque areas on enamel, originated through the loss of mineral from the subsurface layer of the hard tissue, without clinically visible cavitation [3].

Early diagnosis of enamel demineralization allows the adoption of a remineralization therapy, which often stops the progression of the lesion. Currently, the use of fluoride-based compounds represents the main method to prevent tooth caries [4]. The preventive effect of fluoride has been extensively investigated in recent years. Different methods of applying fluoride compounds have been studied, each one with its own recommended concentration, frequency of use and dosage schedule. The use of fluoride through toothpastes, varnishes, gels and mouthwashes is common in cavity prevention programs [1,2,4]. Although the American Dental Association suggests that individuals at high risk for caries should receive fluoride varnish twice a year, the literature is not conclusive on this subject, mainly due to the risk of dental fluorosis in children [5]. Another factor that can be considered a disadvantage is the need to constantly use fluoride compounds so that fluoride levels in saliva remain high and can act in the prevention or progression of carious lesions [6]. For this reason, other non-invasive approaches such as resinous infiltrates, toothpastes containing casein (CPP-ACP) and laser are being studied to reduce the use or even replace fluoride in the oral hygiene process [7,8].

The milk-derived protein casein demonstrated anticariogenic activity through its ability to stabilize calcium and phosphate on the tooth surface, increasing the gradient of these ions and, consequently, promoting remineralization [9]. The casein phosphopeptide binds to amorphous calcium phosphate, constituting the CPP-ACP complex, which is present in mouthwashes and dentifrices. Amorphous calcium phosphate is capable of releasing calcium and phosphate ions, maintaining a supersaturated state on the tooth surface, optimizing the remineralization process [10].

The infiltrant resin is a promising technique of minimally invasive treatment, since its low viscosity and penetrating capacity allow it to infiltrate the body of the WSL and fill the porosities, preventing the diffusion of acids that would lead to greater demineralization [11]. The resinous infiltrant has a refractive index very similar to that of healthy dental enamel (1.46-1.52), which gives this material the ability to mask the whitish appearance that the incipient lesion presents due to the increased diameter of its pores [12].

High-intensity lasers have also been evaluated to increase the acid resistance of tooth enamel. Literature reports have demonstrated that irradiation of dental enamel with high-intensity lasers has promoted a significant reduction in mineral loss [13]. Different hypotheses have been proposed to explain the changes in enamel that result in the preventive effect and all of them are related to the increase in temperature on the irradiated tissue, which decreases the solubility and permeability of the enamel [14,15].

As discussed above, there are several methods proposed to remineralize tooth enamel. In most cases, studies on caries prevention are carried out on healthy enamel [16,17]. However, it would be interesting to understand the effectiveness of these approaches in preventing the progression of caries lesions, that is, to evaluate the effectiveness of different treatments in controlling initial dental caries lesions. Therefore, the objective of the present study is to evaluate the microhardness of white spot lesions treated with different caries prevention methods. The null hypothesis is that treatments are not able to prevent the demineralization of WSL.

## 6. MATERIALS AND METHODS

Fifty bovine incisors without enamel defects were obtained. They were cleaned with a Robinson brush, pumice paste and water, and then stored in distilled water at 4°C until use. To prepare the 50 enamel samples, the crowns were separated from the roots at the cementoenamel junction, and subsequently sectioned under water cooling with a diamond disc mounted on a cutting machine (Cortadora Metalográfica de Precisão ISOMET 1000, Buehler), resulting in 1 sample per tooth crown (5x5x3 mm). To standardize the enamel surface, samples were polished with 1200 grit sandpaper for 1 minute and, for adequate surface smoothness, polished with a felt disc and 0.3 and 0.05μm grit alumina. Shortly after, the experimental area of 16.0 mm² was delimited using red nail polish (Colorama) and subsequently, all specimens were stored in distilled water and placed in an incubator at 37°C until the next stage.

*6.1 Surface Microhardness Measurement*

Initially, samples were evaluated for their surface Knoop microhardness (μKHN) before any intervention (baseline). Specimens were mounted individually in 1-inch acrylic blocks using sticky wax. Five indentations were made in the central area of the sample with a load of 50g, during 10s and a distance of 100 μm between the markings. The Knoop microhardness number (μKHN) for each sample was obtained by calculating the average length of the longest diagonal of each of the five indentations [18]. Other μKHN analyzes were performed, similarly to what was described above, after the induction of WSL (pH cycling), after the treatments and after the second cariogenic challenge (2nd pH cycling).

*6.2 Cariogenic Challenge*

White spot lesions were induced on dental enamel using a pH cycling model previously described [14,19]. Each sample was fixed on the bottom of 24-well cell culture plates, so that the delimited area was exposed to the demineralizing and remineralizing solutions. The demineralization solution (pH = 5.0) consisted of 2.0 mmol/l Ca and 2.0 mmol/l phosphate in a buffering solution of 0.075mol/l acetate. The remineralization solution (pH= 7.0) consisted of 1.5 mmol/l Ca, 0.9 mmol/l phosphate, 150 mmol/l of potassium chloride. Each specimen was cycled in 3.0 mL of both solutions, being 6 h in the demineralizing solution and 18 h in the remineralizing solution, at 37°C, for a period of 14 days. At the end of each 5 consecutive days of cycling, the samples were immersed in the remineralizing solution for 2 days. Subsequently, a new pH cycle was carried out after the proposed treatments to analyze the therapeutic effectiveness in controlling dental caries.

*6.3 Treatments*

The treatments applied are described below (Table 1)

| Groups (n=10) | Treatments |
|---|---|
| 1 | Fluoride Gel (2% NaF) |
| 2 | Fluoride Varnish (5% NaF) |
| 3 | MI Paste (CPP-ACP) Dentifrice |
| 4 | ICON Infiltrant Resin |
| 5 | Er,Cr:YSGG Laser (ED= 8.92 J/cm$^2$, P= 0.5 W) |

Table 1. Experimental groups (ED – Energy Density; P- Power)

The samples of Group 1 (G1) were treated with 2% neutral sodium fluoride gel (2% NaF; Flugel, DFL, Rio de Janeiro, RJ, Brazil), which was placed on demineralized enamel for 3 minutes and then removed with gauze [20]. The samples remained in distilled water at 37ºC. This procedure was repeated after 7 and 14 days, totaling 3 applications.

The samples of group 2 (G2) were treated with fluoride varnish (5% NaF; Duraphat, Colgate-Palmolive, São Bernardo do Campo-SP). The product (0.50g) was placed on the demineralized area using a microbrush for 60 seconds [21]. Excess varnish was removed with gauze and the samples were stored in distilled water for 7 days at 37ºC. This procedure was repeated after 7 and 14 days, totaling 3 applications.

In group 3 (G3), brushing was carried out with a CPP-ACP based dentifrice (MI PASTE, GC America, Alsip-IL, United States). Brushing was performed with an electric brush (Oral-B Pro-Saúde Power, Procter and Gamble), which was attached to a standardized fixed support. The brush head has 3 sets of bristles of different shapes and positioned at different angles and heights. During brushing, the soft bristles of the brush came into contact with the surface of the samples for 210 seconds, with a force of 1.96N, at room temperature. Taking into consideration that an individual brushes each tooth 3 times a day, during 5 seconds on each side of the tooth, this brushing protocol simulated a total toothbrushing period of 14 days [22,23]. A solution (slurry) was obtained by mixing the dentifrice (Table 1) and distilled water in a 1:2 weight ratio (200 mL of distilled water and 100 g of dentifrice – ISO Specification #145669-1), respectively. This solution was prepared just before use. During brushing, 1.0 mL of the slurry was injected laterally to the sample, between the restorative material and the toothbrush, every 30 seconds. At the end, the excess dentifrice was removed under running water and the samples were stored in distilled water at 37ºC.

The application of the infiltrant resin (ICON - DMG, Chemisch - Pharmazeutische Fabrik GmbH, Hamburg, Germany) was done on specimens of group 4 (G4) according to the manufacturer's instructions. White spot lesions were cleaned and etched 15% hydrochloric acid (Icon-Etch) for 2 minutes. The acid was removed with water jets and the enamel surface was dried with air jets. Ethanol (99%, Icon-Dry) was applied on WSL for 30 seconds. Then, the surface was dried with air jets and the Icon-Infiltrant resin was applied during 3 minutes and, then, light cured for 40s. A second application of the infiltrant resin was carried out for a period of 1 minute. The surface was polished with felt discs and the samples were immersed in distilled water at 37°C.

The samples of group 5 (G5) were irradiated with an Er,Cr:YSGG laser ($\lambda$= 2.78 µm; Waterlase™, Biolase™ Technology Inc., San Clemente, CA, USA), with an energy density of 8.92J/cm2, power of 0.5 W, 20 Hz repetition rate, with water cooling, using a sapphire tip of 600µm diameter in a non-contact mode [14]. To standardize the irradiation distance, a device was used that attached the laser handpiece to a pre-established distance from the surface of the sample. Irradiation was carried out for 30s, with the fiber positioned perpendicular to the enamel surface and moved in the horizontal and vertical directions, so that the entire pre-established area of $16mm^2$ received the irradiation. Finally, the samples were placed in distilled water and kept at 37ºC.

At the end of the treatments, all samples were subjected to a second cariogenic challenge, in the same way as was done previously. The µKHN of the samples was analyzed after the treatments and after this second pH cycling.

*6.4 Statistical analyses*

The data were submitted to the D´Agostino normality test. One-way ANOVA and Tukey´s test, when necessary, were used to analyze the differences in Knoop microhardness numbers (µKHN) of all groups. The level of statistical significance was set at 5%. All analyses were done using Bioestat 5.3 (Instituto Mamirauá, Tefe-AM, Brazil).

## 7. RESULTS

Table 2 shows the (µKHN) of enamel, in the initial situation (baseline) and after the white spot lesion induction (1st Cariogenic Challenge). The values obtained in all samples before treatments were compared. This table shows that the method used to demineralize the enamel samples was effective. It should be noted that no cavitation was observed in the samples after the acid challenge. However, an opaque white area was created, characteristic of the white spot lesion.

| | Baseline | 1st Cariogenic Challenge | p-value |
|---|---|---|---|
| µKHN | 498±90 | 334±71 | < 0.0001 |

Table 2. Mean Knoop microhardness numbers (µKHN) ± standard deviation of enamel found in all groups, considering the initial analyzes (Baseline) and after artificial white spot lesion induction (1st cariogenic challenge).

The longitudinal analysis of enamel hardness in each group is shown in table 3. It was observed, for group 1 (fluoride gel), that enamel µKHN after treatment (453±137) was similar to that found in baseline (457±150), suggesting that this method was effective in remineralizing enamel. µKHN analysis after the second cariogenic challenge indicated that fluoride gel increased the acid resistance of the white spot lesion (428±79), as in that situation the values were similar to that of the post-treatment situation, but different from those found after the first cariogenic challenge (289±44). The analysis of the enamel µKHN treated with fluoride varnish (group 2) showed that the treatment recovered the enamel hardness (472±38). As observed for group 1, enamel hardness after the second cariogenic challenge (420±63) was similar to the value found after treatment, but lower than that observed at baseline (498±37). This indicated that the varnish was effective in increasing the acid resistance of the white stain. Samples from group 3 were treated with a dentifrice composed by the bioactive remineralizing agent casein phosphopeptides-amorphous calcium phosphate (CPP-ACP). This group also demonstrated an increase in microhardness post-brushing (448±107ab) followed by a reduction in the face of a new acidogenic challenge (439±111b). The infiltrant resin (ICON) was used in samples of group 4. Similar to what was observed for group 1, the infiltrant recovered the hardness of the demineralized enamel (480±40), and also prevented the demineralization of the white spot lesions (431±66). The same situation was observed in samples irradiated by the Er,Cr:YSGG laser (group 5), where µKHN after treatment (445±47) was statistically similar to baseline (507±85), as well as after the second cariogenic challenge (443±40).

| Groups | Baseline | 1st Cariogenic Challenge | Treatment | $2^{nd}$ Cariogenic Challenge | p-value |
|---|---|---|---|---|---|
| 1 | 457± 150$^{a}$ | 289±44$^{c}$ | 453±137$^{a}$ | 428±79$^{a}$ | 0,009 |
| 2 | 498±37$^{a}$ | 339±51$^{c}$ | 472±38$^{ab}$ | 420±63$^{b}$ | < 0,0001 |
| 3 | 516±51$^{a}$ | 356±92$^{c}$ | 448±107$^{ab}$ | 439±111$^{b}$ | 0,0046 |
| 4 | 490±76$^{a}$ | 334±82$^{c}$ | 480±40$^{a}$ | 431±66$^{a}$ | 0.0003 |
| 5 | 507±85$^{a}$ | 343±41$^{c}$ | 445±47$^{a}$ | 443±40$^{a}$ | < 0,0001 |

Table 3. Mean Knoop microhardness numbers (µKHN) ± standard deviation of enamel found in each group. Different letters on the table represent differences statistically significant ($p<0.05$).

Table 4 shows the percentage of hardness recovery of the enamel samples in all groups. This percentage was calculated by comparing the hardness of the treated enamel with that of the sound enamel (baseline). In general, the recovery of enamel hardness was above 86%, that is, the baseline µKHN was always greater than the treated value. It was observed that gel (98.9±0.9%) and icon (97,9±0.5%) presented slightly better results than the CPP-ACP dentifrice (86,7±2.0%) and Er,Cr:YSGG laser (87.7±0.5%). The percentage of acid resistance of the white spot lesion is also shown in table 3. This percentage was calculated by comparing the hardness of the treated enamel with that of the 2nd cariogenic challenge. The data showed that the µKHN of the enamel after the cariogenic challenge was always lower than after treatments (values less than 100%). In groups 1 (94.4±0.5%), 3 (97.9±1.2%) and 5 (99.5±0.8%) the loss of hardness was lower than that observed in groups 2 (88.9±1.6%) and 4 (89.7±1.6%).

| Group | Recovery of initial µKHN (%) | Acid resistance of white spot lesion (%) |
|---|---|---|
| 1 | 98.9±0.9$^{a}$ | 94.4±0.5$^{a}$ |
| 2 | 94.7±1.0$^{ab}$ | 88.9±1.6$^{b}$ |
| 3 | 86.7±2.0$^{b}$ | 97.9±1.2$^{a}$ |
| 4 | 97.9±0.5$^{a}$ | 89.7±1.6$^{b}$ |
| 5 | 87.7±0.5$^{b}$ | 99.5±0.8$^{a}$ |
| p-value | 0.004 | 0.03 |

Table 4. Percentage of recovery of initial µKHN (treatment/baseline) and percentage of demineralization resistance of white spot lesions (2nd cariogenic challenge/treatment). Different letters in the same column represent differences statistically significant ($p<0.05$).

## 8. DISCUSSION

This research aimed to evaluate the effectiveness of different caries prevention methods to stop the progression of WSL. It was observed that the methods used here were able to fully or partially recover the microhardness of the demineralized enamel and, in some cases, increase the acid resistance of the WSL. Thus, the null hypothesis that treatments would not be able to prevent the demineralization of white spot lesions was rejected.

The initial manifestation of caries is represented by the formation of WSL on the enamel surface, resulting from the loss of minerals from the hard tissue due to an imbalance in the demineralization and remineralization process [3]. Under a high cariogenic challenge, there is a rapid loss of minerals that leads to the softening of the enamel surface, altering its integrity. The Knoop microhardness test (µKHN) is one method used to quantitatively evaluate the degree of enamel mineralization [24,25].

As previously mentioned, if the white spot lesion is not properly treated, the hard tissue may become cavitated and the tooth will need to be restored [26]. Several methods have been recommended to prevent the initiation or to interrupt the progression of white spot lesions [27,28]. The present study evaluated five different treatments for controlling or reversing the initial lesions of dental caries, and found that gel fluoride, infiltrant resin and laser were capable of recovering the baseline numbers of enamel hardness (Table 3).

Fluoride represents the most used preventive method to control the caries process. The mechanism of action of fluoride in caries prevention is well described in the literature. Fluoride compounds promote enamel remineralization, preventing the destruction of tooth structure or even the need for tooth restoration. It is a simple, non-invasive and low-cost treatment and can be applied by the dentist (high concentrations) or by the patient (low concentrations) [4]. The present study evaluated the application of fluoride gel (group 1 - 2% NaF) and varnish (group 2 - 5% NaF) on demineralized enamel. It was observed that both compounds, after their application on demineralized enamel, were able to recover the μKHN of the hard tissue. Similarly, after the second cariogenic challenge, it was observed that the white spot lesion treated with both fluoridate compounds resisted demineralization (Table 3), which is in accordance with the literature [4].

Studies have shown that the effectiveness of fluoride arises from the ability of the ion to form fluoridated apatite instead of hydroxyapatite in the enamel remineralization process. It is assumed that this new surface containing fluoride is less soluble than the original enamel surface, since this newly formed mineral reduces the critical pH of enamel to 4.5, when the sound enamel begins to demineralize when the salivary pH is 5.5 or under. The continuous presence of fluoride in saliva throughout the individual's life is, therefore, essential for the preventive effect, with the formation of calcium fluoride in the remineralization stage [29]. In the present work, fluoride compounds were applied at 3 different times, and between these times the samples remained immersed in distilled water. However, as both the gel and varnish were removed with gauze, it is possible that an excess of fluoride remained in the test tube, and this may have contributed to the remineralizing effect on the surfaces.

The literature suggests that higher concentrations of fluoride mainly allow the remineralization of the superficial layer of the carious lesion, preventing the penetration of the ion into the body of the lesion due to external obstruction of the porosity [29,30]. This information explains the results of the present research because, when compared, the fluoride gel obtained superior results in relation to the varnish after a new cariogenic challenge (table 4).

The CPP-ACP (casein phosphopeptide-amorphous calcium phosphate) is a complex formed from the milk protein casein. These compounds were created to replace fluoride in toothpastes, since fluoridated compounds, if used indiscriminately, can cause intoxication, promoting hypermineralization and staining on teeth (fluorosis) [31]. According to the literature, it binds to enamel and releases calcium and phosphate when needed. The released calcium and phosphate ions penetrate the enamel crystals and increase the density and hardness of the hydroxyapatite crystals it maintains the stability of these ions, ensuring supersaturated levels, thus favoring the formation of hydroxyapatite and promoting remineralization [10,27].

However, when compared to other treatments, this group showed lower performance, as also identified in other researches [24,32]. This can be justified by the short exposure of the specimens to CPP-ACP, with a single brushing using the material for a period of 3 minutes and 50 seconds. A previous study suggested that prolonged use of MI Paste to ensure remineralization should last up to 4 weeks [33]. However, Table 4 suggests that CPP-ACP acts better on demineralized tissue than on sound tissue. The studies that present the best results are those that use CPP-ACP associated with F-, thus forming CPP-ACPF (MI Paste Plus) [34,35]. In the present study, this association was not made because the objective here was to test a compound other than fluoride.

One of the most promising therapeutic methods for treating WSL is the infiltrant resin technique. This low viscosity resin penetrates the porosities of the demineralized enamel and restores the morphology of the tissue, making it difficult for acids to penetrate the enamel. Furthermore, the resinous infiltrant restores the color of the tooth, as it masks the whitish appearance of the demineralized enamel [12]. Previous studies have demonstrated that the microhardness of demineralized enamel is increased after application of the infiltrant. This occurred because the infiltrant fills the porosities of the WSL, making the enamel more cohesive [36,37]. However, it is important to highlight that, despite the performance observed here, the infiltrant resin showed a decrease in acid resistance and, consequently, in microhardness when subjected to a new acid challenge. This can be attributed to the composition of the material, which predominantly contains low-molecular weight monomer (TEGDMA), known for its high hydrophilicity which contributes to the reduction of the material's hardness when exposed to acidic environments, in addition to presenting greater polymerization contraction [38]. Artificial WSLs induced on bovine enamel were used in this study and it has differences when compared with natural WSLs. As artificial WSLs have smaller depth than natural WSLs, the thickness of the infiltrant is lower, and the effect of its degradation may be more pronounced in artificial lesions [39].

The Er,Cr:YSGG laser is used in the minimally invasive therapy of dental caries due to its affinity with water and hydroxyapatite present in hard tissues [40]. Previous studies have shown that erbium lasers increase the acid resistance of sound enamel and dentin by heating/melting the tissues, and by altering the chemical structure of the enamel, forming more acid-resistant crystals [41,42]. To increase the temperature on the tissue surface and to avoid enamel ablation, irradiation was performed with low energy density ($8.92 J/cm^2$) and without water cooling, as described in the literature [14]. After irradiation, no thermal damage or dark spots were observed on the samples. According to table 4, it was observed that Er,Cr:YSGG laser recovered the initial hardness of the samples by almost 88%, while the resistance to demineralization of the irradiated WSL was 99%. This can be explained by the rapid heating and melting of the enamel during irradiation. Melting changes the morphology of the enamel, causing the obliteration of the pores present in WSLs, which prevents the penetration of acids into the hard tissue. As enamel pores are filled with air, eliminating the porosities and filling them with hard tissue may increase the µKHN numbers of the irradiated area [43].

The results found here showed that all methods were able to recover the hardness of tooth enamel. Fluoride varnish and CPP-ACP showed less potential to prevent the demineralization of the WSL after a new cariogenic challenge. In general, the recovery of the initial µKHN of the enamel after the treatments was higher than 86%, and the methods were able to prevent the demineralization of WSL with performance higher than 88%. The effectiveness of these methods can increase if the treatments are carried out together, for example, irradiating surfaces with a laser or applying an infiltrant resin followed by tooth brushing with dentifrices containing fluoride or CPP-ACP. Therefore, new studies are necessary to evaluate the synergy of these techniques, in order to promote greater results in dental caries prevention.

## 9. CONCLUSIONS

Considering the limitations of this *in vitro* study, it is concluded that all methods tested here were able to recover the hardness of tooth enamel, but fluoride varnish and toothbrushing with a CPP-ACP dentifrice appear not to have the same effectiveness in protecting WSL in a new cariogenic challenge than the other techniques. In turn, fluoride gel, Er,Cr:YSGG laser and the resinous infiltrant were able to keep the hardness of dental enamel to levels similar to its baseline. Although the tested methods did not fully prevent the demineralization of white spot lesions in tooth enamel, their performance was higher than 85%.

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## 11. CONFLICT OF INTEREST

The authors state that there are no conflict of interest.

## 12. ETHICAL APPROVAL

This study was approved by the Animal Experimentation Ethics Committee of the University of Uberaba (process: 002/2023).

**REFERENCES**

1. Cheng L, Zhang L, Yue L, Ling J, Fan M, Yang D, Huang Z, Niu Y, Liu J, Zhao J, Li Y, Guo B, Chen Z, Zhou X (2022) Expert consensus on dental caries management. Int J Oral Sci 14: 17. https:// doi:10.1038/s41368-022-00167-3

2. Pitts NB, Zero DT, Marsh Pd, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A (2017) Dental caries. Nat Rev Dis Primers 3:17030. https://doi:10.1038/nrdp.2017.30

3. Oguro R, Nakajima M, Seki N, Sadr A, Tagami J, Sumi Y (2016) The role of enamel thickness and refractive index on human tooth colour. J Dent 51:36-44. https:// doi:10.1016/j.jdent.2016.05.010.

4. Chan Aky, Tamrakar M, Jiang Cm, Tsang Yc, Leung Kcm, Chu Ch (2022) Clinical evidence for professionally applied fluoride therapy to prevent and arrest dental caries in older adults: A systematic review. J Dent 125:104273. https:// doi: 10.1016/j.jdent.2022.104273

5. Yan IG, Zheng FM, Gao SS, Duangthip D, Lo ECM, Chu CH (2022) A Review of the Protocol of SDF Therapy for Arresting Caries. Int Dent J 72:579-588. https:// doi:10.1016/j.identj.2022.06.006

6. Benedito LCB, Pedro Ricomini-Filho A, Tabchoury CPM, Cury JA (2023) Anticaries potential of a fluoride foam. Braz Dent J 34:89-98. https:// doi:10.1590/0103-6440202305287

7. Doméjean S; Ducamp R; Léger S; Holmgren C (2015) Resin Infiltration of Non-Cavitated Caries Lesions: A Systematic Review. Medical Principles and Practice.

8. Gulec A; Goymen M. Assessment of the resin infiltration and CPP-ACP applications before orthodontic brackets bonding (2019) Dental Materials Journal.

9. Nakamura K, Hamba H, Miyayoshi Y, Ishizuka H, Muramatsu T (2021) In vitro remineralization of enamel with a solution containing casein and fluoride. Dent Mater J 40:1109-1114. https://doi:10.4012/dmj.2020-383

10. Limeback H, Enax J, Meyer F (2023) Improving Oral Health with Fluoride-Free Calcium-Phosphate-Based Biomimetic Toothpastes: An Update of the Clinical Evidence. Biomimetics (Basel) 8:331. https:// doi:10.3390/biomimetics8040331

11. Askar H, Lausch J, Dörfer Ce, Meyer-Lueckel H, Paris S (2015) Penetration of micro-filled infiltrant resins into artificial caries lesions. J Dent 43:832-8. https:// doi:10.1016/j.jdent.2015.03.002

12. Andrade RMPM, Lima TO, Menezes-Oliveira MA, Nogueira R, Lepri CP, Geraldo-Martins V (2020) Clinical evaluation of the immediate masking effect of enamel white spot lesions treated with an infiltrant resin. Int J Esthet Dent 15:306-316.

13. Al-Maliky MA, Frentzen M, Meister J (2020) Laser-assisted prevention of enamel caries: a 10-year review of the literature. Lasers Med Sci 35:13-30. https:// doi:10.1007/s10103-019-02859-5.

14. Santos DM Jr, Nogueira RD, Lepri CP, Gonçalves LS, Palma-Dibb RG, Geraldo-Martins VR (2014) In vitro assessment of the acid resistance of demineralized enamel irradiated with Er, Cr:YSGG and Nd:YAG lasers. Pediatr Dent 36:137-42.

15. Damar S, Gümrü Çelikel AD, Pınar Erdem A (2023) Comparative evaluation of fluoride-free remineralization agents with and without Er,Cr:YSGG laser on artificial enamel remineralization. Lasers Med Sci 38:172. https:// doi:10.1007/s10103-023-03837-8.

16. Taher NM, Alkhamis HA, Dowaidi SM (2012) The influence of resin infiltration system on enamel microhardness and surface roughness: An in vitro study. The Saudi Dental Journal 24:79-84.

17. Abufarwaa M, Noureldinb A, Dziakc R, Jr DC (2022) Efficacy of CPP-ACP fluoride varnish applied with and without acid etching in preventing enamel demineralization compared to light-curable fluoride varnish. Angle Orthodontist, 92.

18. Alkattan R, Lippert F, Tang Q, Eckert GJ, Ando M (2018) The influence of hardness and chemicalcomposition on enamel demineralization and subsequent remineralization. J Dent 75:34-40. https:// doi:10.1016/j.jdent.2018.05.002.

19. ten Cate JM, Duijsters PP (1982) Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 16:201-10. https:// doi:10.1159/00026059

20. Crastechini E, Borges AB, Torres C (2019) Effect of Remineralizing Gels on Microhardness, Color and Wear Susceptibility of Bleached Enamel. Oper Dent 44:76-87. https:// doi:10.2341/17-150-L.

21. Goff SL, Gilson CF, Decou E, Dick AW, Geissler KH, Dalal M, Kranz AM (2023) Barriers and Facilitators to Optimal Fluoride Varnish Application. Acad Pediatr. https:// doi:10.1016/j.acap.2023.09.018

22. Sharma N, He T, Barker ML, Biesbrock AR (2013) Plaque control evaluation of a stabilized stannous fluoride dentifrice compared to a triclosan dentifrice in a six-week trial. J Clin Dent 24:31-6.

23. Hegde S, Rao BH, Kakar RC, Kakar A (2013) A comparison of dentifrices for clinical relief from dentin hypersensitivity using the Jay Sensitivity Sensor Probe. Am J Dent 26:29B-36B.

24. Dhillon SN, Deshpande AN, Macwan C, Patel KS, Shah YS, Jain AA (2020) Comparative Evaluation of Microhardness and Enamel Solubility of Treated Surface Enamel with Resin Infiltrant, Fluoride Varnish, and Casein Phosphopeptide-amorphous Calcium Phosphate: An In Vitro Study. Int J Clin Pediatr Dent 13:S14-S25. https:// doi:10.5005/jp-journals-10005-1833.

25. Leal J, Ferreira R, Tabchoury C, Lingström P, Vale G (2020) Dose-Response Effect of Fluoride Dentifrices on De-/Remineralization of Root Dentine in situ. Caries Res 54:502-508. htpps:// doi:10.1159/000510535.

26. Warreth A (2023) Dental Caries and Its Management. International Journal of Dentistry 2023.

27. Giacaman RA, Maturana CA, Molina J, Volgenant CMC, Fernández CE (2023) Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate Added to Milk, Chewing Gum, and Candy on Dental Caries: A Systematic Review. Caries Research 57:106-118. https:// doi:10.1159/000530638

28. Daneshkazemi P, Sadeghian S, Khodaei M (2021) Shear bond strength of orthodontic brackets on intact and demineralized enamel after application of resin infiltrant, fluoride varnish and casein phosphopeptide-amorphous calcium phosphate remineralizing agents: in-vitro study. International Orthodontics 19:259-268.

29. Twetman S, Keller MK (2016) Fluoride Rinses, Gels and Foams: An Update of Controlled Clinical Trials. Caries Res 50:38-44. https:// doi:10.1159/000439180.

30. Tenuta LMA, Nóbrega DF, Mei ML (2023) Chapter 9.1: The Use of Fluorides in the Control of Coronal Caries. Monogr Oral Sci 31:129-148. https:// doi:10.1159/000530564.

31. Goldberg M. Fluoride: Double-Edged Sword Implicated in Caries Prevention and in Fluorosis (2018) J Cell Dev Biol 1:10-22.

32. Oliveira PRA, Barboza CM, Barreto LSDC, Tostes MA (2020) Effect of CPP-ACP on remineralization of artificial caries-like lesion: an in situ study. Braz Oral Res 34. https:// doi:10.1590/1807-3107.

33. Thepyou R, Chanmitkul W, Thanatvarakorn O, Hamba H, Chob-Isara W, Trairatvorakul C, Tagami J (2013) Casein phosphopeptide-amorphous calcium

phosphate and glass ionomer show distinct effects in the remineralization of proximal artificial caries lesion in situ. Dent Mater J 32:648-53. https:// doi:10.4012/dmj.2012-253.

34. Escalante-Otárola WG, Castro-Núñez GM, Leandrim TP, Alencar CM, de Albuquerque Jassé FF, Kuga MC (2021) Effects of Remineralizing Agents Based on Calcium Phosphate, Sodium Phosphate, or Sodium Fluoride on Eroded Cervical Dentin. Oper Dent 46:E296-E306. https:// doi:10.2341/20-209-L.

35. Heshmat H, Kazemi H, Hoorizad Ganjkar M, Chaboki F, Shokri M, Kharazifard MJ (2023) Effect of Two Remineralizing Agents on Dentin Microhardness of Non-Caries Lesions. J Dent (Shiraz) 24:417-421. https:// doi:10.30476/dentjods.2023.95663.1883.

36. Yazkan B, Ermis RB (2018) Effect of resin infiltration and microabrasion on the microhardness, surface roughness and morphology of incipient carious lesions. Acta Odontol Scand 76:473-481. https:// doi:10.1080/00016357.2018.1437217.

37. Chindane AA, Patil AT, Sandhyarani B (2022) Effect of CPP-ACPF, resin infiltration, and colloidal silica infiltration on surface microhardness of artificial white spot lesions in primary teeth: An in vitro study. Dent Res J (Isfahan) 19:52.

38. Alrahlah A, Khan R, Al-Odayni AB, Saeed WS, Bautista LS, Alnofaiy IA, de Vera MAT (2023) Advancing Dimethacrylate Dental Composites by Synergy of Pre-Polymerized TEGDMA Co-Filler: A Physio-Mechanical Evaluation. Biomimetics (Basel) 8:577. https:// doi:10.3390/biomimetics8080577.

39. Nedeljkovic I, Abdelaziz M, Feilzer AJ, Szafert S, Gulia N, Dawaa M, Krejci I, Kleverlaan CJ (2022) Novel hybrid-glass-based material for infiltration of early caries lesions. Dent Mater 38:1015-1023. https:// doi:10.1016/j.dental.2022.04.008.

40. Almaz ME, Ulusoy NB, Oba AA, Dogan ÜM (2020) Thermal, morphological, and spectral changes after Er, Cr:YSGG laser irradiation at low fluences on primary teeth for caries prevention. Microscopy Research&Techquine 1-10.

41. Geraldo-Martins VR, Lepri CP, Palma-Dibb RG (2013) Influence of Er,Cr:YSGG laser irradiation on enamel caries prevention. Lasers Med Sci 28:33-9. https:// doi:10.1007/s10103-012-1056-9.

42. Ulusoy NB, Akbay Oba A, Cehreli ZC (2020) Effect of Er,Cr:YSGG Laser on the Prevention of Primary and Permanent Teeth Enamel Demineralization: SEM and EDS Evaluation. Photobiomodul Photomed Laser Surg 38:308-315. https:// doi:10.1089/photob.2019.4772

43. Olivi G, Angiero F, Benedicenti S, Iaria G, Signore A, Kaitsas V (2010) Use of the erbium, chromium:yttrium-scandium-gallium-garnet laser on human enamel tissues. Influence of the air-water spray on the laser-tissue interaction: scanning electron microscope evaluations. Lasers Med Sci 25:793-7. https:// doi:10.1007/s10103-009-0689-9.**7**.

## 14. CONCLUSÃO

Considerando as limitações deste estudo *in vitro*, conclui-se que todos os métodos aqui testados foram capazes de recuperar a dureza do esmalte dentário, mas o verniz fluoretado e a escovação com dentifrício CPP-ACP parecem não ter a mesma eficácia na proteção do WSL em um novo desafio cariogênico do que as outras técnicas. Entretanto, o gel fluoretado, o laser Er,Cr :YSGG e o infiltrante resinoso foram capazes de manter a dureza do esmalte dentário em níveis semelhantes à sua linha de base.

Embora os métodos testados não tenham evitado totalmente a desmineralização das lesões de manchas brancas no esmalte dentário, o desempenho deles foi superior a 85%.

**REFERÊNCIAS**

ABBASI, M.; NAKHOSTIN, A.; NAMDAR, F.; CHINIFORUSH, N.; HASANI TABATABAEI, M. The Rate of Demineralization in the Teeth Prepared by Bur and Er:YAG Laser. **Journal of Lasers in Medical Sciences**, v.9, n.2, p.82-86, 2018.

ABDULLAH, Z.; JOHN, J. Minimally Invasive Treatment of White Spot Lesions--A Systematic Review. **Oral Health & Preventive Dentistry**, v.14, n.3, p.197-205, 2016.

ABUFARWAA, M.; NOURELDINB, A.; DZIAKC, R.; JR, D.C. Efficacy of CPP-ACP fluoride varnish applied with and without acid etching in preventing enamel demineralization compared to light-curable fluoride varnish. **Angle Orthodontist**, v.92, n.2, 2022.

ALKATTAN, R.; LIPPERT, F.; TANG, Q.; ECKERT, G.J.; ANDO, M. The influence of hardness and chemicalcomposition on enamel demineralization and subsequent remineralization. **Journal of Dentistry**, v.75, p.34-40, 2018.

AL-MALIKY, M.A.; FRENTZEN, M.; MEISTER, J. Laser-assisted prevention of enamel caries: a 10-year review of the literature. **Lasers in Medical Science**, v.35, n.1, p.13-30, 2020.

ALMAZ, M.E.; ULUSOY, N.B.; OBA, A.A.; DOGAN, Ü.M.; Thermal, morphological, and spectral changes after Er, Cr:YSGG laser irradiation at low fluences on primary teeth for caries prevention. **Microscopy Research&Techquine**, p.1-10, 2020.

ALRAHLAH, A.; KHAN, R.; AL-ODAYNI, A.B.; SAEED, W.S.; BAUTISTA, L.S.; ALNOFAIY, I.A.; VERA, M.A.T. Advancing Dimethacrylate Dental Composites by Synergy of Pre-Polymerized TEGDMA Co-Filler: A Physio-Mechanical Evaluation. **Biomimetics (Basel, Switzerland)**, v.8, n.8, 2023.

ANDRADE, R.M.P.M.; LIMA, T.O.; MENEZES-OLIVEIRA, M.A.; NOGUEIRA, R.; LEPRI, C.P.; GERALDO-MARTINS, V. Clinical evaluation of the immediate masking effect of enamel white spot lesions treated with an infiltrant resin. **The International Journal of Esthetic Dentistry**, v.15, n.3, p.306-316, 2020.

ARSLAN, S.; ZORBA, Y.O.; ATALAY, M.A. ÖZCAN, S.; DEMIRBUGA, S.; PALA, K.; PERCIN, D.; OZER, F. Effect of resin infiltration on enamel surface properties and

Streptococcus mutans adhesion to artificial enamel lesions. **Dental Materials Journal**, v.34, n.1, p.25-30, 2015.

ASKAR, H.; LAUSCH, J.; DÖRFER, C.E.; MEYER-LUECKEL, H.; PARIS, S. Penetration of micro-filled infiltrant resins into artificial caries lesions. **Journal of Dentistry** v.43, n.7, p.832-8, 2015.

AYAD, A.H.; ABDELHAFEZ, M.I.; ALGHANDOUR, R.N.; MUSTAFA, D.S.; NOUR, K.A. Effect of different surface treatments on the microhardness and colour change of artificial enamel lesions. **Australian Dental Journal**, v.67, n.3, p.230-238, 2022.

BAKRY, A.S.; ABBASSY, M.A. Increasing the efficiency of CPP-ACP to remineralize enamel white spot lesions. **Journal of Dentistry**, v.76, p.52-57, 2018.

BEHROUZI, P.; HESHMAT, H.; HOORIZAD, G.M.; TABATABAEI, S.F.; KHARAZIFARD, M.J. Effect of Two Methods of Remineralization and Resin Infiltration on Surface Hardness of Artificially Induced Enamel Lesions. **Journal of Dentistry (Shiraz, Iran)**, v.21, n.1, p.12-17, 2020.

BENEDITO, L.C.B.; PEDRO RICOMINI-FILHO, A.; TABCHOURY, C.P.M.; CURY, J.A. Anticaries potential of a fluoride foam. **Brazilian Dental Journal**, v.34, n.1, p.89-98, 2023.

BUZALAF, M.A.R; PESSAN, J.P; HONÓRIO, H.M; TEN CATE, J.M. Mechanisms of action of fluoride for caries control. **Monographs in Oral Science**, v.22, p.97-114, 2011.

CERQUEIRA, G.A.; DAMASCENO, J.E.; PEDREIRA, P.R.; SOUZA, A.F.; AGUIAR, F.H.B.; MARCHI, G.M. Roughness and Microhardness of Demineralized Enamel Treated with Resinous Infiltrants and Subjected to an Acid Challenge: An in vitro Study. **The Open Dentistry Journal**, v.17, 2023.

CHAN, A.K.Y.; TAMRAKAR, M.; JIANG, C.M.; TSANG, Y.C.; LEUNG, K.C.M.; CHU, C.H. Clinical evidence for professionally applied fluoride therapy to prevent and arrest dental caries in older adults: A systematic review. **Journal of Dentistry**, v.125, 2022.

CHENG, L.; ZHANG, L.; YUE, L.; LING, J.; FAN, M.; YANG, D.; HUANG, Z.; NIU, Y.; LIU, J.; ZHAO, J.; LI, Y.; GUO, B.; CHEN, Z.; ZHOU, X. Expert consensus on dental caries management. **International Journal of Oral Science**, v.14, n.1, 2022.

CLARK, M.B.; KEELS, M.A.; SLAYTON, R.L. Section On Oral Health. Fluoride Use in Caries Prevention in the Primary Care Setting. **Pediatrics**, v.146, n.6, 2020.

CRASTECHINI, E.; BORGES, A.B.; TORRES, C. Effect of Remineralizing Gels on Microhardness, Color and Wear Susceptibility of Bleached Enamel. **Operative Dentistry**, v.44, n.1, p.76-87, 2019.

DAMAR, S.; GÜMRÜ ÇELIKEL, A.D.; ERDEM, A.P. Comparative evaluation of fluoride-free remineralization agents with and without Er,Cr:YSGG laser on artificial enamel remineralization. **Lasers in Medical Science**, v. 38, n.1, 2023.

DANESHKAZEMI, P.; SADEGHIAN, S.; KHODAEI, M. Shear bond strength of orthodontic brackets on intact and demineralized enamel after application of resin infiltrant, fluoride varnish and casein phosphopeptide-amorphous calcium phosphate remineralizing agents: in-vitro study. **International Orthodontics**, v.19, n.2, p.259-268, 2021.

DHILLON, S.N.; DESHPANDE, A.N.; MACWAN, C.; PATEL, K.S.; SHAH, Y.S.; JAIN, A.A. Comparative Evaluation of Microhardness and Enamel Solubility of Treated Surface Enamel with Resin Infiltrant, Fluoride Varnish, and Casein Phosphopeptide-amorphous Calcium Phosphate: An *In Vitro* Study. **International Journal of Clinical Pediatric Dentistry,** v.13, p.S14-S25, 2020.

DÍAZ-MONROY, J.M.; CONTRERAS-BULNES, R.; OLEA-MEJÍA, O.F.; GARCÍA-FABILA, M.M.; RODRÍGUEZ-VILCHIS, L.E.; SÁNCHEZ-FLORES, I.; CENTENO-PEDRAZA, C. Chemical changes associated with increased acid resistance of Er:YAG laser irradiated enamel. **ScientificWorldJournal**, 2014.

DOMÉJEAN, S.; DUCAMP, R.; LÉGER, S.; HOLMGREN, C. Resin Infiltration of Non-Cavitated Caries Lesions: A Systematic Review. **Medical Principles and Practice**, 2015.

EBRAHIMI, M.; MEHRABKHANI, M.; AHRARI, F.; PARISAY, I.; JAHANTIGH, M. The effects of three remineralizing agents on regression of white spot lesions in children: A two-week, single-blind, randomized clinical trial. **Journal of Clinical And Experimental Dentistry**, v.9, n.5, p.641-648, 2017.

ESCALANTE-OTÁROLA, W.G.; CASTRO-NÚÑEZ, G.M.; LEANDRIM, T.P.; ALENCAR, C.M.; JASSÉ, F.F.A.; KUGA, M.C. Effects of Remineralizing Agents Based on Calcium Phosphate, Sodium Phosphate, or Sodium Fluoride on Eroded Cervical Dentin. **Operative Dentistry** v.46, n.6, p.E296-E306, 2021.

FARIAS, J.O.; CUNHA, M.C.A.; MARTINS, V.L.; MATHIAS, P. Microinvasive esthetic approach for deep enamel white spot lesion. **Dental Research Journal**, v.19, 2022.

FRENCKEN, J.E.; GIACAMAN, R.A.; LEAL, S.C. An assessment of three contemporary dental caries epidemiological instruments: a critical review. **British Dental Journal**, 2020 v.228, n.1, p.25-31.

GERALDO-MARTINS, V.R.; LEPRI, C.P.; PALMA-DIBB, R.G. Influence of Er,Cr:YSGG laser irradiation on enamel caries prevention. **Lasers in Medical Science**, v.28, n.1, p. 33-9, 2013.

GIACAMAN, R.A.; MATURANA, C.A.; MOLINA, J.; VOLGENANT, C.M.C.; FERNÁNDEZ, C.E. Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate Added to Milk, Chewing Gum, and Candy on Dental Caries: A Systematic Review. **Caries Research**, v.57, n.2, p.106-118, 2023.

GOFF, S.L.; GILSON, C.F.; DECOU, E.; DICK, A.W.; GEISSLER, K.H.; DALAL, M.; KRANZ, A.M. Barriers and Facilitators to Optimal Fluoride Varnish Application. **Academic Pediatrics**, 2023.

GOLDBERG, M. Fluoride: Double-Edged Sword Implicated in Caries Prevention and in Fluorosis. **Journal of Cell and Devolopmental Biology**, v.1, p.10-22, 2018.

GROHE, B.; MITTLER, S. Advanced non-fluoride approaches to dental enamel remineralization: The next level in enamel repair management. **Biomaterials and Biosystems**, v.4, 2021.

GULEC, A.; GOYMEN, M. Assessment of the resin infiltration and CPP-ACP applications before orthodontic brackets bonding. **Dental Materials Journal**, 2019.

GURDOGAN, E.B.; OZDEMIR-OZENEN, D.; SANDALLI, N. Evaluation of Surface Roughness Characteristics Using Atomic Force Microscopy and Inspection of Microhardness Following Resin Infiltration with Icon®. **Journal of Esthetic and Restorative Dentistry**, v.29, n.3, p.201-208, 2017.

HEGDE, S.; RAO, B.H.; KAKAR, R.C.; KAKAR, A. A comparison of dentifrices for clinical relief from dentin hypersensitivity using the Jay Sensitivity Sensor Probe. **American Journal of Dentistry**, v.26, p.29B-36B, 2013.

HESHMAT, H.; KAZEMI, H.; GANJKAR, M.H.; CHABOKI, F.; SHOKRI, M.; KHARAZIFARD, M.J. Effect of Two Remineralizing Agents on Dentin Microhardness of Non-Caries Lesions. **Journal of Dentistry (Shiraz, Iran)**, v24, n.4, p. 417-421, 2023.

LEAL, J.; FERREIRA, R.; TABCHOURY, C.; LINGSTRÖM, P.; VALE, G. Dose-Response Effect of Fluoride Dentifrices on De-/Remineralization of Root Dentine in situ. **Caries Research**, v.54, n.5-6, p.502-508, 2020.

LEPRI, C.P.; DE CASTRO, D.T.; GERALDO-MARTINS, V.R.; FARAONI, J.J.; PALMA-DIBB, R.G. Laser irradiation prevents root caries: Microhardness and scanning electron microscopy analysis. **Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research,** v.33, n.2, p.198-202, 2022.

LIMEBACK, H.; ENAX, J.; MEYER, F. Improving Oral Health with Fluoride-Free Calcium-Phosphate-Based Biomimetic Toothpastes: An Update of the Clinical Evidence. **Biomimetics**, v.8, n.4, 2023.

LUK, K.; ZHAO, I.S.; YU, O.Y.; ZHANG, J.; GUTKNECHT, N.; CHU, C.H. Effects of 10,600 nm Carbon Dioxide Laser on Remineralizing Caries: A Literature Review. **Photobiomodulation, Photomedicine, and Laser Surgery**, v.38, n.2, p.59-65, 2020.

MUNTEANU, A.; HOLBAN, A.M.; PĂUNA, M.R.; IMRE, M.; FARCAȘIU, A.T.;, FARCAȘIU, C. Review of Professionally Applied Fluorides for Preventing Dental Caries in Children and Adolescents. **Applied Sciences**, v.12, n.3, 2022.

NAKAMURA, K.; HAMBA, H.; MIYAYOSHI, Y.; ISHIZUKA, H.; MURAMATSU, T. In vitro remineralization of enamel with a solution containing casein and fluoride. **Dental Materials Jornal,** v.40, n.5, p.1109-1114, 2021.

NEDELJKOVIC, I.; ABDELAZIZ, M.; FEILZER, A.J.; SZAFERT, S.; GULIA, N.; DAWAA, M.; KREJCI, I.; KLEVERLAAN, C.J. Novel hybrid-glass-based material for infiltration of early caries lesions. **Dental Materials: Official Publication of The Academy of Dental Materials**, v.38, n.6, p.1015-1023, 2022.

NÓBREGA, M.T.C.; DANTAS, E.L.A.; ALONSO, R.C.B.; ALMEIDA, L.F.D.; PUPPIN-RONTANI, R.M.; SOUSA, F.B. Hydrolytic degradation of different infiltrant compositions within different histological zones of enamel caries like-lesions. **Dental Materials Journal**, v.39, n.3, p.449-455, 2020.

OGURO, R.; NAKAJIMA, M.; SEKI, N.; SADR, A.; TAGAMI, J.; SUMI, Y. The role of enamel thickness and refractive index on human tooth colour. **Journal of Dentistry**, v. 51, p.36-44, 2016.

OLIVEIRA, P.R.A.; BARBOZA, C.M.; BARRETO, L.S.D.C.; TOSTES, M.A. Effect of CPP-ACP on remineralization of artificial caries-like lesion: an in situ study. **Brazilian Oral Research**, v.34, 2020.

OLIVI, G.; ANGIERO, F.; BENEDICENTI, S.; IARIA, G.; SIGNORE, A.; KAITSAS, V. Use of the erbium, chromium:yttrium-scandium-gallium-garnet laser on human enamel tissues. Influence of the air-water spray on the laser-tissue interaction: scanning electron microscope evaluations. **Lasers in Medical Science**, v.25, n.6, p.793-7, 2010.

O'MULLANE, D.M.; BAEZ, R.J.; JONES, S.; LENNON, M.A.; PETERSEN, P.E.; RUGG-GUNN, A.J.; WHELTON, H.; WHITFORD, G.M. Fluoride and Oral Health. **Community Dental Health**, v.33, n.2, p.69-99, 2016.

PITTS, N.B.; ZERO, D.T.; MARSH, P.D.; EKSTRAND, K.; WEINTRAUB, J.A.; RAMOS-GOMEZ, F.; TAGAMI, J.; TWETMAN, S.; TSAKOS, G.; ISMAIL, A. Dental caries. **Nature Reviews. Disease Primers**, v.3, 2017

SANTOS, D.M.J.R.; NOGUEIRA, R.D.; LEPRI, C.P.; GONÇALVES, L.S.; PALMA-DIBB, R.G.; GERALDO-MARTINS, V.R. In vitro assessment of the acid resistance of demineralized enamel irradiated with Er, Cr:YSGG and Nd:YAG lasers. **Pediatric Dentistry**, v.36, n.7, p.137-42, 2014.

SEOW, W.K. Early Childhood Caries. **Pediatric Clinics of North America**, v.65, n.5, p.941-954, 2018.

SHARMA, N.; HE, T.; BARKER, M.L.; Biesbrock AR. Plaque control evaluation of a stabilized stannous fluoride dentifrice compared to a triclosan dentifrice in a six-week trial. **The Journal of Clinical Dentistry**, v.24, n.1, p.31-6, 2013.

SCHIFFNER, U. Verwendung von Fluoriden zur Kariesprävention [Use of fluorides for caries prevention]. **Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz**, v.64, n.7, p. 830-837, 2021.

SOARES, L.E.S.; NAHÓRNY, S.; DE FARIA BRAGA, V.; MARCIANO, F.R.; BHATTACHARJEE, T.T.; LOBO, A.O. Raman spectroscopy-multivariate analysis related to morphological surface features on nanomaterials applied for dentin coverage. **Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy**, v.228, 2020.

SONGPAISAN, Y.; TWETMAN, S. Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective. Int **International Journal of Pediatric Dentistry**, v.29, n.3, p.238-248, 2019

TAHER, N.M.; ALKHAMIS, H.A.; DOWAIDI, S.M. The influence of resin infiltration system on enamel microhardness and surface roughness: An in vitro study. **The Saudi Dental Journal**, v.24, p.79-84, 2012

TAO, S.; ZHU, Y.; YUAN, H.; TAO, S.; CHENG, Y.; LI, J.; HE, L. Efficacy of fluorides and CPP-ACP vs fluorides monotherapy on early caries lesions: A systematic review and meta-analysis. **PloS One**, v.13, n.4, 2018.

TEN CATE, J.M.; DUIJSTERS, P.P. Alternating demineralization and remineralization of artificial enamel lesions. **Caries Research**, v.16, n.3, p.201-10, 1982.

TENUTA, L.M.A.; NÓBREGA, D.F.; MEI, M.L. Chapter 9.1: The Use of Fluorides in the Control of Coronal Caries. **Monographs in Oral Science**, v.31, p.129-148, 2023.

THEPYOU, R.; CHANMITKUL, W.; THANATVARAKORN, O.; HAMBA, H.; CHOB-ISARA, W.; TRAIRATVORAKUL, C.; TAGAMI, J. Casein phosphopeptide-amorphous calcium phosphate and glass ionomer show distinct effects in the remineralization of proximal artificial caries lesion in situ. **Dental Materials Journal**, v.32, n.4, p.648-53, 2013.

TINANOFF, N.; BAEZ, R.J.; DIAZ GUILLORY, C.; DONLY, K.J.; FELDENS, C.A.; MCGRATH, C.; PHANTUMVANIT, P.; PITTS, N.B.; SEOW, W.K.; SHARKOV, N.; SONGPAISAN, Y.; TWETMAN, S. Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective. **International Journal of Paediatric Dentistry**, v.29, n.3, p.238–248, 2019.

TWETMAN, S.; KELLER, M.K. Fluoride Rinses, Gels and Foams: An Update of Controlled Clinical Trials. **Caries Research**, v.50, p.38-44, 2016.

ULUSOY, N.B.; AKBAY, O.B.A.A.; CEHRELI, Z.C. Effect of Er,Cr:YSGG Laser on the Prevention of Primary and Permanent Teeth Enamel Demineralization: SEM and EDS Evaluation. **Photobiomodulation, photomedicine, and Laser Surgery**, v.38, n.5, p.308-315, 2020.

WARRETH, A. Dental Caries and Its Management. **International Journal of Dentistry**, v.2023, 2023.

YAN, I.G.; ZHENG, F.M.; GAO, S.S.; DUANGTHIP, D.; LO, E.C.M.; CHU, C.H. A Review of the Protocol of SDF Therapy for Arresting Caries. **International Dental Journal**, v.72, n.5 p.579-588, 2022.

YAZKAN, B.; ERMIS, R.B. Effect of resin infiltration and microabrasion on the microhardness, surface roughness and morphology of incipient carious lesions. **Acta Odontologica Scandinavica**, v.76, n.7, p. 473-481, 2018.

ZAKIZADE, M.; DAVOUDI, A.; AKHAVAN, A.; SHIRBAN, F. Effect of Resin Infiltration Technique on Improving Surface Hardness of Enamel Lesions: A Systematic Review and Meta-analysis. **The Journal of Evidence-Based Dental Practice**, v.20, n.2, 2020.

# APÊNDICES

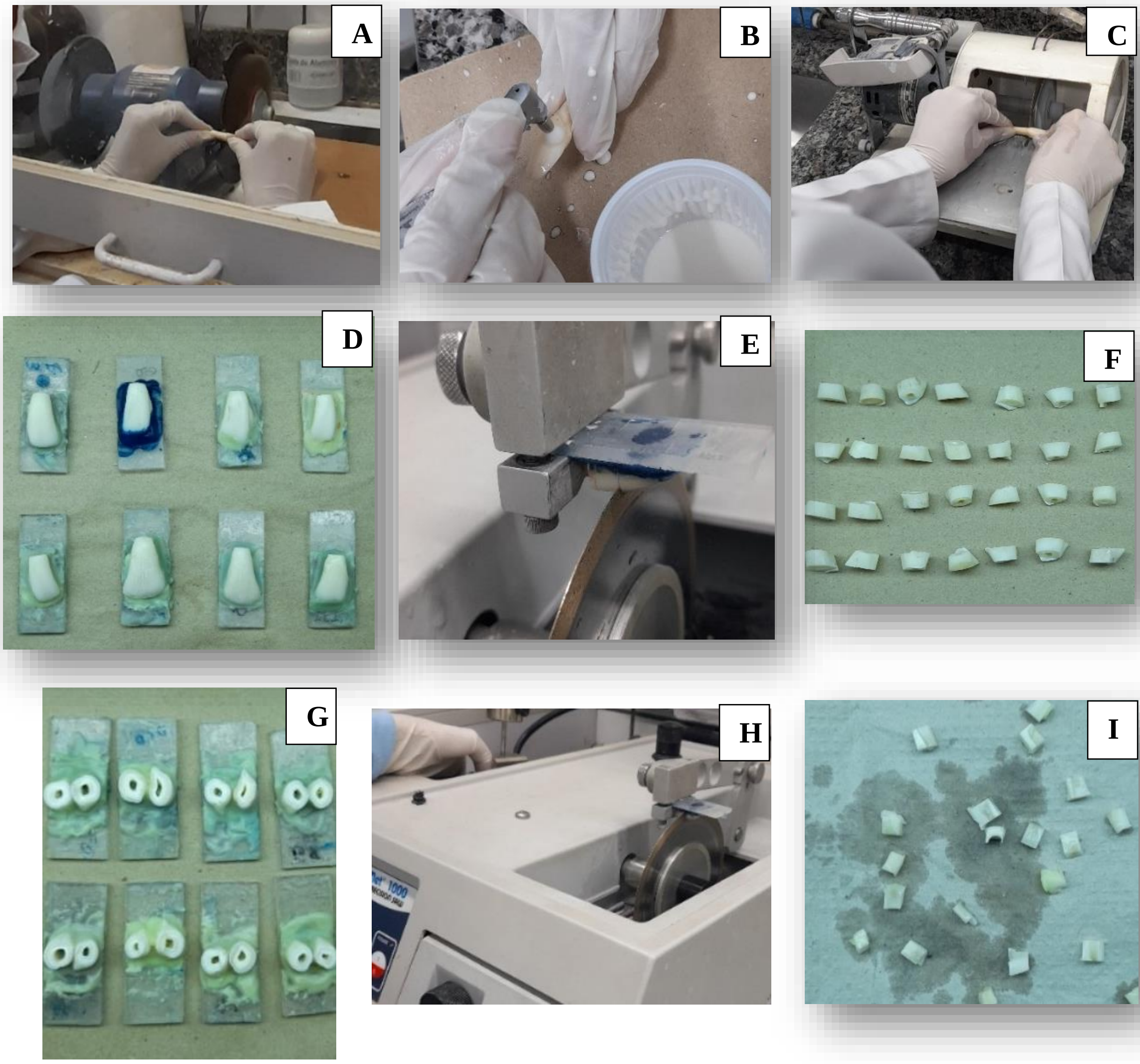



**Figura 1.** Preparo das amostras. **A-** Remoção dos restos de ligamento periodontal e osso alveolar dos dentes bovinos com a máquina Moto Esmeril. **B-** Profilaxia da cora dental com pasta de pedra pomes e água utilizando a escova Robson em baixa rotação. **C-** Corte na junção amelocementária para separação de raiz e coroa. **D-** Fixação das coroas em lâminas com cera pegajosa. **E-** Corte da coroa dental utilizando a máquina de corte ISOMET 1000 com disco diamantado. **F-** Resultado parcial dos cortes. **G-** Nova fixação em lâmina com cera pegajosa. **H-** Novos cortes. **I-** Confecção dos espécimes logo após os cortes.

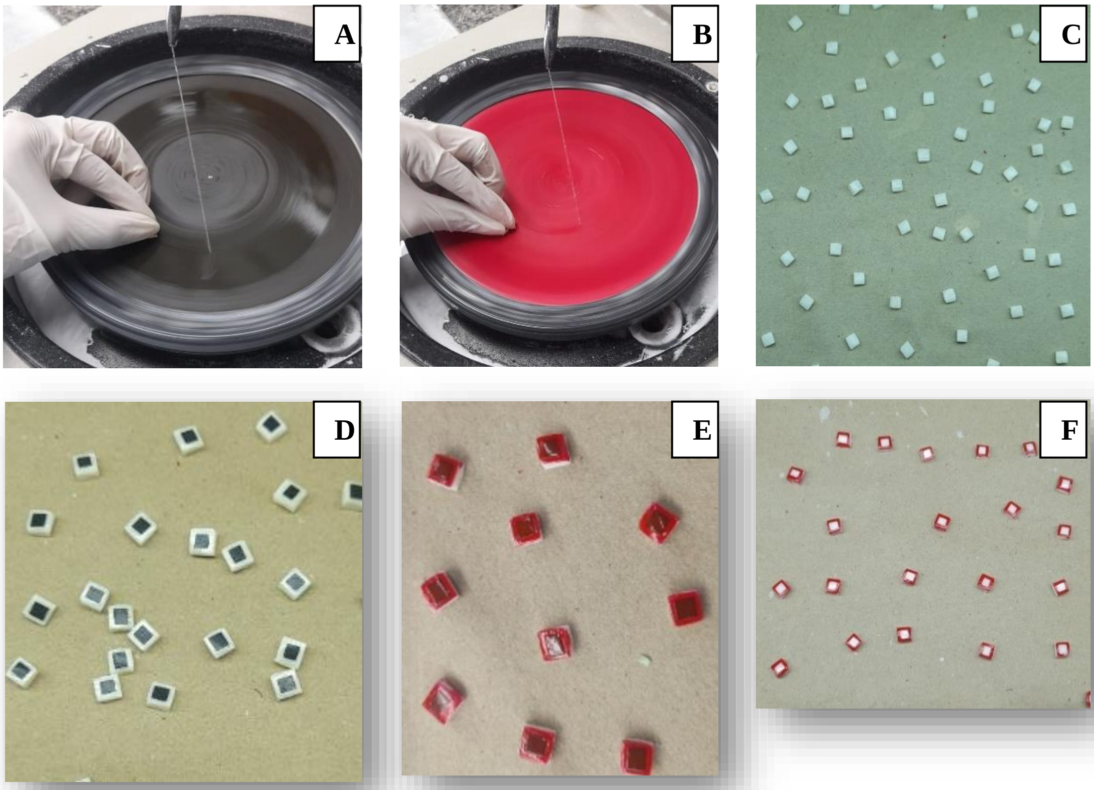


**Figura 2.** Padronização dos espécimes. **A-B-** Polimento das superfícies amostrais. **C-** Amostras padronizadas nas dimensões preconizadas (5x5x3). **D-** Delimitação da área que foi envolvida na pesquisa (16mm$^2$). **E-** Espécimes pintados com esmalte de unha vermelho para a delimitação. **F-** Corpos de prova finalizados com a delimitação.

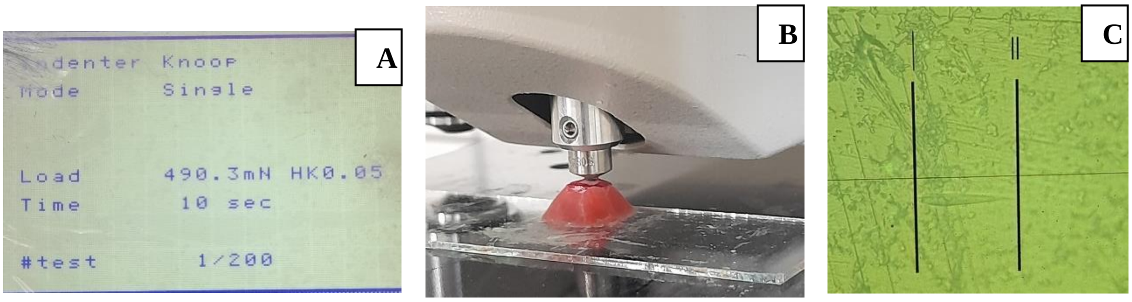



**Figura 3.** Avaliação da microdureza superficial do esmalte. **A-** Dureza Knoop estabelecida no microdurômetro com força de 50g e duração de 10 segundos. **B-** Indentador incidindo sobre a superfície do espécime. **C-** Imagem geométrica de um losango formada após a indentação.


**Figura 4.** Ciclagem de pH. **A-B-** Componentes químicos para produção das soluções desmineralizante e remineralizante. **C-** Amostras nas soluções para simulação da lesão de mancha branca.

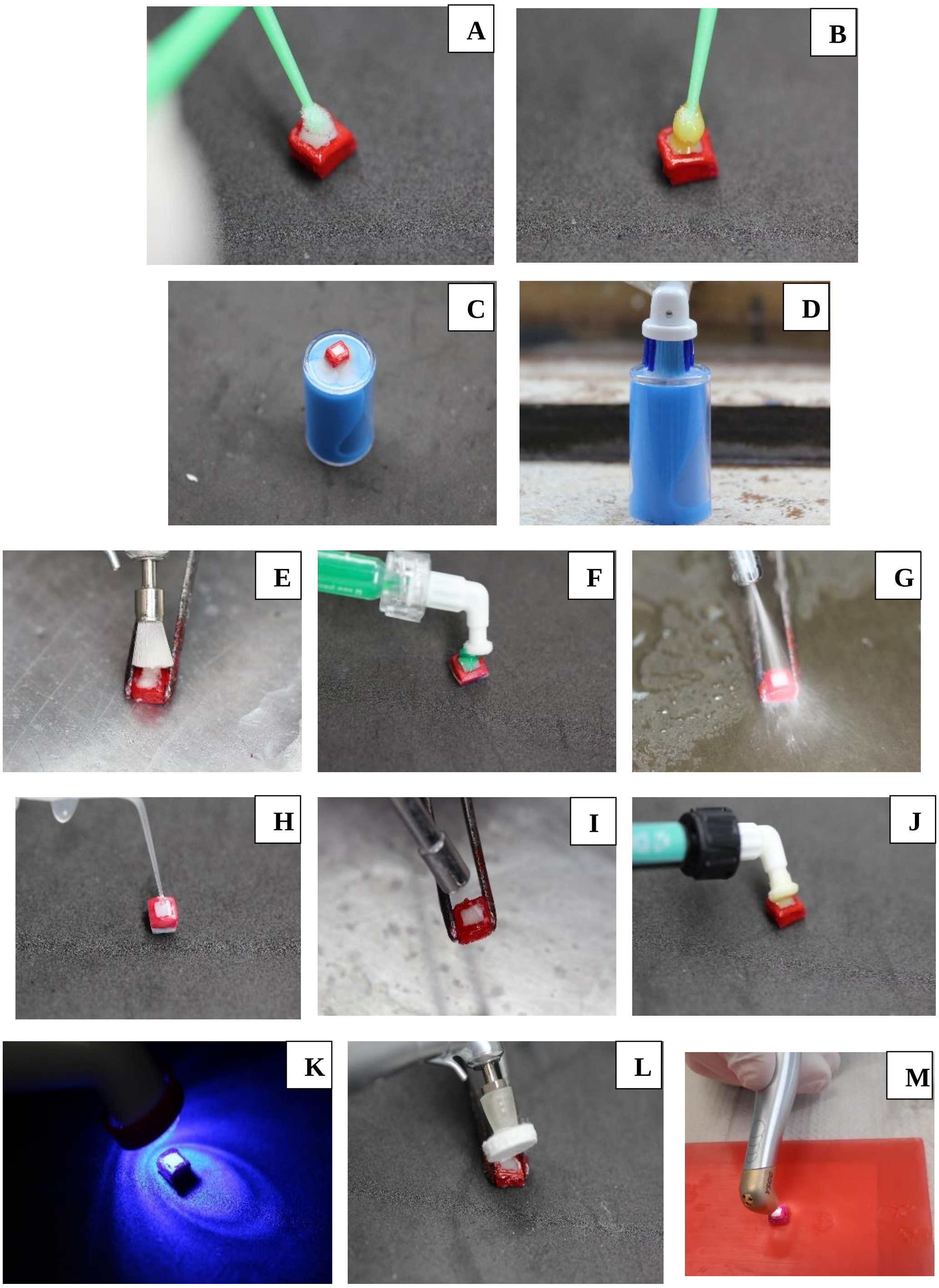



**Figura 5.** Tratamentos. **A-** Aplicação do Flúor Gel Neutro 2% - Flugel. **B-** Aplicação do verniz fluoretado 5% Duraphat. **C-D-** Escovação utilizando o creme dental contendo o CPP-ACP. **E-L-** Aplicação do infiltrante resinoso. **M-** Aplicação do Laser Er,CR:YSGG

# ANEXOS

*Carta de aprovação do Comitê de  Ética em Experimentação Animal*

Uniube Comitê de Ética em Experimentação Animal

Ofício CEEA–001/2023 Uberaba, 10 de abril de 2023.

**CERTIFICADO**

Certificamos que o protocolo n° 002/2023 relativos ao projeto intitulado **"Avaliação da Eficácia dos Tratamentos Preventivos no Controle da Cárie Dental"** que tem como responsável o **Prof. Vinicius Rangel Geraldo Martins,** está de acordo com os Princípios Éticos da Experimentação Animal, adotados pelo Comitê de Ética em Experimentação Animal (CEEA/UNIUBE) regido pela lei n° 11.794/08.

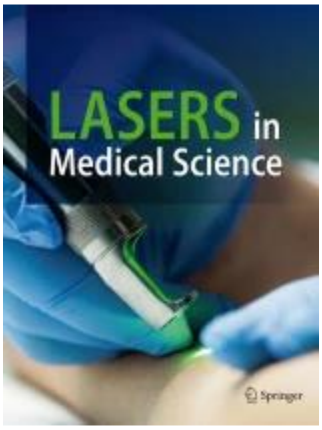
**CERTIFICATE**

We hereby certify that the protocol n° 002/2023 related to the project entitled **"Evaluation of the Effectiveness of Preventive Treatments in the Control of Dental Caries"** under the supervision of **Prof. Vinicius Rangel Geraldo Martins,** is in agreement with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation (CEEA/UNIUBE) according to the law n° 11.794/08.

Atenciosamente,

**Profa. Joely Ferreira Figueiredo Bittar**
Coordenadora do CEEA-UNIUBE

***Anexo 2: Normas para publicação no periódico Lasers in Medical Science***

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- If available, the 16-digit ORCID of the author(s)

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**Abstract**

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

**Keywords**

Please provide 4 to 6 keywords which can be used for indexing purposes.

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The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

**Competing Interests:**

Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to

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