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TAMIRES PASSADORI MARTINS

EFEITO DE GÉIS FLUORETADOS SUPLEMENTADOS COM TRIMETAFOSFATO DE SÓDIO NANOPARTICULADO SOBRE A REMINERALIZAÇÃO DO ESMALTE DENTAL *IN SITU*

Araçatuba

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EFEITO DE GÉIS FLUORETADOS SUPLEMENTADOS COM TRIMETAFOSFATO DE SÓDIO NANOPARTICULADO SOBRE A REMINERALIZAÇÃO DO ESMALTE DENTAL *IN SITU*

Effect of fluoride gels supplemented with nanosized sodium trimetaphosphate on enamel remineralization in situ

Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para a obtenção do título de Mestre em Ciência Odontológica – Área: Saúde Bucal da Criança.

Orientador: Prof. Assoc. Dr. Juliano Pelim Pessan

Coorientador: Prof. Tit. Alberto Carlos Botazzo Delbem

Coorientadora: Dra. Liliana Carolina Báez-Quintero

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Dedicatória

Tamires Passadori Martins

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Por serem meus maiores exemplos de força, fé e amor. Por todo apoio e compreensão durante esta fase e, principalmente, por não medirem esforços para que meus sonhos se realizem. Obrigada por me ensinarem que com humildade, respeito e honestidade podemos ir além e conquistar cada um de nossos objetivos. Não existem palavras que expressem todo meu amor, respeito e gratidão! Sem vocês, nada seria possível! A conquista é nossa! Eu os amo infinitamente!

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Epígrafe

Tamires Passadori Martins

"Os sonhos não determinam o lugar em que você vai estar, mas produzem a força necessária para tirá-lo do lugar em que está".

(Augusto Cury)

Resumo

Tamires Passadori Martins

Martins, TP. **Efeito de géis fluoretados suplementados com trimetafosfato de sódio nanoparticulado sobre a remineralização do esmalte dental *in situ*.** 2022. Dissertação (Mestrado em Ciência Odontológica, área de Saúde Bucal da Criança) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2022.

RESUMO

O objetivo do presente estudo foi avaliar o efeito de géis fluoretados suplementados com nanopartículas de Trimetafosfato de Sódio (TMP) sobre a remineralização de lesões de cárie artificiais *in situ*. Blocos de esmalte dental bovino (*n*=160) foram aleatoriamente divididos entre os grupos de estudo após análise de dureza de superfície (DS) e indução de lesões de subsuperfície. Os géis testados foram: Placebo (sem flúor ou TMP – controle negativo), 9000 µg F/g (9000F – controle positivo), 4500 µg F/g + 5% TMP microparticulado (4500 5%TMPmicro) e 4500 µg F/g + 5% TMP nanoparticulado (4500 5%TMPnano). Dez voluntários utilizaram dispositivos palatinos contendo 4 blocos de esmalte durante 3 dias, após uma única aplicação dos géis, seguindo um protocolo duplo-cego e cruzado. Dois blocos de esmalte foram removidos imediatamente após a aplicação dos géis, para determinar a concentração de fluoreto de cálcio (CaF_2) formado. Após cada fase, determinou-se a porcentagem de recuperação de dureza de superfície (%RDS) e CaF_2 retido no esmalte. Os dados foram submetidos ANOVA de medidas repetidas e teste de Student-Newman-Keuls ($p<0.05$). A maior %RDS foi observada para o gel 4500 5%TMPnano, seguido por 4500 5%TMPmicro, 9000F e Placebo, com diferenças significativas entre os grupos. Em relação ao CaF_2 formado, a maior concentração foi observada para o grupo 9000F. Não foram observadas diferenças significativas entre os grupos 9000F, 4500 5%TMPmicro e 4500 5%TMPnano para concentrações de CaF_2 retido. Conclui-se que a adição de TMP a géis fluoretados melhorou significativamente a remineralização de lesões de cárie *in situ.* O uso de TMP em escala nanométrica potencializou ainda mais este efeito.

Palavras-chave: Fluoretos, Polifosfatos, Cárie Dentária, Nanopartículas.

Abstract

Tamires Passadori Martins

Martins, TP. **Effect of fluoride gels supplemented with nanosized sodium trimetaphosphate on enamel remineralization *in situ***. 2022. Dissertação (Mestrado em Ciência Odontológica, área de Saúde Bucal da Criança) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2022**.**

ABSTRACT

The present study aimed to evaluate the effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate (TMP) on the remineralization of artificial caries lesions *in situ*. Bovine enamel blocks (*n*=160) were randomly distributed among study groups after surface microhardness (SH) analysis and induction of subsurface lesions. Test groups included: Placebo (without F and TMP – negative control), 9000 µg F/g (9000F – positive control), 4500 µg F/g + 5% micrometric TMP (4500 5%+ TMPmicro) and 4500 µg F/g + 5% nano-sized TMP (4500 + 5%TMPnano). Ten volunteers used palatal devices containing 4 enamel blocks during 3 days, after a single application of gels, following a double-blind and crossover protocol. Two enamel blocks were removed immediately after topical application of F to determine calcium fluoride (CaF_2) formed on enamel. After each phase, the samples were analyzed by percentage of surface hardness recovery ($\%SH_R$) and CaF_2 retained on enamel. Data were analyzed by repeated-measures ANOVA and Student-Newman-Keuls test ($p<0.05$). The highest $\%SH_R$ was observed for 4500 5%TMPnano gel, following by 4500 5%TPMmicro, 9000F, and Placebo, with significant differences among all groups. Regarding CaF_2 formed, the highest concentration was observed in the 9000F group. No significant differences were observed among 9000F, 4500 5%TMPmicro and 4500 5%TMPnano groups for concentrations of CaF_2 retained. It was concluded that the addition of TMP to gels improved the remineralization of caries lesions *in situ*. The use of nano-sized TMP further enhanced this effect.

Key-words: Fluorides, Polyphosphates, Dental Caries, Nanoparticles.

Lista de abreviaturas e símbolos

Tamires Passadori Martins

LISTA DE ABREVIATURAS E SÍMBOLOS

ANOVA	Analysis of Variance/Análise de Variância
CaF_2	Calcium Fluoride/Fluoreto de Cálcio
°C	Degrees Celsius/Graus Celsius
DS	Dureza de superfície
F	Fluoride/Fluoreto
h	Hour/Hora
HCl	Hydrochloric Acid/Ácido clorídrico
KOH	Potassium Hydroxide/Hidróxido de potássio
L	Liter/Litro
Log_{10}	Logarithm, base 10/Logaritmo na base 10
mL	Milliliter/Mililitro
M	Molar
mm	Millimeter/Milímetro
mg	Milligram/Miligrama
mmol	Milimol
NaF	Sodium Fluoride/Fluoreto de sódio
nm	Nanometer/Nanômetro
µg	Microgram/Micrograma
µg F/g	Microgram of fluoride per gram/Micrograma de fluoreto por grama
µm	Micrometer/Micrômetro
µM	Micromolar/Micro molar
p	Probability/Probabilidade
pH	Hydrogenionic Potential /Potencial Hidrogeniônico
SD	Standard Deviation/Desvio padrão

SH	Surface hardness
s	Seconds/*segundos*
TISAB	Total Ionic Strenght Adjustment Buffer/Tampão de Ajuste da Força Iônica Total
TMP	Sodium Trimetaphosphate**/**Trimetafosfato de sódio
%RDS	Porcentagem de recuperação de dureza de superfície
%SHR	Percentage of surface hardness recovery

SUMÁRIO

Effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate on enamel remineralization in situ

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Short Title: Effect of nano-sized TMP in enamel remineralization

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Abstract

This study evaluated the effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate (TMP) on the remineralization of artificial caries lesions in situ. Bovine enamel blocks (n=160) were randomly distributed among study groups after surface microhardness (SH) analysis and induction of subsurface lesions. Test groups included: Placebo (without F and TMP – negative control), 9000 µg F/g (9000F – positive control), 4500 µg F/g + 5% micrometric TMP (4500 5%+ TMPmicro) and 4500 µg F/g + 5% nano-sized TMP (4500 + 5%TMPnano). Ten volunteers used palatal devices containing 4 demineralized enamel blocks during 3 days, after a single application of gels, following a double-blind and crossover protocol. Two enamel blocks were removed immediately after topical application of F to determine calcium fluoride (CaF_2) formed. After each phase, the samples were analyzed by percentage of surface hardness recovery (%SHR) and CaF_2 retained in enamel. The data showed normal and homogeneous distributions, and were submitted to one-way ($\%SH_R$) or two-way (CaF_2), repeated-measures ANOVA. Student-Newman-Keuls test was used for multiple comparisons. The highest %SHR was observed for 4500 5%TMPnano gel, followed by 4500 5%TPMmicro, 9000F, and Placebo, with significant differences among all groups. Regarding CaF_2 formed, the highest concentration was observed in the 9000F group. No significant difference was observed among 9000F, 4500 5%TMPmicro and 4500 5%TMPnano groups for concentrations of CaF_2 retained. It was concluded that the addition of TMP to gels improved the remineralization of caries lesions in situ. The use of nano-sized TMP further enhanced this effect.

Keywords: Fluorides, Polyphosphates, Dental Caries, Nanoparticle.

1. Introduction

Dental caries is a chronic, multifactorial and dynamic disease, characterized by a diet-biofilm misbalance, resulting in the progressive mineral loss of dental hard tissues [Machiuskiene et al., 2020; Cugini et al., 2021]. Despite dental caries still affects several populations worldwide [Kassebaum *et al*., 2015], a substantial decline in its prevalence has been observed over the years [Lagerweij & Loveren, 2015]. The widespread use of self- or professional-applied fluoridated vehicles is one of the main reasons to justify such a decline, due to fluoride's ability to prevent the onset and development of caries lesions, by reducing the enamel solubility in acid media, promoting enamel remineralization, and harming the use of glucose by bacteria [Buzalaf et al., 2011].

Amongst the modalities of professionally-applied fluoridated vehicles, fluoride gels can be regarded as one of the most effective caries preventive measures, by reducing 20% and 28% caries lesions development in primary and permanent teeth, respectively [Marinho et al., 2015]. However, despite the proven benefits, the indiscriminate use of fluoride gels deserves attention, since the high concentrations of fluoride in these products may lead to acute toxicity [Whitford, 2011]. To minimize risks from this source, alternatives have been proposed, including the reduction of fluoride concentration in these products [Danelon et al., 2013; Akabane et al*.,* 2018; Gonçalves et al*.,* 2018].

Other alternatives include the addition of calcium and phosphate salts to fluoridated products. Sodium trimetaphosphate (TMP) is cyclic phosphate that has been added to fluoridated products such as gels, varnishes, mouthwash solutions, and dentifrices, promoting synergistic effects on enamel de- and re-mineralization [Takeshita et al., 2009, 2015; Danelon et al., 2013,2014; Favretto et al., 2013] and erosive tooth wear [Moretto et al., 2010; Manarelli et al., 2013; Pancote et al., 2014; Cruz et al., 2015].

In order to enhance the effects of products containing TMP and considering the above-mentioned results, studies with nano-sized TMP have been conducted. A 1000 µg F/g dentifrice containing nano-sized TMP reduced the enamel demineralization [Danelon et al., 2017; Emerenciano et al., 2018] and enhanced remineralization process *in situ* [Danelon et al., 2015]. This dentifrice was also shown

to be more effective against erosive tooth wear than formulations with micrometric TMP or without TMP [Danelon et al., 2018]. The association between fluoride and nano-sized TMP was also tested for fluoride gels containing 1% NaF, which promoted a similar enamel remineralization rate compared with the positive control (2%NaF) *in vitro* [Nagata et al., 2017]. Moreover, the gel containing nano-sized TMP showed a superior protective effect on erosive tooth wear when compared to positive control [Capalbo et al., 2020].

Considering the promising results of the association of nano-sized TMP with F and the limitations inherent to *in vitro* protocols, this study aimed to evaluate the effect of fluoride gels supplemented with nano-sized TMP on enamel remineralization *in situ.* The study's null hypotheses were that the remineralizing effect of the fluoride gels would not be affected by the addition of TMP, and that particle (*i.e.*, micrometric or nanosized) size would not influence the parameters analyzed.

2. Materials and Methods

Ethical aspects and inclusion criteria

This study was reviewed and approved by the Human Ethics Committee of the School of Dentistry, Araçatuba, São Paulo State University (CAAE: 36353320.0.0000.5420). All participants received written and verbal instructions on the research protocol, as well as signed an informed consent statement before the beginning of the study. Ten subjects aged 20-35 years, living in Araçatuba-SP (Brazil), were included in the study. The inclusion criteria involved participants in good general and oral health, who presented normal salivary flow and did not use fixed orthodontic appliances, cigarettes, or drugs that could interfere with the formation of the dental biofilm [Danelon et al., 2013].

Experimental Design

This was a double-blind and crossover *in situ* study performed in four phases of 3 days each, with a 7 day washout period. Sample size (n=8) was determined with data from a pilot study with 4 subjects assessing the percentage of surface hardness recovery as the primary response variable, considering α-error of 5%, β-error of 10%, minimum detectable difference of 8.5, and standard deviation of 4.7. Assuming a 20% drop-out rate, the sample size was established at 10 subjects. The participants

wore palatal appliances with four demineralized bovine enamel blocks each, and were randomly divided into 4 groups: Placebo (without F or TMP), 9000 µg F/g (9000F), 4500 µg F/g + 5% micrometric TMP (4500 5%TMPmicro) and 4500 µg F/g + 5% nano-sized TMP (4500 5%TMPnano). Immediately after topical fluoride application, two blocks were removed for the analysis of CaF_2 formed on the enamel surface. After a 3-day experimental period, surface hardness was again assessed (SH_2) for analysis of mineral gain, evaluated in terms of percentage of surface hardness recovery ($\%SH_R$). In addition, the concentrations of CaF_2 retained were also determined.

Enamel blocks preparation and induction of subsurface lesions

Bovine lower incisors were kept in formaldehyde solution 2% for 30 days [Delbem & Cury, 2002]. The enamel blocks (4×4×2mm) were obtained from tooth crowns, using an ISOMET Low Speed Saw (Buehler Ltd., Lake Bluff, Illinois, USA) under water-cooling and then serially polished with BETA-grinder polisher (Buehler, Lake Bluff, Illinois, USA). Thereafter, the specimens were selected based on their surface hardness (SH, 320-380 Knoop).

To induce enamel subsurface lesions, all surfaces of each block were coated with acid-resistant varnish, except the enamel surface. The specimens were immersed in 32 mL of a solution with 1.3 mmol/L calcium nitrate tetrahydrate, 0.78 mmol/L sodium dihydrogen phosphate monohydrate in 0.05 mol/L acetate buffer, pH 5.0; 0.08 mL F, for 16h at 37 ºC [Queiroz et al., 2008; Báez-Quintero et al., 2017].

Synthesis and characterization of nano-sized TMP particles

The process of synthesis and characterization was carried out at the Federal University of São Carlos, based on Danelon et al. (2015), for the study by Emerenciano et al (2018). Conventional sodium trimetaphosphate ($Na_3O_9P_3$, Aldrich, purity ≥95% CAS 7785-84-4) were ball milled using zirconia spheres in 1 litter of isopropanol for 48 h. Subsequently, the powders were separated from the alcoholic medium, dried, and ground in a mortar. The resulting powder was characterized by X-ray diffraction (XRD), to identify the structure crystal, as well as to estimate the size of the particles. The milling processing did not affect the crystalline structure of TMP. The XRD showed broader peaks due to the smaller crystallites and the average particle size was 27.7 nm.

Gels formulation and determination of fluoride in products

All gels were prepared in a laboratory and had de following ingredients: carboxymethylcellulose (Sigma-Aldrich Co., St. Louis, MO, USA), sodium saccharin (Sigma-Aldrich Co., St. Louis, MO, USA), glycerol (Sigma-Aldrich Co., St. Louis, MO, USA), peppermint oil (Distriol, São Paulo, Brazil), and water. Sodium fluoride (NaF) was added to the gels at concentrations of 4500 µg F/g and 9000 µg F/g. To the gels containing 4500 µg F/g, TMP was added at 5%, either as micrometric or nano-sized particles. Furthermore, a gel without F and TMP (Placebo) was prepared. To determine the concentration of fluoride in these products, it was used a fluoride ion-specific electrode (9609 BN, Orion, USA) attached to an ion analyzer (Orion, 720 A+), and calibrated with standards 0.5-8.0 µg F/mL, as previously described [Delbem et al., 2003; Manarelli et al., 2015]. The mean (SD) pH of gels was 6.5 (0.2), ranging from 6.2 to 6.7.

Clinical phases of experimental groups

Fluoridated dentifrices (1100 µg F/g as NaF, Sorriso Fresh plus gel, Colgate-Palmolive, São Paulo, Brazil) and toothbrushes (Bitufo Class P Extra Soft Toothbrush, Bitufo) were provided to volunteers throughout the study [Manarelli et al., 2015]. Instructions regarding the amount of dentifrice (transversal technique) and frequency of brushing (three times/day) were given. Oral hygiene procedures, following the above parameters, started 7 days before each experimental period [Manarelli et al., 2015]. In addition, during the 3-day experimental period, the volunteers were instructed to initially brush their natural teeth to form a natural toothpaste:saliva slurry, followed by three brushing strokes in each enamel blocks on the palatal appliance [Danelon et al., 2015].

The palatal appliances were inserted in the mouth on the night before each experimental phase to allow the formation of acquired pellicle [Cheung et al., 2005]. Posteriorly, professional application topical fluoride was performed on all natural teeth of the subjects for each experimental gel [Delbem et al., 2010; Danelon et al., 2013], following a double-blind, crossover protocol. At the same time, the enamel blocks on palatal appliances were treated for 1 minute with the same gels. Following, two blocks of each appliance were removed for the determination of CaF_2 formed [Danelon et al., 2013]. At the end of the 3-day experimental phase, the remaining two

blocks were removed from the appliance and cleaned, for determination of CaF_2 retained on the blocks and surface hardness [Danelon et al., 2013].

Analysis of enamel hardness

The enamel microhardness was measured using a Shimadzu hardness tester (HMV-2000, Shimadzu, Kyoto, Japan) with a diamond indenter under a 25 g load for 10 s [Vieira et al., 2005]. Five indentations, separated 100 µm from each other, were made in the center of blocks at the baseline for determining initial surface hardness (SH), and after the induction of subsurface lesions (SH_1). In addition, five other indentations were made (SH_2) spaced 100 µm apart from SH_1 after each experimental phase [Vieira et al., 2005]. The percentage of surface hardness recovery ($\%SH_R$) was calculated as $\%SH_R = [(SH_2 – SH_1)/(SH – SH_1)] \times 100$.

Analysis of loosely-bound fluoride on enamel

The amount of loosely-bound fluoride (Calcium fluoride, CaF_2) on enamel was quantified immediately after topical application gels (CaF_2 formed), and at the end of each phase *in situ* (CaF_2 retained). A digital caliper (Mitutoyo CD-15B) was used to measure the surface area of the enamel. Extraction of loosely bound fluoride was performed following the methodology of Caslavska et al. (1975). All surfaces of each specimen, except the enamel surface, were coated with wax. The specimens were then immersed in 0.5 mL of 1.0 mol/L KOH solution, under constant agitation, for 24h. The solution was neutralized and buffered with 0.5 mL of TISAB II modified with HCl.

Statistical analysis

SigmaPlot 12.0 software was used for statistical analysis, and the significance level was established at 5%. Data analysis considered the values of $\%SH_R$ and CaF_2 content on enamel before (formed) and after (retained) 3 days. The data showed normal and homogeneous distributions, and were submitted to one-way ($\%SH_R$) or two-way (CaF_2), repeated-measures ANOVA ($\%SH_R$), followed by Student-Newman-Keuls test.

3. Results

The mean (SD) fluoride concentrations (µg F/g) in the gels were 50.5 (13.0), 9,091.2 (284.7), 4,131.7 (98.7) and 4,387.4 (228.2), respectively for Placebo, 9000F,

4500 + 5%TMPmicro and 4500 + 5%TMPnano. The mean (SD) of initial surface hardness of all enamel blocks was 353.4 (8.8). After induction of subsurface lesions, the mean (SD) percentage of hardness loss was 83.9 (4.51), ranging from 83.5 (4.6) to 84.1 (4.2) without significant differences among the groups (p=0.932).

Significant differences were observed among all groups regarding surface hardness recovery (%SH_R), as shown in Figure 1. The highest %SHR was observed for the gel containing nano-sized TMP (4500 5%TMPnano), followed by 4500 5%TMPmicro, 9000F, and Placebo.

Regarding loosely bound fluoride data (Table 1), significant differences in CaF_2 formed (after topical application) were observed among all groups, with the highest concentration observed for the 9000F. After the 3-day experimental phase, a significant decrease was observed for all groups. No significant difference was observed among 9000F, 4500 5%TMPmicro, and 4500 5%TMPnano groups; values for the 4500 5%TMPnano group were not significantly different from the Placebo. The Placebo gel promoted the lowest CaF_2 values in both periods.

4. Discussion

The limited action of conventional fluoridated products on the onset and development of dental caries, along with concerns of unwanted side-effects, has stimulated the search for actives that act synergistically with fluoride, thus allowing the use of formulations with lower fluoride content without compromising the preventive therapeutic effects. The present study demonstrated that gels containing TMP were more effective than a conventional formulation containing twice as much fluoride (9000 µg F/g) in promoting the remineralization of caries-like lesions using an *in situ* model. In addition, the highest %SH_R was observed for the gel with nano-sized TMP when compared to the formulation containing micrometric TMP. Thus, both null hypotheses were rejected.

Nanotechnology has been widely used in dentistry [Jandt & Watts, 2020], especially for the control of dental caries, aiming to develop formulations that are more effective in enhancing mineral gain and preventing deminaralization [Hanning et al., 2012; Moothedath et al., 2019]. As previously mentioned, additional effects against enamel de-/re-mineralization and on erosive tooth wear have been attained

by the use of TMP nanoparticles when compared to its micrometric counterpart [Danelon et al., 2015, 2017, 2018; Emerenciano et al., 2018]. Interestingly, an *in situ* study showed that a conventional (1100 µg F/g) toothpaste containing 3% nano-sized TMP promoted superior remineralizing effects compared to counterparts without TMP or supplemented with microparticles [Danelon et al., 2015]. It was noteworthy that the toothpaste containing nanoparticles promoted a 43% reduction in the subsurface lesion (IMR) compared with the conventional formulation [Danelon et al., 2015].

In line with the data above, in the present study, the addition of 5% nano-sized TMP to a fluoride gel increased the $\%SH_R$ by 32% compared to its micrometric counterpart, which reinforces the hypothesis of an additional effect of nano-sized TMP over conventional particles. These results can be explained by some physicochemical properties of nanoparticles, which make the nano-sized TMP more reactive than micrometric TMP [Danelon et al., 2015; Jandt & Watts, 2020]. Nanoparticles, by convention, are smaller than 100 nm and present a high ratio of surface area to volume. The lower size of the nanoparticles (compared with micrometric ones) results in marked changes in the atomic arrangement within the nanoparticle, with their atoms located at the surface or a few atomic distances from the surface. This confers a large chemical potential, resulting in a high atomic diffusion and reactivity [Cao et al., 2011; Jandt & Watts, 2020]. Although the present study did not assess mineral content in-depth, it seems plausible to assume that the higher remineralizing effect of the gel containing nanosized TMP is associated with its effects on the lesion body, similarly to that described by Danelon et al. (2015). This is extremely desirable from a clinical standpoint, as it may suggest a higher degree of lesion recovery in contrast to what is usually observed for conventional fluoridated formulations (*i.e.*, hypermineralization of enamel surface with lesser effects on the subsurface).

Regarding loosely bound fluoride (CaF_2), a dose-response relationship between fluoride content in the products and CaF_2 formed on the enamel was observed, which is in line with the existing literature [Buzalaf et al., 2011]. Nonetheless, despite the 9000 µg F/g gel promoted the highest CaF_2 concentration, the resulting %SHR values were lower than those related to the TMP-containing formulations. This pattern was also observed for TMP-containing varnishes, whose remineralizing

effects have been associated with lower deposition of CaF_2 and F [Manarelli et al., 2014, 2015, 2017], which is somehow unexpected when considering the existing knowledge on the mechanisms of action of fluoride on caries control [Buzalaf et al., 2011]. This reinforces the concept that different mechanisms are related to the effects of TMP on caries dynamics. In fact, the mechanism of action of TMP has been related to its capacity to adsorb to enamel and to retain calcium and fluoride ions [Souza *et al*., 2013], which are released upon acidic pH [Manarelli et al., 2014]. As TMP and F compete for the same binding sites on the enamel surface, higher fluoride concentrations can interfere on TMP action [Souza et al., 2014], which justifies the need to determine an ideal TMP:F molar ratio to achieve maximum synergistic effects [Takeshita et al., 2009].

The discrepancies between the current results and the data reported by Nagata et al. (2017) on enamel remineralization should be further addressed, and are likely to have resulted from the different protocols employed. While in the *in vitro* protocol the remineralization process was produced by static immersion in solutions and a single topical fluoride application was performed, the present *in situ* study included interactions between enamel and salivary ions, the presence of the acquired enamel pellicle and salivary buffers, bringing it closer to reality. Furthermore, in the present *in situ* study, the volunteers brushed their teeth with fluoridated toothpaste three times daily, which provided an additional F source to interact with enamel (coated by a TMP layer). Another reason for differences observed was the duration of the experimental periods, respectively 3 and 7 days for *in situ* and *in vitro* protocols [Afonso et al., 2013].

In summary, the present study demonstrated that the addition of nano-sized TMP to fluoridated gel led to the highest remineralization rate of artificial caries lesions under *in situ* conditions*,* surpassing that achieved by the use of a conventional formulation containing twice as much fluoride. Considering these results, the low-fluoride gel with TMPnano could be a safe and promising alternative in clinical practice, since it has a higher remineralizing capacity and offers a lower risk of intoxication compared with a conventional formulation. Clinical trials are needed to confirm the effects of nano-sized TMP.

5. Statement of Ethics

The present study was reviewed and approved by the Human Ethics Committee of the School of Dentistry, Araçatuba, São Paulo State University (CAAE: 36353320.0.0000.5420). All participants signed an informed consent statement before the beginning of the study.

6. Conflict of Interest Statement

The authors have no conflicts of interest to declare.

7. Author Contributions

Conceived and designed the experiments: JPP and ACBD. Performed the experiments: TPM, MD and LCBQ. Analyzed the data: TPM, JPP, ACBD, MD, and LCBQ. Wrote/revised the paper: TPM, JPP, ACBD, MD, and LCBQ.

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Figure Legend

Fig. 1. Mean percentage of surface hardness recovery (%SHR) according to each gel. Different letters indicate significant differences among the groups; bars indicate standard deviations. One-way, repeated measures ANOVA and Student-Newman-Keuls' test ($p<0.05$, $n=10$).

Table Legend

Table 1. Mean values of loosely bound fluoride (CaF_2) formed immediately after gel application ("formed") and after the 3-day experimental period ("retained"). Upper-case letters show significant differences between CaF_2 formed and retained; lower-case letters indicate significant differences among groups within each row. Two-way, repeated-measures ANOVA and Student-Newman Keuls' test ($p<0.05$; $n=10$).

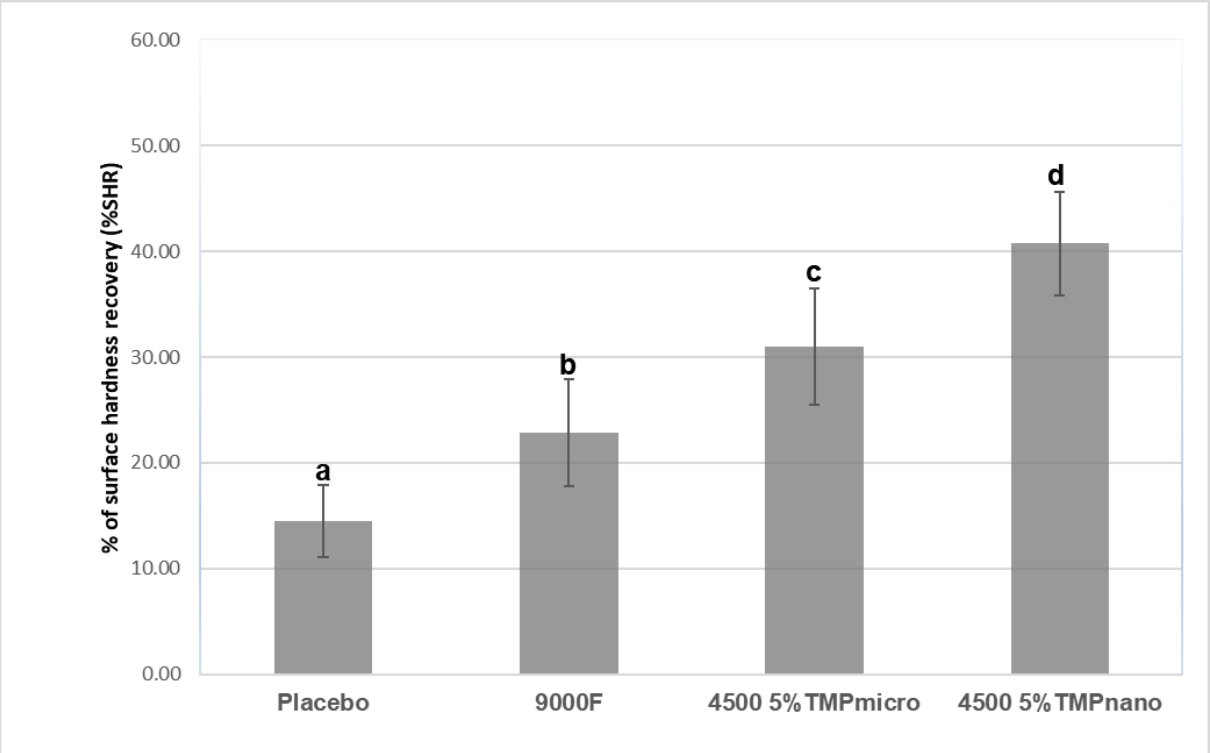

Figure 1. *Mean percentage of surface hardness recovery (%SHR) according to each gel. Different letters indicate significant differences among the groups; bars indicate standard deviations. One-way, repeated measures ANOVA and Student-Newman-Keuls' test (p<0.05, n=10).*

Table 1. Mean values of loosely bound fluoride (CaF_2) formed immediately after gel application ("formed") and after the 3-day experimental period ("retained")

Groups	CaF_2 (µg/mm^3)	
	Formed	**Retained**
Placebo	0.97 (0.57) ^{A,a}	0.83 (0.25) ^{A,a}
9000F	12.44 (3.20) ^{A,b}	2.84 (1.43) ^{B,b}
4500 5%TMPmicro	7.29 (2.78) ^{A,c}	1.67 (0.81) ^{B,b}
4500 5% TMPnano	5.62 (1.86) ^{A,d}	1.53 (1.27) ^{B,ab}

Upper-case letters show significant differences between CaF_2 formed and retained; lower-case letters indicate significant differences among groups within each column. Two-way, repeated-measures ANOVA and Student-Newman Keuls' test ($p<0.05$; n=10).

Anexos

ANEXO A

CARIES RESEARCH

INSTRUÇÕES AOS AUTORES

Aims and Scope

Caries Research publishes epidemiological, clinical, and laboratory studies in dental caries, fluorosis, erosion, and related dental diseases. Some studies build on the considerable advances already made in caries prevention, e.g. through fluoride application. Some aim to improve understanding of the increasingly important problem of dental erosion and the associated tooth wear process. Others monitor the changing pattern of caries in different populations, explore improved methods of diagnosis, or evaluate methods of prevention or treatment. Studies using genetic methods to identify human genes or mutations associated with caries prevalence are welcome as are manuscripts using modern high-throughput sequencing methods to characterize microbial biofilms associated with oral health and active caries. The broad coverage of innovative research into dental caries is unique and has given the journal an outstanding international reputation as an indispensable source for both basic scientists and clinicians engaged in understanding, investigating, and preventing dental diseases.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Efeito de géis fluoretados suplementados com Trimetafosfato de Sódio nanoparticulado sobre a remineralização do esmalte dental in situ

Pesquisador: TAMIRES PASSADORI MARTINS

Área Temática: Equipamentos e dispositivos terapêuticos, novos ou não registrados no País;

Versão: 3

CAAE: 36353320.0.0000.5420

Instituição Proponente: Universidade Estadual Paulista Júlio de Mesquita Filho

Patrocinador Principal: FUND COORD DE APERFEICOAMENTO DE PESSOAL DE NIVEL SUP
UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

DADOS DO PARECER

Número do Parecer: 4.981.952

Apresentação do Projeto:

Serão utilizados no estudo incisivos centrais bovinos, mantidos em solução de formol 2% (pH 7,0) durante 30 dias (Delbem & Cury, 2002). Os blocos de esmalte (4 mm x 4 mm) serão obtidos a partir da porção mais plana da superfície vestibular das coroas, utilizando cortadeira ISOMET (Buehler,
Lake Bluff, Illinois, USA). A dentina será ajustada para obtenção de superfícies paralelas entre esmalte e dentina (espessura ± 2 mm). Os blocos serão fixados em bases de resina acrílica, a fim de realizar o polimento das superfícies de esmalte e dentina, por meio de lixas de diferentes granulações. Posteriormente, será determinada a microdureza do esmalte, utilizando-se o microdurômetro Micromet 5114 (Buehler, Lake Bluff, EUA) e o software BuehlerOmniMet (Buehler, Lake Bluff, EUA), com um penetrador de diamante Knoop sob carga de 25 g por 10 s. Serão incluídos na pesquisa blocos de esmalte com dureza de superfície (DS) entre 320-380 Knoop (KHN), que serão distribuídos aleatoriamente entre os grupos após a indução de lesões de cárie.Os géis a serem testados serão: Placebo (sem flúor ou TMP – controle negativo), 9000 ppm de F (controle positivo), 4500 ppm de F + 5% TMP microparticulado e 4500 ppm de F + 5% TMP nanoparticulado. Voluntários utilizarão dispositivos palatinos contendo 4 blocos de esmalte. Para o

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uso dos dispositivos intrabucais, orientações verbais e por escrito serão fornecidas aos voluntários antes do início da pesquisa. A não utilização de produtos fluoretados, com exceção da água fluoretada, também será instruída. Os hábitos de escovação serão padronizados quanto ao dentifrício com 1100ppm F, quantidade de dentifrício, frequência de escovação (3 vezes ao dia, sendo uma ao acordar, outra antes de dormir e a terceira de livre escolha do voluntário). Na manhã do primeiro dia experimental, os voluntários serão instruídos a utilizar os dispositivos palatinos por 2 h, a fim de promover a formação da película adquirida do esmalte. Em seguida, os géis serão aplicados uma única vez sobre os blocos, por 1 minuto. Imediatamente após as aplicações, dois blocos de cada dispositivo serão aleatoriamente removidos para a determinação de CaF2 formado e a mensuração do conteúdo de F, Ca e P presentes no esmalte. A partir deste momento, os dispositivos deverão ser utilizados por 3 dias inteiros, inclusive durante a noite, exceto durante as refeições e ingestão de líquidos (exceto água). Durante estes momentos, os dispositivos deverão permanecer armazenados em estojo próprio, coberto com gaze umedecida em água. Transcorridos 3 dias após aplicação dos géis, os blocos serão retirados dos dispositivos e serão limpos utilizando escova macia, gaze e NaOCl 5%. Em seguida, avaliar-se-á a dureza de superfície e, a seguir, os blocos serão seccionados no sentido longitudinal no centro dos blocos, para análise da dureza em secção longitudinal e dosagem de CaF2 retido e o fluoreto, cálcio e fósforo presente no esmalte (Delbem et al., 2005). Será adotado um protocolo duplo-cego (pesquisador e voluntário) e cruzado (para minimizar fatores relacionados à cooperação do paciente, bem como fatores externos desconhecidos).

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo do presente estudo será avaliar o efeito de géis fluoretados suplementados com TMP nanoparticulado remineralização de lesões de cárie artificialmente induzidas in situ.

Avaliação dos Riscos e Benefícios:

Riscos:

Até o presente momento não há na literatura indícios de riscos do uso de dispositivos intrabucais em metodologias in situ. No estudo, os voluntários continuarão com hábitos de higiene oral, sendo entregues dentifrícios fluoretados e escovas para as quatro fases experimentais, portanto não há chances de desenvolverem cárie. Quanto ao uso dos dispositivos intrabucais, ressalta-se que o aparelho é semelhante ao utilizado com finalidade ortodôntica, sendo confeccionado com os

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Telefone: (18)3636-3200 **Fax:** (18)3636-3332 **E-mail:** andrebertoz@foa.unesp.br

mesmos materiais e critérios de biossegurança. Assim, os riscos podem ser considerados como mínimos.
Benefícios:
Os voluntários não terão benefícios diretos quanto à participação na pesquisa. Entretanto, receberão profilaxia dentária e instruções de higiene bucal previamente ao início do estudo. Caso haja necessidade de restaurações dos dentes, será oferecido tratamento na Faculdade de Odontologia de Araçatuba-UNESP. Espera-se que este estudo resulte em informações importantes sobre as vantagens do uso de géis suplementados com TMP nanopartículado sobre a remineralização de lesões iniciais de cárie.

Comentários e Considerações sobre a Pesquisa:
Pesquisa apresenta-se apta para a sua realização.

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos foram adicionados de acordo com a resolução 466/12 do CNS.

Recomendações:
Não há.

Conclusões ou Pendências e Lista de Inadequações:
Pesquisa apresenta-se apta para a sua realização.

Considerações Finais a critério do CEP:
Salientamos que, de acordo com a Resolução 466 CNS, de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/11/2021.

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

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1777498_E1.pdf	02/09/2021 07:05:32		Aceito
Outros	CARTADERESPOSTA.docx	02/09/2021 07:04:50	TAMIRES PASSADORI MARTINS	Aceito
Projeto Detalhado	ProjetoDePesquisa_Modificado.pdf	11/07/2021	TAMIRES	Aceito

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/ Brochura Investigador	ProjetoDePesquisa_Modificado.pdf	20:22:45	PASSADORI MARTINS	Aceito
Outros	TermoDeCompromisso_MODIFICADO_2021.pdf	02/07/2021 00:02:00	TAMIRES PASSADORI MARTINS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_MODIFICADO_2021.pdf	02/07/2021 00:00:59	TAMIRES PASSADORI MARTINS	Aceito
Folha de Rosto	FolhaDeRosto_Atualizada.pdf	02/07/2021 00:00:06	TAMIRES PASSADORI MARTINS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ATUALIZADO.pdf	21/07/2020 16:31:14	TAMIRES PASSADORI MARTINS	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDePesquisa_CEP.pdf	15/07/2020 09:09:41	TAMIRES PASSADORI MARTINS	Aceito
Outros	TermoDeCompromisso.pdf	15/07/2020 08:22:38	TAMIRES PASSADORI MARTINS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	15/07/2020 08:05:07	TAMIRES PASSADORI MARTINS	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Sim

ARACATUBA, 17 de Setembro de 2021

Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))

Endereço: JOSE BONIFACIO 1193
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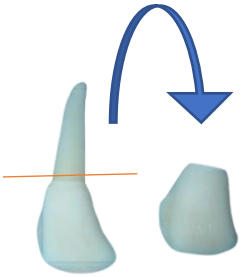
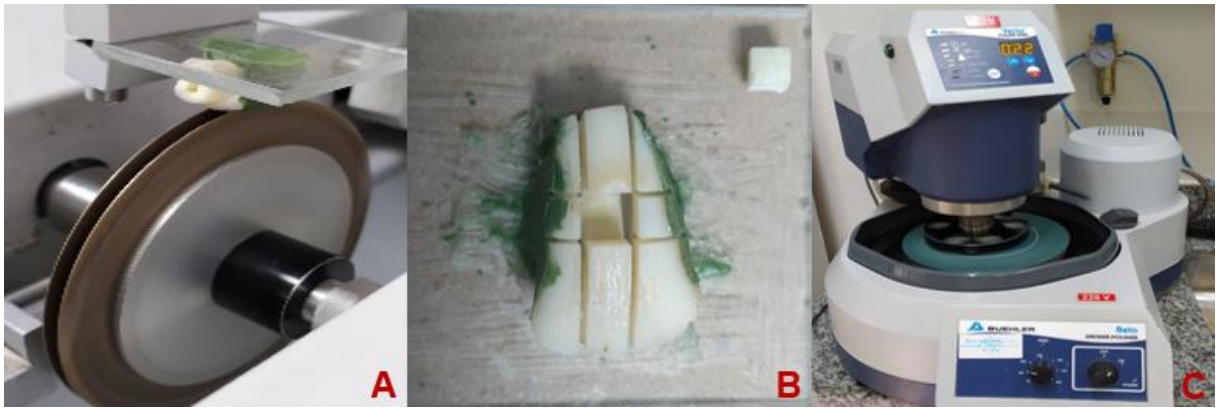
ANEXO C

OBTENÇÃO DOS BLOCOS DE ESMALTE



1. Separação coroa-raiz

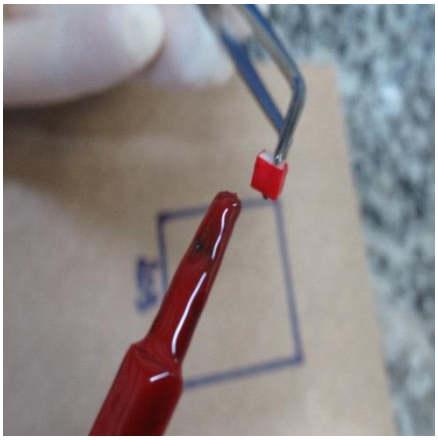


2. Coroas dentárias foram fixadas a uma base acrílica e levadas à cortadeira **(A)**. Os blocos foram obtidos da superfície mais plana da coroa **(B)** e posteriormente polidos **(C)**.

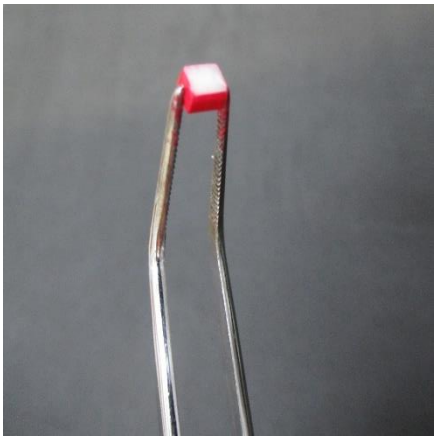
3. Os blocos foram submetidos à análise de dureza inicial, utilizando Microdurômetro. Foram inclusos no estudo blocos com valores de dureza 320-380 Knoop.

ANEXO D

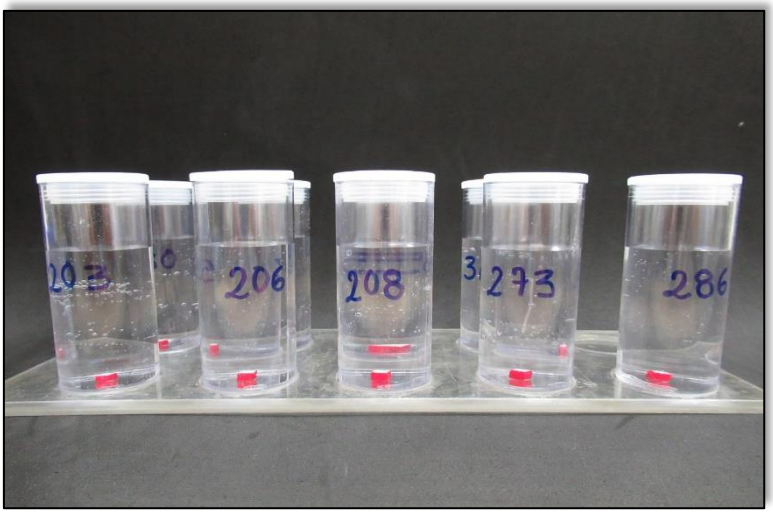
INDUÇÃO DE LESÃO DE CÁRIE ARTIFICIAL



1. Aplicação de verniz ácido-resistente.

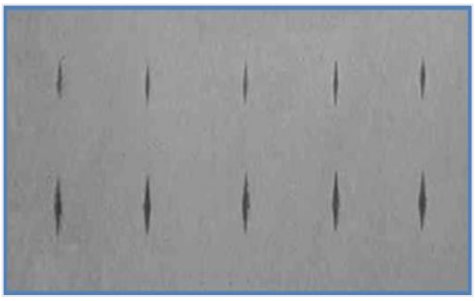


2. Apenas a camada de esmalte não foi recoberta.



3. Imersão em solução desmineralizadora (pH 5,0 – 32ml/ bloco)

Incubação = 16 horas, a 37ª C.



4. Análise de dureza de superfície pós indução de lesão de cárie (DS_1).

Foram inclusos blocos com perda entre 76-90%.

ANEXO E

DOSAGEM DE FLUORETO NOS GÉIS EXPERIMENTAIS

Dissolução de 100-110mg de gel em água deionizada.

↓

Transferência do conteúdo para balões volumétricos, completando-se o volume para 100mL

↓

Retirada de 1mL de cada amostra e tamponamento com TISSAB II (triplicata)

↓

Análise utilizando eletrodo íon específico (9409 BN-Orion, USA) acoplado a um analisador de íons (Orion 720, USA)

ANEXO F

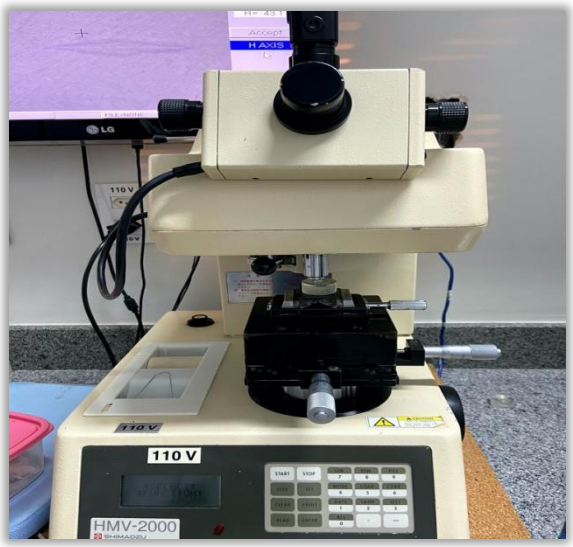
DISPOSITIVO PALATINO E APLICAÇÃO DE GEL FLUORETADO

Dispositivo palatino com 4 blocos de esmalte e kit de higiene bucal fornecido aos voluntários.

Aplicação tópica de flúor no voluntário **(A)** e posterior aplicação em blocos de esmalte bovino **(B).** Remoção de dois blocos para dosagem de CaF_2 formado **(C).**

ANEXO G

ANÁLISE DE PORCENTAGEM DE RECUPERAÇÃO DE DUREZA DE SUPERFÍCIE



Microdurômetro Shimadzu

Carga 25 gramas

Tempo: 10 s

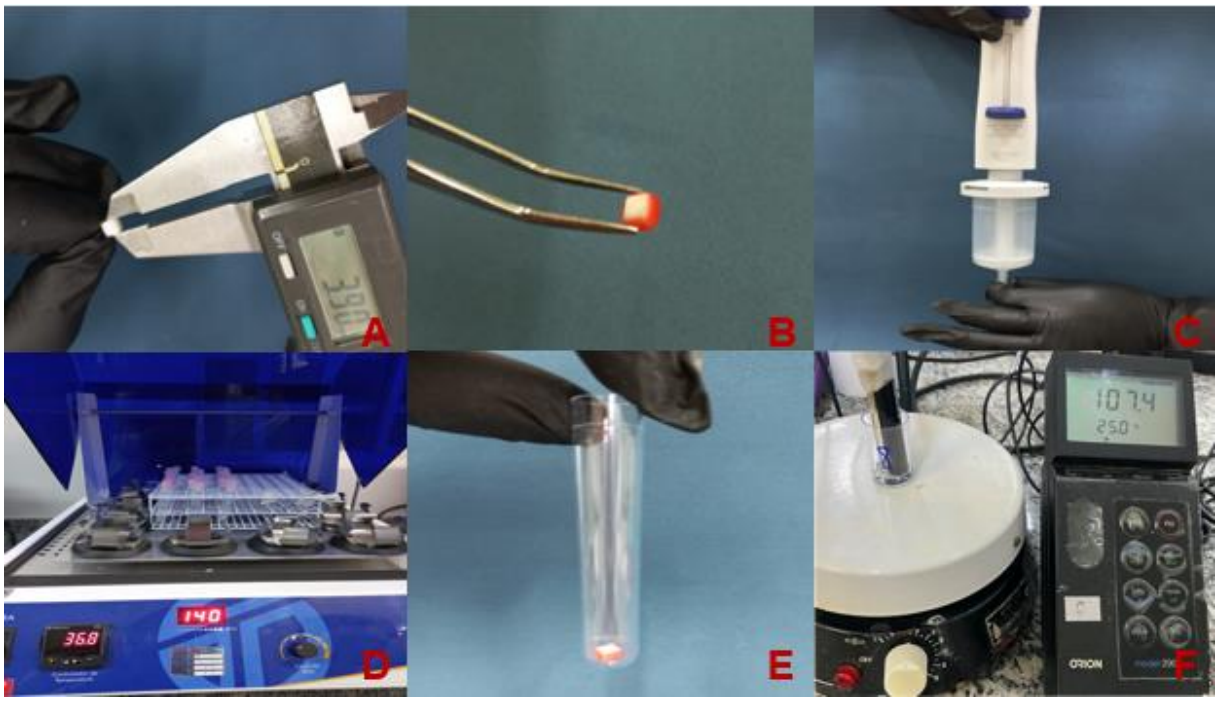
DS

DS_1

DS_2

ANEXO H

DOSAGEM DE FLUORETO DE CÁLCIO (CaF_2)



A área de superfície foi mensurada **(A)** e os blocos foram cobertos com cera rosa, exceto a superfície de esmalte **(B).** Foram adicionados 0,5 mL de KOH 1,0 mol L^{-1} **(C)** e as amostras submetidas à agitação porr 24h **(D).** Posteriormente, adicionou-se 0,5 mL de TISAB II modificado por HCl a cada amostra **(E).** As leituras foram realizada através de um eletrodo íon-específico **(F).**