

Amanda Scarpin Gruba

**Efeito de géis clareadores formulados com
nanopartículas de trimetafosfato de sódio sobre a
alteração de cor, propriedades mecânicas e morfológica
do esmalte dentário**

Araçatuba – SP
2023

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Dissertação apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção de título de Mestre em Ciências - Área de Concentração: Saúde Bucal da Criança.

Orientador: Profº. Titular. Alberto Carlos Botazzo Delbem

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Dados Curriculares

Amanda Scarpin Gruba

Nascimento	25.11.1997 – Laranjeiras do Sul - PR
Filiação	Sandro José Gruba Tarcys Scarpin
2016/2021	Curso de Graduação em Odontologia pela Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP.
2018/2020	Desenvolvimento de Projeto de Iniciação Científica
2021/2023	Desenvolvimento de Projeto de Mestrado com auxílio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP).
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Dedicatória

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*“Mas, buscai primeiro o reino de Deus, e a sua justiça,
e todas estas coisas vos serão acrescentadas.”*

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Resumo

GRUBA, AS. **Efeitos de géis clareadores formulados com nanopartículas de trimetafosfato de sódio sobre alteração de cor, propriedades mecânicas e morfológicas do esmalte dentário.** 2023 56f. Dissertação (Mestrado) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2023.

O objetivo deste estudo foi avaliar *in vitro* os efeitos do gluconato de cálcio (CaGlu), fluoreto de sódio (F), trimetafosfato de sódio nanométrico (TMPnano) e F/TMPnano adicionados a um gel clareador de peróxido de hidrogênio (H_2O_2) a 35% sobre a alteração da cor, propriedades mecânicas e morfológicas do esmalte e difusão transamelodentinal. Discos de esmalte/dentina bovino ($n = 180$) foram divididos de acordo com o gel clareador: 35% H_2O_2 (HP); 35% H_2O_2 + 0,1% NaF (HP/F); 35% H_2O_2 + 1% TMPnano (HP/TMPnano); 35% H_2O_2 + 0,1% NaF + 1% TMP nano (HP/F/TMPnano) e 35% H_2O_2 + 2% CaGlu (HP-Ca). Os géis clareadores foram aplicados três vezes (40 min/sessão) em intervalos de 7 dias entre cada aplicação. Em seguida, determinou-se a alteração da cor (ΔE_{ab} e ΔE_{00}), o índice de clareamento (ΔWI_D), a dureza de superfície final (SH), a dureza transversal (ΔKHN), a rugosidade superficial (Ra) e a difusão transamelodentinária. A morfologia e composição química do esmalte foi determinada por Microscopia Eletrônica de Varredura (MEV) e Energia Dispersiva de Raios X (EDX). Os dados foram submetidos à ANOVA, seguida do teste de Student-Newman-Keuls ($p < 0,05$). Todos os géis clareadores apresentaram alterações significativas de cor após o procedimento clareador ($p < 0,001$). ΔE_{ab} , ΔE_{00} e ΔWI_D foram semelhantes entre os géis avaliados. A perda mineral (SH e ΔKHN), rugosidade superficial e difusão transamelodentinal de peróxido de hidrogênio foram menores para HP/F/TMPnano; os grupos HP e HP-Ca apresentaram os maiores valores em relação aos demais grupos ($p < 0,001$). HP/F/TMPnano apresentaram menos alterações morfológicas no esmalte. É possível concluir que a adição de F/TMPnano ao agente clareador em consultório não interferiu na eficácia do clareamento e reduziu a desmineralização do esmalte, a alteração da rugosidade superficial, a difusão de H_2O_2 e as alterações morfológicas. Como significado clínico obteve que a associação de F/TMPnano ao gel clareador pode ser utilizada como uma nova estratégia para aumentar a segurança do produto e com desempenho clínico semelhante.

Palavras-chave: Clareadores; Peróxidos; Fosfatos; Fluoreto de Sódio; Estética dentária; Nanopartículas.

Abstract

GRUBA, AS. **Effects of bleaching gels formulated with sodium trimetaphosphate nanoparticles on color change, mechanical and morphological Properties of tooth enamel.** 2023 56f. Dissertação (Mestrado) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2023.

This study evaluated *in vitro* the effects of calcium gluconate (CaGlu), sodium fluoride (F), sodium trimetaphosphate nano-sized (TMPnano), and F/TMPnano added to a 35% hydrogen peroxide (H_2O_2) bleaching gel on the color alteration, enamel mechanical and morphological properties, and trans-amelodentinal diffusion. Enamel discs / bovine dentin ($n = 180$) were divided according to the bleaching gel: 35% H_2O_2 (HP); 35% H_2O_2 + 0,1% NaF (HP/F); 35% H_2O_2 + 1% TMPnano (HP/TMPnano); 35% H_2O_2 + 0,1% NaF + 1% TMP nano (HP/F/TMPnano) e 35% H_2O_2 + 2% CaGlu (HP-Ca). The bleaching gels were applied three times (40 min/session) at 7-day intervals between each application. Then, color alteration (ΔE_{ab} and ΔE_{00}), whitening index (ΔWI_D), surface hardness (SH), cross-sectional hardness (ΔKHN), surface roughness (Ra), and trans-amelodentinal diffusion were determined. The morphology and chemical composition of enamel were determined by Scanning Electron Microscopy (SEM) and X-ray Dispersive Energy (EDX). Data were submitted to ANOVA, followed by the Student-Newman-Keuls test ($p < 0.05$). All bleaching gels showed significant color alterations after the bleaching procedure ($p < 0.001$). ΔE_{ab} , ΔE_{00} , and ΔWI_D were similar among the evaluated gels. Mineral loss (SH and ΔKHN), surface roughness, and transamelodentinal diffusion of hydrogen peroxide were lower for PH/F/TMPnano; the PH and HP Blue groups presented the highest values about the other groups ($p < 0.001$). PH/F/TMPnano showed less morphological changes in enamel. It is possible to conclude that the addition of F/TMPnano to the in-office bleaching agent did not interfere with the bleaching efficacy and reduced enamel demineralization, surface roughness alteration, H_2O_2 diffusion and morphological changes. The association of F/TMPnano to the bleaching gel can be used as a new strategy to increase the safety of the product and with similar clinical performance.

Keywords: Tooth Bleaching Agents; Peroxides; Phosphates; Sodium Fluoride; Esthetics; Nanoparticles.

Summary

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Article: Influence of bleaching gels formulated with sodium trimetaphosphate nano-sized and fluoride on the physicochemical, mechanical, and morphological properties of dental enamel

*Manuscrito preparado nas normas do periódico *Clinical Oral Investigations*

ABSTRACT

Objective: The aim of this study was to evaluate *in vitro* the effects of sodium fluoride (F) and sodium trimetaphosphate nano-sized (TMPnano) added to a 35% hydrogen peroxide (H_2O_2) bleaching gel on the color alteration, enamel mechanical and morphological properties, and H_2O_2 transamelodentinal diffusion.

Materials and methods: Enamel discs / bovine dentin ($n = 180$) were divided according to the bleaching gel: 35% H_2O_2 (HP); 35% H_2O_2 + 0.1% F (HP/F); 35% H_2O_2 + 1% TMPnano (HP/TMPnano); 35% H_2O_2 + 0.1% F + 1% TMPnano (HP/F/TMPnano) and 35% H_2O_2 + 2% calcium gluconate (HP/Ca). The bleaching gels were applied three times (40 min/session) at 7-day intervals between each application. Then, color alteration (ΔE and ΔE_{00}), whitening index (ΔWI_D), surface (SH) and cross-sectional hardness (ΔKHN), surface roughness (Ra), and transamelodentinal diffusion were determined. The morphology and chemical composition of enamel were evaluated by Scanning Electron Microscopy (SEM) and X-ray Dispersive Energy (EDX). Data were submitted to ANOVA, followed by the Student-Newman-Keuls test ($p < 0.05$).

Results: All bleaching gels showed significant color alterations after the bleaching procedure ($p < 0.001$). ΔE , ΔE_{00} , and ΔWI_D were similar among the evaluated gels. Mineral loss (SH and ΔKHN), surface roughness, and transamelodentinal diffusion of H_2O_2 were lower for HP/F/TMPnano; the HP and HP/Ca groups presented the highest values about the other groups ($p < 0.001$). For SEM/EDX, surface changes were observed in all bleached groups, but less intense with TMPnano in the gel composition.

Conclusion: It is possible to conclude that the addition of F/TMPnano to the bleaching agent did not interfere with the bleaching efficacy and reduced enamel demineralization, surface roughness alteration, H_2O_2 diffusion, and morphological changes.

Clinical significance: The association of F/TMPnano to the bleaching gel can be used as a new strategy to increase the safety of the product and with a similar clinical performance to the conventional bleaching agent.

Keywords: Tooth Bleaching Agents; Peroxides; Phosphates; Sodium Fluoride; Esthetics; Nanoparticles.

1. Introduction

A pleasant or attractive smile is the key to acceptance in interpersonal relationships between individuals in society [1]. In addition, the smile is regarded as one of the most important facial expressions and denotes essentiality to express friendship, agreement, and appreciation [1,2]. Thus, dental bleaching is one of the most requested clinical procedures [3,4] and has become a popular option for patients with dental discoloration [3,5]. In the office technique, hydrogen peroxide (H_2O_2) is used as a high-concentration bleaching agent (35-40%) [6,7]. However, despite the great aesthetic satisfaction that the procedure provides, it is known that whitening can also cause undesirable changes in the dental structure [5,8,9].

Studies have shown that the greater the concentration of H_2O_2 and the time of exposure of the dental tissue to the bleaching gel [10,11], the greater the changes in mechanical properties [5,9], such as mineral loss [6,9,12], morphological changes [12,13], increased roughness [5] of dental enamel, as well as effects that extend to the pulp tissue [8,14,15], mainly due to dissociation into reactive oxygen species and other powerful oxidizing agents [10,11], which culminates clinically in the high rates of sensitivity reported by patients [3,16]. Even with such inconveniences, in-office bleaching remains the main aesthetic clinical conduct required by patients [1]. In this context, new whitening protocols and approaches have been sought to minimize such therapy harms. Among possible alternatives, the addition of remineralizing agents, such as phosphate salts, to bleaching gels has been studied [6,9,17].

Among the available phosphate salts, sodium trimetaphosphate (TMP) has good adsorption on tooth enamel and benefits such as the ability to reduce H^+ diffusion and facilitate calcium and phosphate diffusion [18,19] and the ability to bind to OH^- groups [6,19]. Thus, micrometric TMP associated with sodium fluoride (F) has been tested in bleaching gels [6,17,20], where it was possible to observe a reduction in mineral loss, diffusion, and transamelodentinal cytotoxicity of H_2O_2 and with similar whitening efficacy of the product [6,17]. A strategy to further optimize the effect of TMP would be its use in nanoparticulate size, as shown in model studies of caries and dental erosion [18,21,22], where the combination of TMP on the nanometer scale (TMP nano) with F provided a superior effect than the use of micrometric TMP [21,22]. Thus, considering the promising effects of TMP nano-sized on tooth structure, it is assumed that TMP nano could further reduce the diffusion of products derived from H_2O_2 degradation and consequently minimize changes in dental substrates resulting from the bleaching procedure.

Furthermore, it is known that the use of fluoride toothpaste during bleaching treatment has the potential to reduce adverse effects on the morphology and properties of dental tissues,

whether applied before [23] or during the bleaching regimen [17,24]. However, no study so far has evaluated the effect of associating TMPnano with F to the whitening gel, as well as being subjected to a clinical condition in which patients make daily use of fluoridated toothpaste. Thus, the aim of this study was to evaluate, *in vitro*, the effects of F and TMPnano added to a 35% H₂O₂ bleaching gel on color alteration, enamel mechanical and morphological properties, and H₂O₂ transamelodentinal diffusion. The null hypothesis of the study was that bleaching gels containing 35% H₂O₂ and F/TMPnano showed no difference in promoting the bleaching effect, reducing mineral loss, morphological appearance, roughness, and transamelodentinal diffusion compared to the bleaching gel containing only 35% H₂O₂.

2. Materials and Methods

2.1. Formulation of fluoride toothpaste, bleaching gels, and determination of experimental groups

The toothpaste had the following composition: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate sodium, saccharin, peppermint oil, glycerol, abrasive silica, sodium lauryl sulfate, deionized water, and F at the concentration of 1100 ppm fluoride (Merck, Darmstadt, Germany) [18,21]. The total (TF) and ionic (IF) fluoride concentrations were evaluated [18,21] using a fluoride ion-specific electrode (Orion 9609 BN, Orion Research Inc., Beverly, Mass., USA) coupled to an ion analyzer (Orion 720 A⁺, Orion Research Inc.) previously calibrated with five standard solutions (0.25, 0.5, 1.0, 2.0, and 4.0 µg F/mL). The total and ionic fluoride concentrations (TF and IF) (mean [SD]; ppm F; n = 3) were, respectively, 1117.1 [26.2] and 1105.2 [19.8]. All of the experimental and commercial bleaching gels had a final pH of approximately 7.0. The pH (mean [SD]; n = 3) of the 1100 ppm fluoride toothpaste was 7.0 [0.2].

The bleaching gels were prepared in each application session since there were no stabilizers in their composition. The basic components of the gels were comprised of thickener (12% Carbopol), bleaching agent (35% H₂O₂), glycerin and water (qs), and NaOH (4 molar) required to maintain a pH of approximately 7.0. Depending on the experimental group, 0,1% sodium fluoride (F) and/or 1% nano-sized sodium trimetaphosphate (TMPnano) was added (synthesis and characterization of the TMP nano based on the study by Danelon et al. [21]; and the concentration used based on the previous study by Akabane et al. [6]). A commercial (marketplace) bleaching gel containing 35% H₂O₂ and 2% calcium gluconate, neutral pH (Whiteness HP Blue, FGM, Joinville, SC, Brazil) was used as the positive control. Thus, five

experimental bleaching gels were defined: 1) 35% hydrogen peroxide (HP); 2) H_2O_2 + 0.1% F (HP/F); 3) H_2O_2 + 1% TMP nano (HP/TMPnano); 4) H_2O_2 + 0.1% F + 1% TMPnano (HP/F/TMPnano); 5) H_2O_2 + 2% calcium gluconate (HP/Ca). The sample size per group was 12 enamel/dentin discs, which was based on a previous study [Akabane et al., 2021], adopting surface and cross-sectional hardness as primary outcomes, mean the difference between groups (10 and 2800, respectively), standard deviation (4 and 1500, respectively) an α error of 5% and a β error of 10%. Disks were randomly divided (Excel, Microsoft Corporation, USA) among the 5 experimental groups ($n = 12$).

2.2. Color alteration analysis

2.2.1. Preparation and pigmentation of enamel/ dentin discs

Enamel/ dentin discs (5.7 mm diameter x 3.5 mm thick) were obtained from bovine incisor teeth using an 8 mm diameter grinding wheel under water cooling (4° C). Then, the discs were cleaned in deionized water and stored in a physiological saline solution containing 0,1% thymol at 4°C [6].

After the initial reading of the color values, determined according to the Commission Internationale de l'Eclairage (CIE $L^* a^* b^*$ color system), the discs were stored in microtubes (Kasvi K6-0150, 1.5 mL, São José dos Pinhais, PR, Brazil) containing 1 mL of room temperature black tea infusion for pigmentation. The infusion was made with 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) for every 100 mL of deionized water [25]. The pigmentation process was monitored for 6 days, and the infusion was changed daily. Then, a new reading of the color values was determined by the CIE $L^* a^* b^*$ [6,25].

2.2.2. Bleaching gel and toothpaste treatments

The enamel/dentin discs were treated with a slurry of toothpaste prior to the study and during the 21-day time of the experiment. To prepare the slurry, the toothpaste was weighed daily, placed in a glass beaker, added to deionized water at a ratio of 1:3, and shaken to obtain a homogeneous suspension. Each enamel disc was immersed in the slurry (2 mL) in individual vials under agitation on an orbital shaker (SK300, Nova Analítica, São Paulo, SP, Brazil) for 1 min. Then, bleaching gels were applied to the enamel surface (0.04 mL) for 40 min, using a dosing syringe and a micro brush (KG Sorensen, Cotia, SP, Brazil). For 14 days, 3 bleaching sessions were performed at 7-day intervals. The gels were removed with gauze, followed by

washing with deionized water for 30 s. Between bleaching sessions, the discs were kept in individual plastic containers containing 2 mL of artificial saliva (1,5 mmol/L of Ca (NO₃)₂.H₂O; 0,9 mmol/L of NaH₂PO₄.4H₂O; 150 mmol/L de KCl; in 0.1 mol/L of sodium cacodylate buffer, pH 7.0); and renewed every day [6].

2.2.3. Calculation of total color alteration and whiteness index for dentistry

The enamel/ dentin discs were fixed in black silicone supports with a diameter of 5.7 mm and a thickness of 3.5 mm, standardizing the incidence of the light beam in the Visible Ultraviolet Reflection (UV-2450 Model, Shimadzu, Kyoto, Japan), with wavelength ranging from 400 nm to 700 nm, under standard lighting D65 and an illumination/observation angle of 45/0°. The measurement of colors was performed on the vestibular surface of the enamel after preparation of the discs and their pigmentation, 1st, 2nd, and 3rd bleaching sessions, as well as on the 7th and 14th days after the end of bleaching. Then, the absolute differences (Δ) of the colors coordinates (L*, a*, b*) were calculated between the time analysis (post-staining, post-bleaching, and after 7 and 14 days) and the initial values: ΔL^* (positive value indicates more brightness, a negative value indicates darker), Δa^* (positive value indicates redder, a negative value indicates greener) and Δb^* (positive value indicates more yellow, negative indicates more blue). To determine the total color change between the three coordinates, the following formula was used: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [6,25]. Subsequently, the whiteness index for dentistry (WI_D) was determined according to the following equation: $\Delta WI_D = 0.511 L^* - 2.324 a^* - 1.100 b^*$ [26]. The CIEDE2000 system was used and the values of ΔE_{00} were calculated by the formula [27]: $E_{00} = (\Delta L/K_L \times S_L) + (\Delta C/K_C \times S_C)^2 + (\Delta H/K_H \times S_H)^2 + R_T \times (\Delta C/K_C \times S_C) \times (\Delta H/K_H \times S_H)^{0.5}$, where ΔL^* , ΔC^* and ΔH^* are the differences in brightness, chroma, and hue between two specimens, and R_T (rotation function) is a function that explains the interaction between chroma and hue differences in the blue region. S_L , S_C , and S_H are the weighting functions for the luminance, chroma, and hue components, respectively. K_L , K_C , and K_H are the parametric factors according to different visualization parameters that were defined as 1.

2.3. Surface and cross-sectional hardness and roughness analyses

The enamel surface of the enamel/dentin discs was flattened and polished, to remove approximately 120 μ m of the surface enamel, according to a previous study [6]. After

polishing, the samples were submitted to initial surface hardness readings (SH) (Micromet 5114, Buehler, Lake Bluff, IL, USA), using a Knoop diamond under static vertical load with 25g for 10 s [6,9], and also to initial roughness (Ra) using a profilometer (model SJ-401, Mitutoyo, Kawasaki, Japan) with operating with a radius of 2 mm, constant speed of 0.1 mm/s with a load of 5 N and a cut value of 0.25 mm [28]. Enamel/dentin discs, with initial SH values between 342.0 and 378.0 KHN, were randomly distributed (Excel, Microsoft Corporation, USA) in the 5 previously defined experimental groups ($n = 12$). After the 3 bleaching sessions, as described above, surface hardness was done again (final SH), and final Ra was also measured.

After SH and Ra determination, the enamel/dentin discs were sectioned in half, and one of the halves was embedded in acrylic resin and polished [6]. Knoop hardness in the cross-sectional was determined with a load of 5 g/10 s at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160 e 180 μm from the surface. The integrated area (AI) under the curve of hardness values ($\text{KHN} \times \mu\text{m}$) was calculated by the trapezoidal rule using the software GraphPad Prism (Prism 7 for Windows, version 7.00, San Diego, CA, USA) [9].

2.4. Determination of transamelodentinal diffusion of H_2O_2

To quantify the amount of H_2O_2 that permeated the dental tissues, enamel/dentin discs ($n = 10/\text{group}$) were placed in an artificial pulp chamber (APC) between two silicone rings (5.60 mm internal diameter; 1.78 mm thick; Ref. OR 008 - Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) and sealed with melted pink wax nº 7 (Wilson®, Polidental, Cotia, SP, Brazil) restricting the lateral penetration of the bleaching agent, according to a study by Briso et al. [29]. APCs were placed individually in 24-well cell culture plates (Costar Corp., Cambridge, MA, USA). Each well was filled with 1 mL of sodium acetate buffer solution (2.0 mol/L, pH 4.5 by acetic acid, Sigma Chemical Co, St Louis, MO, USA) and subsequently received the APCs, already containing the disks of enamel/dentin. Thus, the dentin surface remained in contact with the acetate solution during the bleaching protocol, where the bleaching gels were applied once as described above (item 2.2.2) [29]. Then, the amount of 500 μL of buffer solution plus extract was transferred to experimental tubes to react with leuco-crystal violet (0.5 mg/mL; Sigma Chemical Co, St Louis, MO, USA) and horseradish peroxidase enzyme (1 mg/mL; Sigma Chemical Co, St Louis, MO, USA) [29]. The final reaction volume was adjusted to 3 mL with deionized water inserted into a cuvette and the optical density of the solutions was measured at a wavelength of 596 nm (UV-2450,

Shimadzu, Kyoto, Japan) [30]. A standard curve (0.5–5.0 $\mu\text{g/mL}$) was used to convert the optical density obtained in the samples into lg/mL of H_2O_2 .

2.5. Scanning electron microscopy surface examination

Two specimens from each group were examined by scanning electron microscopy (SEM - Carl Zeiss, EVO ILS15, Carl Zeiss NTS LTD, Germany). All discs were gradually dehydrated in an ascending ethanol series (50, 60, 70, 80 and 100%) and coated in gold (Quorum - Q150T E), and then analyzed in SEM at 20 kV under $\times 5000$ magnification [31]. Chemical elemental quantification (atomic %) of calcium (Ca), phosphorus (P), oxygen (O) and fluoride (F) on the enamel surface [32] was determined using energy dispersive X-rays (EDX, Oxford Instruments, INCAx - act, 133 eV, England), spatial resolution at $\sim 2 \mu\text{m}$, and the count time was 7000 s.

2.6. Statistical analysis

The Sigmaplot® for Windows version 12.0 statistical program was used, with a significance level of 5%. For the color alteration data, the different bleaching gels and the time of analysis were considered as variation factors and, as variables, the parameters ΔE , ΔE_{00} , ΔWI_D , SH, and Ra. The data were homogeneous and submitted to analysis of variance (ANOVA) of repeated measurements, followed by the Student-Newman-Keuls multiple comparison test. For the analysis of the integrated area (AI) and diffusion of H_2O_2 , the values were considered as variables, and the bleaching gels as a factor of variation. The values were homogeneous and were submitted to one-way ANOVA followed by the Student-Newman-Keuls test.

3. Results

The bleaching agents evaluated promoted a significant change in ΔE after bleaching sessions ($p < 0.001$) in previously pigmented enamel (Fig 1A-C). No differences in ΔE , ΔE_{00} , and ΔWI_D were observed among the groups at any bleaching application, or throughout the bleaching sessions ($p > 0.05$) (Fig 1A-C). All treatments showed similar bleaching effects, regardless of the period evaluated ($p > 0.05$). The color change was constant from the first bleaching session (Fig. 1A-C). In ΔWI_D , only the HP group showed significant partial

regression in whiteness values 7 days after the bleaching procedures ($p < 0.05$), however, similar values were found between groups 14 days after bleaching completion (Fig. 1B).

All bleaching gels showed a reduction in hardness after the bleaching procedure ($p < 0.001$; Table 1), and the greatest reduction in hardness value was for the HP group. Compared to the HP group, the addition of F reduced the loss of surface hardness (SHf: $p < 0.001$), and the bleaching gel containing HP/F/TMPnano presented the highest final surface hardness value ($p < 0.001$; Table 1). The integrated hardness ($\text{KHN} \times \mu\text{m}$) showed a higher value when the HP/F/TMPnano bleaching gel was applied to enamel ($p < 0.001$), being 13% and 15% higher compared to the HP and HP/Ca groups, respectively. Furthermore, the addition of F and/or TMPnano promoted the smallest changes in enamel roughness; and the HP group provided higher roughness values after bleaching (Table 1). The bleaching gels HP and HP/Ca showed the highest values of transamelodentinal diffusion of H_2O_2 and the HP/F/TMP nano group had the lowest value, being 27% and 23% inferior to the values of the mentioned gels, respectively ($p < 0.001$; Table 1).

The enamel surfaces of the teeth, observed before carrying out any treatment (sound enamel), were seen to be uniform or with fewer irregularities (Fig. 2A), and a Ca/P ratio of 1.59 (Fig. 2B). The samples subjected to the bleaching protocol showed changes and moderate irregularities in enamel, mainly for the HP group (Fig. 2C) which presented Ca/P ratio of 1.52 (Fig. 2D). Enamel prism exposure with protruding interprismatic enamel was observed in the bleached groups, mainly for the HP/TMPnano group (Fig. 2G); nevertheless, the groups with TMPnano had fewer changes on the surface compared to other groups (Fig. 2G and 2I). HP/F/TMPnano group (Fig. 2I) presented a Ca/P ratio of 1.58 (Fig. 2J), and a surface similar to that observed in the unbleached enamel (Fig. 2A).

4. Discussion

Although dental bleaching is considered a traditional and conservative aesthetic treatment, there are guidelines, precautions, and certain concerns regarding its biocompatibility, especially when highly concentrated bleaching agents are applied. Currently, novel bleaching protocols are being developed to increase the clinical safety and use of the procedure. In the present study, the addition of F/TMPnano did not change the aesthetic performance of the bleaching gel at 35% H_2O_2 ; however, the presence of F/TMPnano in the gel reduced mineral loss, surface roughness, transamelodentinal diffusion of H_2O_2 and morphological changes. Thus, the null hypothesis was partially rejected.

The results of this study indicate that the incorporation of F and/or TMP nano did not interfere with the whitening efficacy of the product, as the analyses showed that in all bleaching gels evaluated the mean total color change (ΔE) was higher than 3.3 (Fig. 1A), which is considered a clinically accepted standard value for noting color difference per perception with the naked eye [33]. Furthermore, for all evaluated groups, ΔWID values (Fig. 1B) were positive after bleaching sessions and ΔE_{00} was also greater than 3 (Fig. 1C). This is important data, since for a clinically perceptible color change, $\Delta E_{00} > 0.8$ is required [34], which reinforces the whitening capacity of the gels, regardless of the addition of tested agents. As observed in previous studies [6,9], the color change is intense in the first bleaching session and less constant in the subsequent ones; this is because the darker chromophores react more easily with free radicals, so it provides a great visual effect immediately in the first application of the bleaching agent with high concentration. It is important to report that testing previously pigmented enamel makes it possible to observe such findings, as well as to directly compare the performance of gels with supplemented agents. Furthermore, it was verified that the gels maintained chromatic stability (without regression) and no differences were observed between the experimental groups. A recent review showed that individual ΔE values and ΔE_{00} values are compatible with the visual perceptibility as well as the effectiveness of the bleaching procedure [34]. Therefore, the inclusion of these three objective parameters (ΔE , ΔE_{00} , and ΔWID) provides a more precise and broader discussion.

To obtain a bleaching effect, it is essential that the hydrogen peroxide is able to penetrate through the enamel and dentin, reaching the chromogenic molecules in the tooth structure [8,25]. However, this substance can promote changes in mineralized dental tissues, as well as being toxic to the pulp tissue, since the greater the penetration of hydrogen peroxide inside the pulp chamber, the more aggressive the bleaching treatment [8,15]. Several studies suggest that bleaching leads to changes in enamel surface structure (erosion and mineral loss) and these, in turn, alter the biomechanical properties of enamel [6,13,28]. Thus, the mechanical (Table 1) and topographic (Fig. 2) alterations observed in the present study reaffirm the conception that bleaching also promotes an erosive process [31]. Furthermore, bleaching also directly affects the organic protein components of the teeth [35], due to the penetration of hydrogen, as well as aggressive treatment that can also lead to changes in the mineral phase, resulting in visible morphological changes of the tooth surface [12,13], as well decreased Ca/P ratio in enamel chemical composition as observed in high-concentrated peroxides (Fig. 2C and 2D), and indicated by previous reports in the literature [32,36].

The addition of F/TMPnano to the bleaching gel produced a smaller reduction in hardness, enamel roughness, and hydrogen peroxide diffusion when compared to the HP group. These results were expected since the synergistic action of F/TMP has already been reported in relation to its beneficial effect on erosion processes [22] and dental caries [18,37,38]. Furthermore, its protective capacity during bleaching may be due to the fact that TMP is adsorbed on enamel by binding to the OH^- groups of hydroxyapatites [19] forming a $\text{TMP-Ca}^{2+}\text{-PO}_4^-$ and/or $\text{TMP-Ca}^{2+}\text{-F}$ layer on the enamel [39]. Thus, the TMP layer can bind to anions (HO_2^- ; OH^-) and cations (H^+) derived from H_2O_2 dissociation, which explains the more pronounced effect on enamel demineralization, mainly in depth ($\text{KHN} \times \mu\text{m}$; Table 1). However, it is not able to completely prevent alterations in enamel roughness and morphology.

Furthermore, the HP group had the lowest KHN values, the greatest positive variation in roughness (Ra) values, topographic alteration, and reduction in enamel calcium and phosphorus values when compared to the other bleached groups. These alterations are probably related to gels with concentrated H_2O_2 , capable of causing a modification/dissolution of mineralized structures when in contact with tooth enamel [9,13]. The change in physical properties after bleaching is associated with the effects of demineralization caused by diffusion H_2O_2 and also by the acidic effect of bleaching gels [6,40]. The SEM images confirmed the results observed for the HP group, showing greater demineralization and an increase in the number of striations, scratch marks, and pitting on the enamel surface, when compared to the groups bleached with F and/or TMP nano. On the other hand, the addition of TMPnano to the bleaching gel showed an enamel surface with fewer irregularities and deformities than the HP group, possibly allowing less attack and promoting a protective effect on the enamel surface (Fig. 2G and 2I). Although such an effect minimized the topographic alteration, it was not enough to prevent the loss of hardness and roughness, which can be related to the apparent exposure of the enamel prisms observed also observed in the SEM (Fig. 2G). Moreover, it is interesting to note that the HP/F group showed greater topographic changes than the HP/TMPnano group, however, the presence of F in the gel composition allowed a higher hardness value (Table 1), probably attributable to the protective activity surface (Des/Re processes) that fluoride exerts on mineralized dental tissues [9,38]. The HP/F/TMPnano group, on the other hand, presented the best results in all analyzed variables, possibly due to the combination of the good properties of TMP in the nanometer scale associated with the synergistic effect with F [18,21]. In addition, this protective effect is also evidenced by the higher proportion in the calcium/phosphorus ratio (atomic %) verified in the EDX analysis (Fig. 2J).

The nanotechnology is also attributable to the good results because composites containing nanoparticles have better physical and mechanical properties when compared to traditional composites [41] and are attractive to the development of more composites acting in the processes of demineralization and remineralization of dental enamel [21]. In previous studies, it was verified that the addition of TMP nano to fluoridated vehicles showed a superior effect than conventional products and TMP counterparts on the micrometer scale [18,21,22,37], as particles reduced to nanometer size optimized their effect on the formulation compared to larger particles, which makes them more reactive [18,21].

It has been shown that after tooth bleaching, using fluoride products minimizes mineral loss and roughness [23] or during bleaching treatment [17,28]. It is noteworthy that the present study adopted a whitening protocol simulating a clinical condition where treatment with fluoride toothpaste is performed twice daily. Possibly, the consideration of the clinical aspect reflected in minor alterations of the dental substrate could probably be more pronounced. Although enamel roughness may be associated with mineral loss, alteration of topography, or modification of light reflectance [28]; changes in roughness values, when compared to baseline (unbleached) data, were considered like previously reported results [42,43]. This variation in roughness is probably less intensified due to the daily use of fluoride toothpaste as already reported in the literature [44], and possibly may be clinically less pronounced, although it is necessary to study the effects of clinical bleaching on the dental structure. In addition, it is speculated that the change in roughness can be recovered by the action of human saliva, although such an effect was not observed with the use of artificial saliva.

Previous studies [6,9,43,45] indicated that the addition of Ca and F to bleaching gels did not confer additional protection on the enamel. These findings are corroborated in the present study, where the HP/Ca group presented similar results to the HP group, not having shown an effect in minimizing the alterations caused in the enamel as observed for the HP/F/TMPnano group. Thus, saturating the medium with a calcium source does not seem to guarantee an effect. It is known that the incorporation of remineralizing agents must be able to interact with the enamel in the presence of hydrogen peroxide and at the same time oppose the redox release of its degradation products. Fluoride and TMPnano have been shown to have the ability to be adsorbed to enamel [18,21], which possibly also justifies our good results for the gel formulated with F/TMPnano. Furthermore, our findings related to SH, KHN $\times$ μ m, and H₂O₂ diffusion values resemble the study conducted by Akabane et al., [6] who did not use TMPnano, but noted that the F/TMP association at the micrometer scale reduce enamel

changes to a greater extent than HP/Ca. Under similar experimental conditions, other studies using higher concentrations of fluoride (0.2% or 0.5%) in 35% hydrogen peroxide (office bleaching agents) also reported less loss of surface hardness compared to using fluoride-free gels without reversal of surface demineralization [40,46], and no effects were observed deep into the enamel [40]. This particularity of fluoride having a limiting action in depth on dental substrates has already been described in the literature [38,47].

In general, the data obtained in the present study must be interpreted with caution, seeing that an *in vitro* model was used to evaluate esthetic clinical procedures widely applied in vital teeth. Within this context and knowing that under physiological conditions the vital tooth presents continuous exudation of fluid through the dentinal tubules, we may suggest that transdameleontinal diffusion of H_2O_2 may be reduced, limiting its deleterious effects on the dental tissue. Furthermore, some differences are observed between laboratory conditions and general aspects involving the oral cavity. As with many other interventions, some discolorations and some continuous bleaching processes can be observed in the natural condition of the oral cavity. Therefore, it is recommended to conduct clinical trials to confirm the optimization of the bleaching product containing F/TMP nano, as well as a more accurate assessment of the biological responses to mineralized and pulpal tissues.

5. Conclusion

According to the methodology used in the present study, it was concluded that the addition of F/TMPnano to the bleaching gel does not interfere with bleaching efficacy and significantly reduces the loss of surface and cross-sectional hardness, surface roughness, transamelodontinal diffusion of H_2O_2 , as well as the morphological changes in enamel. Thus, future studies should be performed to assess the aesthetic outcome and biocompatibility of the different in-office bleaching gels.

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Declarations

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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Informed consent: Not applicable.

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Figure 1. Mean values of the alteration of: (A) total color alteration (ΔE), (B) whitening index in dentistry (ΔWI_D), and (C) color alteration by CIEDE2000 (ΔE_{00}) according to bleaching gels and time of analysis (n=12). Distinct superscript lowercase letters indicate statistical difference among the bleaching gels and each moment of analysis (Student-Newman-Keuls; $p < 0.05$). Vertical bars mean the standard deviation.

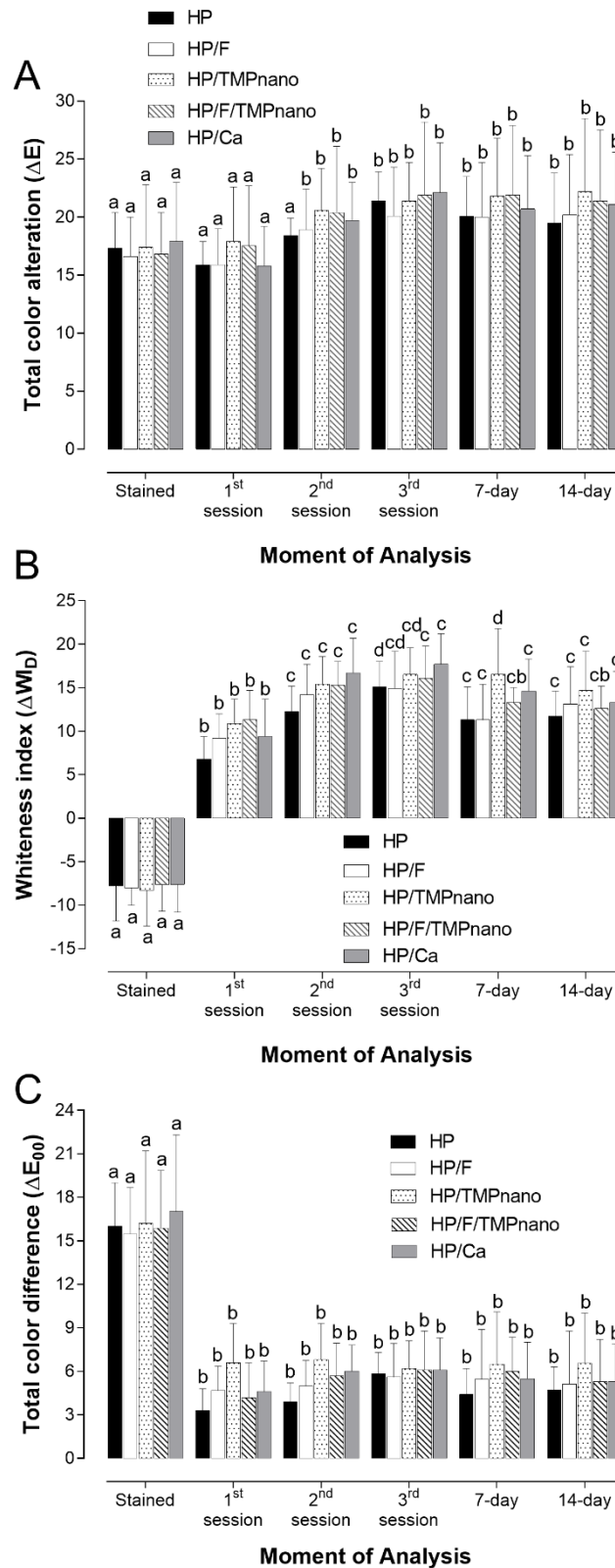


Figure 2. Photomicrographs of surface topography of enamel under SEM ($\times 5000$) and EDX representative of the (A and B) sound enamel, and experimental groups: (C and D) HP, (E and F) HP/F, (G and H) HP/TMP nano, (I and J) HP/F/TMP nano and (K and L) HP/Ca; respectively.

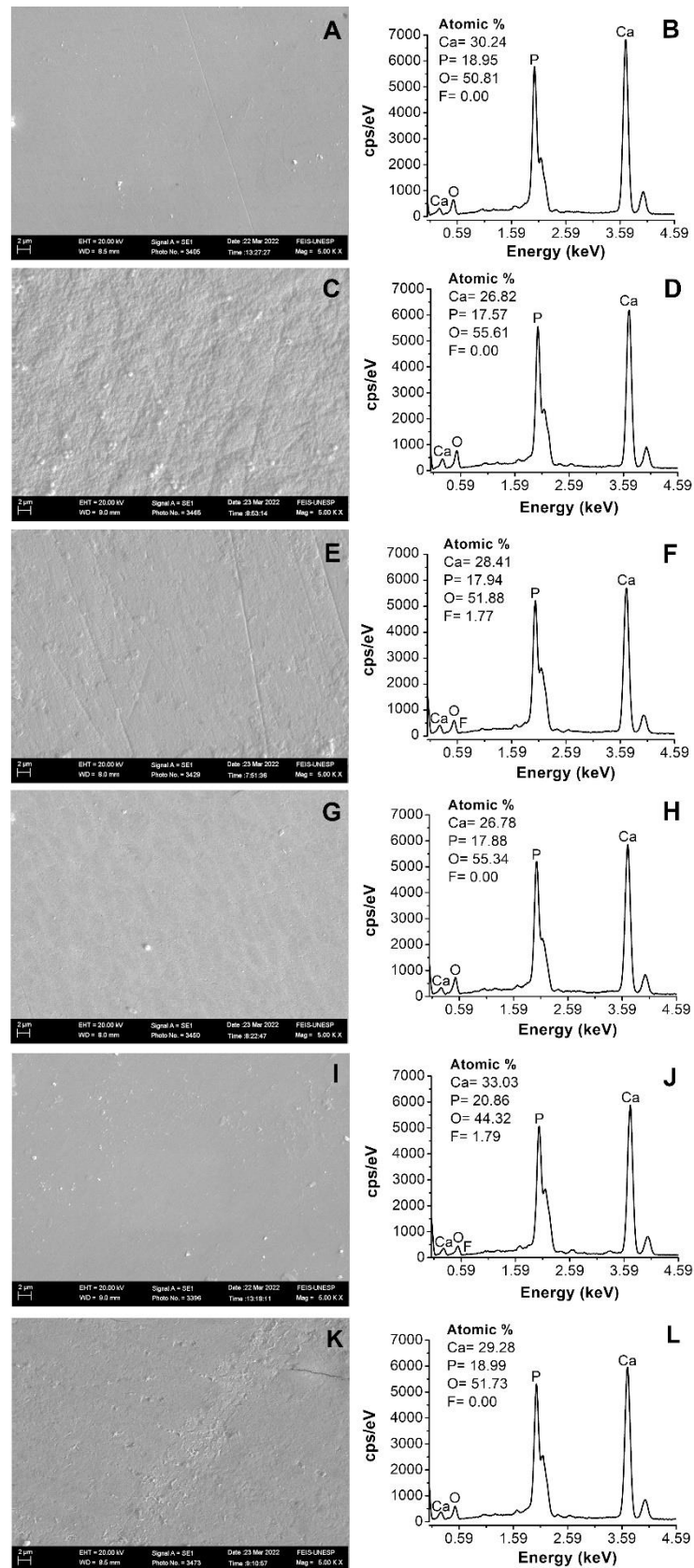


Table 1. Mean values ($\pm$ SD) of roughness (Ra) and surface hardness (SH): initial and final, integrated area (KHN $\times$ μ m), and trans-amelodentinal penetration of H₂O₂, according to experimental gels (n = 12).

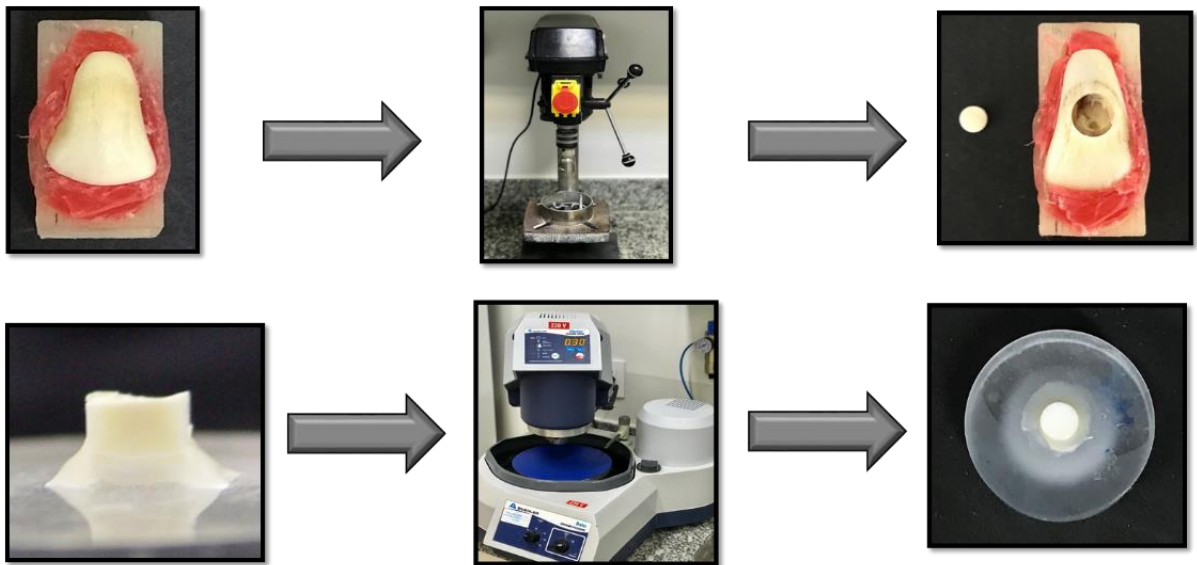
Groups	Variables					
	Ra		SH		H ₂ O ₂ (μ g/mL)	KHN $\times$ μ m
	Initial	Final	Initial	Final		
HP	0.067 ^{aA} (0.033)	0.190 ^{aB} (0.058)	370.1 ^{aA} (3.7)	312.1 ^{aB} (6.3)	7.89 ^a 0.47	18486.9 ^a (491.9)
HP/F	0.065 ^{aA} (0.025)	0.104 ^{bB} (0.038)	370.1 ^{aA} (3.9)	321.2 ^{bB} (5.9)	6.98 ^{bc} 0.75	19005.5 ^b (427.7)
HP/TMPnano	0.065 ^{aA} (0.030)	0.122 ^{bcB} (0.047)	370.2 ^{aA} (4.1)	314.9 ^{cB} (5.4)	6.41 ^c 0.68	18538.0 ^a (410.9)
HP/F/TMPnano	0.066 ^{aA} (0.031)	0.097 ^{bB} (0.034)	370.1 ^{aA} (2.4)	349.3 ^{dB} (4.1)	5.74 ^d 0.73	20919.8 ^c (389.5)
HP/Ca	0.068 ^{aA} (0.032)	0.154 ^{cB} (0.056)	370.2 ^{aA} (2.5)	314.1 ^{cB} (6.6)	7.42 ^{ab} 0.64	18241.2 ^a (503.1)

Distinct superscript lowercase letters indicate statistical differences among bleaching gels in each variable (Student–Newman–Keuls test, $p < 0.001$).

Apêndices

APPENDIX A

PREPARO DOS DISCOS DE ESMALTE

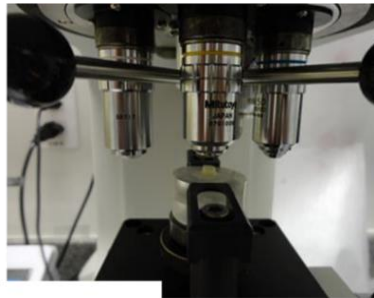


APÊNDICE B

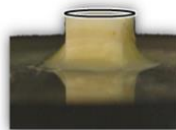
Dureza de Superfície Inicial (SH) e Tratamento com os géis clareadores



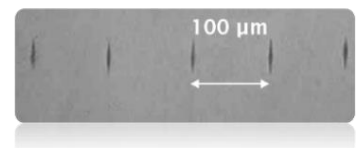
Microdurômetro Buehler



Carga de 25 gramas
Tempo 10 segundos



320-380 KHN



Tratamento com os géis clareadores

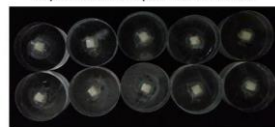


Géis
experimentais

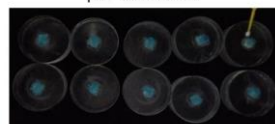


HP Blue 35%

Tratamento com Géis
experimentais por 40 minutos



Tratamento com Gel HP Blue 35%
por 40 minutos



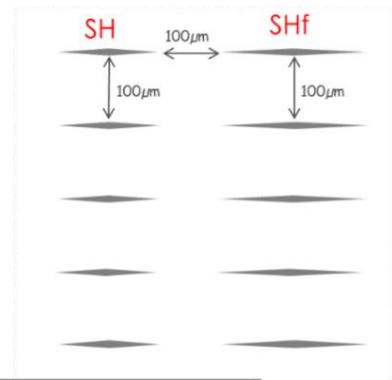
APÊNDICE C

Dureza de Superfície Final e Perda Integrada de Dureza de Subsuperfície (KHN)



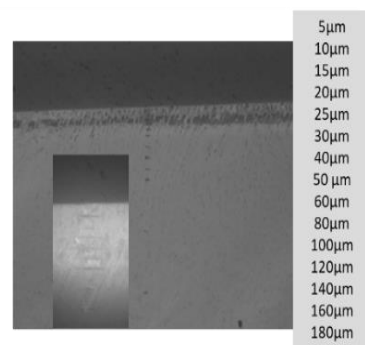
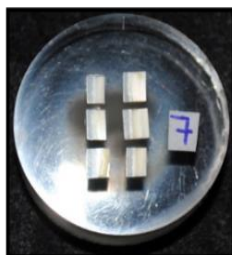
Microdurômetro Buehler

Carga de 25
gramas
Tempo 10
segundos



Cálculo da porcentagem de perda de dureza de superfície $[\%SH = ((SHF - SHI) / SHI \times 100)]$.

Perda Integrada de Dureza de Subsuperfície (KHN)



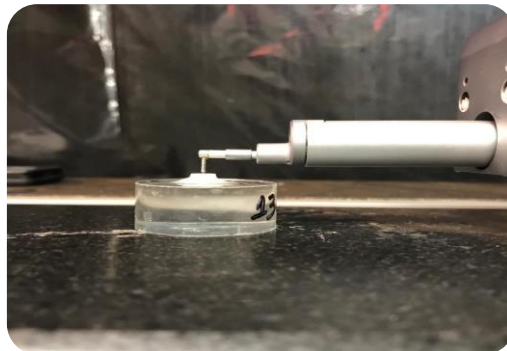
Microduromêtro Buehler
Carga 5g - Tempo 10 seg.

APÊNDICE D

Análise de Rugosidade de Superfície



- ❑ Ponta ativa de diamante de 0,002mm de raio
- ❑ Velocidade constante de 1,25 mm/s
- ❑ Carga de 0,7 mN.



APÊNDICE E

Análise da Alteração de cor

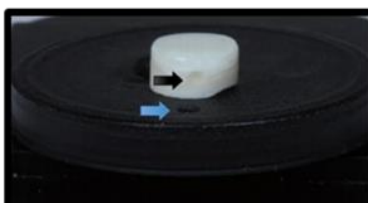
1 – Profilaxia com pedra-pomes



3 - Espectrofotômetro – Leitura Inicial



2 - Marcação para padronização das leituras



4 – Manchamento solução de Chá - Preto



2g de chá /100mL de água fervente
e 100mL por disco; 6 dias a 37°C

Armazenamento em água
durante 7 dias/37°C

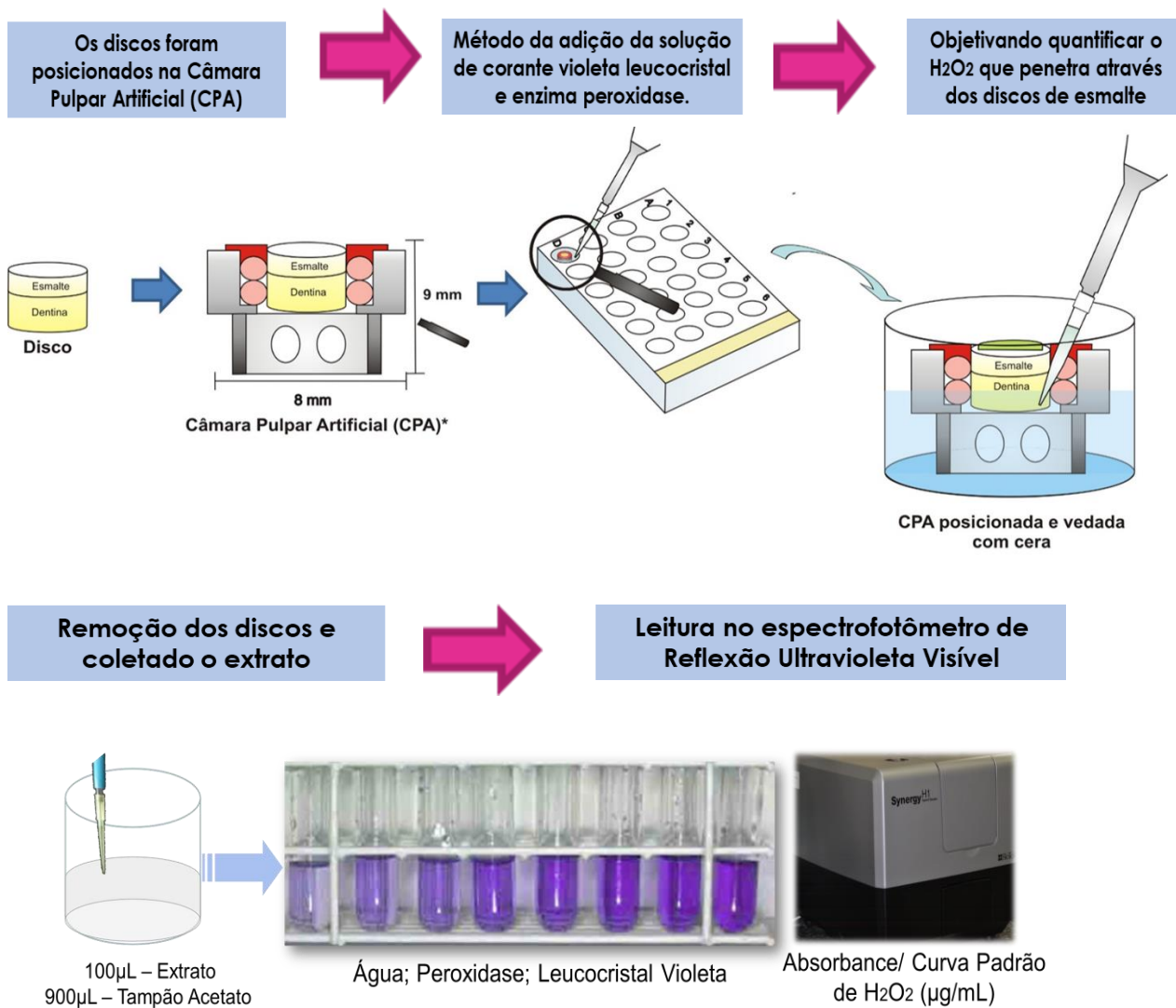
Armazenamento em ambiente úmido
com esmalte em contato com
a saliva artificial a 37°C por 24h

5 – Tratamentos: 3 sessões clareadoras

- ✓ Leitura pós sessão;
- ✓ Leitura pós 7 e 14 dias após o tratamento: estabilização cromática;

APÊNDICE F

Difusão Trans-amelodentinária



APÊNDICE G

Análise da Morfologia e Composição Química do Esmalte (MEV/EDS)



Microscópio – imagem de superfície (3 espécimes / grupo)

