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Avaliação de agente clareador para uso profissional contendo trimetafosfato e fluoreto sobre a alteração de dureza e cor: estudo *in vitro*

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Araçatuba, da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Cirurgião-Dentista

Orientador: Prof. Tit. Alberto Carlos Botazzo Delbem

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RESUMO

A busca por agentes clareadores que permitam minimizar a desmineralização do esmalte tem sido constante na literatura. Os objetivos deste estudo foram avaliar *in vitro* a adição do trimetafosfato de sódio (TMP) na concentração de 0,25% associado ao fluoreto de sódio a 0,05% e ao peróxido de hidrogênio (PH) a 35% sobre: alteração de cor e dureza superficial do esmalte. Os tratamentos foram: PH à 35% (PH); PH + 0,05% NaF (PH/F); PH + 0,25% TMP (PH/TMP); PH + 0,05% NaF + 0,25% TMP (PH/F/TMP) e HP Blue (HP). Os géis clareadores foram aplicados uma vez por sessão, em 3 sessões, com intervalo de 7 dias. A dureza de superfície (antes e após os clareamentos) para o cálculo da % da perda de dureza (%SH) foi determinada. Para analisar o efeito clareador, os blocos foram pigmentados com infusão de chá preto, e a alteração de cor foi determinada por espectrofotometria de reflexão utilizando o modelo de cores CIE L*a*b*. A análise de cor foi realizada antes e após a pigmentação, após cada sessão de clareamento e 7 e 14 dias do último clareamento. Os dados foram submetidos à ANOVA seguido pelo teste Student-Newman-Keuls ($p<0,05$). O grupo PH/F/TMP apresentou menor %SH, seguido pelos grupos PH/FPH>HP ($p<0,05$). Os valores de L* e b* não diferiram entre os tratamentos antes e após a pigmentação e após as sessões de clareamento ou 7 e 14 dias do último clareamento ($p>0,05$). Conclui-se que o gel clareador contendo PH/F/TMP reduziu a perda mineral do esmalte quando comparado a um gel clareador convencional contendo 35% de PH, com similar efeito clareador entre os géis.

Palavras-chave: Clareamento dentário. Peróxido de hidrogênio. Trimetafosfato de sódio. Alteração de cor. Dureza.

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ABSTRACT

This study evaluated the addition of sodium trimetaphosphate (TMP) and/or sodium fluoride (NaF) or calcium gluconate (CaGlu) to a 35% hydrogen peroxide (H_2O_2) bleaching gel on the color change and enamel microhardness. Bovine enamel/dentin were divided according to the bleaching gel: 35% H_2O_2; 35% H_2O_2 + 0.05% NaF; 35% H_2O_2 + 0.25% TMP; 35% H_2O_2 + 0.05% NaF + 0.25% TMP; 35% H_2O_2 + 0.1% NaF + 1% TMP and 35% H_2O_2 + 2% CaGlu. The bleaching gels were applied thrice (40 min/session) at the intervals of 7 days between each application. Then, the color change, percentage of surface hardness loss (%SH), cross-sectional hardness (ΔKHN), transamelodentinal penetration of H_2O_2, cell viability and morphology (MDPC-23 odontoblast-like cells), alkaline phosphatase activity (ALP) and deposition of mineralization nodules were determined. The data were submitted to ANOVA followed by the Student-Newman-Keuls test ($p<0.05$). All bleaching gels showed significant color changes after treatment ($p<0.001$). Mineral loss (%SH and ΔKHN) and H_2O_2 penetration were lower for 35% H_2O_2/0.1% NaF/1% TMP, and 35% H_2O_2/2% CaGlu showed higher values, compared to the other groups ($p <0.001$). ALP was higher for groups containing TMP compared to others whitening gels ($p<0.05$). The formation of mineralization nodules was greater for gels containing NaF/TMP or CaGlu ($p<0.05$). The alterations of cell morphology were intense for all bleaching gels. It was concluded that the bleaching gel containing PH/F/TMP reduced the enamel demineralization when compared to a conventional 35% PH contained bleaching gel.

Keywords: Demineralization. Dental enamel. Fluoride. Phosphate. Dental bleaching.

LISTA DE ABREVIATURAS

ACP Amorphous calcium phosphate

ALP Alkaline phosphatase activity

APC Artificial pulp chamber

ANOVA Analysis of Variance

ºC Degrees Celsius

Ca Calcium

$CaCl_2$ Calcium Chloride

CaGlu Calcium gluconate

Ca^{+2} Calcium ion

CaF^{+} Calcium fluoride ion

$CaHPO_4^0$ Phosphate hydrogenated calcium neutral

$Ca(NO_3)_2.H_2O$ Calcium nitrate tetrahydrate

CM Centimeters

CIE Commissione international de l´Eclairage

F Fluoride

g Gram

h Hour

HF^o Neutral hydrogen fluoride

KCl potassium chloride

KHN Knoop hardness unit

H_2O Water

H_2O_2 Hydrogen peroxide

Min Minutes

mg Milligram

ml Milliliter

mm Millimeter

mmol/l Milimol per liter

Mol/l Mol per liter

NaF Sodium Fluoride

NaOH Sodium hydroxide

$NaH_2PO_4.H_2O$ Sodium phosphate monobasic monohydrate

nm Nanometers

pH Hydrogen potential

ROSs Reative oxygen species

s Seconds

SHi Initial surface hardness

SH_f Final surface hardness

% SH Percentage of loss surface hardness

TMP Sodium trimetaphosphate

LISTA DE TABELAS

LISTA DE FIGURAS

SUMÁRIO

1. INTRODUCTION

The current dentistry has been strongly affected by great aesthetic demand, with teeth whitening being one of the most required clinical procedures by patients. This treatment can be carried out using the at-home technique, which is characterized by the daily exposure to low concentration peroxides or by the in-office technique, which uses peroxides highly concentrated in the elements to be cleared [1]. Both techniques are based on the release of reactive oxygen species (ROSs), which, due to their extreme instability, end up reacting and cleaving the chromophoric agents present in the dental structure, transforming them into smaller molecules and making the teeth lighter. However, the great challenge of whitening treatment is to offer the patient a safe, fast, comfortable and satisfactory technique [2]. Some studies report that whitening agents can cause changes in enamel morphology [3,4] and decrease in enamel hardness [5,6]. Furthermore, an enamel demineralized would facilitate the ROSs penetration leading to dental sensitivity, since it can also reach the dentin-pulp complex [5,7], resulting in inflammatory responses, cellular changes and possible pulp damage [2,8]. Therefore, it is essential to seek strategies using remineralizing agents, which added to the bleaching gels, can somehow reduce or minimize mineral loss and dental sensitivity.

New agents with remineralizing potential phosphate-based associated or not to sodium fluoride (NaF) have been studied. They can be a calcium or phosphate source [5] as calcium chloride ($CaCl_2$), calcium gluconate (CaGlu) or amorphous calcium phosphate (ACP), however without clear evidence to minimize the side effects of the bleaching agents. The other phosphate-based agents act as a barrier against acid diffusion and works as nucleators of calcium phosphate apatite-like [9-12], as the cyclophosphates. Sodium trimetaphosphate (TMP) is a cyclotriphosphate that have been demonstrated a great effect against enamel demineralization [10,13] when associated to low-fluoride toothpaste [9,10]. According to studies on enamel demineralization, TMP can assist in the mineral retention of hydroxyapatite, forming a more stable crystal [11]. Therefore, it would be valid to evaluate whether the addition of TMP to bleaching agents with a high concentration of hydrogen peroxide (H_2O_2) can inhibit mineral loss.

Based on previous studies [9,23,24], TMP would have the ability to reduce the H^+ ions diffusion into the enamel, reducing demineralization, as well as it would bind to anions from H_2O_2 minimizing the dentin-pulp complex damages. However, it is necessary to verify whether TMP would not reduce the whitening ability when added to H_2O_2. Given the above, knowing the good properties of TMP and NaF, it would be interesting to evaluate the formulation of a new bleaching gel for professional use with these agents that aims to potentiate the inhibiting effect on the enamel demineralization, maintaining the bleaching activity of the H_2O_2.

2. MATERIALS AND METHODS

2.1 Experimental Design

Enamel/dentin disks (5.7 mm in diameter × 3.5 mm in thickness) were obtained from bovine incisor teeth. The disk were cleaned in deionized water and stored in physiological saline solution containing 0.1% thymol at 4°C [14]. Next were randomly into six experimental groups whitening gel (n=24/group) containing 35% H_2O_2 and NaF and/or TMP. After pigmentation of the enamel disks, bleaching gels were applied once (40 min/session), every 7 days, totaling 3 sessions (21 days). The color alteration was analyzed after pigmentation, 1st, 2nd and 3rd whitening session, and 7 and 14 days after whitening.

2.2 Bleaching Gels Formulation and Experimental Groups

The whitening gels were manufactured by Oralls - Innovation in Oral Health Company (São José dos Campos, SP, Brazil), without pigmentation and packaged in applicator syringes. The base components of the gels were composed of thickener (12% Carbopol), bleaching agent (35% H_2O_2), glycerin and water (q.s.), and NaOH required maintaining pH of approximately 7.0. Depending on the experimental group, 0.05% or 0.1% NaF and/or 0.25% or 1% TMP was added [9,10]. A market bleaching gel containing 35% H_2O_2 and 2% CaGlu, neutral pH (Whiteness HP Blue, FGM, Joinville, SC, Brazil), was used as a positive control. Thus, six experimental groups were defined: 1) whitening gel containing 35% hydrogen peroxide (35% H_2O_2); 2) whitening gel containing 35% H_2O_2 + 0.05% NaF (35% H_2O_2/0.05% NaF); 3) whitening gel containing 35% H_2O_2 + 0.25% TMP (35% H_2O_2/0.25% TMP); 4) whitening gel containing 35% H_2O_2 + 0.05% NaF + 0.25% TMP (35% H_2O_2/0.05% NaF/0.25% TMP); 5) whitening gel containing 35% H_2O_2 + 0.1% NaF + 1% TMP (35% H_2O_2/0.1%

NaF/1% TMP) and; 6) commercial whitening gel containing 35% H_2O_2 + 2% calcium gluconate (35% H_2O_2/2% CaGlu).

2.3 Color Alteration Analysis

2.3.1 Selection and Distribution of Enamel/Dentin Disks

After obtaining the disks, a preliminary initial reading of the color values was carried out, according to the Commissione Internationale de l'Eclairage (CIE) which uses the CIE L*a*b* color system [14,15]. The enamel/dentin disks were fixed on 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 spectrophotometer (Model UV-2450, Shimadzu, Kyoto, Japan), with wavelength ranging from 400 nm to 700 nm, D65 standard illumination and illumination/observation angle 45/0º. The readings were performed on the enamel face, defining the initial L*a*b* values of the enamel/dentin disks as standard in this experiment. The disks were randomly divided (Excel, Microsoft Corporation, USA) into 6 experimental groups (n=12) with mean values (SD) varying between: L* = 62.72 (2.95) and 65.01 (1.18) ($p = 0.170$); a* = -1.21 (0.28) and -0.86 (0.26) ($p = 0.087$); and b* = 5.02 (1.50) and 6.10 (1.24) ($p = 0.396$).

2.3.2 Pigmentation of Enamel/Dentin Disks

For the pigmentation of the enamel/dentin disks, these were stored in microtubes (Kasvi K6-0150, 1.5 mL, São José dos Pinhais, PR, Brazil) containing 1 mL of black tea infusion at room temperature. The infusion was made using 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) for each 100 mL of distilled/deionized water [15]. The pigmentation process was monitored for 6 days, and the infusion was changed daily. After pigmentation was complete, the color alteration was determined as previously described.

2.3.3 Bleaching Gels Treatments and Color Alteration Measurement

After pigmentation, the whitening gels were applied to the enamel surface (0.04 mL), for 40 min, utilizing dosing syringe and microbrush (KG Sorensen, Cotia, SP, Brazil). During 21 days, 3 whitening sessions were performed with an interval of 7 days.

The gels were removed with gauze followed by washing with deionized water for 30 s. Amongst whitening sessions, the disks were kept in individual plastic containers containing 2 mL of artificial saliva [14] with the following formulation: 1.5 mmol/L de $Ca(NO_3)_2 \cdot H_2O$; 0.9 mmol/L de $NaH_2PO_4 \cdot H_2O$; 150 mmol/L de KCl; in 0.1 mol/L of sodium cacodylate buffer, pH 7.0 [9]. Artificial saliva was renewed every day. The measurement of the colors were performed after the 1st, 2nd and 3rd whitening session, as well as at 7 and 14 days after the whitening was finished. After that, it were calculated the absolute differences (Δ) of the color coordinates (L*, a*, b*) between the time analysis (post-stained, post-bleaching sessions and post 7 and 14-days) and initial values: ΔL* (positive value indicate more light; negative indicate more dark), Δa* (positive indicate more red; negative indicate more green) and Δb* (positive indicate more yellow; negative indicate more blue). To determine the total color alteration among the three coordinates, the following formula was used: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [14,15].

2.4 Surface and Cross-sectional Hardness Analysis

The enamel/dentin disks had their enamel surface flatted. After polishing, the initial surface hardness (SHi) was performed using Micromet 5114 hardness tester (Buehler, Lake Bluff, USA) with Knoop diamond indenter under 25 g for 10 s. In each enamel/dentin disk, five indentations were made with a distance of 100 µm from each other [9,13]. The enamel/dentin disks were selected by SHi and randomly divided (Excel, Microsoft Corporation, USA) into the 6 previously defined experimental groups (n=12) with mean values (SD) ranging from 363.9 (6.3) to 366.7 (5.6) KHN ($p = 0.642$). Hereafter, the disks were submitted to the three bleaching sessions as previously described. After the 21-day period, the final surface hardness (SHf) was determined to calculate the percentage of loss of surface hardness ($\%SH = [[SHf–SHi]/SHi] \times 100$) [10].

After determining of SHf, the enamel/dentin disks were sectioned in the center, and one of the halves were included in acrylic resin and polished as previously described. The cross-sectional hardness was performed using the Micromet 5114 Knoop hardness tester, with a load of 5 g for 10 s. Three sequences of equidistant indentations were performed 100 µm from each other, and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160 and 180 µm of the surface. The mean values at all 3 measuring points at each distance from the surface were then averaged. The integrated area below the curve (KHN × µm) using the hardness values was calculated by the trapezoidal rule [9,13].

2.5 Statistical Analysis

The statistical program Sigmaplot® for Windows version 12.0 was used, with a significance level of 5%. For the color alterations data, the different bleaching gels and the time analysis were considered as variation factors and, as variables, the CIE parameters: L*, a*, b* and ΔE. The data were heterogeneous and were subjected to two-way analysis of variance (ANOVA) of repeated measures followed by the Student-Newman-Keuls multiple comparison test. For hardness analysis, the values were considered as variables; and bleaching gels as a variation factor. The values were homogeneous and were submitted to one-way ANOVA followed by the Student-Newman-Keuls test.

3. RESULTS

The process of pigmentation of the enamel sample reduced the luminosity (Figure 1A), increased the red and yellow chromatography (Figure 1B and 1C), with great rise in the total difference of color among the coordinates (Figure 1D) and no difference among the groups. After the 1st bleaching session, the enamel became lighter, less red and bluer. With the 2nd and 3rd sessions bleaching, lighter enamel was established with a low content of red and a higher content of blue, differing the 1st session. This was reflected in the increase in the total color alteration (Figure 1D). Chromatic stability did not differ statistically after 7 and 14 days from last bleaching session, except the 35% H_2O_2/0.05% NaF group.

All bleaching gels led to hardness loss (Table 1) and the greatest loss of hardness was approximately between the depths of 0 and 40 µm into the enamel for all groups tested (Figure 2). Compared to the 35% H_2O_2 group, the addition of NaF reduced the surface hardness loss (SHf: $p<0.001$; %SH: $p<0.001$), the TMP reduced the hardness loss in the inner part of the enamel ($p=0.036$), and CaGlu had the highest losses ($p=0.004$; $p<0.001$; $p<0.001$, respectively). Bleaching gel containing 35% H_2O_2/0.1% NaF/1% TMP led to the lowest values of hardness loss ($p<0.001$; $p<0.001$; $p<0.001$;

respectively) followed by 35% H_2O_2/0.05% NaF/0.25% TMP (Table 1). In addition, it can be noted that the hardness values of the 35% H_2O_2/0.1% NaF/1% TMP group were higher in all profiles (Figure 2).

4. DISCUSSION

It is extremely important to maintain the functional properties and clinical effectiveness of a product when adding and/or changing its chemical composition. The addition of NaF/TMP did not alter the bleaching properties of 35% H_2O_2 gel; however, reduced the mineral loss. All bleaching gels, regardless the addition of NaF, TMP, CaGlu or association NaF/TMP, maintained the bleaching capacity of H_2O_2 producing clinically relevant results. The increase in the ΔL* values (Fig. 1A) and a decrease in the Δa* and Δb* values (Fig. 1B and 1C) clearly indicates the color change to white and a decrease in the redwish and yellowish hue, producing the whitening effect on the tooth structure [16]. These outcomes are in agreement with the Borges et al. [14] for 35% H_2O_2. For the patients, whitening satisfaction is more related with variations in the b* coordinate than a* and L* [14]. Despite being considered less important in bleaching, in the present study a* component showed great variation, probably due to the pigmentation previously produced. In this case, the reduction in a* values had a great impact on the total color change.

All gels produced the most pronounced changes in the first whitening session. These findings are in agreement with those of Kwon et al. [4], who also observed a marked whitening effect after the first session with 35% H_2O_2 and less chromatic changes in subsequent sessions. According to Sun [17], this is because the darker molecules react more easily with free radicals, causing great visual impact even in the first application of tooth whitening with a highly concentrated agent. As important as the whitening capacity, color sustaining or chromatic stability over time (7 days and 14 days after whitening) was maintained for all whitening gels. According to Bizhang et al. [18], color degradation is minimal and linear over time showing suitable color stability throughout 18-month post-treatment. The color alteration and its maintenance over time is a consequence of a large penetration of H_2O_2 in the enamel prisms and reaction with the chromophore molecules resulting in more reflection and less absorption of light [4].

The presence of remineralizing agents in the bleaching gels as calcium [5] or fluoride [5] or its associations [5] can reduce the penetration of H_2O_2. Reducing the diffusion of peroxide through enamel and dentin can lead to less whitening effect, but clinically noticeable to the naked eye [2,5]. Nonetheless, ΔE values greater than 3 would indicate an acceptable bleaching as long as it is associated with a reduction of b* values and increase of L* values [19]. This was observed for all experimental gels tested after treatment sessions (Fig. 1). Except after pigmentation that, despite the ΔE values being greater than 12, the positive Δb^* and negative ΔL^* values indicate a darkened enamel. Notwithstanding the reduction in the diffusion of H_2O_2 in the groups with TMP (Table 1), it was not possible to verify a relationship in the whitening effect (Fig. 1). These reductions were around 14% to 26%; half of the percentage observed in the study by Soares et al. [2], when there was less lightening effect. Unlike previous studies [5,20], the addition of calcium and fluoride did not reduce the penetration of H_2O_2 as well as surface and cross-sectional hardness loss (Table 1). Probably the best results with the addition of fluoride are due to a higher concentration (0.2% to 1.1% NaF) [5,20,21] than the present study (0.05% and 0.1% NaF). For calcium, there are formulations with similar concentrations to the present study (2% CaGlu) that have been shown to reduce mineral loss [22], contrary to the present data and other studies [5].

During the diffusion of the bleaching agent into the enamel prisms, the products of the degradation of H_2O_2 (reactive oxygen molecules and hydrogen peroxide anions) not only react with the dark-colored chromophore molecules, but also on the organic component (as proteins, lipids and teeth-staining substances) of the enamel [1]. Removal or degradation of the protein matrix located between the enamel prisms and hydroxyapatite crystallites can reduce the enamel's mechanical properties such as hardness, since they act as “glue” between them [1]. According to previous studies, the changes in the mineral component of the enamel produced by peroxide free radicals would only occur in the outermost part of the enamel [1,6] at 10 µm below the surface [6]. Here, it is noteworthy that all experimental gels had their pH adjusted to 7.0 to avoid enamel demineralization due to the acidity of the whitening gel [3]. Differently, the results of the present study showed a great reduction of the hardness in depth mainly in the region up to 50 µm of the surface (Fig. 2), in agreement with the study by Cavalli et al. [20] that observed hardness loss up to 60 µm depth from the outer enamel surface. This shows that H_2O_2 has not only an oxidizing effect, but also an acid effect [1]. It should be noted that in the present study, unlike previous studies, three 45-minute whitening sessions were performed, which may explain the greater mineral loss. Treatment time and number of sessions have an impact on observing changes in enamel hardness. Thus, the reduction in the values of surface and cross-sectional hardness was not only due to the degradation of the organic component of the enamel, but also the loss of its inorganic one. According to ISO 28399, a reduction of up to 10% in the enamel hardness is acceptable for whitening procedures [5]. Considering this information, only the gel containing 0.1% NaF and 1% TMP shows an acceptable surface hardness loss. We can infer that the gel with 0.05% NaF and 0.25% TMP is in the border area (Table 1). This effect is based on altering the selective permeability and diffusion of positively charged ions of CaF^+ and Ca^{2+} and/or neutral species ($CaHPO_4^0$ and HF^0), and reducing of H^+ diffusion in the enamel produced by NaF/TMP association [12]. It is possible to consider that in the innermost part of the enamel (> 80 µm; Fig. 2), the reduction of hardness derives more from the degradation of the protein matrix than the demineralization of the hydroxyapatite crystals, and the association NaF/TMP minimized these structural changes in the matrix. Taking into account what was said earlier, the addition of remineralizing agents to whitening gels should aim not only to reduce the demineralization of hydroxyapatite, but also to minimize the degradation of the enamel protein component.

Thus, the scientific evidence obtained in the present investigation for the gel with the associations of TMP and NaF can be considered promising. The bleaching gels evaluated are presented as interesting alternatives to be tested in future clinical research, which may result in the development of aesthetically viable office whitening therapies that are biologically compatible with the dentin-pulp complex, so as to effectively reduce side effects teeth whitening increased the safety of the procedure.

5. CONCLUSION

Taking into account the data obtained and the limitations of the experimental model, we can conclude that: (1) the addition of TMP and NaF in the composition of the bleaching gel to 35% hydrogen peroxide significantly reduces the loss of surface and cross-sectional hardness on the enamel; (2) the addition of NaF/TMP to the bleaching gel does not interfere in the bleaching effectiveness;

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Table 1 – Means values (SD) of final surface hardness (SHf), percentage of loss surface hardness (%SH) and integrated area (KHN × µm), according experimental gels (n=12)

Bleaching gels	**Variables**		
	SHf	%SH	KHN × µm
35% H_2O_2	294.0[a] (5.1)	-19.7[a] (1.0)	55,035.8[a] (2,678.7)
35% H_2O_2/ 0.05% NaF	306.3[b] (2.1)	-16.1[b] (0.9)	53,852.3[a,b] (1,216.9)
35% H_2O_2/ 0.25% TMP	296.0[a] (4.4)	-18.6[a] (1.3)	53,223.4[b] (950.4)
35% H_2O_2/0.05% NaF/0.25% TMP	324.6[c] (4.7)	-11.8[c] (1.1)	57,928.2[c] (1,895.5)
35% H_2O_2/0.1% NaF/1% TMP	339.0[d] (9.1)	-7.1[d] (2.1)	61,400.5[d] (1,180.8)
35% H_2O_2/ 2% CaGlu	287.6[e] (3.1)	-21.6[e] (1.1)	51,455.5[e] (1,979.2)

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

Figure 1. Means values (SD) of the alteration of: (A) lightness degree (ΔL*), (B) green/red degree (Δa*), (C) blue/yellow degree (Δb*) and total color (ΔE) by Comission Internacionale de l'Eclairage (CIE) accordint to bleaching gels and moment of analysis (n=12). Equal lowercase letters indicate that there is no difference among the bleaching gels at each moment of analysis and among the moments of analysis for each bleaching gel (Student-Newman-Keuls; $p < 0.05$).

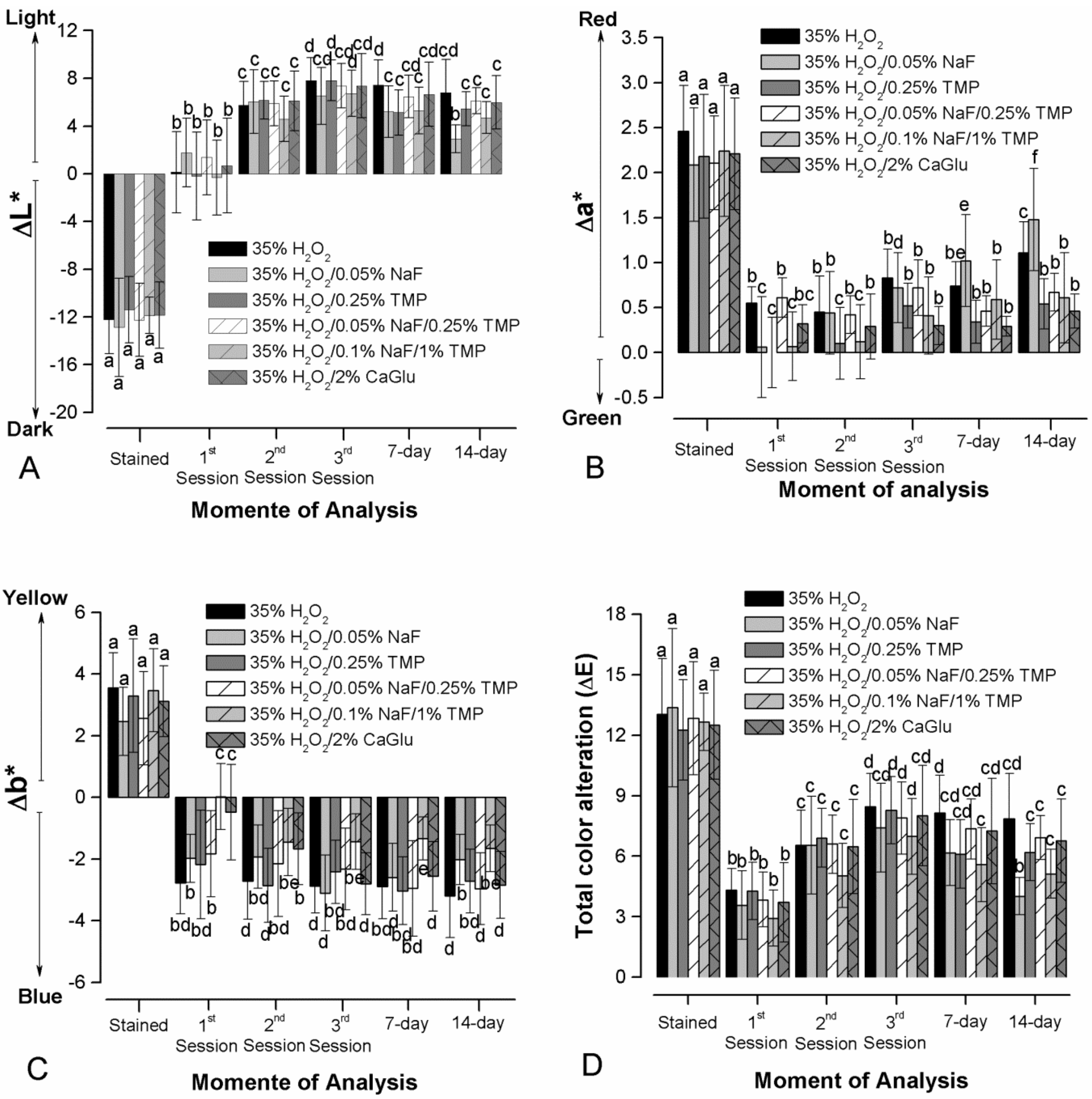
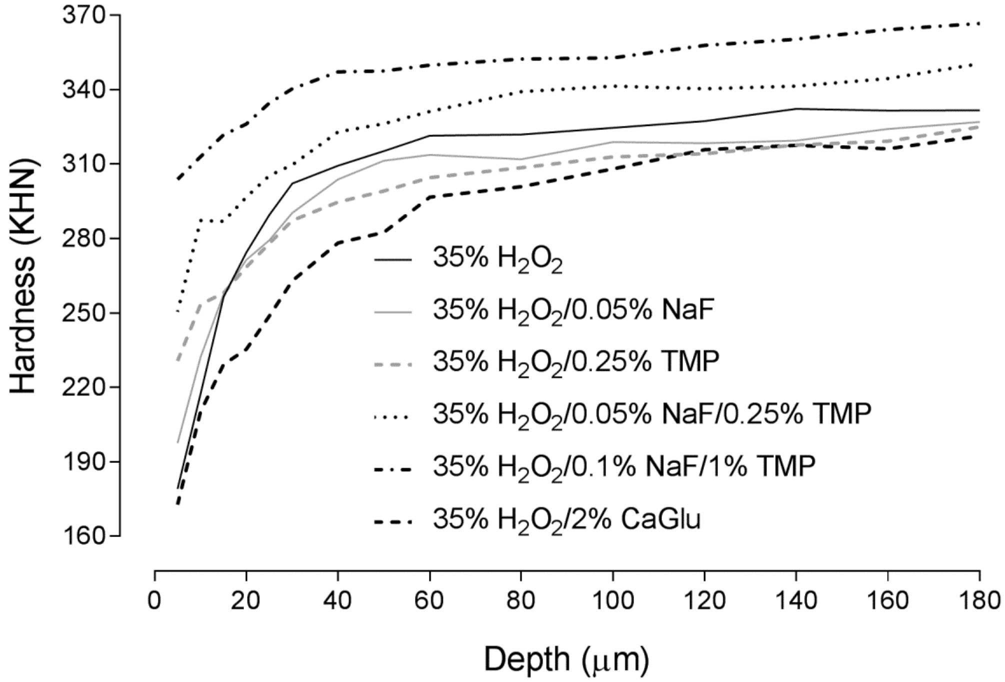
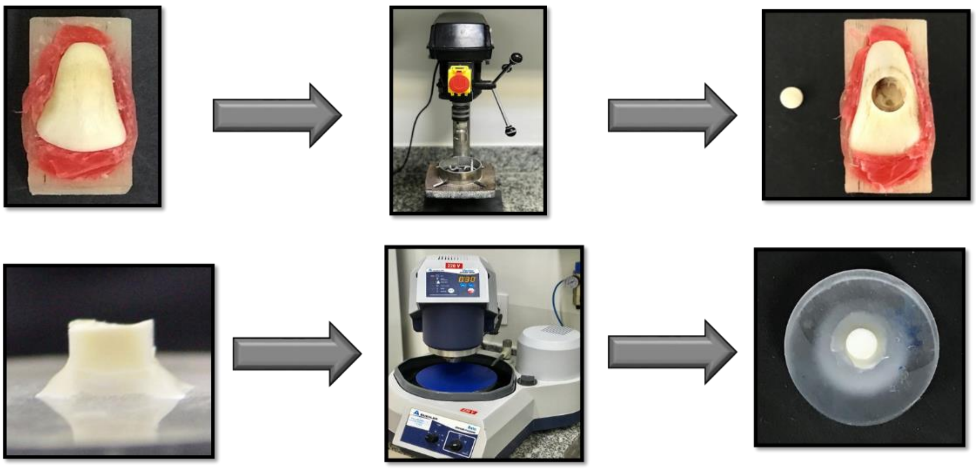


Figure 2. Cross-sectional hardness profiles at different depths in the enamel (n=12) according to the bleaching gels.

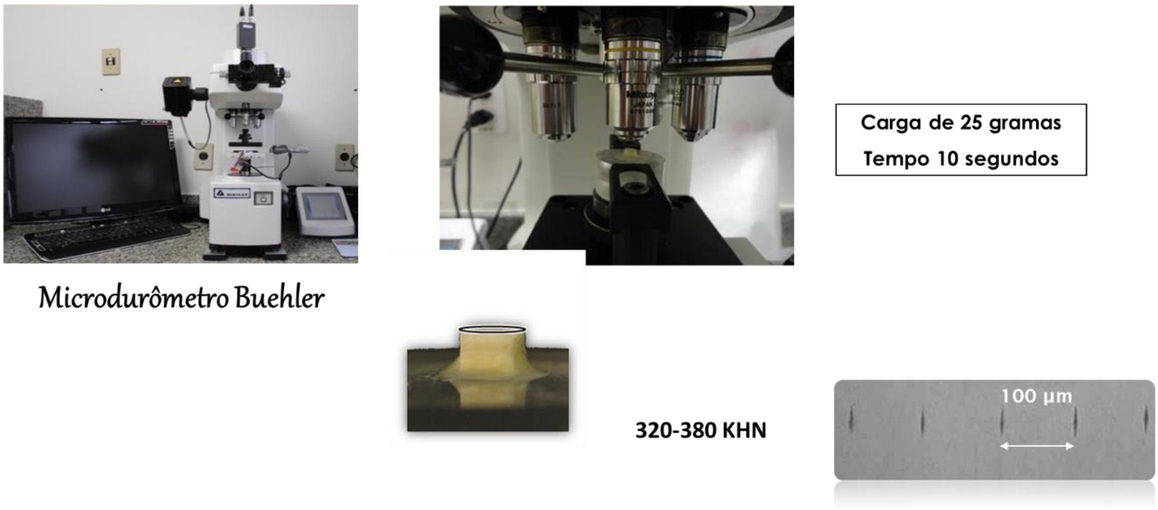


ANEXOS

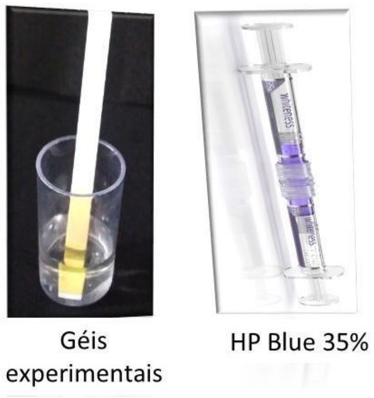
ANEXO A – Preparo dos discos de esmalte

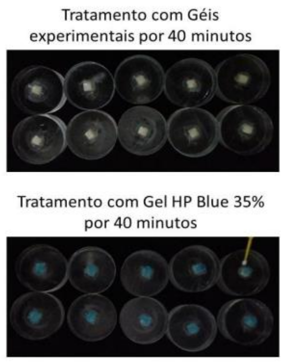


ANEXO B - Dureza de Superfície Inicial (SH)



ANEXO C - Tratamento – Sessões Clareadoras



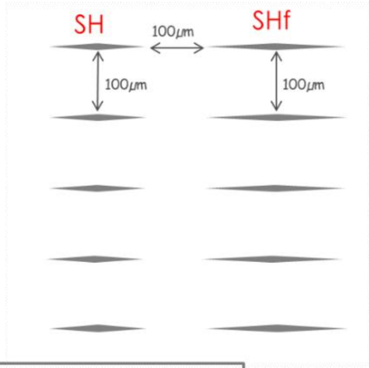


ANEXO D - Dureza de Superfície Final

Microdurômetro Buehler

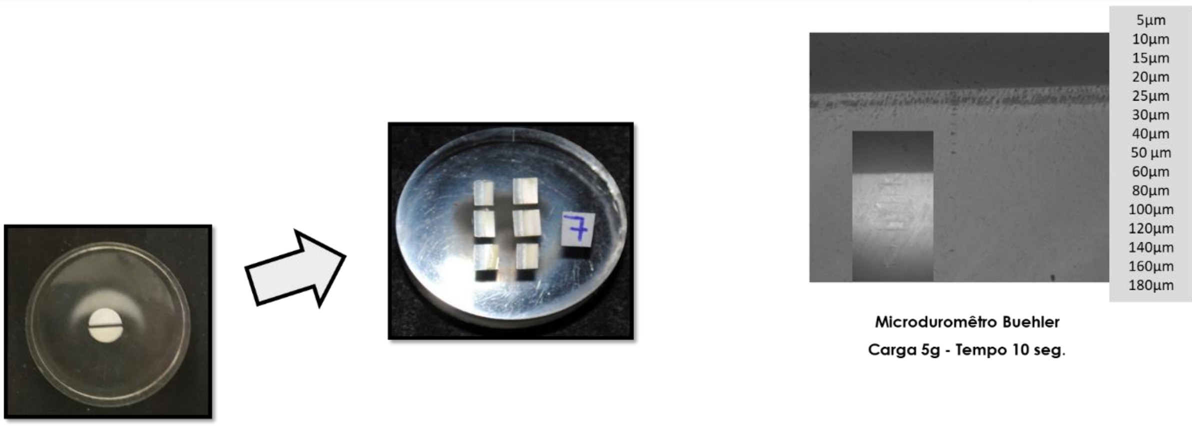
Carga de 25 gramas

Tempo 10 segundos



Cálculo da porcentagem de perda de dureza de superfície [%SH = ((SHF– SHI)/SHI x 100)].

ANEXO E - Perda Integrada de Dureza de Subsuperfície (KHN)

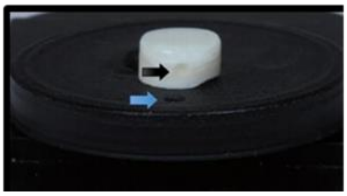


ANEXO F - Análise da Alteração de cor

1 – Profilaxia com pedra-pomes

3 - Espectrofotômetro – Leitura Inicial

2 - Marcação para padronização das leituras



4 – Manchamento solução de Chá - Preto