Ana Laura Esteves dos Santos

Avaliação de uma nova composição de agente clareador sobre a microdureza do esmalte: associação do trimetafosfato de sódio e fluoreto de sódio ao peróxido de hidrogênio a 10%

Araçatuba – SP
2014

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AVALIAÇÃO DE UMA NOVA COMPOSIÇÃO DE AGENTE CLAREADOR SOBRE A MICRODUREZA DO ESMALTE: ASSOCIAÇÃO DO TRIMETAFOSFATO DE SÓDIO E FLUORETO DE SÓDIO AO PERÓXIDO DE HIDROGÊNIO A 10%

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

Orientadora: Profª Drª Mirela Sanae Shinohara

Araçatuba - SP
2014

Dados Curriculares

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Dedicatória

Ana Laura Esteves dos Santos

À DEUS, SEMPRE EM PRIMEIRO LUGAR

Elevo meus olhos para o monte, de onde vem meu socorro?
O meu socorro vem do Senhor, que fez o céu e a terra,
Não deixará o teu pé vacilar, aquele que te guarda não dormitará,
Eis que não dormitará, nem dormirá aquele que guarda a Israel.
O Senhor é quem te guarda, o Senhor é a tua sombra á tua mão direita,
De dia o sol não te ferirá, nem a lua de noite,
O Senhor te guardará de todo mal, ele guardará a tua vida,
O Senhor guardará a tua saída e a tua entrada, desde agora e para sempre.
(Salmos 121)

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Muito obrigada pela sincera amizade que construímos, com vocês aprendi o que é uma amizade verdadeira. Obrigada por todos momentos!

"A única amizade que vale é aquela que nasce sem nenhum motivo".

(Van Shendel)

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Epígrafe

"Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós"

Antoine de Saint-Exupéry

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Sumário

Avaliação de uma nova composição de agente clareador sobre a microdureza do esmalte: associação do trimetafosfato de sódio e fluoreto de sódio ao peróxido de hidrogênio a 10%

1 Resumo

Objetivos: avaliar a ação do trimetafosfato de sódio (TMP)+fluoreto de sódio (NaF) em duas concentrações 3%TMP+0,1%NaF e 0,3%TMP+0,05%NaF, adicionados ao peróxido de hidrogênio (PH) 10%, em inibir a desmineralização do esmalte em contato com dentifrício fluoretado (DF) ou não (DP). *Métodos*: Blocos de esmalte bovino (4.0 x 4.0 mm) foram obtidos, planificados e polidos para leitura da microdureza superficial Knoop (SH) inicial (25g/5seg). 72 blocos selecionados (320-380KHN) foram divididos aleatoriamente em 6 grupos (n=12), de acordo com o gel clareador e o dentifrício: PH+DF; PH+3TMP+0,1NaF+DF; PH+0,3TMP+0,05NaF+DF; PH+DP; PH+3TMP+0,1NaF+DP; PH+0,3TMP+0,05NaF+DP. O clareamento foi realizado por 30min/dia, seguido da imersão das amostras em dentifrício (1min) durante 14 dias e entre as sessões, mantidos em saliva artificial à 37^{o}C. Em seguida, foi realizada a leitura da SH final e os blocos foram seccionados ao meio para análise da dureza em profundidade (ΔKHN) (5g/5seg). O cálculo da perda de dureza foi realizado a partir dos valores de SH/ΔKHN e submetidos à análise estatística. Imagens de Microscopia de Luz Polarizada (MLP) foram obtidas do corte longitudinal das amostras. *Resultados:* O PH+3TMP+0,1NaF+DF demonstrou os melhores resultados, seguido pelo PH+0,3TMP+0,05NaF+DF. O PH+DF e PH+DP apresentaram os menores valores de SH/ΔKHN. No entanto, a ΔKHN do PH+DF foi estatisticamente superior ao do PH+DP. As imagens qualitativas de MLP mostraram nitidamente uma desmineralização subsuperficial para os grupos PH+DF e PH+DP. *Significância:* A adição do TMP+NaF ao gel de PH foi eficaz na diminuição da perda de dureza. A aplicação do SF foi benéfica à ação do TMP+NaF no gel clareador.

Palavras-chave: clareamento dental, polifosfatos, peróxido de hidrogênio, testes de dureza, esmalte dental, desmineralização do dente.

Artigo Científico

Ana Laura Esteves dos Santos

Evaluation of a new composition of bleaching agent on enamel microhardness: an association of sodium trimetaphosphate with sodium fluoride at 10% hydrogen peroxide

Short title: Evaluation of a new bleaching agent on enamel microhardness

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Key words: tooth bleaching, polyphosphates, hydrogen peroxide, hardness tests, dental enamel, tooth demineralization.

The manuscript is according to the guide for authors of Journal of Dentistry (Anexo)

Evaluation of a new composition of bleaching agent on enamel microhardness: an association of sodium trimetaphosphate with sodium fluoride at 10% hydrogen peroxide

2.1 Abstract

Objectives: To evaluate the effects of sodium trimetaphosphate (TMP) + sodium fluoride (NaF) in two concentrations 3%TMP+0.1%NaF and 0.3%TMP+0.05%NaF, added to hydrogen peroxide (HP) 10% by inhibiting demineralization of enamel in contact with fluoridated dentifrice (FD) or not (PD). *Methods*: Bovine enamel blocks (4.0 x 4.0 mm) were flat and polished in order to perform the initial Knoop surface microhardness (SH) analysis (25g/5s). Seventy two selected blocks (320-380KHN) were randomly assigned into 6 groups (n=12), according to the bleaching gel and slurry: HP+FD; HP+3TMP+0.1NaF+FD; HP+0.3TMP+0.05NaF+FD; HP+PD; HP+3TMP+0.1NaF+PD; HP+0.3TMP+0.05NaF+PD. Bleaching was carried out 30min/day, followed by immersing the samples in dentifrice (1min) for 14 days and between sessions, stored in artificial saliva at 37^{o}C. Then, the final SH reading was taken and the blocks were cut in halves to analyze cross-sectional hardness (ΔKHN) (5g/5s). The calculation of the loss of microhardness (%SH) was carried out from the values of SH/ΔKHN and subjected to statistical analysis. Images from Polarized Light Microscopy (PLM) were obtained from the longitudinal section of the samples. *Results*: HP+3TMP+0.1NaF+FD showed the best results, followed by HP+0.3TMP+0.05NaF+FD. HP+FD and HP+PD showed the lowest values of %SH/ΔKHN. However, ΔKHN of HP+FD was statistically higher than HP+PD. Qualitative PLM images clearly showed subsurface demineralization for groups HP+FD and HP+PD. *Conclusion*: The addition of TMP+NaF to the gel of HP was effective in reducing the loss of hardness. Applying FD was beneficial to the action of TMP+NaF of the bleaching gel.

Clinical Significance: The new formulation of bleaching gel using TMP+NaF can minimize the structural alteration of enamel and consequently decrease tooth sensitivity.

2.2 Introduction

Considered as a relatively simple and non-invasive treatment, bleaching has been the first alternative to stained teeth from extrinsic discolouration to achieve a pleasing aesthetic smile.[1] The nightguard vital bleaching with carbamide peroxide (CP) 10%[2-4] has been widely studied and considered a consolidated and effective technique. However, prolonged use of the tray overnight can cause discomfort to the patient. Thus, the technique of daytime bleaching with hydrogen peroxide has been a trend,[5, 6] allowing to reduce the daytime treatment and consequently lessen the time of contact of the gel with the tooth structure.[7]

CP has a slower action and requires a long time of use, due to the breakdown of the composition of the molecules, which dissociate in urea and hydrogen peroxide (HP).[2, 5] In contrast, in HP - based gels the oxidation process occurs more rapidly due to its ready active form, penetrating in the tooth structure (enamel and dentin) and acting directly on the breakdown of macromolecules of pigments.[7, 8]

Studies show that the use of the CP or HP is effective in the home bleaching technique.[5, 7] However, they can generate sensitivity,[5, 7, 9] changes in hardness [10, 11] and in the morphology of enamel.[6, 7] The process of remineralization of enamel occurs by the uptake of calcium and phosphate ions present in saliva, which help prevent and reduce mineral loss[12] mainly due to caries[13] and erosion[14] as well as from the bleaching process.[15] Mineral loss can be effectively minimized by using these additional ion sources,[12] as has been demonstrated in studies[16-23] with inorganic phosphates such as sodium trimetaphosphate (TMP) associated with sodium fluoride (NaF).[12, 16, 20] TMP

when added in some dental materials can remineralize and prevent enamel demineralization.[16-18] These effects probably occur due to the ability of polyphosphate to adsorb to the enamel surface, altering the permeability of acids and calcium, phosphate and fluoride ions.[18, 24, 25]

Given the above, it is very important to study the action of supplements added to the bleaching agents in order to reduce mineral loss and consequently tooth sensitivity due to bleaching. The aim of this study was to evaluate the effects of TMP associated with NaF, at two different concentrations: 3% TMP+0.1%NaF[16] and 0.3%TMP+0.05%NaF[26], added in the composition of the 10% HP bleaching gel in an attempt to inhibit *in vitro* demineralization of enamel in contact with fluoridated dentifrice or not, after each daily bleaching session. The null hypotheses tested were that: 1) there is no difference in enamel demineralization exposed to different bleaching gels; 2) there is no influence of the use of fluoridated dentifrice associated with bleaching treatment in enamel hardness.

2.3 Materials and Methods

2.3.1. Experimental Design

Seventy-two bovine enamel blocks were used as experimental units for this study. The factors under study were: (1) different compositions of experimental bleaching agents, with or without TMP+NaF and (2) the use of fluoridated dentifrice or not after daily bleaching, having as a variable response the enamel microhardness on the surface and cross-sectional hardness.

2.3.2 Selection and Preparation of Specimens

Two hundred recently extracted bovine teeth were cleaned and maintained in a solution of 0.1% thymol until the beginning of the experiment. Teeth with cracks, stains and excessive wear were excluded.

The roots were separated from the crowns with the aid of a diamond blade disc coupled to an engine bench (Bethil Indústria e Comércio Ltda, Marília, SP, Brazil) and discarded . The crowns were bonded with thermoplastic material (Godiva Exata, Nova DFL, Jacarépagua, RJ, Brazil) on an acrylic base and sectioned with a diamond disc (Extec, Enfield, CT, USA) at constant water cooling coupled to a metallographic cutter (Isomet 1000 Precision Saw - Buehler, Lake Bluff, IL, USA) to obtain enamel blocks (4.0 x 4.0 mm) from the buccal middle third.

The enamel surface was flattened using silicon carbide (SiC) sandpaper in grits 600, 800 and 1200 (Extec, Enfield, CT, USA), respectively, coupled on a polisher (APL- 4, Arotec, Cotia, SP, Brazil) under constant water cooling. The final surface polishing was carried out with felt and diamond pastes in granules 1.0 and 0.5 µm (Extec, Enfield, CT, USA). Between exchanges of sandpaper

and diamond pastes, the specimens were placed in ultrasonic washer (Cristófoli Equipamentos de Biossegurança, Campo Mourão, PR, Brazil) for 5 minutes to remove debris.

After polishing the enamel surface of the blocks, analysis of the surface hardness was performed and the specimens with average hardness between 320 and 380 KHN (Knoop hardness) were selected, totaling 72 blocks to the experiment, randomly assigned into 6 groups (n=12).

2.3.3. Bleaching Treatment (Manipulation and application of Experimental Gels)

Bleaching gels were daily handled prior to their application, for they do not have stabilizers in their composition. The amounts of the components were determined in pilot studies to achieve optimal consistency and homogenous mixing of the gel. Their solid components (Table 1) were weighed on a precision scale (Adventurer, Ohaus Corp., Parsippany, NJ, USA), placed in a plastic flask where 10 % HP was added with a pipette and then mixed thoroughly. The pH of the bleaching gels was adjusted between 6-7 with pH indicator strips (©Merck KGaA, Darmstadt, Germany). In total, three different compositions were handled according to the concentrations of TMP (Sigma Aldrich Co., St. Louis, MO, USA) and NaF (Merck KGaA, CAS 7681-49-4, Darmstadt, Germany).

The experimental bleaching gel was applied with the aid of an adhesive applicator (WMRB100, Microbrush International, Grafton, WI, USA) in a standardized amount to cover the entire surface of the enamel of each block. The sides of the blocks were isolated to avoid contact and penetration of the bleaching in other areas.

Table 1. Composition of the materials used

Materials		Composition	pH
Experimental Bleaching Gels	HP	Hydrogen peroxide, Carbopol (Sodium hydroxide to adjust the pH)	6~7
	HP+3TMP+0.1NaF	Hydrogen peroxide, Carbopol, Sodium trimetaphosphate, Sodium fluoride (Sodium hydroxide to adjust the pH)	6~7
	HP+0.3TMP+0.05NaF	Hydrogen peroxide, Carbopol, Sodium trimetaphosphate, Sodium fluoride (Sodium hydroxide to adjust the pH)	6~7
Dentifrices	Fluoridated (1100 ppm F)	Carboxymethyl cellulose, Sodium methyl-*p*-hydroxybenzoate, Sodium saccharin, Peppermint oil, Glycerol, Hydrated silica (Tixosil 73.15%), Sodium lauryl sulfate, Water and Sodium fluoride	7.3
	Placebo	Carboxymethyl cellulose, Sodium methyl-*p* -hydroxybenzoate, Sodium saccharin, Peppermint oil, Glycerol, Hydrated silica (Tixosil 73.15%), Sodium lauryl sulfate and Water	7.3
Artificial Saliva		1.5 mmol/l Calcium, 0.9 mmol/l Phosphate, 0.15mmol/l Calcium chloride and 0.02 mol/l Cacodylic buffer	7.0

Treatment and permanence of gel in contact with the tooth was 30 minutes daily for 14 days (Figure 1), as indicated by manufacturers of products in the same concentration of HP. During each bleaching session, the blocks were stored at 37^{o}C. At each session, the bleaching gel was removed from the enamel surface with the aid of gauze, washed with deionized water and placed in ultrasound for 5 minutes to completely remove any residue of the material.

2.3.4 Treatment with Dentifrice

Two dentifrices with the same composition were manipulated in the laboratory (Table 1), differing only in NaF concentration, one with 1100 ppm F and the other without sodium fluoride (placebo - negative control). The quantities of total fluoride and fluoride ion[27] were determined in triplicate using a fluoride ion specific electrode (Orion 9609 -BN, Orion Research Inc., Beverly, MA, USA) connected to an ion analyzer (Orion 720 A +; Orion Research Inc., Beverly, MA, USA). The pH levels of dentifrices (Table 1) were determined using a pH electrode (2A09E, Analyser, São Paulo, SP, Brazil) calibrated with standard pH levels 7.0 and 4.0.[16]

To prepare the slurry, each dentifrice was daily weighed, placed in a glass beaker and added to deionized water at a ratio of 1:3 (w/w). With the aid of a magnetic stirrer (NT -101, Nova Técnica Equipamentos, Piracicaba, SP, Brazil), the components were mixed to obtain a homogeneous solution. Each specimen was individually immersed in 4 ml of slurry placed in containers under agitation on shaker table (SK300, Lab Companion, Minesota, MN, USA) for 1 minute (Figure 1). After contact with the dentifrice, the specimens were washed for 20 seconds with the aid of a wash bottle containing deionized water and placed in ultrasound bath for 5 minutes to remove any residue of the dentifrice. Permanence time in ultrasound bath and washing the specimens with deionized water were standardized.

After the treatment with dentifrice, the specimens were immersed in individual containers containing 4 ml of fresh artificial saliva (Table 1) and kept at 37^{o}C until the next session (approximately 22 hours).

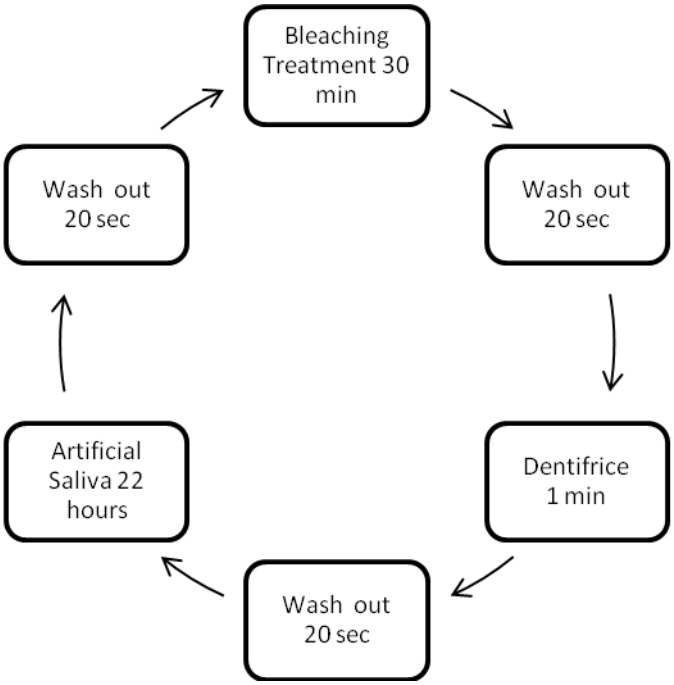


Figure 1. Flowchart – Sequence of bleaching treatment and dentifrice

2.3.5 Analysis of Surface Microhardness

Prior to treatment, the specimens were subjected to the initial microhardness measurement (SHi) with indenter type Knoop under a load of 25 g / 5 s (Micromet 5114, Buehler, Lake Bluff, IL, USA and Mitutoyo Corporation, Kanagawa, Japan), in which a row was performed with 5 indentations[17, 28-30] at the center of each specimen with a distance of 100 µm. After the treatments, the analysis of final microhardness (SHf) was performed in parallel with the initial indentations at a distance of 100 µm. With the mean values of the initial and the final readings of each specimen, the percentage of microhardness loss (%SH) was calculated by the formula: ((SHf-SHi)/SHi*100).

2.3.6 Analysis of Cross-sectional Hardness

After analyzing the SH, the specimens were sectioned in halves, crosswise, with the aid of diamond disc coupled on metallographic cutter under constant water cooling to obtain 2 halves. Each part of the specimen was embedded in acrilic resin, abraded with SiC sandpaper in grits 400, 600, 800 and 1200, respectively, and polished with felts and diamond pastes in grains 1.0, 0.5 and 0.25 µm.

To read the hardness in depth a load of 5 g was used for 5 seconds, making the indentations in a single row[15, 28] at depths of 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160 and 180 µm on the surface of the enamel. Integrated hardness area (IAH; KHN × µm) for the lesion into sound enamel was calculated by the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the IAH for sound enamel to obtain the integrated area of the subsurface regions in the enamel, which was termed integrated loss of subsurface hardness (ΔKHN; KHN × µm).[27]

2.3.7 Polarized Light Microscopy (PLM)

Images were obtained from enamel blocks slices (300 µm thick) which were ground to a thickness of approximately 150 µm. The initial wear of the slices was performed with SiC sandpaper grit 400 on a polisher with abundant water cooling. Subsequently, the slices were hand and carefully ground, in SiC sandpaper grits 600, 800 and 1200, respectively. The specimens were placed on glass slides, immersed in water for viewing and retrieval of images in 400X magnification in PLM (Axiophot, Zeiss, Germany).

2.3.8 Statistical Analysis

For the statistical analysis, the Sigma Plot 12.0 software, at 5% significance level was used. Variable values were SHi, SHf, ΔKHN and as variation factor, bleaching treatments and dentifrice (slurry). Data were first checked for normal distribution and homogeneity of variances. The results of %SH and ΔKHN calculation were submitted to two-way ANOVA, followed by multiple comparison Student-Newman-Keuls Method for SHi/SHf and Tukey Test for ΔKHN.

2.4 Results

2.4.1 Surface and Cross-sectional Hardness of Enamel

Groups without TPM+NaF (HP+PD and HP+FD) in the composition of the bleaching gel had greater loss of hardness in both %SH and ΔKHN when compared with HP containing TMP+NaF groups. However, ΔKHN of HP+FD was statistically lower than HP+PD (Table 2).

Table 2. Percentage means values of surface microhardness loss and integrated subsurface microhardness loss in enamel after fluoride or placebo dentifrice

Dentifrices	Experimental Bleaching Gels	%SH (kg/mm^2)	ΔKHN (kgf/mm^2 x μm)
Fluoridated	HP	-22.4 (3.8) ^{A}	591.9 (247.8) ^{B}
	HP+3TMP+0.1NaF	-3.1 (2.8) ^{D}	143.4 (56.2) ^{E}
	HP+0.3TMP+0.05NaF	-5.0 (2.1) ^{C}	231.2 (75.4) ^{D}
Placebo	HP	-28.9 (7.3) ^{A}	931.4 (409.3) ^{A}
	HP+3TMP+0.1NaF	-5.9 (1.8) ^{B}	247.4 (121) ^{C}
	HP+0.3TMP+0.05NaF	-8.1 (3.5) ^{B}	328.5 (121.4) ^{C}

Distinct superscript letters indicate statistical difference in each row ($p<0.05$).

The group HP+3TMP+0.1NaF+FD showed significantly higher results in both SH and ΔKHN, followed by the group HP+0.3TMP+0.05NaF+FD (Table 2). Therefore, the application of fluoridated dentifrice after treatment with bleaching gel containing TPM+NaF, regardless of concentrations, positively affected the values of %SH / ΔKHN.

Figure 2 shows that there was a greater loss hardness to approximately the depth of 20 μm for all groups tested. Furthermore, it can be noted that the hardness values of the groups treated with fluoridated dentifrice were higher in general.

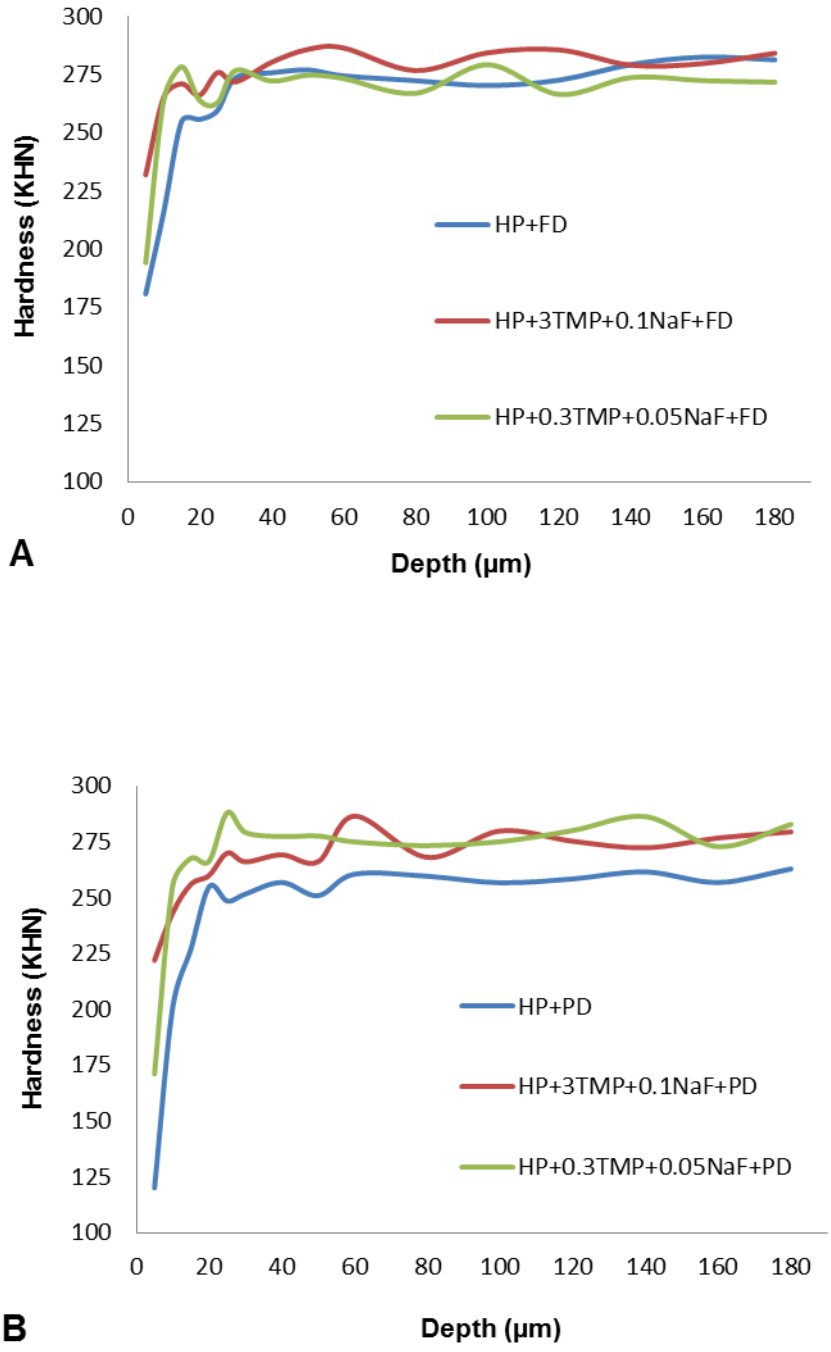


Figure 2. Cross-sectional hardness profiles at different depths in enamel specimens treated with experimental bleaching gel. **A** Fluoridated dentifrice – 1100 ppm F. **B** Placebo dentifrice.

2.4.2 Polarized Light Microscopy Analysis

Regarding qualitative PLM images, one can clearly observe a subsurface demineralization for groups HP+FD (Figure 3A) and HP+PD (Figure 3D). However, in the HP+PD group, the demineralized area is more evident than in the HP+FD group; while the groups treated with bleaching gels containing TMP+NaF showed minimal subsurface alteration (Figures 3B, 3C, 3E and 3F), consistently with the results of hardness.

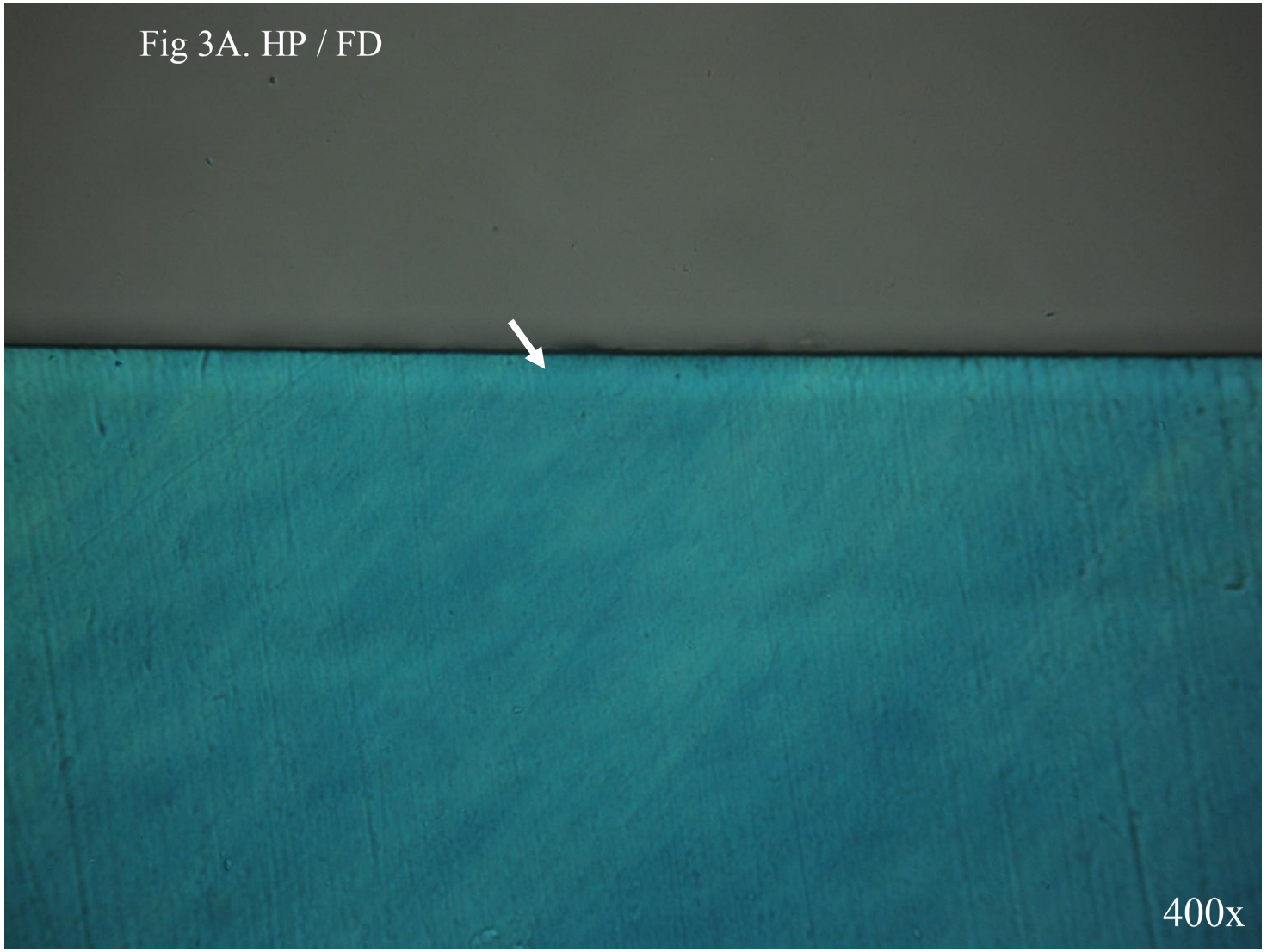


Figure 3 - Images of Polarized Light Microscopy. (A) HP + fluoridated dentifrice. Arrow indicate subsurface demineralized area.

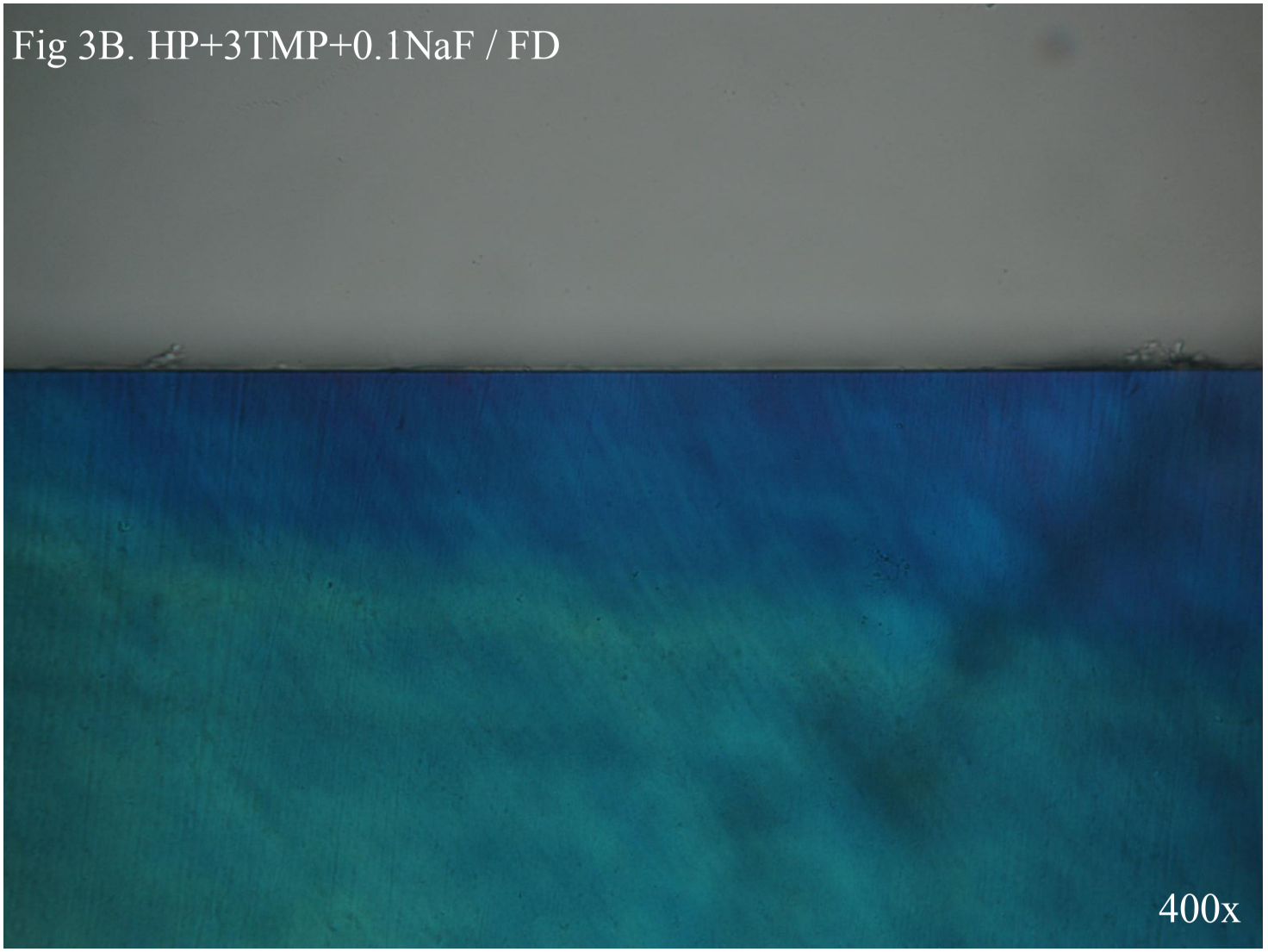
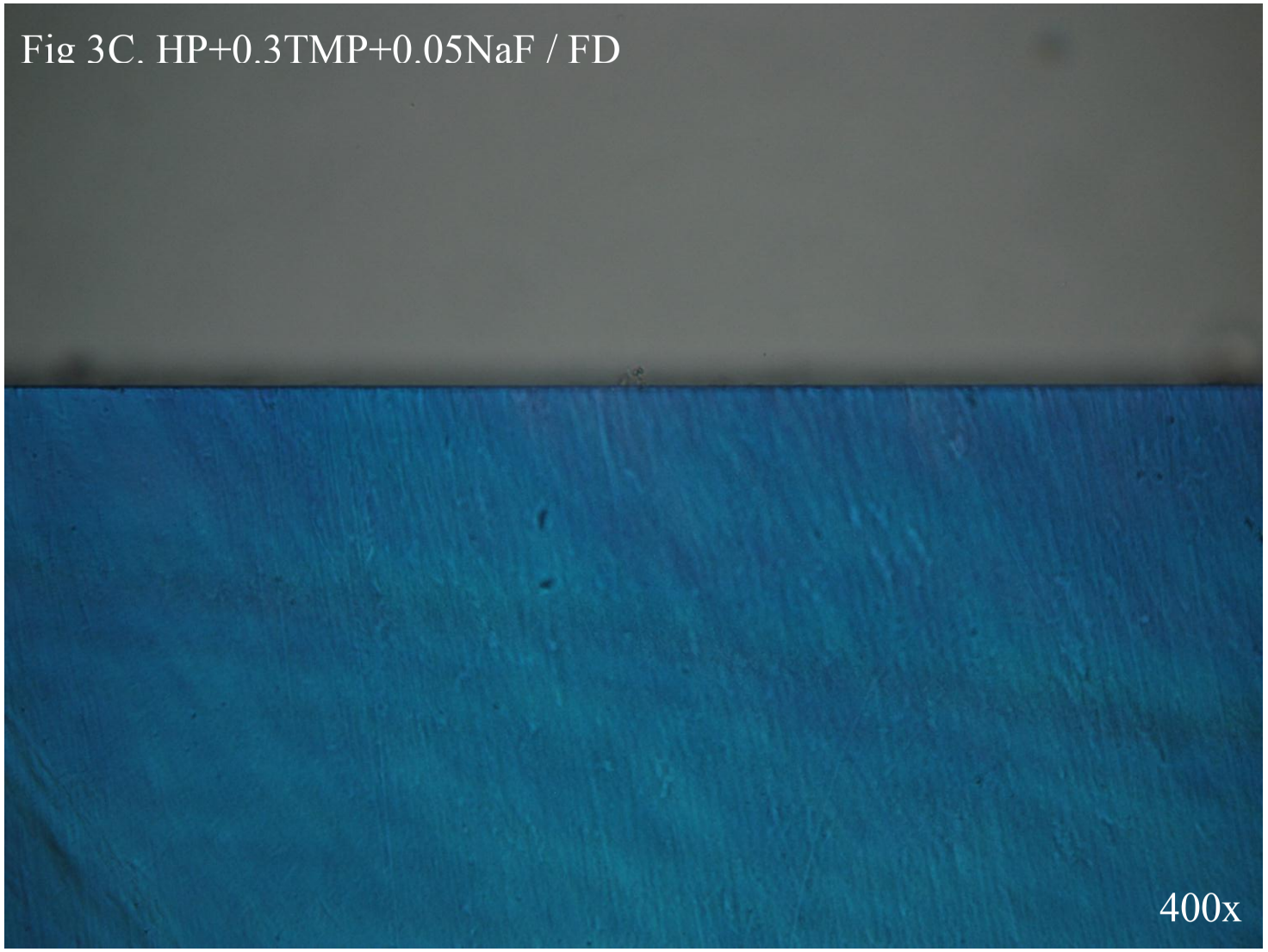


Figure 3 - Images of Polarized Light Microscopy. (B) HP + 3% TMP + 0.1% NaF + fluoridated dentifrice.



Figures 3 - Images of Polarized Light Microscopy. (C) HP + 0.3% TMP + 0.05% NaF + fluoridated dentifrice.

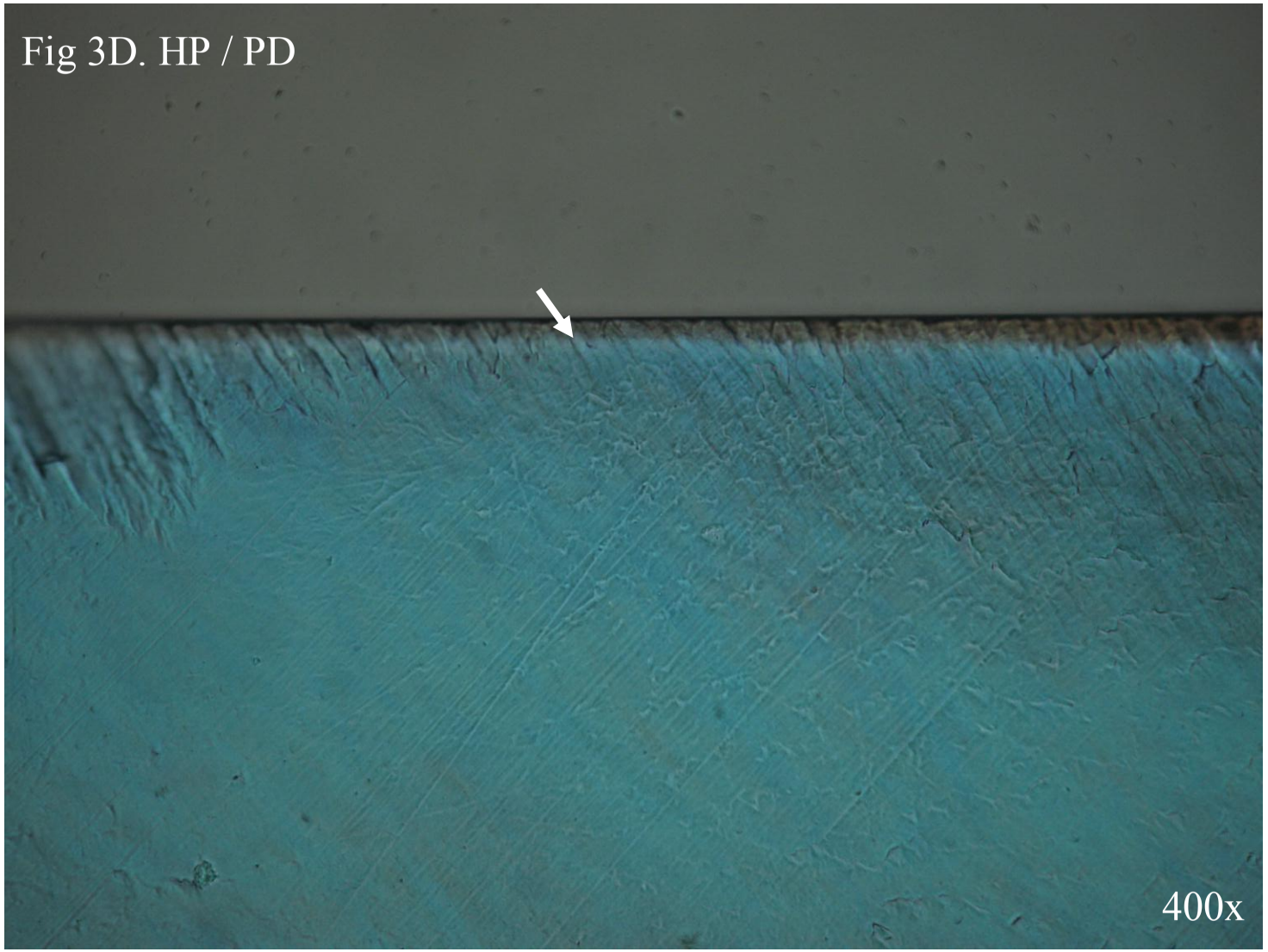


Figure 3 - Images of Polarized Light Microscopy. (D) HP + placebo dentifrice. Arrow indicate subsurface demineralized area.

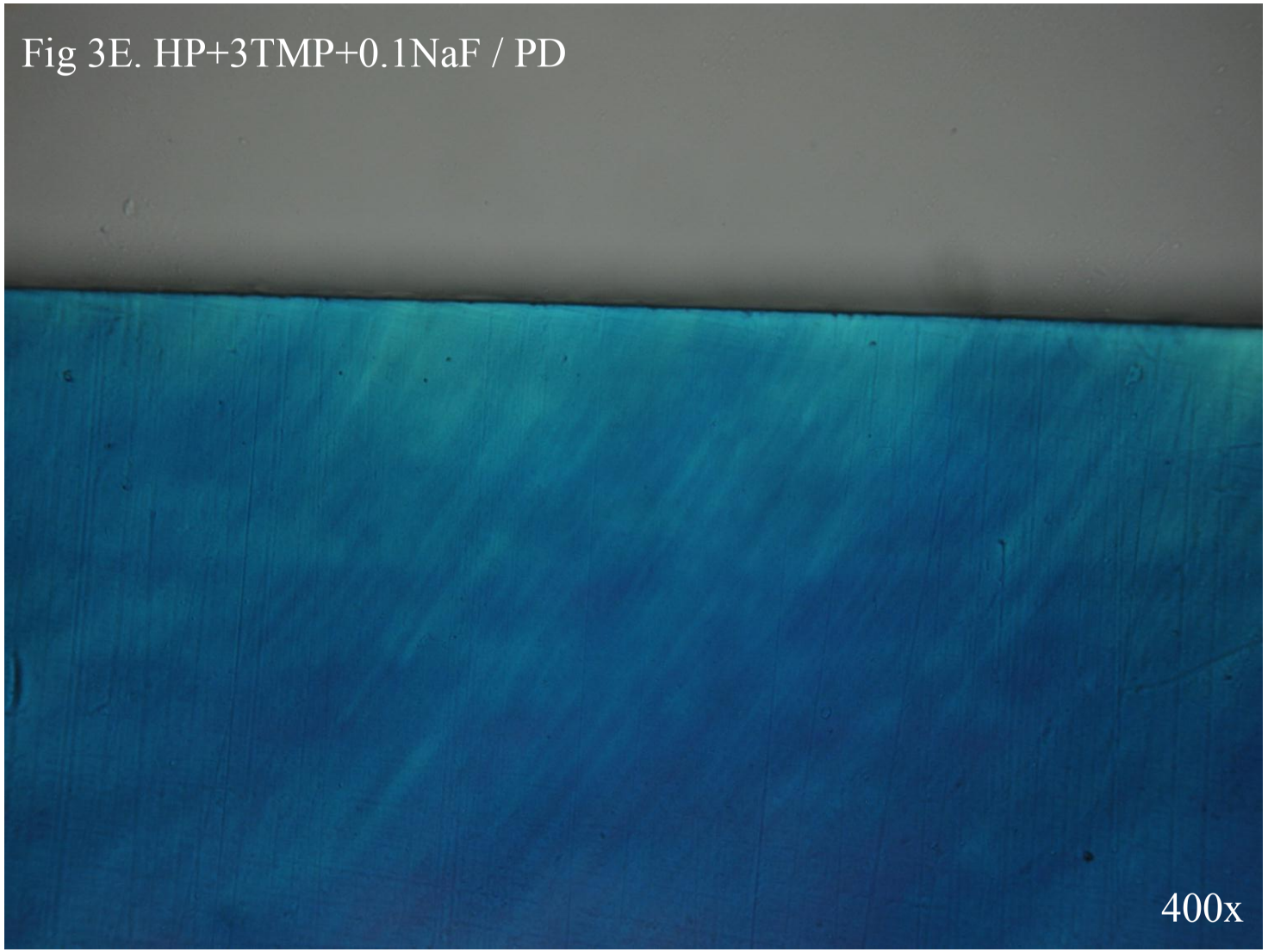


Figure 3 - Images of Polarized Light Microscopy. (E) HP + 3% TMP + 0.1% NaF + placebo dentifrice.

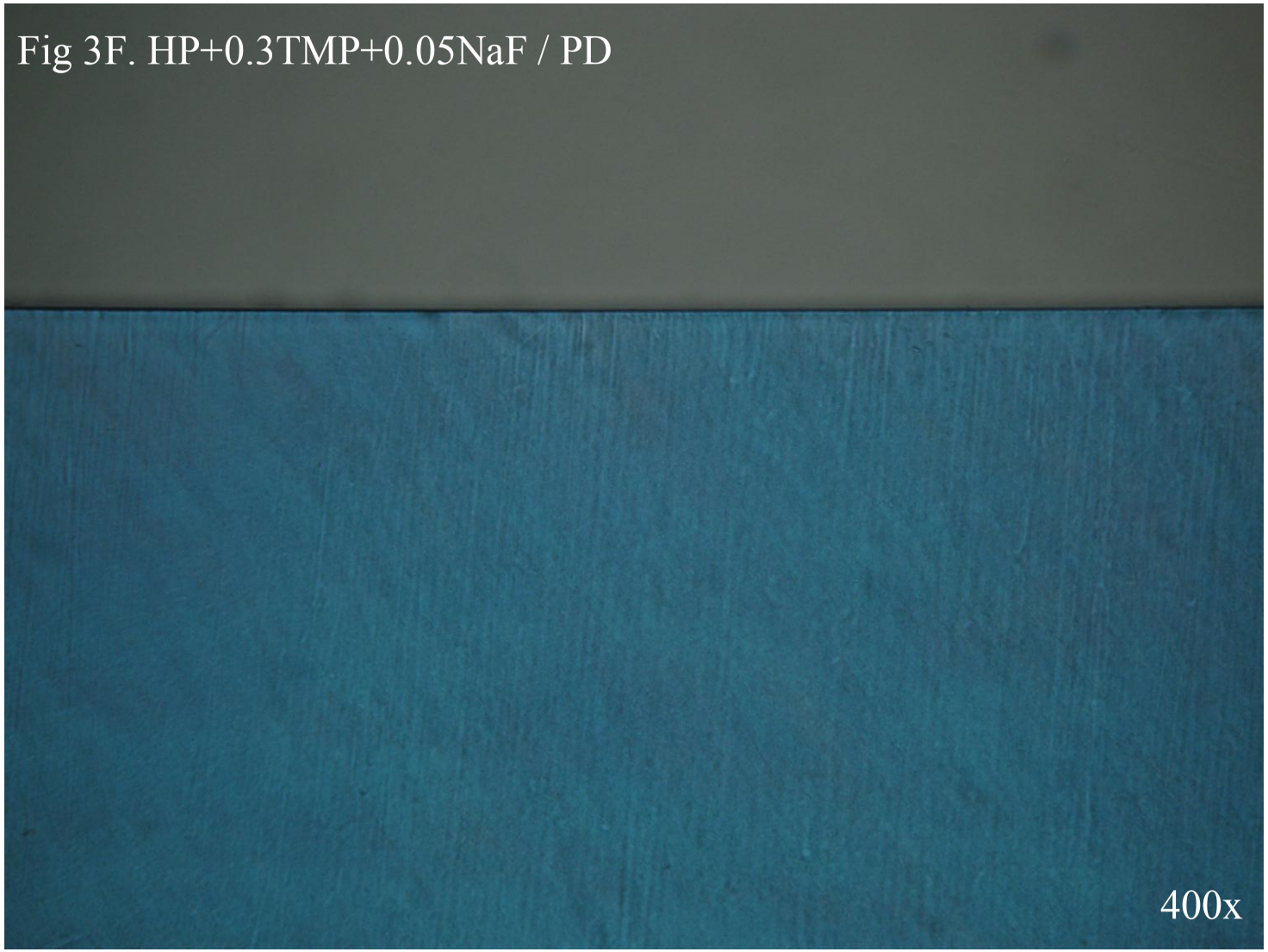


Figure 3 - Images of Polarized Light Microscopy. (F) HP + 0.3% TMP + 0.05% NaF + placebo dentifrice.

2.5 Discussion

The quest for lighter more youthful teeth with healthy appearance has increased demand for dental bleaching.[31, 32] Although the treatment is considered effective and safe, it provides an unusual and troubling clinical response: temporary dental sensitivity.[5, 9, 33-35] This adverse effect seems to be related to the ease of penetration of the bleaching agent components through the enamel and dentin[29, 36, 37] and this fact can be enhanced by possible mineral loss[37, 38] resulting from the treatment. Although not clinically visible, several *in vitro* studies show possible morphological[15, 22] and structural changes after bleaching,[10, 11, 15, 39] which may jeopardize the strength of enamel.[40, 41] It is necessary to develop a new formulation of whitening gel for home and daytime use that minimize the alteration of enamel and consequently decrease tooth sensitivity.

The trend toward home bleaching treatments has been to decrease the time of usage of the tray at daytime.[5, 6, 9, 40] For this, different concentrations of HP have emerged in the market. Currently, the use of HP at concentrations between 3.5% - 10%[9, 40] has been recommended by the manufacturers and the higher the concentration, the shorter the use.[42] We chose the formulation with 10 % HP for being among the highest concentrations for home usage and the least investigated. In this study, all bleaching gels were adjusted to neutral pH 6-7 to avoid a possible demineralization due to the acidity of gel,[43] since the low pH is a major cause for enamel mineral loss.[43-46]

The bleaching gels containing only HP, *i.e.* without addition of TMP+NaF had significantly lower values of microhardness on the surface and in depth, results indirectly complemented by qualitative PLM images obtained from

longitudinal section, where it can be observed the formation of an area of the subsurface demineralization of the enamel (Figures 3A and 3D). Possibly this clear mineral loss observed in the PLM images was the reason that the gels were handled daily prior to their application, which may have kept the action of HP more active than is presented by the commercial bleaching gels containing stabilizers.

The association of TMP+NaF to HP was positive regarding the preservation of hardness values in the enamel on the surface and in depth, proving to be most effective in preventing demineralization, rejecting the null hypothesis that bleaching gels containing TMP+NaF do not influence the hardness of enamel after treatment. However, regardless the presence or absence of TMP+NaF in the composition, all experimental bleaching gels tested caused a mineral loss of enamel, even minimal for groups with TMP+NaF, corroborating previous studies found in the literature.[15, 40] It can be qualitatively demonstrated in the PLM images minimal change in the subsurface groups containing TMP+NaF (Figures 3B, 3C, 3E and 3F).

Among other components[47, 48] added to the bleaching agents in previous investigations, sodium fluoride[22, 49] has been inserted in order to prevent tooth sensitivity and demineralization caused by the bleaching treatment.[22, 23, 50] However, the addition of NaF alone is controversial, due to its effectiveness required amount in the enamel microstructures.[22] It is known that high concentrations of NaF can be harmful to both the dental substrate and the effect of the bleaching agent.[49] However, different concentrations of TMP associated with low NaF concentrations have shown favorable results in case of dynamic imbalance on demineralization and remineralization of the dental

substrate,[12, 20, 21] when added to dentifrices,[12, 16, 19] gels,[20] varnishes[28] and mouth rinses,[17] preventing mineral loss.

The mechanism of action of bleaching involves a redox reaction, which occurs in the release of free radicals that work on breaking down macromolecules of pigments that bleach teeth, but some of these radicals can alter the inorganic structure of the dental substrate.[1] During the bleaching process, ions H^+ may react with the inorganic material from substrate causing enamel dissolution,[51] possibly responsible for the structural changes previously reported in scientific researches.[11, 15, 52] Studies have shown that TMP is capable of binding to the mineral and is also adsorbed on partially demineralized enamel.[25, 53] This action of TMP on the enamel substrate reduces the amount of deposited mineral and causes qualitative changes in the mineral formed, leading to a lower solubility of enamel connected to the TMP.[24, 54] Importantly, the association of TMP and NaF is simultaneously positive, they act for themselves forming minerals with low solubility.[54] Therefore, TMP+NaF added to the HP base bleaching gel made possible a minor structural alteration of enamel.

The two concentrations of TMP+NaF tested were based on previous studies of Moretto *et al*. 2010[16] and Cruz *et al.* 2012,[26] whose results were satisfactory and significant in the remineralization of enamel. However, larger studies are needed to find an optimal concentration of these additives to the bleaching gel, in which the addition of TMP+NaF is functional without interfering with the reaction of the bleaching.

In most of the *in vitro* bleaching research published, the samples are subjected to bleaching and directly stored in distilled water[55] or artificial saliva[22,

[40, 56] between treatment sessions. However, clinically, patients perform home bleaching sessions immediately after the teeth come into contact with saliva and then use dentifrice for cleaning. Following this sequence and *in vitro* attempt to represent what occurs *in vivo*, we chose to perform the immersion of the specimens in dentifrice solution under agitation after each bleaching session, simulating the direct contact of the enamel surface with dentifrice. In addition, the samples were immersed in artificial saliva containing components such as calcium and phosphorus in order to simulate the oral environment between intervals of daily sessions of bleaching treatment.[57]

Among all groups evaluated, the group with 3% TMP + 0.1% NaF immersed in fluoridated dentifrice showed the best results of the experiment, with minor loss of surface and cross-sectional hardness. Followed by groups with 0.3% TMP and 0.05% NaF, which in contact with fluoridated dentifrice also showed a positive influence, indicating that an external fluoride may be favorable to the action of TMP+NaF present in the bleaching gel. In contrast, in groups of bleaching gel containing only HP, contact with fluoridated dentifrice was not significant on surface hardness, being significant only in depth but still showing loss of hardness greater than the groups with addition of TMP+NaF. One reason for this is that during the period of contact with NaF and saliva some phenomena can occur: (1) precipitation of minerals in the demineralized enamel[58] and (2) the growth of apatite crystals.[59] This suggests that in this study there was a greater deposition of fluoride in depth of demineralized enamel by HP. Probably the time of contact with fluoridated dentifrice was not enough for it to happen on its surface.

The results of this study demonstrate that the analysis of hardness in depth is of paramount importance, through the different responses to the same treatment for surface and cross-sectional hardness. In most studies of hardness of bleached teeth, evaluations are limited to surface,[6, 22, 29, 45, 46, 60] underestimating the possible influence of the tested materials in depth. In a previous study,[61] which evaluated a bleaching gel containing calcium in its composition, it was observed that the surface did not lose hardness, but in depth it did significantly. There was probably calcium deposition only on the surface, preventing a remineralizing action in depth. In this study, a higher demineralization was observed up to 20 μm deep for all groups (Figure 2), indicating the occurrence of change in subsurface enamel, as shown in PLM images (Figures 3A and 3D). It was possible to identify in the graphs that the values of hardness after bleaching were not homogeneous, probably due to the bleaching cause specific changes in the structure of the enamel, *i.e.* the HP penetrates the enamel causing changes related to mineral loss in milder and heterogeneous forms than those caused by caries lesions and erosion.

It was possible to visually note that all specimens treated with HP, with or without TMP+NaF, bleached, indicating that the addition of TMP+NaF to the bleaching gel does not prevent their action. However, further studies regarding the penetration and influence of these components in dental substrate are key.

Although the demineralization from the bleaching is not clinically detected, it is essential to lessen the mineral loss of tooth substrate, which can cause an increase in permeability and enhance the tooth sensitivity commonly reported by patients during and shortly after the treatment.[35]

Further investigation on this new formulation of bleaching gel containing TMP+NaF should be held to effectively decrease the side effects from the bleaching treatment and increase the safety of the procedure.

2.6 Conclusion

Within the limitations of this study we can conclude that:

- The bleaching gel containing only 10% hydrogen peroxide (without TMP+NaF) increase loss of hardness of the enamel on surface and in depth;

- The addition of TMP and NaF in the composition of the bleaching gel significantly reduced the loss of hardness in both surface and depth;

- Contact with fluoridated dentifrice showed to positively influence the action of TMP+NaF contained in the bleaching gel, minimizing further loss of enamel hardness.

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Anexos

ANEXO A

Guide for Authors (Journal of Dentistry)

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Book chapters

Phillips SJ, Whisnant JP. The role of dentine under restorations. In: Laragh JH, Brenner BM, editors. The science of restorative dentistry. 2nd ed. Oxford: Elsevier; 2003. p.266-78.

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ANEXO B

OBTENÇÃO E PREPARO DOS BLOCOS DE ESMALTE

Confecção dos blocos de esmalte bovino (4.0 x 4.0 mm)

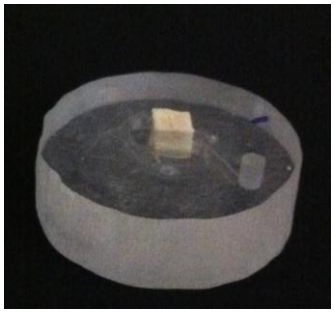
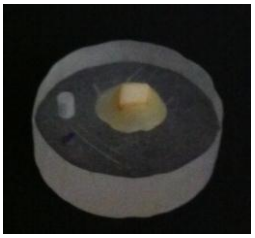
Coroa do dente bovino incisivo central inferior separada da raiz através de disco diamantado montado em motor de bancada, mantido sob refrigeração (água destilada/deionizada).

Coroa fixada na placa de acrílico com material termoplástico (Godiva).

Secção no sentido longitudinal, na porção mais plana, utilizando-se 1 disco diamantado (Buehler), montado em cortadeira sob refrigeração com água deionizada. Foi realizado o corte no sentido transversal e horizontal para obtenção do bloco com 4.0 x 4.0 mm.

ANEXO C

PLANIFICAÇÃO DA DENTINA E POLIMENTO DO ESMALTE

	Bloco de esmalte fixado em base de resina acrílica com auxílio de cera pegajosa (Kota Ind. e Com. LTDA). Superfície dentinária voltada para cima.
	Ajuste da dentina para obtenção de superfícies paralelas entre esmalte e dentina, utilizando Politriz e lixas de granulação 320 (BUEHLER), durante 40 segundos sob baixa rotação e refrigeração.
	Bloco fixado com a superfície do esmalte voltada para cima, a qual foi polida para análise de dureza.

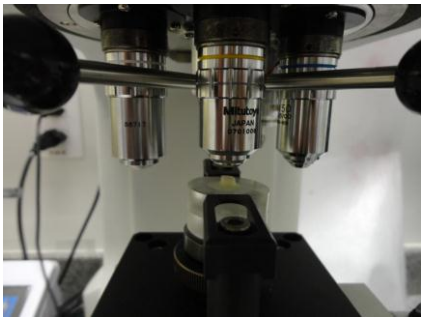
Sequência do polimento de esmalte

1. Na Politriz - lixa de granulação 600, 800 e 1200 (2 minutos) e refrigeração a água.
2. Na Politriz - acabamento final com disco de papel feltro (Buehler Polishing Cloth) (2 minutos) e suspensão de diamante 1.0 e 0.5μm base-água (Buehler).
3. Limpeza em lavadora ultrassônica e água deionizada por 5 minutos, entre cada lixa e feltros.

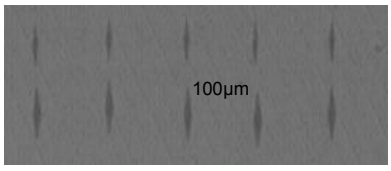
ANEXO D

ANÁLISE DE DUREZA SUPERFICIAL

Microdurômetro Micromet 5114 Hardness Tester (Buehler), com penetrador tipo Knoop, acoplado ao Software para análise de imagem (Buehler OminMet).



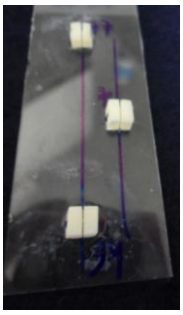
Bloco de esmalte sendo submetido à determinação de dureza no microdurômetro, carga estática de 25 gramas e tempo de 5 segundos, para análise da dureza de superfície.



Fotomicrografia das impressões para análise de dureza de superfície inicial (SHi), e final (SHf) -(Aumento: 100x)

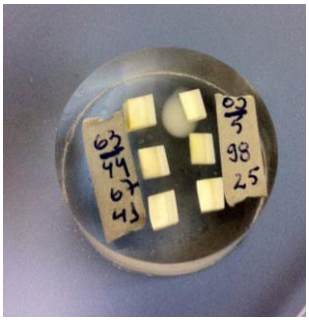
ANEXO E

PREPARO DOS BLOCOS PARA ANÁLISE DA DUREZA EM SECÇÃO LONGITUDINAL



Secção longitudinal do bloco para leitura de dureza interna

Embutidora (AROTEC PRE 30S) – utilizada para inclusão dos blocos de esmalte em 7 gramas de resina acrílica, pressão de 150 kgf/cm^2, tempo de aquecimento de 7 minutos e 7 minutos de resfriamento. Os blocos foram fixados em posição com cola adesiva (Super Bonder – Loctite).



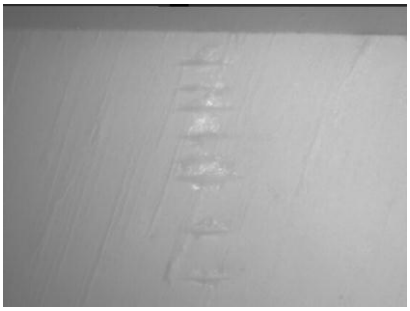
Blocos embutidos - plano longitudinal voltado para a superfície da resina acrílica.

Na Politriz - lixa de granulação 400, 600, 800 e 1200 com refrigeração a água. Limpeza em lavadora ultrassônica e água destilada/deionizada por 5 minutos, entre cada lixa. Acabamento final com disco de papel feltro (Buehler Polishing Cloth) (3 minutos) e suspensão de diamante 1/4 micron base-água (Buehler); lavagem em lavadora ultrassônica por 5 minutos.

ANEXO F

ANÁLISE DE DUREZA EM SECÇÃO LONGITUDINAL

Microdurômetro Micromet 5114 Hardness Tester (Buehler, Lake Bluff, USA e Mitutoyo Corporation, Kanagawa, Japan), com penetrador tipo Knoop, acoplado ao Software para análise de imagem Buehler OminMet (Buehler, Lake Bluff, USA). Carga de 5g e tempo de 5 segundos

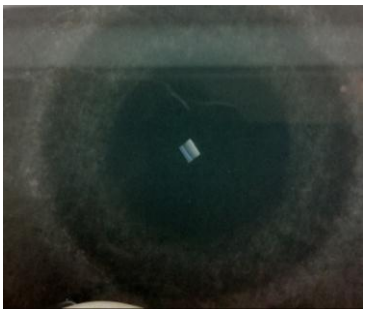


Fotomicrografia das impressões. (Aumento: 1000x). Lesão de subsuperfície. Profundidades de: 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180 µm

ANEXO G

ANÁLISE DE MICROSCOPIA DE LUZ POLARIZADA (MLP)

Corte de fatia dos blocos embutidos



Desgaste dos espécimes nas lixas SiC #400, 600, 800, e 1200 até estar na espessura de 150 μm

Análise qualitativa das imagens em microscópio de luz polarizada

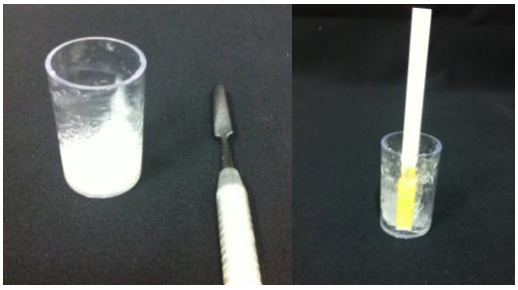
ANEXO H

MATERIAIS, MANIPULAÇÃO E APLICAÇÃO DOS GÉIS CLAREADORES EXPERIMENTAIS

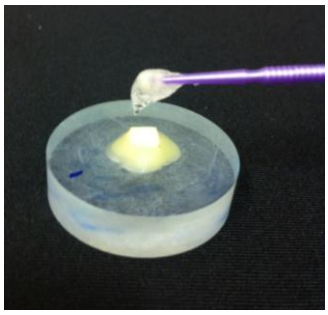
A B C D

Materiais:
A – Peróxido de hidrogênio;
B – Carbopol;
C- Trimetafosfato de sódio;
D- Fluoreto de sódio

Balança de precisão para pesagem dos componentes dos géis experimentais



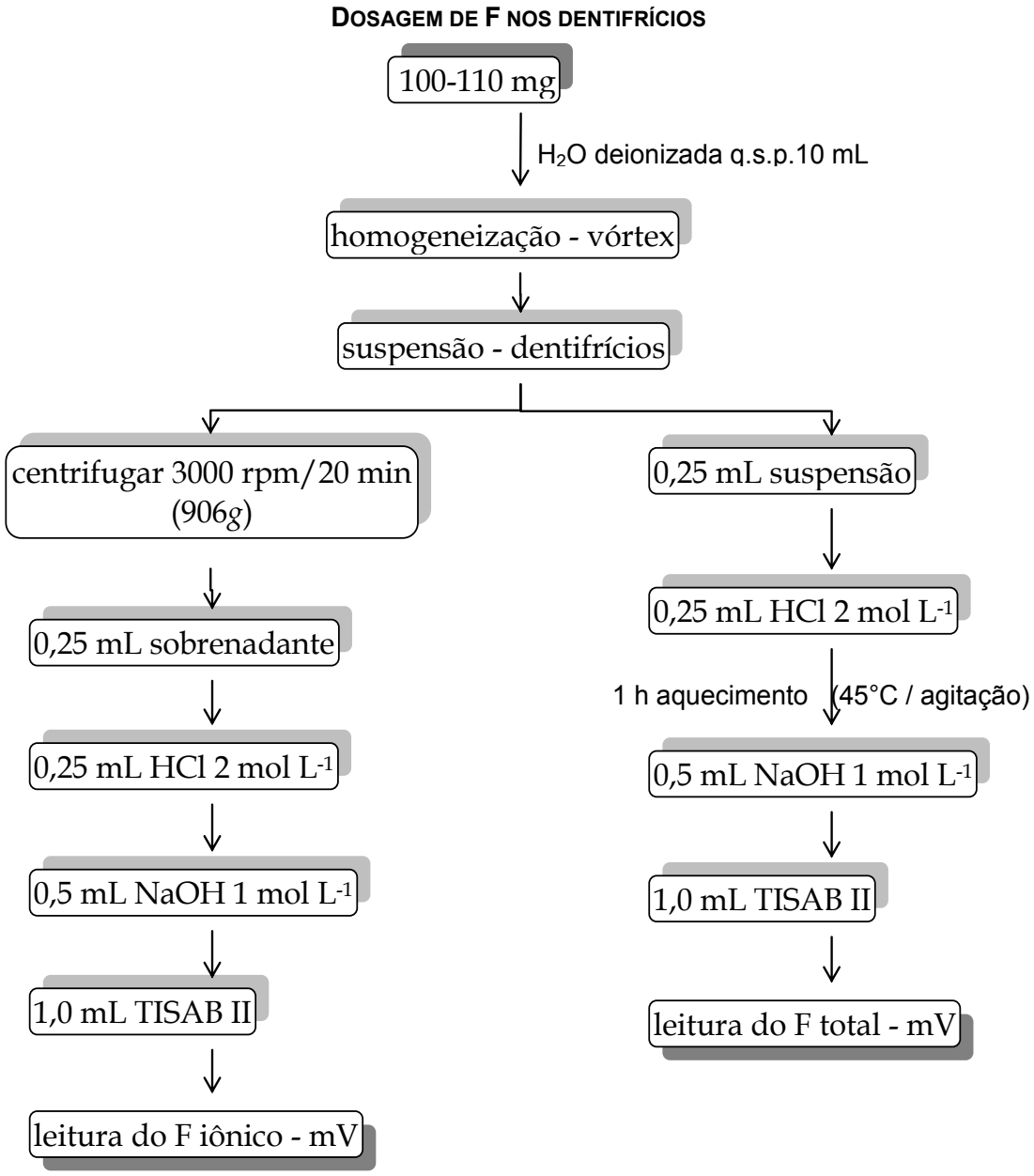
Manipulação dos géis com espátula metálica, ajuste do pH com hidróxido de sódio e medição com tiras indicadoras de pH.



Aplicação padronizada do gel sob a superfície do esmalte

ANEXO I

DOSAGEM DE FLUORETO NOS DENTIFRÍCIOS



ANEXO J

EQUIPAMENTO PARA MEDIÇÃO DE QUANTIDADE DE FLUORETO DOS DENTIFRÍCIOS

Equipamento para medição de quantidade de fluoreto dos dentifrícios: Eletrodo específico Orion 9409-BN (Orion Research). Microeletrodo de referência (Analyser Comércio e Indústria LTDA). Analisador de íons Orion 720A (Orion Research).

ANEXO K

VALORES DE FLUORETO NOS DENTIFRÍCIOS

Tabela 1. Valores de fluoreto iônico (FI) e fluoreto total (FT) (média ± dp, *n* = 2) nos dentifrícios.

Dentifrícios	FI (ppm F)	FT (ppm F)
Placebo	18,0 ± 4,1 (2)	17,5 ± 3,2 (2)
1100 ppm F	1152,0 ± 9,9 (2)	1142,9 ± 8,8 (2)

DETERMINAÇÃO DO pH NOS DENTIFRÍCIOS EXPERIMENTAIS

Tabela 2. Valores de pH (média ± dp, *n* = 2) nos dentifrícios experimentais

Dentifrícios	pH
Placebo	7,3 ± 0,1 (2)
1100 ppm F	7,3 ± 0,1 (2)

ANEXO L

PREPARO DO DENTIFRÍCIO (*SLURRY*)

Preparo dos dentifrícios em laboratório contendo 1100 ppm F ou sem fluoreto (placebo)

Armazenamento dos dentifrícios em frascos vedados com tampa.

A pesagem dos dentifrícios foi realizada diariamente.

Proporção – 1g de dentifrício para cada 3 ml de água deionizada. Solução homogeneizada com auxílio de agitador magnético.

4 ml de dentifrício em cada recipiente para imersão dos blocos por 1 min sob agitação.

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Serviço Técnico de Biblioteca e Documentação – FOA / UNESP

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S237a Avaliação de uma nova composição de agente clareador sobre a microdureza do esmalte : associação do trimetafosfato de sódio e fluoreto de sódio ao peróxido de hidrogênio a 10%. / Ana Laura Esteves dos Santos. – Araçatuba, 2014

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