UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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INFLUÊNCIA DO PERÓXIDO DE HIDROGÊNIO A 7,5% CONTENDO CÁLCIO, ACP, FLÚOR E HIDROXIAPATITA NA MICRODUREZA, RUGOSIDADE SUPERFICIAL, ALTERAÇÃO DE COR E MICROMORFOLOGIA DE ESMALTE HUMANO

Dissertação de Mestrado apresentada à Faculdade de Odontologia de Piracicaba da UNICAMP para obtenção do Título de Mestre em Clínica Odontológica, na Área de Dentística.

Orientador: Prof. Dr. Flávio Henrique Baggio Aguiar

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“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis."

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RESUMO

O objetivo deste estudo *in vitro* foi determinar a microdureza, rugosidade superficial, micromorfologia e alterações de cor de esmalte dental humano submetido a tratamentos clareadores caseiros com peróxidos de hidrogênio a 7,5% (PH) adicionados de cálcio, fosfato de cálcio amorfo (ACP), flúor e/ou hidroxiapatita (HA). Três agentes de tratamento comerciais: (Pola Day 7,5% (PD), Day White ACP 7,5% (DW), White Class Calcium 7,5% (WC)), três agentes clareadores experimentais (PH 7,5% + NaF (PHF), PH 7,5% + HA (PHH) e PH 7,5% + NaF + HA (PHFH)) e os grupos controle positivo (gel de peróxido de hidrogênio a 7,5% - PC) e controle negativo (gel sem peróxido de hidrogênio a 7,5% - NC), foram avaliados (n=10). Os agentes de tratamento comerciais foram aplicados pelo prazo recomendado pelos fabricantes e os agentes experimentais por 1,5 hora por dia. Todas as amostras foram imersas em solução de saliva artificial pelo resto do dia, num total de 21 dias de tratamento. Após, ficaram armazenados em saliva artificial por 14 dias. Ensaios de microdureza, rugosidade superficial, micromorfologia do esmalte e análise de alteração de cor foram realizados nos tempos *baseline*, 7, 14, 21 dias de tratamento, 7 e 14 dias de pós-tratamento. Para análise estatística, as médias foram comparadas utilizando o teste de Tukey-Kramer com um nível de significância de 5%. Como resultados, no teste de rugosidade superficial (Ra) foi observado aumento em todos os grupos experimentais (PHF, PHH e PHFH) e nos grupos comerciais DW e WC ao longo do tempo. No teste de microdureza (KHN) observou-se um aumento significativo para o grupo PHH e redução significativa do grupo DW, enquanto os outros grupos não apresentaram alteração significativa ao longo do tempo de estudo. Para o teste de análise de alteração de cor, todos os agentes apresentaram efeito clareador, exceto o grupo NC, sendo que o grupo PHH foi o único que apresentou valores similares aos agentes clareadores comerciais em todos os tempos de estudo. Na análise morfológica observou-se alterações como a presença de porosidades na maioria dos grupos, exceto no grupo NC, além de deposição de cristais de hidroxiapatita nos grupos PHH e PHFH. Concluiu-se que o tratamento clareador contendo hidroxiapatita proporcionou alterações de cor similares aos agentes clareadores comerciais, foi capaz de aumentar a microdureza, e que quando este componente é formulado com ou sem flúor, pode levar a um aumento da rugosidade superficial, devido a deposição de cristais de hidroxiapatita na superfície de esmalte, como observado pela análise de microscopia eletrônica de varredura.

Palavras-chave: Clareamento dental, Agentes remineralizadores, Microdureza, Rugosidade superficial, Análise de cor, Microscopia eletrônica de varredura

ABSTRACT

The aim of this *in vitro* study was to determine the microhardness, surface roughness, micromorphology and color change analysis of enamel submitted to with home-use bleaching treatment 7.5% hydrogen peroxide (HP) added with calcium, amorphous calcium phosphate (ACP), fluoride and/or hydroxyapatite (HA). Three commercial treatment agents: (Pola Day 7.5% (PD), Day White ACP 7.5% (DW), White Class 7.5% Calcium (WC)), three experimental bleaching agents (7.5% HP + NaF (PHF), HP + 7.5% HA (PHH) and PH + 7.5% NaF + HA (PHFH)), a negative control (Gel without 7.5% hydrogen peroxide - NC) and a positive control (Gel with 7.5% hydrogen peroxide - PC) groups were evaluated (n=10). The treatment agents were applied according to the manufacturer's recommendation and experimental agents were applied for 1,5 hour daily. Then all the specimens were immersed in artificial saliva for the rest of the day, in a total of 21 days of treatment. After the treatment phase, the specimens were stored in artificial saliva for 14 days. Microhardness tests, surface roughness, micromorphology of the enamel and color change analysis were performed at baseline, 7, 14, 21 days of treatment, 7 and 14 days post-treatment. For the statistical analysis, means were compared using the Tukey-Kramer test at a significance level of 5%. As results, the surface roughness test (Ra) presented increase in all experimental groups (PHF, PHH and PHFH) and commercial groups DW and WC in function of time. The microhardness test (KHN) showed a significant increase for the group PHH and a significant reduction on DW group, while the other groups did not show statistical difference in function of timespan study. For the color change analysis test, all the groups presented bleaching effectiveness, except the group NC, and the PHH group was the only one that showed similar values compared to the commercial bleaching agents at all times of study. In the morphological analysis, changes were observed as the presence of porosities in most groups, except on NC group, and deposition of hydroxyapatite crystals on groups PHH and PHFH. It was concluded that the bleaching treatment containing hydroxyapatite provided color changes similar to commercial bleaching agents, was able to increase the microhardness values, and that when this component is formulated with or without fluoride, it can lead to an increase in surface roughness due to deposition of hydroxyapatite crystals over the enamel surface, as observed by analysis of scanning electron microscopy.

Key Words: Tooth bleaching, Remineralizing agents, Microhardness, Surface roughness, Color analysis, Scanning electron microscopy

LISTA DE ABREVIATURAS E SIGLAS

ΔE	-	Variação de alteração de cor
ACP	-	Fosfato de cálcio amorfo
CMC	-	Carboximetilcelulose
CPP-ACP	-	Fosfopeptídeo de caseína - Fosfato de cálcio amorfo
DW	-	Day White ACP 7,5%
F	-	Flúor
F-HA	-	Fluorapatita
HA	-	Hidroxiapatita
KHN	-	Knoop Hardness Number (Número de Dureza Knoop)
MEV	-	Microscopia eletrônica de varredura
NaF	-	Fluoreto de sódio
NC	-	Grupo controle negativo
PC	-	Grupo controle positivo
PH	-	Peróxido de hidrogênio
PD	-	Pola Day 7,5%
PHF	-	Peróxido de hidrogênio a 7,5% + Flúor
PHH	-	Peróxido de hidrogênio a 7,5% + Hidroxiapatita
PHFH	-	Peróxido de hidrogênio a 7,5% + Flúor + Hidroxiapatita
PVC	-	Policloreto de vinil
Ra	-	Rugosidade superficial média
WC	-	White Class Calcium 7,5%

SUMÁRIO

INTRODUÇÃO

A demanda por tratamentos estéticos, como o clareamento dental caseiro, vem crescendo muito nas últimas décadas, sendo considerado um método eficaz para clareamento de dentes escuros (Matis *et al*., 2002). O método tem como princípio ativo o peróxido de hidrogênio, que pode ser aplicado diretamente ou obtido por uma reação química do peróxido de carbamida (Jiang *et al*., 2008). O peróxido de hidrogênio atua como agente oxidante, de modo que as moléculas reativas de oxigênio interagem com moléculas cromóforas, oxidando macromoléculas pigmentadas (Dahl *et al*., 2003; Joiner, 2006).

Entretanto, estudos apontam para efeitos adversos como alterações na micromorfologia superficial da estrutura dental observadas por meio de imagens de microscopia eletrônica de varredura com a presença de erosões e porosidades (Basting *et al*., 2007; Oliveira & Mansur, 2007; Chen *et al*., 2008; Jiang *et al*., 2008; Cavalli *et al*., 2009; Dudea *et al*., 2009; Sasaki *et al*., 2009) alterações em microdureza (Basting *et al*., 2001; Freitas *et al*., 2002; Basting *et al*., 2003; Attin *et al*., 2005; Costa & Mazur, 2007; Jiang *et al*., 2008; Leandro *et al*., 2008; Maia *et al*., 2008; Cavalli *et al*., 2011; De Abreu *et al*., 2011; Borges *et al*., 2011) e rugosidade superficial (Cavalli *et al*., 2009; Azrak *et al*., 2010; Martin *et al*., 2010).

Com o intuito de se evitar estes efeitos adversos, componentes remineralizadores como o flúor, cálcio e fosfato de cálcio amorfo (ACP) têm sido recentemente desenvolvidos e disponibilizados no mercado, sendo formulados juntamente com os agentes clareadores.

O efeito de agentes clareadores caseiros adicionados de flúor e cálcio vêm sendo estudado e apresentado resultados benéficos quanto a remineralização do esmalte dental humano, prevenindo a redução da microdureza, minimizando os efeitos adversos de desmineralização da superfície e sub-superfície, bem como reduzir a progressão da profundidade da lesão em estudo *in vitro* (Cavalli *et al*., 2011).

O ACP foi criado pela ADA Foundation's Paffenbarger Research Center e adicionado em alguns produtos de clareamento dental disponíveis no mercado. Costa & Mazur (2007) avaliaram o efeito do peróxido de carbamida a 10% com ACP, na dureza do esmalte durante e após o tratamento clareador e verificaram que o ACP não foi capaz de prevenir a redução dos valores de microdureza no esmalte. De Abreu *et al.* (2011) também não observou resultados benéficos quanto a prevenção da redução da microdureza em esmalte e não relatou alterações quanto a rugosidade superficial.

A hidroxiapatita (HA), representada pela fórmula $Ca_{10}(PO_4)_6(OH)_2$ é o principal componente biomineral dos tecidos duros, e quando sintetizado artificialmente, é um material biocompatível. O esmalte dental humano é formado por cristais de hidroxiapatita que são compostos, em peso, por 17,4% de fósforo (Lazzari, 1976) e 37,1% de cálcio (Spector & Curzon, 1978) e, quando os íons de flúor substituem os íons hidroxila, dá-se origem à fluorapatita (FA) - $Ca_{10}(PO_4)_6F_2$. O uso de hidroxiapatita sintética na Odontologia tem sido estudado, sendo indicado para uso em situações de lesões de cárie incipientes, prevenção de cárie como selante de sulcos, fossas e fissuras, proteção contra cáries recorrentes em paredes de cavidades restauradas e balanceio das perdas minerais em tratamentos clareadores (Oliveira & Mansur, 2007), podendo ser utilizado juntamente com o agente clareador.

De acordo com Li *et al.* (2010), a presença de conteúdo mineral presente em saliva artificial e de flúor na composição de agente clareador podem ser fatores para regressão de cor após tratamentos clareadores devido a remineralização do tecido mineral. Além disso, o flúor poderia apresentar interação adversa com o peróxido de carbamida, de modo que o flúor poderia reduzir a eficácia clareadora do peróxido de carbamida e que este agente clareador poderia prejudicar o potencial remineralizador do flúor (Tschoppe *et al.*, 2009). Desta forma, é necessário avaliar a alteração de cor quando da adição de agentes remineralizadores na formulação de um agente clareador.

No entanto, ainda não se sabe se a adição destes componentes remineralizadores resultam em um efeito benéfico ao esmalte dental humano, assim como se ocorrerá comprometimento no clareamento dental. Desta forma, o objetivo deste estudo *in vitro* foi avaliar a influência do peróxido de hidrogênio a 7,5% contendo agentes remineralizantes cálcio, ACP, flúor e hidroxiapatita na microdureza, rugosidade superficial, alteração de cor e micromorfologia de esmalte dental humano.

CAPÍTULO 1: Influence of 7.5% hydrogen peroxide containing calcium, ACP, fluoride and hydroxyapatite on microhardness and color change of sound human enamel

ABSTRACT

Objectives: Determine the microhardness and the color change of enamel submitted to bleaching treatment with 7.5% hydrogen peroxide (HP) added with calcium, amorphous calcium phosphate (ACP), fluoride and hydroxyapatite. Methods: Eighty dental enamel slabs were embedded, ground flat, and divided into eight groups (n=10). Three commercial agents (Pola Day 7.5% (PD), Day White ACP 7.5% (DW), White Class Calcium 7.5% (WC)) three experimental agents (7.5% PH + NaF (PHF), 7.5% PH + HA (PHH) and 7.5% PH + NaF + HA (PHFH)), a positive control group (no PH (PC)) and a negative control group (7.5% PH (NC)) were assessed. The commercial treatment agents were applied according to manufacturer's recommendations and the experimental ones were applied for 1,5 hour daily, and in the rest of the hours the specimens were stored in artificial saliva, in a total of 21 days. After the treatment phase, the specimens were stored in artificial saliva for 14 days. The microhardness and color change (ΔE) tests were performed at baseline, 7, 14 and 21 days of treatment, 7 and 14 days post-treatment. For the statistical analysis, means were compared using the Tukey-Kramer test at a significance level of 5%. Results: DW presented significant lower microhardness values and PHH was the only one to present significant higher values, also it was the only experimental group that presented color change similar to the commercial agents (PD, DW and WC) at all times. Conclusion: Bleaching treatment with 7.5% hydrogen peroxide containing hydroxyapatite was capable of increase the enamel microhardness and it also was the only to present color change similar to the commercial bleaching agents.

Clinical significance: Home-use bleaching with 7.5% hydrogen peroxide added with hydroxyapatite may lead to an increase of the microhardness of human enamel and present color change similar to those obtained by commercial bleaching agents.

INTRODUCTION

The bleaching agents used in home-bleaching techniques may lead to morphological changes on mineralized structures, that is, enamel.[1,2] Such changes are attributed to the modification of the organic and inorganic composition of enamel after treatment with bleaching agents based on peroxides.[3] Such treatments substantially reduce the amount of calcium and phosphorus in the enamel structure, and modify the morphology of most superficial crystals when compared to enamel surfaces not submitted to bleaching treatment.[4,5] Some remineralizing components, such as fluoride, calcium, and ACP, are available to minimize the adverse effects of bleaching treatment on enamel structure.

Costa and Mazur[6] evaluated the effects of new formulations of bleaching products, including a 10% carbamide peroxide with ACP formulation, on the hardness of enamel during and after bleaching treatment and found that the ACP was unable to prevent the reduction of microhardness values of enamel. Only high concentrations of fluoride after bleaching treatment could restore the values of hardness to levels similar to enamel that was not bleached. De Abreu et al.[7] assessed home-use and in-office dental bleaching treatments with or without ACP and found that all groups showed decreases in microhardness values during treatment with values only returning to their baseline values post-treatment, due to the presence of artificial saliva.

Hydroxyapatite, represented by the formula $Ca_{10}(PO_4)_6(OH)_2$ is the main component of hard tissues and, when artificially synthesized, is a biocompatible material. If the fluoride ions replace hydroxyl ions, it gives rise to fluorapatite (FA) - $Ca_{10}(PO_4)_6F_2$. The composition of human dental enamel consists of hydroxyapatite crystals, which correspond to a weight of 17.4% phosphorus[8] and 37.1% calcium.[9]

According to Yamagishi et al.,[10] synthetic hydroxyapatite is similar in composition and density to tooth enamel; therefore, this biomineral, which adheres to the dental substrate, can be used widely in dentistry for purposes such as: protecting enamel caries, recurrent cavities, and restored cavities; sealing grooves, pits and fissures in deciduous and permanent teeth and balancing the mineral losses provided by bleaching treatments.[11]

Thus, the aim of this *in vitro* study was to assess the influence of 7.5% hydrogen peroxide containing calcium, ACP, fluoride, and hydroxyapatite on the microhardness and color change of sound human enamel. The null hypothesis of the present study was that the addition of calcium, ACP, fluoride, and hydroxyapatite would not significantly affect bleaching effectiveness and also not present a beneficial effect on the microhardness of enamel.

MATERIALS AND METHODS

1. Preparation of Tooth Slabs

After approval from the Research Ethics Committee of FOP-Unicamp (protocol 059/2009), caries-free human third molars were extracted and stored in a thymol solution (0.1%, pH 7.0). The teeth were debrided with scalpel blades and periodontal curettes and polished with rubber cups, pumice, and distilled and deionized water at low speed. A transversal section was made, dividing the root and coronal portions. Longitudinal sections allowed enamel slabs measuring 4 mm x 4 mm to be obtained. The height of the slab was 3 mm, comprising 1 mm of enamel and 2 mm of dentin. Teeth with cracks or stains were excluded.

The dental slabs were covered with wax except on the enamel surface and embedded in polystyrene resin using 2 cm-diameter PVC molds, leaving the enamel surface uncovered by the resin. After 24 h, the slabs were removed from the molds and ground flat to obtain the smooth surfaces required for microhardness testing.

The enamel specimens were flattened with aluminum oxide discs (Norton, São Paulo, SP, Brazil) of sequentially decreasing granulation (400, 600, 1200), cooled under running water, and polished with diamond pastes of 6, 3, 1⁄2, and 1⁄4 µm and felt discs (Top, Ram and Gold, Arotec, Osasco, SP, Brazil) under mineral oil cooling (Red mineral oil, Arotec, Osasco, SP, Brazil). The specimens were ultra-sound washed (Ultrasound washer, Unique, Brazil) by placing the specimens in distilled and deionized water for a 10-min period to eliminate residues. Then, the enamel slabs were removed from the polystyrene resin and, the wax was removed from the surfaces. The specimens were randomly divided into 8 groups of 10 and kept in a humid environment for 1 day until the beginning of the treatment application.

2. Treatment Agent Specification

The treatment agents used in this experiment are specified in Table 1, with their compositions, manufacturers, protocols of application, lot numbers, and mean pH values. The pH was measured 3 times with a pHmeter (PG 1400, Gehaka, São Paulo, Brazil), and the mean was obtained for each treatment agent.

3. Bleaching Treatment Phase

For the negative control (NC), positive control (PC), Pola Day (PD), Day White ACP (DW), White Class Calcium (WC), and PH + NaF (PHF) groups, application of the bleaching agents was performed using a calibrated syringe for the placement of 0.02 ml of each treatment agent on the dental fragments. For the PH + HA (PHH) and PH + NaF + HA (PHFH) groups, 0.01 ml of hydroxyapatite paste and 0.01 ml of 15% hydrogen peroxide gel were mixed in a dappen pot and applied to the enamel slab. The commercial treatment agents (PD, DW, and WC) were applied according to manufacturers' recommendations, and the experimental agents (PHF, PHH, and PHFH) were applied daily for 1.5 h. After the application period, the bleaching agent was removed from the slabs manually with a soft toothbrush using 5 back-and-forth movements with light pressure, and each slab was washed with distilled and deionized water.

During the remaining hours of each day, the slabs were stored in 1.5 ml of artificial saliva solution in Eppendorf tubes at 37ºC (±1ºC). The artificial saliva solution was changed every 2 days. The artificial saliva used was the remineralizing solution originally described by Featherstone et al.[12] and modified by Serra and Cury.[13] The specimens were stored for a total of 35 days (corresponding to 21 days of treatment and 14 days post-treatment).

4. Microhardness Test

The microhardness tests (HMV-2, Shimadzu Corporation, Tokyo, Japan) were performed prior to the application of treatment agents to obtain baseline values, after 7, 14, and 21 days of bleaching treatment, and 7 and 14 days post-treatment. Three microhardness indentations were performed for 5 s at each measurement using a Knoop indenter and a static load of 25 g.

5. Color Change Analysis

The enamel slabs were placed in a white Teflon matrix 5 mm in height and 5 mm in diameter. Color was measured inside a day-light chamber to standardize ambient light for all specimens. The enamel slabs were subjected to an initial color analysis, using a CM-700 UV-Visible Spectrophotometer Reflectance (Konica Minolta Sensing, Inc., Osaka, Japan). The dental slabs with values far above or far below average were excluded from the sample. The color change was determined by the difference (ΔE) between the coordinates obtained at baseline and those obtained from the timespan study at 7, 14, and 21 days during bleaching treatment and 7 and 14 days post-bleaching, using the CIE*L*a*b system. The total change in color, ΔE, is often used to represent a difference in color and is calculated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$. In this study, the comparison of the difference was between the timespan study values and the baseline value.

6. Statistical Analysis

After exploratory analysis, the data were analyzed in SAS using the MIXED repeated measures procedure. For the covariance matrix it was used the Akaike information criterion, selecting the matrix the lowest value for this parameter. Means were compared using the Tukey-Kramer test at a significance level of 5%.

RESULTS

The results of microhardness and color change tests are presented in Table 2 and Table 3, respectively. For the microhardness test, in function of timespan study, no group demonstrated statistically significant differences, except groups DW and PHH at 21 days of treatment. The group DW presented statistically lower microhardness values, and the group PHH presented statistically higher values, when compared to baseline values.

Between groups, at 14 days of treatment, the group NC was similar to groups PHF, PHH, and PHFH. At 21 days of treatment, NC was similar only to groups that contained fluoride in its composition (PHF and PFHF). At 7 days post-treatment, the group DW differed from all other groups, presenting lower means of KHN. At all other times (baseline, 7 days of treatment and 14 days post-treatment) the groups were statistically similar to each other.

For the color change test, in function of the timespan study, the groups NC, DW, and WC showed color stability at all times, although the NC group presented low values of ΔE, showing that there was no bleaching. The groups NC and PD had statistically significant differences at 14 and 21 days during treatment and 14 days post-treatment, with similar values between 7 days during and 7 days post-treatment. The group PHF showed statistical difference only at 7 days compared with other periods of study. The group PHH presented statistical differences at 14 and 21 days of bleaching whereas, at 7 days during treatment and 7 and 14 days post-treatment, color measurements were statistically similar. The 14 days post-treatment values were similar to the values obtained at 7 days of treatment for the group PHFH as well.

Between groups, the NC group differed statistically from all groups at all times of study. Only the group PHF was similar to experimental groups PHH and PHFH at 7 days of treatment. At 14 days, the commercial groups PD, DW, and WC were similar to the experimental group PHH, while PHFH was not similar to PD or the experimental group NC. The NC group differed from the commercial group DW and the groups containing fluoride (PHFH and PHF) in the composition. At 21 days, the group PHF differed from NC and PD, but differed only from the NC group at 7 days post-treatment. At 14 days post-treatment, only the group PHFH differed from the other groups.

DISCUSSION

The hypothesis that remineralizing agents such as hydroxyapatite, fluoride, ACP, and calcium could be beneficial to the microhardness of dental enamel in addition to providing a bleaching effect was tested. It was observed that only hydroxyapatite confirmed this hypothesis, showing a remineralizing effect on microhardness and a bleaching effect similar to the commercial bleaching agents at all times during the study.

Although previous studies have been conducted to evaluate the effects of bleaching agents on enamel microhardnes,[2,4,7,14-29] the effects of individual components in the formulation of bleaching agents may affect the mineral content of dental structures and the bleaching effect. Thus, it is necessary to study the effects of components added to the bleaching agent, such as hydroxyapatite, fluoride, amorphous calcium phosphate, and calcium, on the surface of human enamel.

Acidic properties in the composition of bleaching agents can affect the physical and chemical structure of the enamel[16,30,31] if the pH is below 5.5.[12,32] In this study, only the group that was treated with Day White ACP presented statistical differences compared to control groups for the microhardness test.

Interestingly the measured pH of this bleaching agent was the highest pH among all tested agents (pH 7.7), and the bleaching agent Pola Day, which had the lowest pH (pH 6.0), showed no statistically significant effects. This effect can be explained by the observation in Basting et al.'s study,[33] which showed that, in the presence of glycerol (a component present in the Day White ACP and all the experimental bleaching agents in this study), small changes in enamel microhardness were observed due to the increased pH of the solution by raising the hydrogen ion concentration of the solution. In addition, studies showed a reduction in enamel microhardness depending on the thickening agent used, such as carbopol and glycerin.[16,34] However, this demineralization effect may not be significant in in vivo conditions, as reported by an in situ study conducted by Maia et al.,[35] which reported that home-bleaching agents like 7.5% hydrogen peroxide caused no changes in the microhardness of enamel.

On the microhardness test, Day White ACP showed statistical differences at 21 days of treatment, as the only bleaching agent to suffer a significant decrease in microhardness in this study. This result corroborates those obtained by Costa and Mazur,[6] who evaluated a 10% carbamide peroxide with ACP formulation and observed that the amorphous calcium phosphate was unable to prevent decreases in enamel microhardness values, and De Abreu et al.,[7] who observed changes during the bleaching treatment, concluding that the presence of amorphous calcium phosphate did not offer significant benefits. However, Borges et al.[36] assessed the use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste mixed with 10% and 16% carbamide peroxide on the microhardness of bleached enamel and found an increase of microhardness as well as whitening efficacy. These varying results may be related to some factors like the concentration of ACP, mixture of bleaching agent with ACP, time of exposure of the bleaching agent with the enamel surface, and the higher stability of CPP over ACP.

On the other hand, the experimental composition containing hydroxyapatite (PHH) was the only one with significant increases in enamel microhardness, which was observed at 21 days, an effect also observed by Jiang et al.,[37] who tested 30% hydrogen peroxide combined with hydroxyapatite to prevent and minimize the loss of enamel mineral content during bleaching. However, the experimental compositions containing fluoride (PHF, PHFH) did not show statistical differences, confirming the positive results obtained by Leandro et al.[23], who tested 10% carbamide peroxide followed by application of solutions of sodium fluoride and acidulated phosphate fluoride for which there were significant differences compared with the control group, differently from results obtained by Chen et al.[38] which observed that only at post-treatment phase the values returned to baseline due to the artificial saliva. Cavalli et al.[39] related that the addition of fluoride and calcium to home-applied bleaching agents was effective in minimizing demineralization as was observed with compositions that contained calcium and fluoride in the present study. All other groups in this study (NC, PC, and PD) showed no statistical differences in microhardness.

The artificial saliva, which contained calcium and phosphate ions[12,32] and was used in this study because of its remineralizing potential as reported in previous studies,[7,16,21,30,33,40-45] seems to have contributed to the post-treatment values of microhardness returning to baseline. In addition to this beneficial effect of artificial saliva observed in vitro, replacement of enamel mineral content is also expected in vivo due to important factors such as salivary flow and the buffering capacity of saliva, oral hygiene, and topical fluoride,[20] which can increase the remineralization of enamel.[21,33] In addition, Attin et al.[14] concluded that studies that simulated intraoral conditions, such as temperature and the presence of saliva, obtained smaller reductions in microhardness values than those that did not simulate intraoral conditions.

For the color change test, the groups NC, DW, and WC showed color stability across all times. In a study conducted by Li et al.,[46] after enamel bleaching and immersion in an artificial saliva and fluoride solution, a color regression of the bleached teeth was observed at post-treatment after color analysis, which could be explained by the increased density of the enamel. This could also be justified in the present study because the PHFH composition, which was composed of two remineralizing components, fluoride and hydroxyapatite, demonstrated. The groups testing compositions that did not contain remineralizing components, PC (experimental) and PD (commercial), presented values similar to each other at all times.

One can assume that, according to the timespan study, all the groups that were submitted to dental bleaching had a change of color, except the NC group. The color values of the DW and WC groups remained stable over time and the groups that were exposed to hydroxyapatite presented values in the post-treatment period similar to values at 7 days of treatment. All 3 commercial bleaching agents (PD, DW, and WC) had similar values, when compared to each other, at all times of study. The same was observed for the 3 experimental bleaching agents containing remineralizing components (PHF, PHH, and PHFH). In this study, compositions that contained fluoride presented bleaching effectiveness in contrast to the results reported by Tschoppe et al.,[47] who reported that fluoride could have adverse interactions with carbamide peroxide, reducing the bleaching effectiveness of carbamide peroxide, and that, conversely, this bleaching agent could compromise the remineralizing potential of fluoride.

The PHH values were similar to the commercial bleaching agents (PD, DW, and WC) at all tested times. A similar result was found by Jiang et al.,[37] who noted that bleaching with hydrogen peroxide added to hydroxyapatite was statistically similar to bleaching with hydrogen peroxide alone. Dabanoglu et al. [48] assessed nano- and micro-hydroxyapatite materials applied as a suspension or dissolvable polymer films to the enamel surface and reported whitening effects and durability against hydrodynamic shearing forces with FE-SEM observations.

Synthetic hydroxyapatite, which has particles with dimensions similar to those found in enamel, could react with the natural hydroxyapatite due to its chemical proximity to the tooth structure[10,48] without affecting or chemically altering the deeper tooth tissue.[10] The use of 7.5% hydrogen peroxide associated with hydroxyapatite presented an effective bleaching result, probably due to the formation of a thin layer of this material over the enamel surface. This layer of hydroxyapatite would also reflect more light than the more translucent natural enamel,[48] giving the enamel a whiter appearance. Hydroxyapatite also led to an increase in microhardness, proving its remineralizing effect. Thus, although hydroxyapatite is a promising component with many applications in dentistry, its use needs further studies to establish criteria for appropriate protocols for bleaching treatment.

CONCLUSION

It was concluded that the bleaching treatment with 7.5% hydrogen peroxide containing hydroxyapatite was capable of increasing the enamel microhardness, the group containing ACP presented significant reduction while the other groups presented no statistical difference along the timespan study. For the color change analysis, all the groups presented bleaching effectiveness, except the group NC, and the PHH group was the only one that showed similar values compared to the commercial bleaching agents at all times of study.

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Table 1: Treatment agents used in this study, compositions, manufacturers, protocol of treatment, lot numbers and pH

Treatment agents	Composition	Manufacturer	Protocol of application	Lot #	pH
Gel without 7.5% Hydrogen peroxide (NC)	Water, CMC, nipagin	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.5
7.5% Hydrogen peroxide (PC)	Water, CMC, nipagin, hydrogen peroxide	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.2
Pola Day 7,5% (PD)	Hydrogen peroxide, additives, glycerol, water, flavoring.	SDI Limited, Bayswater, Victoria, Australia	30 minutes, twice daily	*P090712*	6.0
Day White ACP 7,5% (DW)	Water, poloxamer 407, glycerin, hydrogen peroxide, propylene glycol, potassium nitrate, flavoring, xylitol, hydroxypropyl methylcellulose, eugenol, potassium hydroxide, calcium nitrate, sodium phosphate, mica.	Discus Dental, Culver City, CA, USA	30 minutes, twice daily	*DBE2688BR*	7.7
White Class Calcium 7,5% (WC)	Neutralized carbopol, potassium nitrate, calcium fluoride, aloe vera, calcium gluconate, stabilizer, deionized water and surfactant.	FGM Produtos Odontológicos, Joinville, SC, Brazil	1,5 hour, once daily	*300710*	7.5
7.5% Hydrogen peroxide + NaF (PHF)	Hydrogen peroxide, nipagin, CMC, water, NaF	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.2
7.5% Hydrogen peroxide + HA (PHH)	Hydrogen peroxide, nipagin, CMC, water, hydroxyapatite	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.3
7.5% Hydrogen peroxide + NaF + HA (PHFH)	Hydrogen peroxide, nipagin, CMC, water, hydroxyapatite and NaF	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.5

Table 2: Mean values for microhardness (KHN) in function of the timespan study

Groups	Timespan study					
	0	7	14	21	28	35
NC	321.0 (21.8)ABa	317.3 (24.8)Ba	400.5 (46.8) Aa	369.3 (26.5)ABab	352.1 (33.7)ABa	344.2 (23.0)ABa
PC	319.9 (76.4)Aa	292.7 (19.8)Aa	276.1 (54.4)Ab	295.6 (42.3)Abcd	306.5 (15.8)Aab	321.0 (12.2)Aa
PD	279.9 (42.2)ABa	264.5 (28.1)Ba	296.9 (81.9)ABb	288.4 (65.7)ABcd	303.8 (53.9)ABab	334.4 (17.3)Aa
DW	295.4 (27.1)Aa	258.8 (26.8)ABa	267.1 (36.5)ABb	233.6 (26.6)Bd	276.1 (37.0)ABb	311.2 (22.3)Aa
WC	286.4 (50.3)Aa	273.8 (14.7)Aa	282.1 (35.3)Ab	263.8 (34.2)Acd	299.3 (9.9)Aab	315.2 (24.9)Aa
PHF	308.7 (32.7)ABa	261.0 (22.4)Ba	321.5 (40.2)ABab	314.7 (34.3)ABabc	318.9 (40.3)ABab	335.0 (34.9)Aa
PHH	304.9 (45.5)Ba	297.0 (36.8)Ba	321.3 (46.8)ABab	384.1 (48.8)Aa	307.5 (56.0)Bab	324.4 (26.9)ABa
PHFH	298.2 (35.8)ABa	279.3 (17.5)Ba	320.2 (23.1)ABab	379.9 (94.2)Aab	323.0 (56.4)ABab	348.0 (33.7)ABa

Means followed by different letters (capital case letters on horizontal line and lower case letters on vertical line) differ among them ($p \leq 0.05$).

Table 3: Mean values for color change analysis (ΔE) in function of the timespan study

Groups	Timespan study				
	7	14	21	28	35
NC	1.61 (0.97) dA	0.81 (0.45) dA	1.92 (0.45) dA	0.59 (0.22) cA	0.71 (0.40) eA
PC	7.91 (2.96) aB	11.04 (2.88) aA	10.72 (2.12) aA	9.51 (1.68) aAB	10.85 (2.67) aA
PD	7.39 (2.30) aB	10.83 (1.25) abA	10.55 (2.12) abA	9.04 (1.82) abAB	9.87 (3.05) abA
DW	7.15 (1.80) abA	8.45 (2.38) bcA	8.03 (1.48) bcA	9.30 (1.73) abA	9.20 (1.66) abcA
WC	8.16 (2.13) aA	8.66 (2.50) abcA	9.01 (1.25) abcA	9.46 (0.99) aA	9.17 (1.35) abcA
PHF	4.33 (1.61) cB	7.02 (1.81) cA	7.53 (1.85) cA	6.93 (1.94) bA	6.90 (1.74) cdA
PHH	6.26 (0.91) abc B	9.51 (1.37) abcA	8.75 (1.85) abcA	7.95 (1.94) abAB	7.61 (1.74) bcdAB
PHFH	4.83 (1.16) bcC	8.20 (1.51) cAB	9.41 (1.76) abcA	7.46 (1.99) abAB	6.33 (1.25) dBC

Means followed by different letters (capital case letters on horizontal line and lower case letters on vertical lines) differ among them ($p \leq 0.05$).

CAPÍTULO 2: Influence of 7.5% hydrogen peroxide containing calcium, ACP, fluoride and hydroxyapatite on surface roughness and micromorphology of sound human enamel

ABSTRACT

Objectives: Determine the surface roughness and analyze the micromorphology of enamel submitted to bleaching treatment with 7.5% hydrogen peroxide (HP) added with calcium, amorphous calcium phosphate (ACP), fluoride, and hydroxyapatite. *Methods:* Eighty (80) dental enamel slabs were embedded, ground flat, and divided into eight groups (n=10). Three commercial agents (Pola Day 7.5% (PD), Day White ACP 7.5% (DW), White Class Calcium 7.5% (WC)) three experimental agents (7.5% HP + NaF (PHF), 7.5% HP + HA (PHH) and 7.5% HP + NaF + HA (PHFH)), a positive control group (gel with 7.5% HP (PC)) and a negative control group (gel without 7.5% HP (NC)) were assessed. The commercial treatment agents were applied according to manufacturers' recommendations and the experimental ones were applied for 1.5 hour daily, and during the rest of the time, the specimens were stored in artificial saliva, for a total of 21 days. After the treatment phase, the specimens were stored in artificial saliva for 14 days. The surface roughness tests (Ra) and SEM analysis were performed at baseline, 7, 14, and 21 days of treatment, and 7 and 14 days post-treatment. For the statistical analysis, means were compared using the Tukey-Kramer test at a significance level of 5%. *Results:* There was a roughness increase in function of time on groups DW, WC, PHF, PHH, and PHFH, while it was observed on SEM analysis the presence of porosities in most groups, and a deposition of hydroxyapatite crystals in groups PHH and PHFH. *Conclusion:* Bleaching treatment can lead to an increase in surface roughness and the hydroxyapatite crystals remain even after the bleaching treatment.

Clinical significance: Home-use bleaching with 7.5% hydrogen peroxide may lead to an increase in surface roughness, mostly due to the deposition of remineralizing components present in the bleaching agent formulas. Deposition of hydroxyapatite crystals was observed on SEM analysis, which remained even after the bleaching treatment.

INTRODUCTION

The demand for aesthetic treatments, such as home-use bleaching, has increased in recent decades because it is an effective and safe treatment.[1] With the evolution of knowledge and materials applied in bleaching treatment, a reduction of the original protocol of application was recommended.[2] This reduction in time use is justified by the greater amount of active ingredients in bleaching agents within early hours of contact with the dental element, and the degradation observed in the early hours of application of hydrogen peroxide,[3] which release free radicals that break down pigmented carbon rings with high molecular weight molecules into smaller and lighter ones.[4]

The bleaching agents used in bleaching techniques may cause morphological changes of mineralized structures.[5,6] Such changes are attributed to the modification of organic and inorganic composition after treatment with bleaching agents based on peroxides,[7] which substantially reduce the amount of calcium and phosphorus, and modify the morphology of most crystals layer compared to the enamel surface that was not submitted to bleaching.[8,9] Analysis by scanning electron microscopy (SEM) reported changes in enamel, such as the presence of erosions, porosities,[6,10] and loss of the prismatic layer of enamel prisms of enamel[11] after bleaching treatments. Studies with bleaching agents in concentrations ranging from 10-35% caused surface changes and reductions in calcium-phosphorus ratio.[12-16]

Recently, remineralizing components have been added to bleaching agents in an attempt to reduce the modifications of mineral content of tooth enamel. Among these components, amorphous calcium phosphate (ACP) was established by the ADA Foundation's Paffenbarger Research Center and added to some tooth bleaching products on the market. However, few studies were conducted to assess the effect of ACP on enamel and beneficial results on microhardness[17] and surface roughness were not observed.[18]

Hydroxyapatite, represented by the formula $Ca_{10}(PO_4)_6(OH)_2$, is the main component of hard tissues and, when artificially synthesized, is a biocompatible material. If the fluoride ions replace hydroxyl ions, it gives rise to fluorapatite (FA) - $Ca_{10}(PO_4)_6F_2$. The human dental enamel consists of hydroxyapatite crystals that are composed, by weight, of 17.4% phosphorus[19] and 37.1% calcium.[20]

As the synthetic hydroxyapatite is similar in composition and density in relation to tooth enamel,[21] the use of this biomineral that adheres to the dental substrate can be widely used in dentistry, such as protection enamel caries recurrent cavities restored cavities, sealing grooves, pits and fissures in the deciduous and permanent teeth and balancing the mineral losses provided by bleaching treatments.[22]

Thus, the aim of this study was to determine the influence of 7.5% hydrogen peroxide containing calcium, ACP, fluoride, and hydroxyapatite on surface roughness and micromorphology of sound human enamel.

MATERIALS AND METHODS

1. Preparation of tooth slabs

After approval by the Research Ethics Committee of FOP-Unicamp (protocol 059/2009), caries-free human third molars were extracted and kept stored in thymol solution (0.1%, pH 7.0). The teeth were debrided with scalpel blades and periodontal curettes and polished with rubber cups, pumice and distilled and deionized water at low speed. A transversal section was made, dividing the root and coronal portions. Longitudinal sections allowed enamel slabs measuring 4 mm x 4 mm to be obtained. The height of the slabs was 3mm, with 1mm of enamel and 2mm of dentin. Those with cracks or stains were excluded.

The dental slabs were covered with wax, except on the enamel surfaces, and embedded in polystyrene resin by using 2.0 cm diameter PVC molds, leaving the enamel surface uncovered by the resin. After 24 hours, the slabs were removed from the molds and ground flat to obtain the smooth surfaces required for surface roughness tests and SEM analysis.

The enamel specimens were flattened with aluminum oxide discs (Norton, São Paulo, SP, Brazil) of sequentially decreasing granulation (400, 600, 1200) cooled under running water and polished with diamond pastes (pastes of 6, 3, 1⁄2 and 1⁄4 µm) and felt discs (Top, Ram and Gold, Arotec, Osasco, SP, Brazil) under mineral oil cooling (Red mineral oil, Arotec, Osasco, SP, Brazil). The specimens were ultra-sound washed (Ultrasound washer, Unique, Brazil) by placing the specimens in distilled and deionized water for a 10-minute period to eliminate residue. Then the enamel slabs were removed from the polystyrene resin and the wax removed from the surfaces that it had covered. The specimens were randomly divided into eight groups (n=10) and kept in a humid environment for 1 day until the beginning of the treatment application.

2. Treatment agent specification

The treatment agents used in this experiment are specified in Table 1, according to the composition, manufacturer, protocol of application, lot numbers, and mean pH value. The pH was measured with a pH meter (PG 1400, Gehaka, São Paulo, Brazil) three times and the mean was obtained for each treatment agent.

3. Bleaching treatment phase

For the application of the bleaching agents of groups NC, PC, PD, DW, WC, and PHF, a calibrated syringe was used for the placement of 0.02 ml of each treatment agent on the dental fragments. For groups PHH and PHFH, 0.01 ml of the hydroxyapatite paste and 0.01 ml of 15% hydrogen peroxide gel were used, which were mixed in a dappen pot and then applied to the enamel slab. After the application period, the bleaching agent was removed by a soft toothbrush making five back-and-forth movements manually with light pressure over the slabs and then washed with distilled and deionized water.

During the remaining hours of the day, the slabs were stored in 1.5 ml of artificial saliva in eppendorf tubes at 37ºC (±1ºC). The artificial saliva solution was changed every 2 days. The artificial saliva used was the remineralizing solution described by Featherstone et al.[23] and modified by

Serra and Cury.[24] The specimens were stored for a total of 35 days (corresponding to 21 days of treatment and 14 days post-treatment).

4. Surface roughness test

The measures of surface roughness were performed using a surface profilometer, Surfcorder SE1700 (Kosaka Lab.) The device was positioned so that the needle could go in parallel and with constant pressure over the polished surface of the enamel slab.

In each measure, the average roughness (Ra) was the arithmetic mean between the peaks and valleys recorded after the respective needle of the profilometer scroll over the surface analysis, a length of 3.0 mm, with filtering (cut-off) of 0.25 mm to maximize filtration of surface ondulation. On each surface, three measures were performed, always with the needle passing through the geometric center of the sample, but in three different positions, obtained by spinning the sample about 120°. Thus, the average of three readings was considered the average roughness of each surface. The value of initial roughness was tabulated for each sample.

5. Micromorphology analysis

To perform the micromorphological analysis of human enamel using scanning electron microscopy (SEM), new slabs were prepared because SEM analysis is a destructive test and the specimens were dehydrated, being immersed in concentrations of ethanol with ascending scales of 70%, 80%, 90%, 95% (5 minutes each, one time) and 100% (5 minutes, four times).The specimens were fixed in metallic stubs with double-sided carbon tape, and metallization of the specimens was performed by covering with gold/palladium alloy, using a sputter apparatus (Denton Vacuum Desk II) with 40 mA for 100 seconds. Then the samples were observed in a scanning electron microscope (PHILIPS - XL30 - F.E.I. Company, Hillsboro, OR, U.S.A.) with acceleration voltage of 20 kV, working distance of 10mm, and aperture of the objective lenses (Spotsize) of 5.4 nm for magnifications of 150X, 1000X, 2000X and

3500X. Analyses of the enamel surfaces were performed in baseline, 7, 14, 21 days of treatment, 7 and 14 days post-treatment for all eight study groups.

6. Statistical analysis

After exploratory analysis, the data were analyzed by repeated measures considering MIXED procedure of SAS. For the choice of the covariance matrix, we used the Akaike information criterion, selecting the one with the lowest value for this parameter. Means were compared using the Tukey-Kramer test at a significance level of 5%.

RESULTS

The results are presented on Table 2. For the surface roughness test, the groups PC, NC, and PD did not show statistical differences compared to baseline. All other groups presented statistical differences represented by an increase in surface roughness of enamel. Among these groups, at the post-treatment phase, the groups DW and WC presented at 28 and 35 days similar values to the baseline, while group PHFH did so only at 35 days.

For the scanning electron microscopy test, it was observed that in most groups, there were morphological changes such as the presence of porosities and, in some cases, exposure of enamel matrix interprismatic, except for the group PC. The most evident demineralization process was observed for the group DW at 21 days of treatment. In the groups containing hydroxyapatite (PHH and PHFH), the deposition of hydroxyapatite crystals over the enamel surface was observed at both treatment and post-treatment phases.

DISCUSSION

The hypothesis that remineralizing agents such as hydroxyapatite, fluoride, ACP, and calcium could be beneficial to the surface roughness of dental enamel were analyzed. It was observed that bleaching treatment with 7.5% hydrogen peroxide may lead to an increase of the surface roughness.

The SEM photomicrographs showed deposition of hydroxyapatite crystals on the surface of the enamel even after the bleaching treatment phase, which could have contributed to the higher Ra mean values observed for the experimental group that contained hydroxyapatite and fluoride in the composition.

Groups NC, PC, and PD did not show statistical differences compared to baseline. All other groups containing components that induce the remineralization of enamel, such as amorphous calcium phosphate (DW), calcium (WC), sodium fluoride (PHF), hydroxyapatite (PHH), and sodium fluoride + hydroxyapatite (PHFH), showed statistical differences represented by an increase in the surface roughness of enamel. This result can be explained by the deposition of these components and their products in the enamel surface, increasing surface roughness.

A study conducted by Martin et al.[25] reported that fluoride, when used separately from the bleaching agent, showed beneficial effect on tooth enamel to reduce the increase in surface roughness after bleaching with 16% carbamide peroxide and 35% hydrogen peroxide. In this study, the use of fluoride was concomitant with the 7.5% hydrogen peroxide, formulated in the same composition, and did not present beneficial results for the surface roughness test.

The group DW, despite having significantly increased the roughness of the enamel at 7 and 21 days of treatment, showed values statistically similar to the baseline values at 14, 28 and 35 days, which could be explained because of the amorphous calcium phosphate that could have precipitated the defects of the enamel surface and hydrolyzed to form apatite, filling these defects, leading to a less rough surface.[26] However, De Abreu et al.[18] reported that bleaching treatment with 7.5% and 9.5% Day White ACP did not change the surface roughness of enamel in all tested times, which was explained by the presence of artificial saliva and the ACP added to the bleaching agent. These different results may be explained by the protocol of treatment, in which De Abreu et al.[18] applied the bleaching agent for a period of 30 minutes once daily, while in this present study the application was 30 minutes twice daily, leaving the product in contact with the enamel for a longer period, which could have led to changes on the surface. It is still unclear whether the ACP would present beneficial results on surface roughness if applied separately from the bleaching agent as a post-treatment therapy. The WC group also returned values statistically similar in the post-treatment, probably due to the precipitation of calcium present in the formulation of the bleaching agent, as well as the calcium and phosphate ions in the solution of artificial saliva used in this study.

The pH could be related to the increase in surface roughness of enamel, but among the groups in which there were significant increases in values, the pH ranged from 7.2 to 7.7. Although some studies indicate that the pH of 7.0 can cause effects similar to those observed with pH below 7.0,[6,27,28] that seems not to have been the main cause in this study. Azrak et al.[29] related a slight increase in the values of surface roughness on the enamel after treatment with bleaching agents with pH ranging from 4.9 to 10.8, the same observed in the present study in a way that groups PC (pH 7.5), NC (pH 7.2) and PD (pH 6.0) presented also a slight increase and a not significant result. In a study conducted by Paula et al.,[30] a bleaching agent with pH 7.0 caused loss of calcium and phosphate after EDX analysis, which the authors related to the oxidation process in which enamel is subjected to the action of bleaching agents, considered as one of the agents responsible for changes in the tooth surface,[30-33] a theory that will probably also be applied in this study. Hegedüs et al.[7] reported that oxygen is capable of increasing the porosity of the tooth surface, so that oxygen free radicals are not specific and can react with organic structures of the dental tissues, where they can diffuse more freely than in mineralized structures.

For the scanning electron microscopy analysis, it was observed that in most groups there were morphological changes, such as the presence of porosities on groups PC (Fig. 2), PD (Fig. 3), DW (Fig. 4), WC (Fig. 5), PHF (Fig. 6) and PHFH (Fig. 8). These changes were more evident in DW (Fig. 4), justifying the significant changes observed for this group on the surface roughness test, although this could not be related to the results for the groups PC (Fig. 2) and PD (Fig. 3). In the PHH group (Fig. 7), there were no morphological changes present, and it is possible to see the deposition of hydroxyapatite crystals over the enamel surface. The same phenomenon would be expected for the PHFH group (Fig. 8); however, it was observed concurrently with deposition of hydroxyapatite, the presence of porosities in enamel, pointing to a possible interaction between fluoride and hydroxyapatite, as being more advantageous to use the latter element separately. The paste of synthetic hydroxyapatite is exactly like the hydroxyapatite crystals of natural enamel and it bonds at the affected site within 15 minutes, showing no gap on the interface between the repaired layer and the natural enamel, with elongated crystals regularly oriented to the tooth surface and developing across the interface, when observed with transmission electron microscopy.[21]

Once the groups DW, WC, PHF, PHH, and PHFH presented increases in surface roughness through the timespan study, it is possible to observe on SEM images that the group DW possibly presented this result due to the reduction of mineral substrate. Groups WC, PHH, and PHFH were possibly the opposite, with the increase of mineral substrate due to the deposition of crystalline structures. The group PHF presented both conditions on the superficial layer of the enamel.

The use of artificial saliva in this study was because of its potential remineralizing as reported in previous studies,[18,32-39] which has in composition calcium and phosphate ions.[23,40] In addition to this consideration of the beneficial effect of artificial saliva in *in vitro* studies, the replacement of the mineral content is also expected *in vivo* due to important factors such as salivary flow and buffering capacity of saliva, oral hygiene, and topical fluoride,[41] which can increase the remineralization of enamel.[32,34]

Recently, it is being studied the use of synthetic hydroxyapatite, which has particles with similar dimensions to those found in enamel and synthetic hydroxyapatite that could react with the natural hydroxyapatite due to its chemical proximity to the tooth structure and could be used as a method of "whitening".[25] This occur due to formation of a thin layer of this material to the tooth surface, which would also have the property to reflect more light than the more translucent natural enamel.[25] However, the use of this component together with the bleaching agent does not seem to have the beneficial effects on surface roughness, because of the deposition of hydroxyapatite crystals that led to an increase of the roughness (Figs. 6 and 8), which could be solved through a simple procedure of polish. The use of smallest particles with the dimension of macro or nano sizes should be investigated in an attempt to reduce the increase of the roughness. Thus, although hydroxyapatite is a promising component with many applications in dentistry, its use needs further studies to establish criteria for appropriate protocols of treatment.

CONCLUSION

It can be concluded that the bleaching treatment containing ACP, fluoride and hydroxiapatite may lead to an increase of the surface roughness and that micromorphological changes were observed as the presence of porosities in most groups, except on NC, and deposition of hydroxyapatite crystals that remains over the enamel surface even after the bleaching treatment.

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Table 1: Treatment agents used in this study, compositions, manufacturers, protocol of treatment, lot numbers and pH

Treatment agents	Composition	Manufacturer	Protocol of application	Lot #	pH
Gel without 7.5% Hydrogen peroxide (NC)	Water, CMC, nipagin	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.5
7.5% Hydrogen peroxide (PC)	Water, CMC, nipagin, hydrogen peroxide	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.2
Pola Day 7,5% (PD)	Hydrogen peroxide, additives, glycerol, water, flavoring.	SDI Limited, Bayswater, Victoria, Australia	30 minutes, twice daily	P090712	6.0
Day White ACP 7,5% (DW)	Water, poloxamer 407, glycerin, hydrogen peroxide, propylene glycol, potassium nitrate, flavoring, xylitol, hydroxypropyl methylcellulose, eugenol, potassium hydroxide, calcium nitrate, sodium phosphate, mica.	Discus Dental, Culver City, CA, USA	30 minutes, twice daily	DBE2688BR	7.7
White Class Calcium 7,5% (WC)	Neutralized carbopol, potassium nitrate, calcium fluoride, aloe vera, calcium gluconate, stabilizer, deionized water and surfactant.	FGM Produtos Odontológicos, Joinville, SC, Brazil	1,5 hour, once daily	300710	7.5
7.5% Hydrogen peroxide + NaF (PHF)	Hydrogen peroxide, nipagin, CMC, water, NaF	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.2
7.5% Hydrogen peroxide + HA (PHH)	Hydrogen peroxide, nipagin, CMC, water, hydroxyapatite	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.3
7.5% Hydrogen peroxide + NaF + HA (PHFH)	Hydrogen peroxide, nipagin, CMC, water, hydroxyapatite and NaF	Laboratory of Pharmacology, Piracicaba School of Dentistry, Piracicaba, SP, Brazil	1,5 hour, once daily	-	7.5

Table 2:Mean values of surface roughness (Ra) in function of time

Groups	Timespan study					
	0	7	14	21	28	35
NC	0.36 (0.04)Aa	0.30 (0.04)Ac	0.33 (0.04)Ab	0.34 (0.03)Ac	0.35 (0.04)Ac	0.34 (0.03)Ab
PC	0.36 (0.03)Aa	0.32 (0.03)Ac	0.32 (0.03)Ab	0.35 (0.06)Ac	0.36 (0.06)Abc	0.36 (0.04)Aab
PD	0.34 (0.05)Aa	0.42 (0.05)Ab	0.35 (0.05)Ab	0.39 (0.04)Abc	0.36 (0.04)Abc	0.36 (0.06)Aab
DW	0.33 (0.03)Ba	0.45 (0.06)Aab	0.40 (0,03)ABb	0.43 (0.04)Aabc	0.40 (0.03)ABabc	0.39 (0.04)ABab
WC	0.38 (0.05)Ba	0.51 (0.06)Aa	0.52 (0.04)Aa	0.51 (0.02)Aa	0.44 (0.05)ABab	0.44 (0.04)ABa
PHF	0.24 (0.02)Db	0.47 (0.05)ABab	0.49 (0.04)Aa	0.41 (0.03)BCbc	0.38 (0.05)Cabc	0.37 (0.03)Cab
PHH	0.30 (0.16)Cab	0.50 (0.06)Aab	0.50 (0.06)Aa	0.41 (0.02)Bbc	0.38 (0.03)Babc	0.38 (0.02)Bab
PHFH	0.29 (0.02)Cab	0.50 (0.03)ABa	0.55 (0.04)Aa	0.46 (0.05)Bab	0.45 (0.07)Ba	0.33(0.04)Cb

Means followed by different letters (capital case letter on horizontal and lower case letter on vertical) differ among them ($p \leq 0.05$).

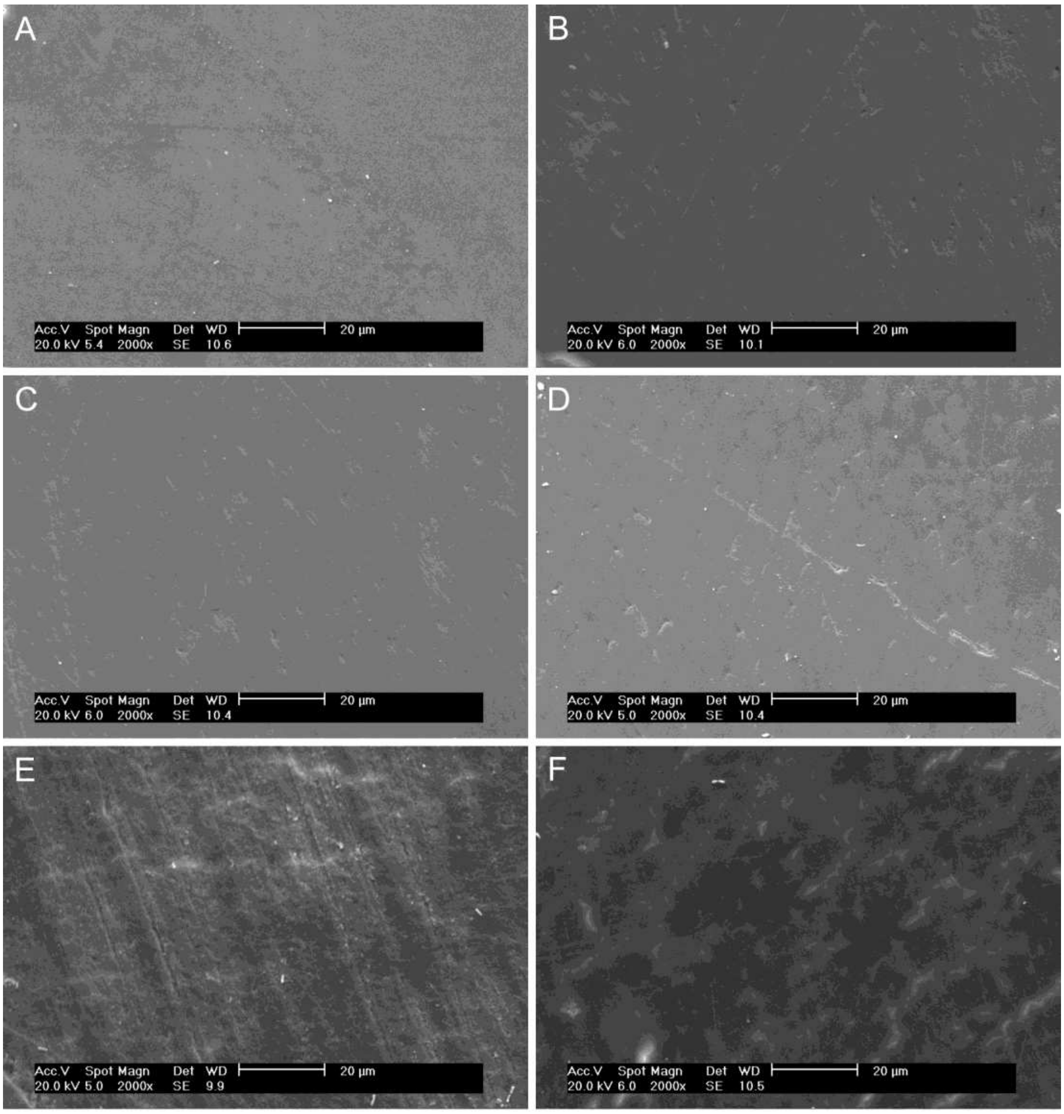

Fig. 1 - SEM photomicrographs of group NC at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. No presence of micromorphologic changes in all times of study due to the absence of bleaching agent.

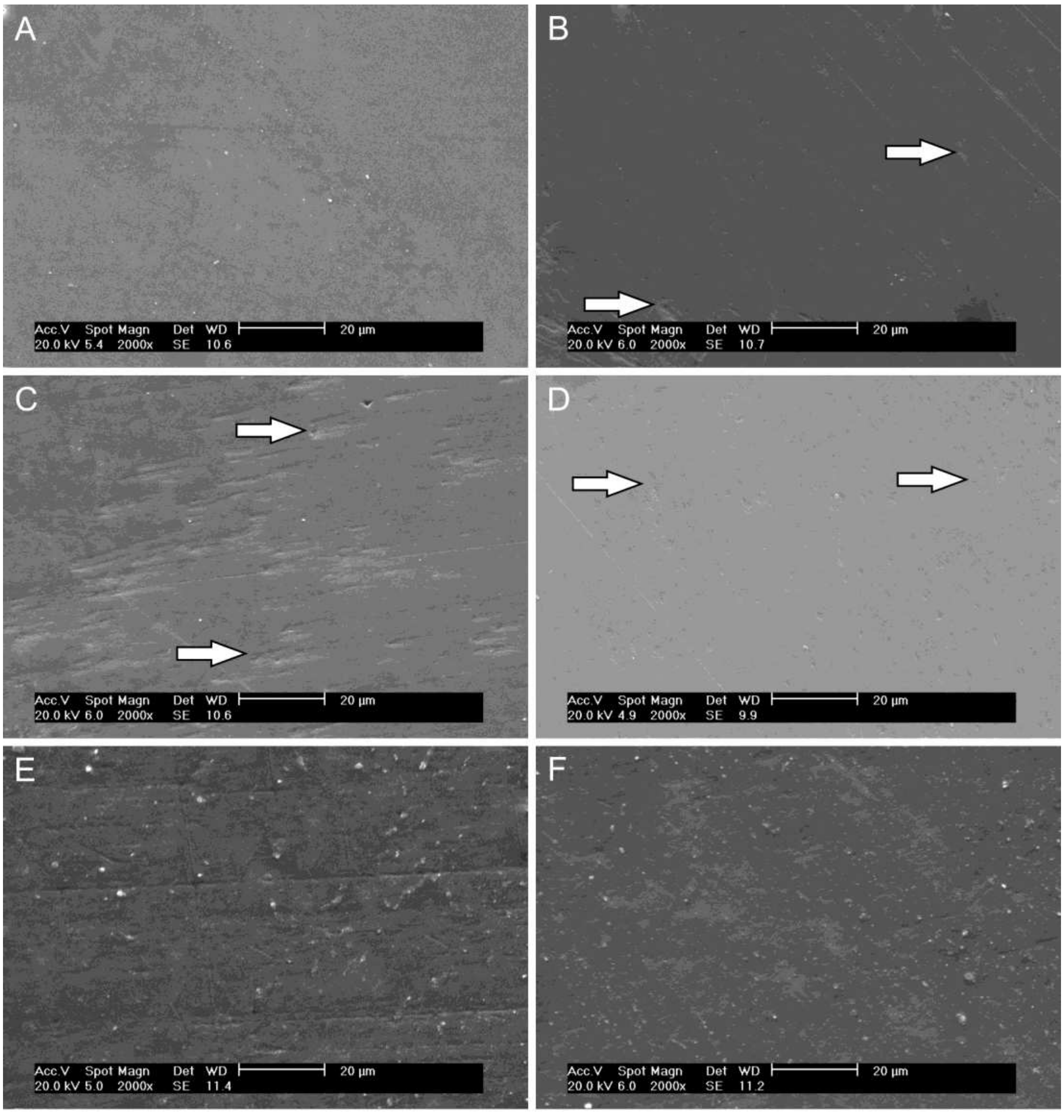

Fig. 2 - SEM photomicrographs of group PC at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. It is possible to see minor presence of irregularities (arrows) over the enamel surface during the treatment phase.

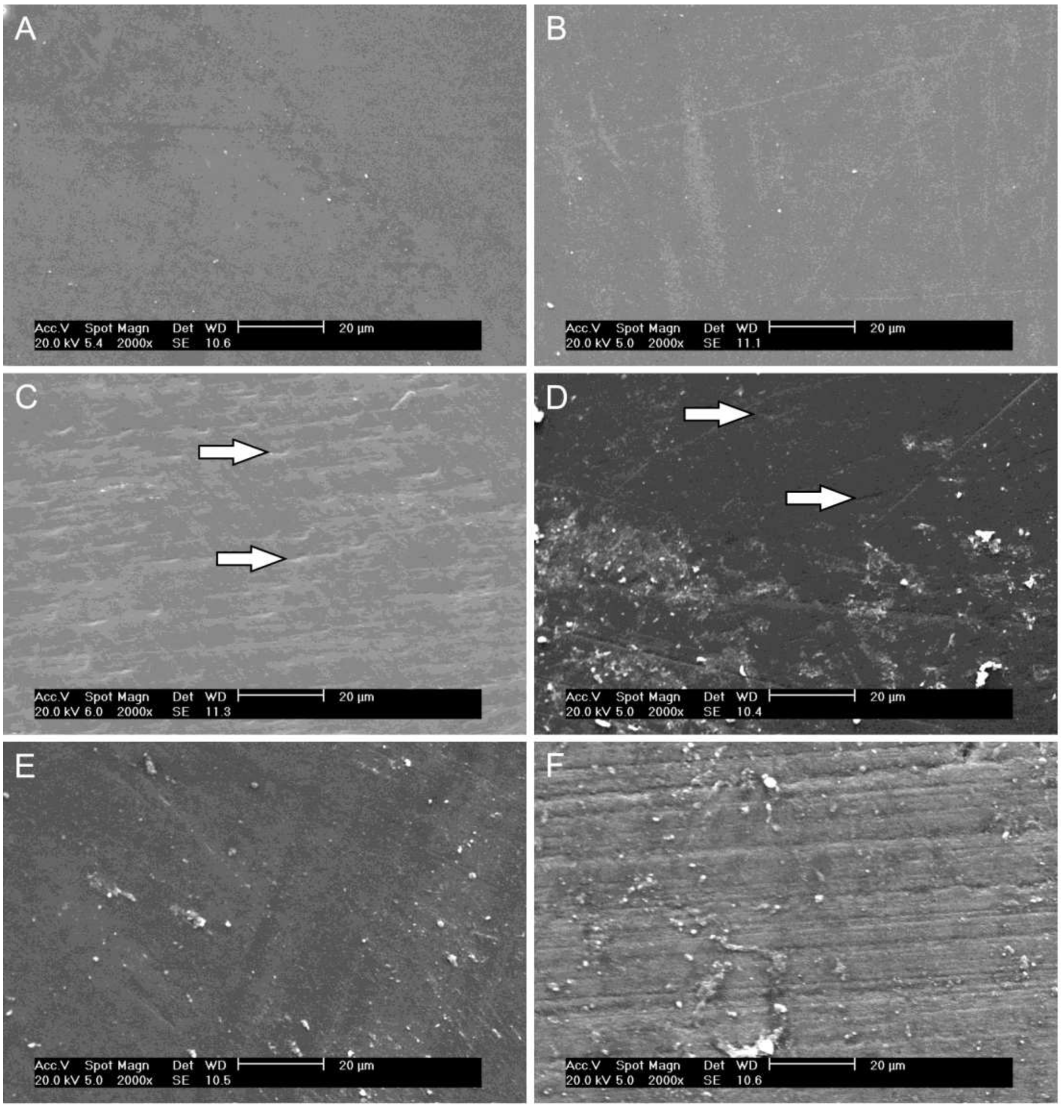

Fig. 3 - SEM photomicrographs of group PD at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Micromorphological changes such as the presence of porosities (arrows) over the enamel surface during the treatment phase.

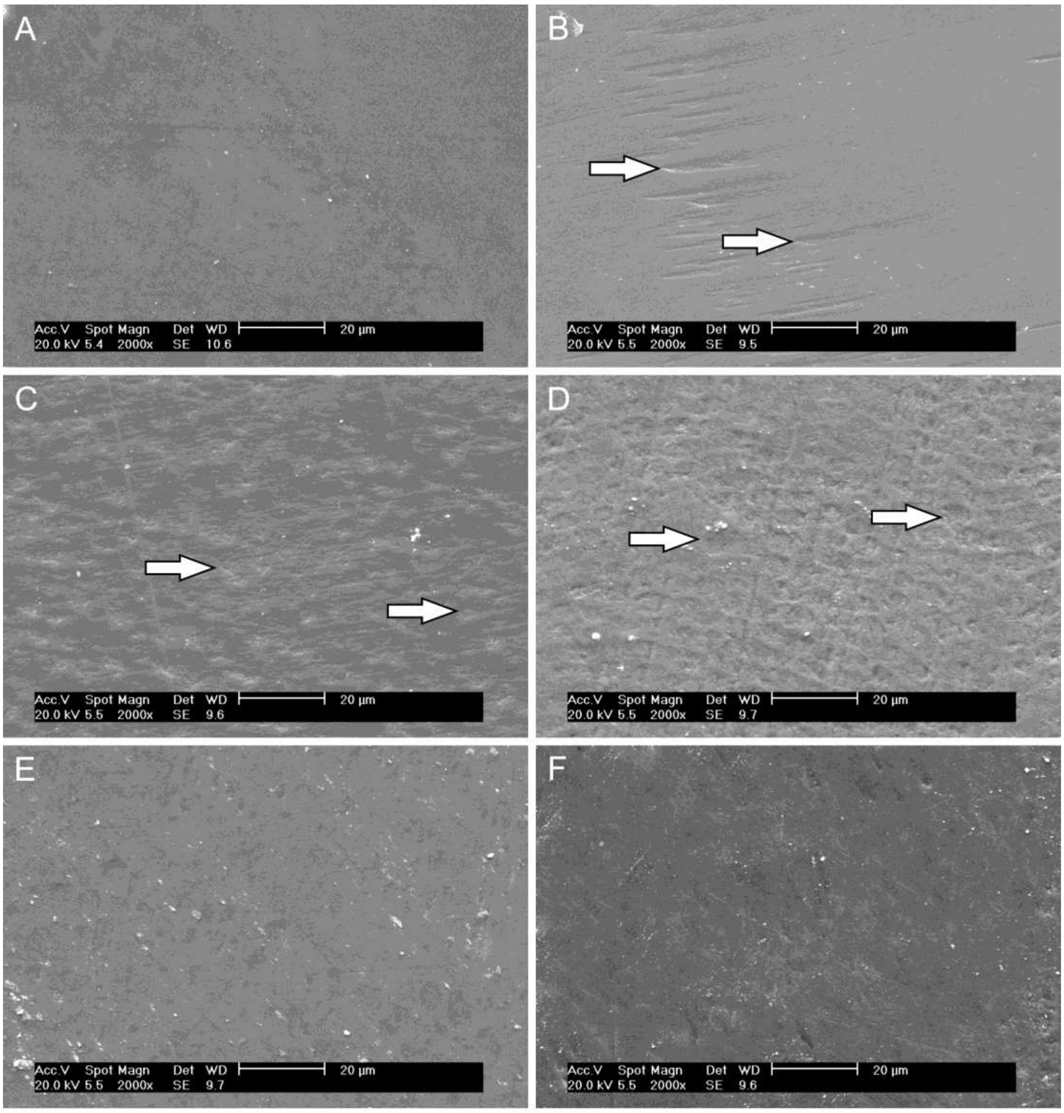

Fig. 4 - SEM photomicrographs of group DW at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Presence of demineralization process such as porosities (arrows) on enamel surface during the treatment phase that was increasing throughout the days and at post-treatment phase it is possible to observe a recovery of mineral substance probably due to the artificial saliva.

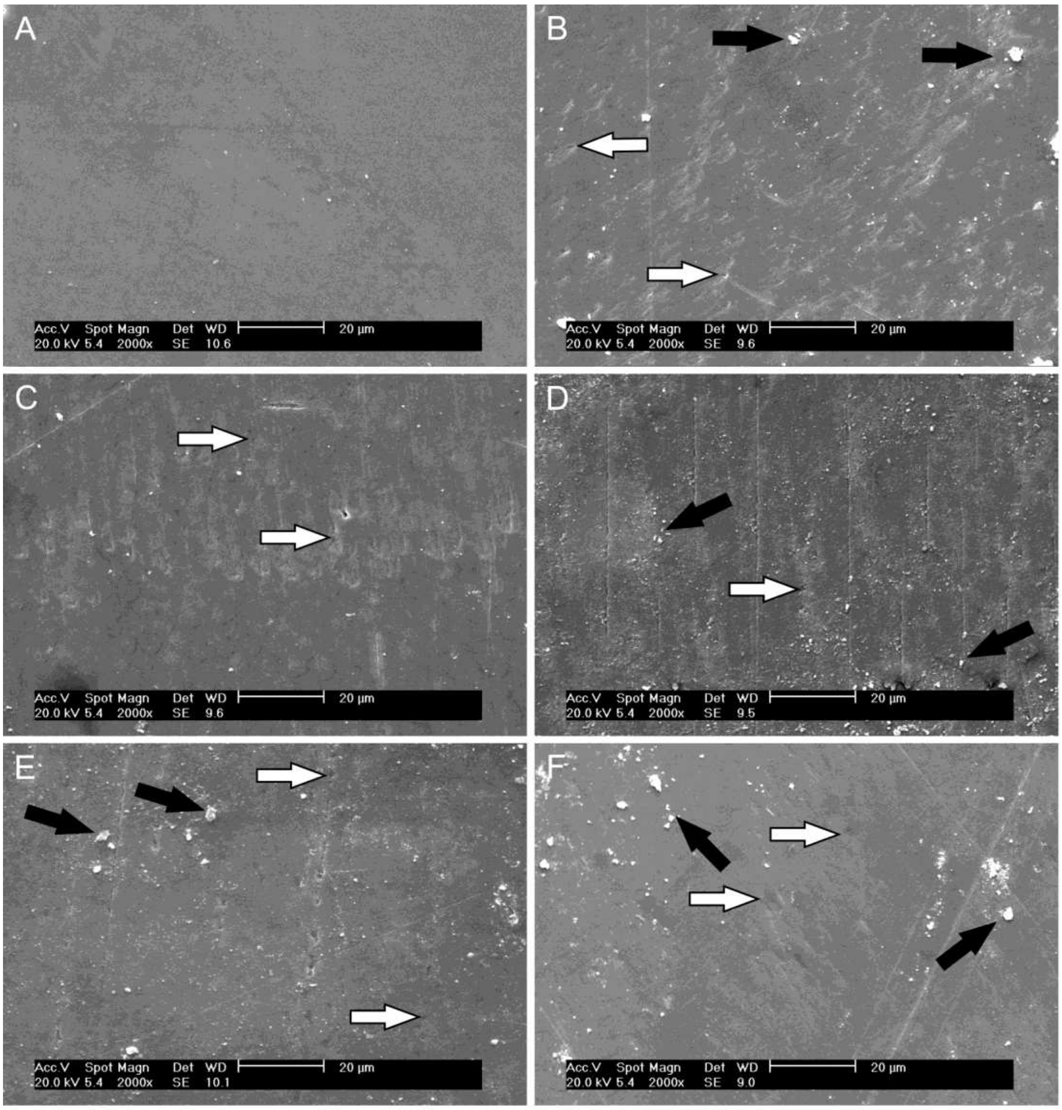

Fig. 5 - SEM photomicrographs of group WC at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Deposition of crystalline structures (black arrows) over the enamel surface probably due to the presence of calcium, along with the presence of porosities (white arrows).

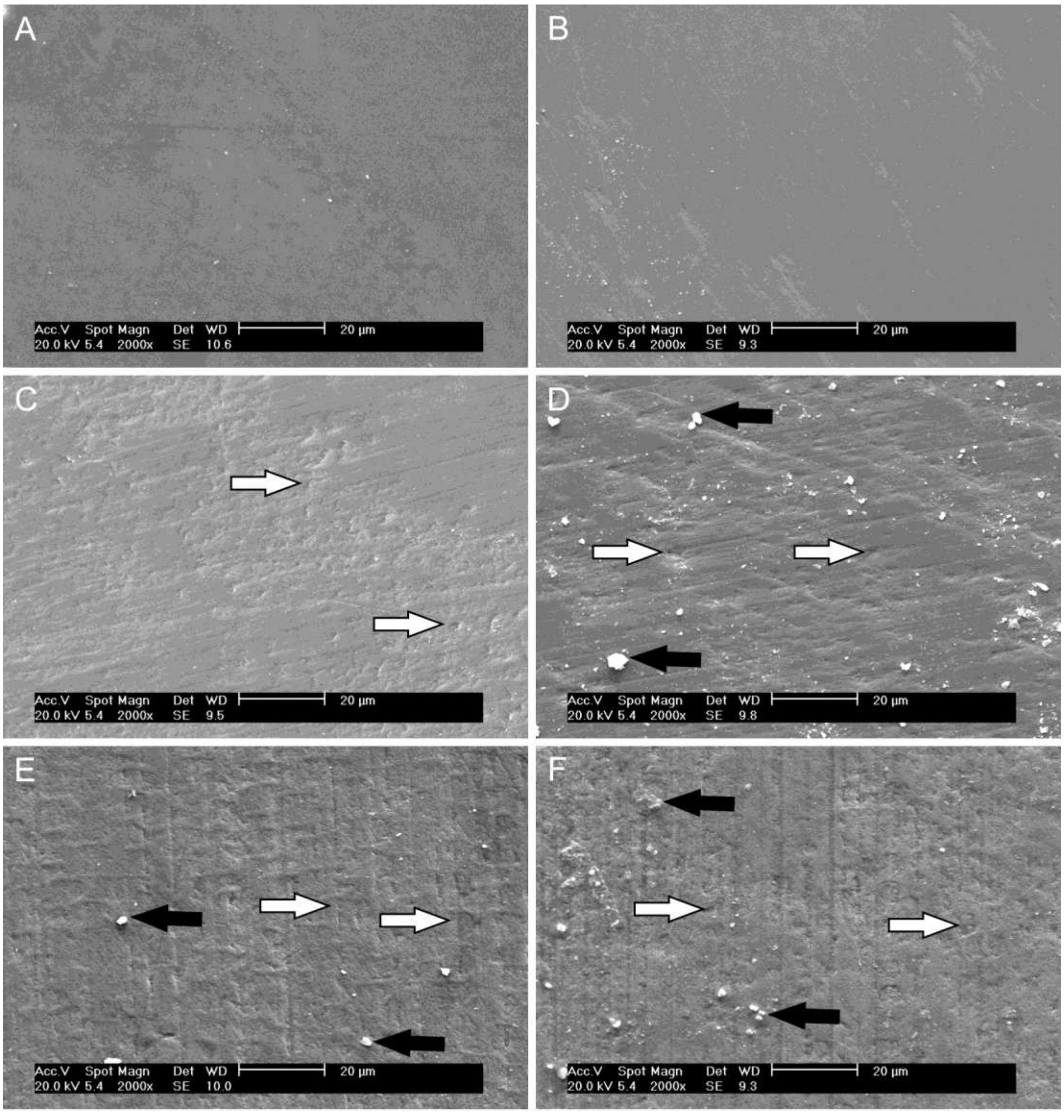

Fig. 6 - SEM photomicrographs of group PHF at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Deposition of crystalline structures (black arrows) over the enamel surface, probably due to the presence of fluoride, along with the presence of porosities (white arrows).

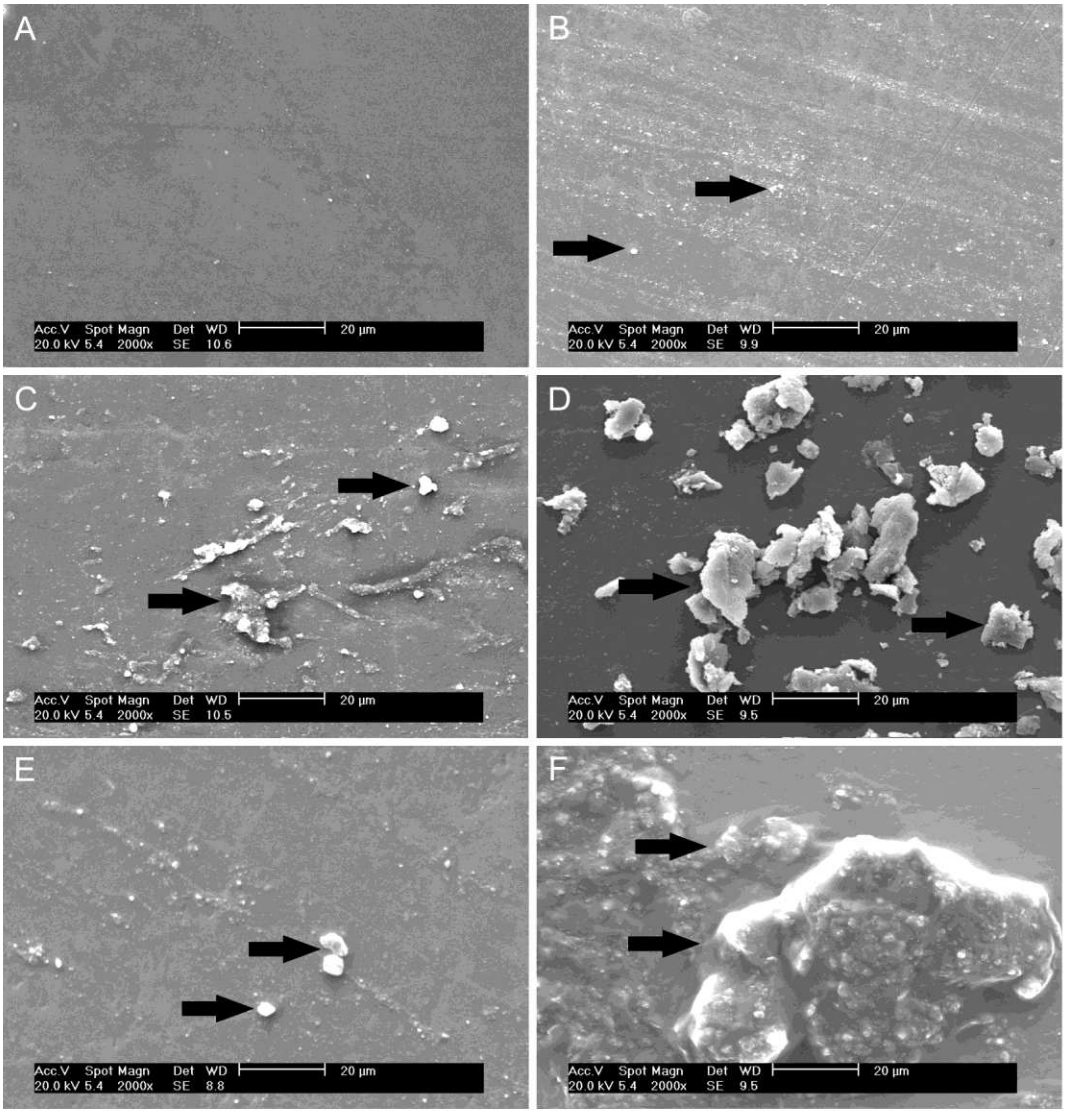

Fig. 7 - SEM photomicrographs of group PHH at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Deposition of hydroxyapatite crystals (arrows) over the enamel surface at both treatment and post-treatment phases.

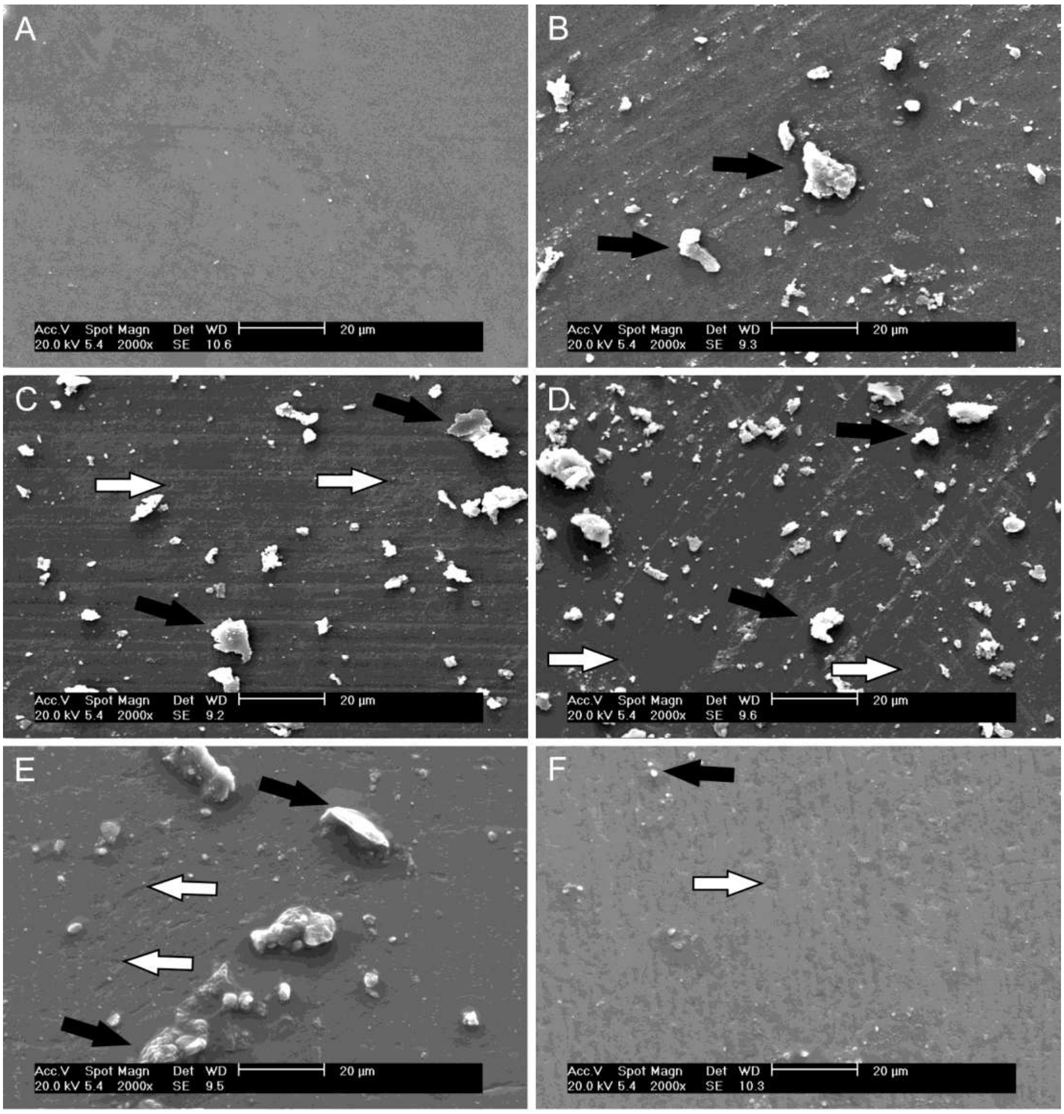

Fig. 8 - SEM photomicrographs of group PHFH at 2000x magnifications: A) Baseline; B) 7 days; C) 14 days; D) 21 days; E) 28 days; F) 35 days. Deposition of hydroxyapatite crystals (black arrows) over the enamel surface and the presence of porosities (white arrows).

CONCLUSÃO

Diante das limitações deste estudo *in vitro*, pôde-se concluir que:

- Para o teste de microdureza, o tratamento clareador contendo hidroxiapatita (PHH) apresentou aumento significativo, enquanto o grupo contendo ACP apresentou redução significativa e todos os outros grupos não apresentaram alterações estatísticas ao longo do tempo de estudo;
- Para o teste de análise de alteração de cor, todos os grupos apresentaram eficácia clareadora, com exceção do grupo NC. O agente clareador contendo hidroxiapatita (PHH) foi o único que apresentou valores similares comparados aos agentes clareadores comerciais em todos os tempos de estudo;
- Os agentes clareadores que continham componentes remineralizadores (ACP, cálcio, flúor e hidroxiapatita) apresentaram aumento da rugosidade superficial ao longo dos tempos de estudo, enquanto os agentes clareadores que não continham estes componentes não apresentaram alteração significativa;
- Foram observadas alterações micromorfológicas na superfície de esmalte como presença de porosidades e erosões em quase todos os grupos de estudo, exceto no grupo NC e também deposição de cristais de hidroxiapatita que permaneciam mesmo após o tratamento clareador nos grupos PHH e PHFH.

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[2] De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors - Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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ANEXO

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Microdureza, rugosidade superficial, análise morfológica e de alteração de cor de esmalte submetido a tratamentos clareadores, utilizando agentes clareadores contendo cálcio, ACP, flúor e/ou hidroxiapatita"**, protocolo nº 059/2009, dos pesquisadores Robson Tetsuo Sasaki e Flávio Henrique Baggio Aguiar, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 10/06/2009.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Microhardness, superficial rugosity, morphologic and color analysis of enamel submitted to bleaching treatments, using bleaching agents containing calcium, ACP, fluoride and/or hydroxyapatite"**, register number 059/2009, of Robson Tetsuo Sasaki and Flávio Henrique Baggio Aguiar, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at .

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.