UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Faculdade de Odontologia - Câmpus de Araçatuba

Nilson Antonio Nunes Junior

EFEITO DE UM NOVO AGENTE CLAREADOR PARA USO PROFISSIONAL CONTENDO HEXAMETAFOSFATO E FLUORETO SOBRE A EFICÁCIA CLAREADORA, DIFUSÃO TRANS-AMELODENTINÁRIA E MICRODUREZA: ESTUDO *IN VITRO*

Araçatuba – SP
2022

Nilson Antonio Nunes Junior

EFEITO DE UM NOVO AGENTE CLAREADOR PARA USO PROFISSIONAL CONTENDO HEXAMETAFOSFATO E FLUORETO SOBRE A EFICÁCIA CLAREADORA, DIFUSÃO TRANS-AMELODENTINÁRIA E MICRODUREZA: ESTUDO *IN VITRO*

Dissertação apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção de título de Mestre em Ciência Odontológica - Área de Concentração: Biomateriais.

Orientador: Profº. Titular. Alberto Carlos Botazzo Delbem

Araçatuba – SP
2022

Catalogação-na-Publicação

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

N972e

Nunes Junior, Nilson Antonio.
Efeito de um novo agente clareador para uso profissional contendo hexametafosfato e fluoreto sobre a eficácia clareadora, difusão trans-amelodentinária e microdureza: estudo *in vitro* / Nilson Antonio Nunes Junior. – Araçatuba, 2022
32 f. : il.2; tab1.

Dissertação (Mestrado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. Alberto Carlos Botazzo Delbem.

1. Clareamento dental 2. Peróxidos 3. Fosfatos 4. Fluoreto 5. Estética dentária 6. Desmineralização I. T.
Black D27
CDD 617.64

Ana Claudia M. Grieger Manzatti CRB-8/6315

Dedicatória

À minha mãe, Clavenice Costa Nunes (*in memoriam*)

Mãe, três letras para representar a maior e melhor pessoa que já passou em minha vida. Quis a vida me dar o privilegio de crescer e viver com minha mãe que sempre foi um poço infinito de amor, mesmo quando os puxões de orelha se fizeram necessário, aos quais, me nortearam como estudante e homem. Lembro-me do seu sonho em ser professora e do quão ficou feliz pelo meu ingresso na faculdade. Por mais que doa não poder apertar sua mão na formatura, eu quero que saiba que suas sementes continuam crescendo, pois ser mãe é ser professora.

Te amo !

Agradecimentos

Ao meu pai, Nilson Antonio Nunes,

A você, minha eterna gratidão. Obrigado por acreditar e confiar que eu poderia ir mais longe. Obrigado por ter me educado, apoiado meus sonhos e permitir que ele se tornasse realidade. Estaremos sempre juntos, pai.

À minha namorada Haruê,

Agradeço por ser companheira, inteligente, simpática, estar sempre pronta para me ajudar e por estar comigo nos momentos difíceis (infelizmente não foram poucos) e nos momentos de alegria. Sou muito grato por tê-la em minha vida e assim espero ter pelo resto dela. Saiba que te amo muito e com você ao meu lado tenho confiança para buscar novas conquistas. Você é muito importante em minha vida, sempre será a minha primavera, “till the end”.

À minha família,

Agradeço a todo o apoio que minhas irmãs (Luciana, Luceime, Nilciane), sobrinhos (Luigi, Lucca, Leon) e sobrinhas (Alice, Sofia) sempre me deram na vida e nos estudos. Ohana!

Ao meu orientador Professor Alberto Carlos Botazzo Delbem,

Agradeço ao digníssimo professor pela oportunidade de poder trabalhar neste departamento tão bem equipado e organizado em nossa faculdade. Obrigado pelos ensinamentos sem com poucas palavras pois os sábios são assim, não precisam de muitas palavras para ensinar. Agradeço por sempre estar pronto para nos ajudar e por ter muita, mas muita paciência comigo. És um professor de ímpar admiração entre nós alunos.

À Amanda Scapin e Lívia Valentim,

Obrigado por todo apoio que me deram nos experimentos. Grandes amizades que o mestrado me proporcionou. Torço pelo sucesso de vocês!

Ao meu amigo Gabriel Pereira Nunes,

Dizem que quem encontra um amigo encontra um tesouro, então “Gabs”, você é uma pessoa de ouro. Fico abismado em ver como uma pessoa de ímpar inteligência consegue ter um coração tão bondoso e altuísta. Agradeço imensamente em poder ter vivido com você durante a moradia, graduação e agora no mestrado. O seu poder de transformação na vida das pessoas é algo gigantesco. Graças a sua ajuda pude buscar vôos maiores. Saiba que sempre poderá contar comigo! Tenho muito orgulho da pessoa que você é e sei que você vai longe nessa vida. “TMJ, Gabs”.

À Faculdade de Odontologia de Araçatuba-UNESP, na pessoa dos professores Dr. Glauco Issamu Miyahara, digníssimo Diretor e Dr. Alberto Carlos Botazzo Delbem, digníssimo Vice-Diretor.

Ao Curso de Pós-Graduação em Ciência Odontológica da Faculdade de Odontologia de Araçatuba-UNESP, na pessoa do Coordenador Prof. Adj. Juliano Pelim Pessan;

A seção de Pós-Graduação,

Cristiane, Lilian e Valéria, muito obrigada por toda atenção e paciência.

Em todas as coisas o sucesso depende de uma preparação prévia, e sem tal preparação o falhanço é certo.

Confúcio

Nunes Junior, NA. **Efeito de um novo agente clareador contendo hexametafosfato e fluoreto sobre a eficácia clareadora, difusão trans-amelodentinária e microdureza: estudo *in vitro*.** 2022 32f. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba 2022.

RESUMO

Este estudo avaliou os efeitos do gluconato de cálcio (CaGlu), fluoreto de sódio (NaF), hexametafosfato de sódio (HMP) e NaF / HMP quando adicionado a um gel clareador de peróxido de hidrogênio a 35% (H_2O_2) na alteração de cor, dureza do esmalte e difusão trans-amelodentinária. Discos de esmalte / dentina bovino (n = 150) foram divididos de acordo com o gel clareador: 35% H_2O_2 (H_2O_2); 35% de H_2O_2 + 0,1% NaF (H_2O_2/NaF); 35% de H_2O_2 + 1% de HMP (H_2O_2/HMP); 35% de H_2O_2+ 0,1% NaF + 1% de HMP (H_2O_2/NaF/HMP) e 35% H_2O_2+ 2% CaGlu (H_2O_2/Caglu). Os géis clareadores foram aplicados três vezes (40 min / sessão) em intervalos de 7 dias entre cada aplicação. Então, a alteração de cor (ΔE), índice de clareamento (ΔWI_D), porcentagem de perda de dureza superficial (%SH), dureza transversal (ΔKHN) e difusão transamelodentinária foram determinadas. Os dados foram submetidos à ANOVA, seguida do teste de Student-Newman-Keuls ($p<0,05$). Todos os géis clareadores apresentaram alterações de cor significativas após o tratamento ($p<0,001$). ΔE e ΔWI_D foram similares entre os geis avaliados. A perda mineral (%SH e ΔKHN) e a difusão transamelodentinarária de peróxido de hidrogênio foram menores para o grupo 35% H_2O_2/NaF/HMP; já o grupo H_2O_2/CaGlu apresentou os maiores valores em relação aos demais grupos ($p <0,001$). Concluiu-se que, a adição de NaF / HMP ao agente clareador de consultório não interferiu na eficácia do clareamento, reduziu a desmineralização do esmalte e a difusão de H_2O_2.

Palavras-chave: Clareamento dentário; Peróxidos; Fosfato; Fluoreto; Estética Dentária; Desmineralização.

Nunes Junior, NA**. Evaluation of the bleaching efficacy, microhardness and trans-dentinal difussion of a new bleaching agent for in-office technique containing hexametaphosphate and fluoride.** 2022 32f. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba 2022**.**

ABSTRACT

Objective: This study evaluated in vitro the effects of calcium gluconate (CaGlu), sodium fluoride (NaF), sodium hexametaphosphate (HMP), and NaF/TMP added to a 35% hydrogen peroxide (H2O2) bleaching gel on the color change, enamel hardness and trans-amelodentinal diffusion. Enamel discs / bovine dentin (n = 150) were divided according to the bleaching gel: 35% H_2O_2 (H_2O_2); 35% H_2O_2 + 0.1% NaF (H_2O_2/NaF); 35% H_2O_2 + 1% HMP (H_2O_2/HMP); 35% H_2O_2 + 0.1% NaF + 1% HMP (H_2O_2/NaF/HMP) and 35% H_2O_2 + 2% CaGlu (H_2O_2/Caglu). The bleaching gels were applied three times (40 min/session) at 7-day intervals between each application. Then, color alteration (ΔE), whitening index (ΔWID), percentage of surface hardness loss (% SH), cross-sectional hardness (ΔKHN), and trans-amelodentinal diffusion were determined. Data were submitted to ANOVA, followed by the Student-Newman-Keuls test ($p < 0.05$). All bleaching gels showed significant color changes after treatment ($p < 0.001$). ΔE and ΔWI_D were similar among the evaluated gels. Mineral loss (% SH and ΔKHN) and trans-amelodentinal diffusion of hydrogen peroxide were lower for H_2O_2/NaF/HMP; the H_2O_2/CaGlu group presented the highest values about the other groups ($p < 0.001$). It is possible to conclude that the addition of NaF/HMP to the in-office bleaching agent did not interfere with the bleaching efficacy and reduced enamel demineralization and H_2O_2 diffusion.

Keywords: Tooth Bleaching; Peroxides; Phosphate; Fluoride; Esthetics; Desmineralization.

LISTA DE ABREVIATURAS

APC	Artificial pulp chamber
°C	Degrees Celsius
CaGlu	Calcium gluconate
Ca^{+2}	Calcium ion
CaF^{+}	Calcium fluoride ion
$Ca(NO_3)_2\ 4H_2O$	Calcium nitrate tetrahydrate
CIE	Commission internationale de I'éclairage
F	Fluoride
HMP	Sodium hexametaphosphate
IA	Integrated area
KCl	potassium chloride
KHN	Knoop hardness unit
H_2O	Water
H_2O_2	Hydrogen peroxide
min	Minutes
mg	Milligram
ml	Milliliter
mm	Millimeter
mmol/l	Milimol per liter
Mol/l	Mol per liter
NaF	Sodium Fluoride
NaOH	Sodium hydroxide
$NaH_2PO_4.H_2O$	Sodium phosphate monobasic monohydrate
nm	Nanometers
pH	Hydrogen potential
ROSs	Reative oxygen species
s	Seconds
SHi	Initial surface hardness
SH_f	Final surface hardness
% SH	Percentage of loss surface hardness
TMP	Sodium trimetaphosphate

SUMÁRIO

1. Introduction

Beauty is a combination of qualities that arouses admiration through the senses, especially vision. One of the main ways to express it is through a smile [1]. The appearance of a smile is associated with self-confidence and social interactions [2]. So, tooth whitening is very required in dental clinics [3]. In the in-office technique, a bleaching agent with a high concentration (35-38%) of hydrogen peroxide (H_2O_2) is used. It is known that the higher the concentration of H_2O_2 and the longer the exposure time of the dental tissue to the bleaching product [4,5], the greater the release of reactive oxygen species and their effects on hard tissues and pulp. Some studies report that bleaching can cause demineralization in teeth that undergo the procedure, as well as a decrease in enamel hardness [6-8]. On the other hand, studies show that bleaching does not cause any structural changes to the enamel [5,9]. However, even with the scientific evidence pointed out, the in-office bleaching aesthetic treatment with a high concentration of H_2O_2 gel is still the most used one by clinicians and preferred by patients [3,10].

Thus, there is a need to seed strategies and adopt new dosages that minimize these undesirable effects resulting from the bleaching procedure. Alternatives have been used, such as the use of cyclic phosphates in the composition of the bleaching gel [8,11,12]. The addition of sodium trimetaphosphate (TMP) in the bleaching gel led to less enamel demineralization, H_2O_2 diffusion, and cytotoxicity [8,11]. Sodium hexametaphosphate (HMP) is another cyclophosphate that has 3 more phosphates sites in its structure than TMP and thus may result in a greater reduction of adverse effects resulting from bleaching therapy. In addition, it was observed that HMP reduces the diffusion of H^+ ions in the dental enamel since it retains this ion in its structure, which also minimizes the propagation of acid in the enamel, contributing to reducing the demineralization process of dental tissues [13,14].

In vitro studies have shown that the effects of the association of HMP and sodium fluoride (NaF) depend on the molar proportion between these compounds [14-16]. Therefore, the ideal concentration of HMP to be used is 1% in toothpaste formulations containing 1100 ppm NaF [14,15]. In addition, several studies have shown that the association of this salt with NaF helps to inhibit demineralization and favors the process of remineralization of dental substrates [15,16].

Thus, it is expected that HMP can reduce enamel demineralization and favor the remineralization process [16], as well as reduce H_2O_2 diffusion, in an attempt to minimize the potential adverse effects of bleaching dental. Therefore, the objective of this study was to evaluate, *in vitro*, the effects of calcium gluconate (CaGlu), sodium fluoride (NaF), sodium hexametaphosphate (HMP), and NaF/TMP added to a 35% hydrogen peroxide (H_2O_2) bleaching gel on the color change, enamel hardness and trans-amelodentinal diffusion. The null hypothesis of the study was that bleaching gels containing 35% H_2O_2 and NaF/HMP or CaGlu do not show a difference in promoting bleaching effect

and in reducing mineral loss and trans-amelodentinal diffusion compared with bleaching gel containing only 35% H_2O_2.

2. Materials and Methods

2.1. Formulation of bleaching gels and determination of experimental groups

The bleaching gels were prepared in each application session since there were no stabilizers in their composition. The basic components of the gels were comprised of thickener (12% Carbopol), bleaching agent (35% H_2O_2), glycerin and water (qs) and NaOH required to maintain a pH of approximately 7.0. Depending on the experimental group, 0,1% sodium fluoride (NaF) and/or 1% sodium hexametaphosphate (HMP) was added [14,15]. A commercial (marketplace) bleaching gel containing 35% H_2O_2 and 2% calcium gluconate (CaGlu), neutral pH (Whiteness HP Blue, FGM, Joinville, SC, Brazil) was used as the positive control. Thus, five experimental bleaching gels were defined: 1) 35% hydrogen peroxide (H_2O_2); 2) H_2O_2+ 0.1% NaF (H_2O_2/NaF); 3) H_2O_2+ 1% HMP (H_2O_2/HMP); 4) H_2O_2+ 0.1% NaF + 1% HMP (H_2O_2/NaF/HMP); 5) H_2O_2 + 2% calcium gluconate (H_2O_2/CaGlu). The sample size per group was 10 enamel/dentin discs, which was based on a previous study [8], adopting surface and cross-sectional hardness as primary outcomes, mean the difference between groups (9 and 3500, respectively), standard deviation (5 and 2000, respectively) an α error of 5% and a β error of 10%. Disks were randomly divided (Excel, Microsoft Corporation, USA) among the 5 experimental groups (n = 10).

2.2. Color alteration analysis

2.2.1. Preparation and pigmentation of enamel/ dentin discs

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

After the initial reading of the color values, determined according to the Commissione Internationale de I'Eclairage (CIE L* a* b* color system), the discs were stored in microtubes (Kasvi K6-0150, 1.5 mL, São José dos Pinhais, PR, Brazil) containing 1 mL of room temperature black tea infusion for pigmentation. The infusion was made with 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) for every 100 mL of deionized water [17]. The pigmentation process was monitored for 6 days, and the infusion was changed daily. Then, a new reading of the color values was determined by the CIE L* a* b* [8, 17].

2.2.2. Bleaching gel treatments and color alteration measurement

After pigmentation, bleaching gels were applied to the enamel surface (0.04 mL) for 40 min,

using a dosing syringe and a microbrush (KG Sorensen, Cotia, SP, Brazil). For 14 days, 3 bleaching sessions were performed at 7-day intervals. The gels were removed with gauze, followed by washing with deionized water for 30 s. Between bleaching sessions, the discs were kept in individual plastic containers containing 2 mL of artificial saliva (1,5 mmol/L of Ca $(NO_3)_2.H_2O$; 0,9 mmol/L of $NaH_2PO_4.H_2O$; 150 mmol/L de KCl; in 0.1 mol/L of sodium cacodylate buffer, pH 7.0); and renewed every day [8].

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

The enamel/ dentin discs were fixed in black silicone supports with a diameter of 5.7 mm and a thickness of 3.5 mm, standardizing the incidence of the light beam in the Visible Ultraviolet Reflection (UV-2450 Model, Shimadzu, Kyoto, Japan), with wavelength ranging from 400 nm to 700 nm, under standard lighting D65 and an illumination/observation angle of 45/0°. The measurement of colors was performed on the vestibular surface of the enamel after preparation of the discs and their pigmentation, 1st, 2nd, and 3rd bleaching sessions, as well as on the 7th and 14th days after the end of bleaching. Then, the absolute differences (Δ) of the colors coordinates (L*, a*, b*) were calculated between the time analysis (post-staining, post-bleaching, and after 7 and 14 days) and the initial values: ΔL* (positive value indicates more brightness, a negative value indicates darker), Δa* (positive value indicates redder, a negative value indicates more green) and Δb* (positive value indicates more yellow, negative indicates more blue). To determine the total color change between the three coordinates, the following formula was used: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [8, 17]. Subsequently, the whiteness index for dentistry (WI_D) was determined according to the following equation: $\Delta WI_D = 0.511\ L^* - 2.324a^* - 1.100b^*$ [18].

2.3. Surface and cross-sectional hardness analysis

The enamel surface of the enamel/dentin discs was flattened and polished, to remove approximately 120 μm of the surface enamel, according to a previous study [8]. After polishing, five impressions, 100 μm equidistant from each other, were made (initial surface hardness: SHi) using a Knoop diamond tip under 25 g for 10 s (Micromet 5114, Buehler, Lake Bluff, IL, USA) [13,16]. Enamel/dentin discs, with SHi values between 342.0 and 378.0 KH, were randomly distributed (Excel, Microsoft Corporation, USA) in the 5 previously defined experimental groups (n = 10). After the 3 bleaching sessions, as described above, the final surface hardness (SHf) was determined to calculate the percentage change in surface hardness (% SH = [[SHf – SHi] / SHi] × 100) [8]. After SHf determination, the enamel/ dentin discs were sectioned in half, and one of the halves was embedded in acrylic resin and polished [8]. Knoop hardness in the cross-sectional was determined with a load of 5 g/10 s at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160 e 180 μm from the

surface. The integrated area (AI) under the curve of hardness values (KHN × μm) was calculated by the trapezoidal rule using the software GraphPad Prism (Prism 7 for Windows, version 7.00, San Diego, CA, USA) [8].

2.4. Determination of trans-amelodentinal diffusion of H_2O_2

To quantify the amount of H_2O_2 that permeated the dental tissues, enamel/dentin discs (n = 10/group) were placed in an artificial pulp chamber (APC) between two silicone rings (5.60 mm internal diameter; 1.78 mm thick; Ref. OR 008 - Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) and sealed with melted pink wax nº 7 (Wilson®, Polidental, Cotia, SP, Brazil) restricting the lateral penetration of the bleaching agent, according to a study by Briso et al. [19]. CPAs were placed individually in 24-well cell culture plates (Costar Corp., Cambridge, MA, USA). Each well was filled with 1 mL of sodium acetate buffer solution (2.0 mol/L, pH 4.5 by acetic acid, Sigma Chemical Co, St Louis, MO, USA) and subsequently received the APCs, already containing the disks of enamel/dentin. Thus, the dentin surface remained in contact with the acetate solution during the bleaching protocol, where the bleaching gels were applied once as described above (item 2.2.2) [19]. Then, the amount of 500 μL of buffer solution plus extract was transferred to experimental tubes to react with leuco-crystal violet (0.5 mg/mL; Sigma Chemical Co, St Louis, MO, USA) and horseradish peroxidase enzyme (1 mg/mL; Sigma Chemical Co, St Louis, MO, USA) [19]. The final reaction volume was adjusted to 3 mL with deionized water inserted into a cuvette and the optical density of the solutions was measured at a wavelength of 596 nm (UV-2450, Shimadzu, Kyoto, Japan) [20]. A standard curve (0.5–5.0 μg/mL) was used to convert the optical density obtained in the samples into lg/mL of H_2O_2.

2.5. Statistical analysis

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

3. Results

The bleaching agents evaluated promoted a significant change in ΔE after bleaching sessions ($p<0.001$) in previously pigmented enamel. All treatments showed similar performance, regardless of the period evaluated ($p>0.05$). The color change was progressive and continuous from the first bleaching session (Fig. 1A). In ΔWID, all groups promoted gradual and continuous color changes after the bleaching sessions, making the samples whiter ($p<0.001$). Only the 35% H_2O_2 group showed significant partial regression in whiteness values 14 days after the bleaching procedures ($p<0.05$) (Fig. 1B).

All bleaching gels led to hardness loss (Table 1) and the greatest hardness loss was approximately between 0 and 40 μm enamel depths for all groups tested (Fig. 2). Compared to the 35% H_2O_2 group, the addition of NaF reduced the loss of surface hardness (SHf: $p<0.001$; %SH: $p<0.001$), and CaGlu had the highest losses on the surface and inside the enamel. The bleaching gel containing 35% H_2O_2/NaF/HMP led to the lowest hardness loss values (SHf: $p<0.001$; %SH: $p<0.001$; KHN × μm: $p<0.001$; respectively) followed by H_2O_2/NaF (Table 1). Furthermore, it can be noted that the hardness values, as a function of depth, were higher for the H_2O_2/NaF/HMP group (Fig.2). The bleaching gels H_2O_2/NaF and H_2O_2/CaGlu showed the highest values of trans-amelodentinal diffusion of H_2O_2 and the 35% H_2O_2/NaF/HMP group had the lowest value ($p<0.001$).

4. Discussion

Currently, new bleaching dosages are being developed to minimize the adverse effects of this therapy without interfering with its clinical effectiveness. In the present study, the addition of remineralizing agents CaGlu, NaF, HMP, and NaF/TMP did not change the aesthetic efficacy of the bleaching gel at 35% H_2O_2, thus the first null hypothesis was accepted; however, the presence of NaF/HMP in the gel it reduced mineral loss and trans-amelodentinal diffusion of H_2O_2, therefore these null hypotheses were rejected.

It has been suggested that ΔE* values above 3.3 show clinically perceptible color changes [21,22]. ΔE values between 3 and 8 are moderately visible and above 8 are extremely noticeable [23]. According to this parameter, in the present study, all evaluated gels showed clinically relevant color change after the adopted bleaching protocol. Several trials have shown that traditional in-office tooth bleaching therapies using agents with high concentrations of H_2O_2 for 30 to 45 minutes produce an intense change in tooth color already in the first clinical session and less chromatic changes in subsequent sessions [8,24]. According to Sun [25], this occurs because the darker molecules react more easily with free radicals, causing a great visual impact in the first application of tooth whitening

with a high concentration agent.

Postoperative color stability is as important as the ability to lighten during treatment. Throughout the post-treatment period (7 and 14 days), there was the maintenance of color for all bleaching gels evaluated. According to Bizhang et al. [26], color degradation is minimal and linear over time, showing adequate color stability after the end of treatment at 18 months. The bleaching effectiveness, as well as its maintenance, is derived from a large diffusion of H_2O_2 in the enamel prisms and reaction with the chromophore molecules, resulting in greater reflection and less absorption of light [27]. The dental whitening index (ΔWI_D) indicates that higher values correspond to whiter teeth, and lower ΔWI_D (including negative values) denotes darker teeth. Positive ΔWI_D results for all groups indicated the bleaching ability of the agents [18], and no differences were observed between the experimental groups after 14 days of bleaching completion (chromatic stabilization). Although ΔE is an important parameter to observe color changes based on L*, a *, b * parameters, we must keep in mind that it should not be interpreted individually, but together with other variables, such as ΔWI_D, which correlates color with the proximity of white [18].

These data are important, given the great interest and expectation of the patient when seeking the bleaching procedure [28]. The improvement of the chromatic and aesthetic characteristics of the teeth has been required since the introduction of whitening agents in the dental clinic [2,28] and is associated with beauty, engagement, and social and mental well-being, positively impacting the patient's self-perception and quality of life [2]. Furthermore, it is reported that disadvantages experienced by a person due to cosmetic dental problems may profoundly affect their self-esteem, interactions, environmental adaptations, personal relationships, job opportunities, and other fundamental aspects affecting their quality of life [29]. Thus, it is essentially fundamental to maintain the functional properties and clinical effectiveness of the bleaching gel when adding and/or changing its chemical composition.

Overall, the results of the color analysis suggest that, even with the addition of NaF and/or HMP agents, H_2O_2 was able to diffuse through enamel and dentin and react with the organic and bioorganic matter, or biological waste products from dental tissues, and provide lightning through the oxidation of pigments. It is worth mentioning that, although promoting a similar whitening effect, the addition of NaF and HMP in the bleaching gel reduced the trans-amelodentinal H_2O_2 diffusion (Table 1). Thus, possibly the number of reactive oxygen species released was lower than for the groups without NaF/HMP, which hypothesizes that clinically it could culminate in lower postoperative sensitivity.

On the other hand, all the gels evaluated in the present study negatively affected the enamel microhardness, promoting its decrease. This is mainly justified because the decomposition products

of H_2O_2 not only react with the dark-colored chromophore molecules but also with the organic ones (such as proteins, lipids, and substances that stain teeth) and inorganic components of enamel [30]. However, the group exposed to the bleaching gel containing NaF/HMP had the highest KHN values, which were higher than the other gels evaluated. In addition, it was the only group that showed a loss of surface hardness within the acceptable range recommended by the ISO 28399 standard [31]. In addition to this, the presence of NaF/HMP in the bleaching gel can also protect the enamel against deep mineral loss (Fig. 2). This result is mainly attributed to the synergistic effect resulting from the association NaF/HMP incorporated in the bleaching agent.

It is noteworthy that HMP forms strong complexes with metal ions [32,33] in the oral environment, which adsorbs to the enamel surface and retains the charged ions CaF^+ and Ca^{2+} through Na^+ substitution in the cyclic structure, leading to a reticular formation [34] through the binding of Ca^{2+} to one or more HMP molecules. Through these multiple bonds, the HMP molecules form a layer of condensed phosphates, altering the selective permeability of enamel [35] and, in this case, increasing the selectivity to cations. Furthermore, HMP is a negatively charged cyclic phosphate that leads to a greater amount of electron donor sites on the enamel surface and, consequently, increases the adsorption of ionic species such as Ca^{2+}, $H_2PO_4^-$ e CaH_2PO_4 [13,16,36], which are important for increasing the diffusion of fluoride, calcium, and phosphate in enamel.

Recently, a study showed that HMP adsorption provides more calcium phosphate-binding sites and promotes the supersaturation of these ions near the enamel surface [36], which explains the ability of HMP to reduce demineralization of this substrate (Table 1; Fig. 2). In addition, the results support that the presence of HMP/NaF in the bleaching gel possibly minimizes changes in the organic matrix of enamel, since the removal or degradation of the organic matrix of enamel has been shown to significantly reduce its mechanical properties [37], as products derived from H_2O_2 in tooth whitening give rise to unstable oxygen free radicals that are capable of inducing structural changes in matrix proteins, located between the lenses and in the area of the stem sheath, as well as significant alteration of enamel interrod proteins [30].

Although even the incorporation of NaF/HMP in the gel minimized changes in hardness, it is important to mention that all bleaching gels evaluated promoted a reduction in hardness in-depth, especially up to 60 μm from the surface (Fig. 2), a finding that has already been reported in the literature [38]. This data reinforces the demineralizing potential that H_2O_2 exerts on dental enamel, since all the gels were manipulated, and the pH was previously adjusted to neutrality (pH = 7.0) to avoid biases resulting from acidic pH that could raise doubts about whether enamel effects are attributable to peroxide action or acid etching secondary to the product's low pH value [5]. In addition, the adoption of a protocol with a high exposure time and with three sessions of bleaching agent

application caused a greater loss of hardness.

Regarding the presence of the other remineralizing agents tested (NaF and CaGlu), there was no decrease in H_2O_2 diffusion, nor changes in enamel hardness (Table 1). The literature has positive antecedents with the addition of these agents in the bleaching gel [7, 31 39]. Possibly, the good results observed in these studies with the incorporation of NaF are related to the use of this ion in a higher concentration (0.2% -1.1% NaF), since in our study 0.1% NaF was used. As for CaGlu, Vieira et al. [7], when using a bleaching gel composed of 2% CaGlu, reported a reduction in mineral loss, unlike the data of the present study and other works reported in the literature [8,31,40]. According to previous studies [39,41], CaGlu is incompatible with strong oxidizing agents and cannot be released during bleaching decomposition.

Thus, it is possible to perform an efficient in-office tooth whitening protocol that is less aggressive to mineralized tissues with NaF/HMP supplementation in bleaching gels. However, the results should be extrapolated to clinical practice with caution, as in vitro studies have limitations, since they do not fully reproduce intra-oral conditions, especially regarding the effect of human saliva and the use of fluoride products, such as the daily use of fluoridated toothpaste. Moreover, some differences are observed between the laboratory conditions and the natural atmosphere of the oral cavity. As well as many other interventions, some discoloration and some continual whitening processes can be observed in the natural condition of the oral cavity. Therefore, clinical studies are needed to confirm the efficacy of using NaF/HMP in the bleaching agent, as well as the biological responses to dental and pulp hard tissues, are necessary to obtain clinical validation of the use of these agents as an additive to bleaching gel.

5. Conclusion

Taking into account the data obtained and the limitations of the experimental model, we can conclude that the addition of NaF/HMP to the bleaching gel does not interfere with bleaching efficacy and significantly reduces the loss of surface and cross-sectional hardness in enamel, as well as the trans-amelodentinal diffusion of H_2O_2. Thus, future studies should be performed to assess the aesthetic outcome and biocompatibility of the different in-office bleaching gels.

Declarations

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

Funding: This study was financially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior - Brazil (CAPES) (CAPES, PROCAD/2013 - Proc. 88881.068437/2014–2001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil (CNPq) (grant number 312934/2018-1).

Ethical approval: Not applicable.

Informed consent: Not applicable.

References

1. Arroyo Cruz G, Orozco Varo A, Montes Luna F, Jiménez-Castellanos E (2021) Esthetic assessment of celebrity smiles. J Prosthet Dent.125(1):146-150. doi: 10.1016/j.prosdent.2019.12.006.
2. Lukez A, Pavlic A, Trinajstic Zrinski M, Spalj S (2015) The unique contribution of elements of smile aesthetics to psychosocial well-being. J Oral Rehabil. 42(4):275-81. doi: 10.1111/joor.12250.
3. Vilela AP, Rezende M, Terra RMO, da Silva KL, Sutil E, Calixto AL, Reis A, D Loguercio A, Farago PV (2021) Effect of topical application of nanoencapsulated eugenol on dental sensitivity reduction after in-office dental bleaching: a randomized, triple-blind clinical trial. J Esthet Restor Dent. 33(4):660-667. doi: 10.1111/jerd.12728.
4. Benetti F, Gomes-Filho JE, Ferreira LL, Ervolino E, Briso ALF, Araujo GS, Dezan-Júnior E, Cintra LTA (2017) Hydrogen peroxide induces cell proliferation and apoptosis in pulp of rats after dental bleaching in vivo. Arch Oral Biol 81:103-109.
5. Sa Y, Chen D, Liu Y, Wen W, Xu M, Jiang T, Wang Y (2012) Effects of two in office bleaching agents with different pH values on enamel surface structure and color: an in situ vs. in vitro study. J Dent 40:26-34.
6. D'Amario M, D'Attilio M, Baldi M, De Angeli S F, Marzo G, Vadini M,; et al. (2012) Histomorphologic alterations of human enamel after repeated applications of a bleaching agent. Int J Immunopathol Pharmacol 25:1021-1027.
7. Vieira I, Vieira-Junior WF, Pauli MC, et al. (2020) Effect of in-office bleaching gels with calcium or fluoride on color, roughness, and enamel microhardness. J Clin Exp Dent. 12(2):e116-e122. doi:10.4317/jced.56006.

8. Akabane STF, Danelon M, Nunes GP, et al. (2021) Evaluation of the aesthetic effect, enamel microhardness and trans-amelodentinal cytotoxicity of a new bleaching agent for professional use containing trimetaphosphate and fluoride. J Mech Behav Biomed Mater. 114:104225. doi:10.1016/j.jmbbm.2020.104225.
9. Sasaki RT, Arcanjo AJ, Flório FM, Basting RT (2009) Micromorphology and microhardness of enamel after treatment with home-use bleaching agents containing 10% carbamide peroxide and 7.5% hydrogen peroxide. J Appl Oral Sci 17:611-616.
10. de Paula EA, Nava JA, Rosso C, Benazzi CM, Fernandes KT, Kossatz S, Loguercio AD, Reis A (2015) In-office bleaching with a two- and seven-day intervals between clinical sessions: A randomized clinical trial on tooth sensitivity. J Dent. 43(4):424-9. doi: 10.1016/j.jdent.2014.09.009.
11. Júnior RATP, Danelon M, Pessan JP, et al. (2021) Effect of daily use of fluoridated dentifrice and bleaching gels containing calcium, fluoride, or trimetaphosphate on enamel hardness: an in vitro study. Clin Oral Investig. 25(3):883-889. doi:10.1007/s00784-020-03375-5.
12. Dos Santos ALE, Delbem ACB, Danelon M, Marcon LN, Shinohara MS (2020) Evaluation of new compositions of 10% hydrogen peroxide-based bleaching agents containing trimetaphosphate and fluoride on enamel demineralization. Eur J Oral Sci. 128(5):450-456. doi:10.1111/eos.12735.
13. Dalpasquale G, Delbem ACB, Pessan JP, Nunes GP, Gorup LF, Neto FNS, de Camargo ER, Danelon M (2017) Effect of the addition of nano-sized sodium hexametaphosphate to fluoride toothpastes on tooth demineralization: an in vitro study. Clin Oral Investig. 21(5):1821-1827. doi: 10.1007/s00784-017-2093-3.
14. Da Camara DM, Pessan JP, Francati TM, Souza JA, Danelon M, Delbem AC (2016) Fluoride toothpaste supplemented with sodium hexametaphosphate reduces enamel demineralization in vitro. Clin Oral Investig 20(8):1981-85.
15. Da Camara DM, Pessan JP, Francati TM, Santos Souza JA, Danelo M, Delbem AC (2015) Synergistic effect of fluoride and sodium hexametaphosphate in toothpaste on enamel demineralization in situ. J Dent 43(10):1249-54.
16. Gonçalves FMC, Delbem ACB, Pessan JP, Nunes GP, Emerenciano NG, Garcia LSG, Quintero LCB, Neves JG, Danelon M (2018) Remineralizing effect of a fluoridated gel containing sodium hexametaphosphate: An in vitro study. Arch Oral Biol 90: 40-44.
17. Sulieman M, Addy M, Rees JS (2003) Development and evaluation of a method in

vitro to study the effectiveness of tooth bleaching. J Dent 31:415-422.

18. Pérez MM, Ghinea R, Rivas MJ, Yebra A, Ionescu AM, Paravina RD, et al. (2016) Development of a customized whiteness index for dentistry based on CIELAB color space. Dent Mater. 32(3):461-7. https://doi.org/10.1016/j.dental.2015.12.008.
19. Briso ALF; Lima APB; Gonçalves RS; Gallinari MO; dos Santos PH (2014) Transenamel and transdentinal penetration of hydrogen peroxide applied to cracked or microabrasioned enamel. Oper Dent 39:166-173.7
20. Mottola HA, Simpson BE, Gorin G (1970) Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leuco crystal violet and peroxidase as catalyst. Anal Chem 42:410-411.
21. Stober T, Gilde H, Lenz P (2001) Color stability of highly filled composite resin materials for facings. Dent Mater. 17(1):87-94. doi: 10.1016/s0109-5641(00)00065-8.
22. Vichi A, Ferrari M, Davidson CL (2004) Color and opacity variations in three different resin-based composite products after water aging. Dent Mater. 20(6):530-4. doi: 10.1016/j.dental.2002.11.001.
23. Araújo LS, Santos PH, Anchieta RB, Catelan A, Briso ALF, Zaze ACF (2013) Mineral loss and color change of enamel after bleaching and staining solutions combination. J Biomed Opt. 18:108004–6.
24. Kossatz S, Dalanhol AP, Cunha T, Loguercio A, Reis A (2011) Effect of light activation on tooth sensitivity after in-office bleaching. Oper Dent. 36(3):251-7. doi: 10.2341/10-289-C.
25. Sun G. The role of lasers in cosmetic dentistry (2000) Dent Clin North Am. 44(4):831-850.
26. Bizhang M, Müller M, Phark JH, Barker ML, Gerlach RW (2007) Clinical trial of long-term color stability of hydrogen peroxide strips and sodium percarbonate film. Am J Dent. 20 Spec No A:23A-27A.
27. Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ (2002) Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. J Oral Rehabil. 29(5):473-477. doi:10.1046/j.1365-2842.2002.00856.x
28. Fiorillo L, Laino L, De Stefano R, D'Amico C, Bocchieri S, Amoroso G, Isola G, Cervino G (2019) Dental Whitening Gels: Strengths and Weaknesses of an Increasingly Used Method. Gels. 5(3):35. doi:10.3390/gels5030035
29. Wong AH, Cheung CS, McGrath C (2007) Developing a short form of Oral Health

Impact Profile (OHIP) for dental aesthetics: OHIP-aesthetic. Community Dent Oral Epidemiol. 35(1):64-72. doi:10.1111/j.1600-0528.2007.00330.x

30. Elfallah HM, Bertassoni LE, Charadram N, Rathsam C, Swain MV (2015) Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel. Acta Biomater. 20:120-128. doi: 10.1016/j.actbio.2015.03.035.
31. Torres C, Zanatta RF, Silva TJ, Borges AB (2019) Effect of Calcium and Fluoride Addition to Hydrogen Peroxide Bleaching Gel On Tooth Diffusion, Color, and Microhardness. Oper Dent. 44(4):424-432. doi: 10.2341/18-113-L.
32. Andreola F, Castellini E, Manfredini T, Romagnoli M (2004) The role of sodium hexametaphosphate in the dissolution process of kaolinite and kaolin. The Journal of the European Ceramic Society 24:2113-24.
33. Cochrane HJ, Saranathan S, Cai F, Cross KJ, Reynolds EC (2008) Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res 42:88-97.
34. van Wazer JR, Campanella DA (1950) Structure and properties of the condensed phosphates. IV. Complex ion formation in polyphosphate solutions. Journal of the American Chemical Society 72:655-63.
35. van Dijk JW, Borggreven JM, Driessens FC (1980) The effect of some phosphates and a phosphonate on the electrochemical properties of bovine enamel. Arch Oral Biol 25:591-595.
36. Neves JG, Danelon M, Pessan JP, Figueiredo LR, Camargo ER, Delbem ACB (2018) Surface free energy of enamel treated with sodium hexametaphosphate, calcium and phosphate. Arch Oral Biol. 90:108-112. doi: 10.1016/j.archoralbio.2018.03.008.
37. Baldassarri M, Margolis HC, Beniash E (2008) Compositional determinants of mechanical properties of enamel. J Dent Res. 87(7):645-9. doi: 10.1177/154405910808700711.
38. Cavalli V, Rodrigues LK, Paes-Leme AF, Soares LE, Martin AA, Berger SB, Giannini M (2011) Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and subsurface. Photomed Laser Surg. 29(5):319-25. doi: 10.1089/pho.2010.2797.
39. Cavalli V, Rosa DAD, Silva DPD, Kury M, Liporoni PCS, Soares LES, Martins AA (2018) Effects of experimental bleaching agents on the mineral content of sound and demineralized enamels. J Appl Oral Sci. 4;26:e20170589. doi: 10.1590/1678-7757-2017-0589.

40. da Costa Soares MU, Araújo NC, Borges BC, Sales Wda S, Sobral AP (2013) Impact of remineralizing agents on enamel microhardness recovery after in-office tooth bleaching therapies. Acta Odontol Scand. 71(2):343-8. doi: 10.3109/00016357.2012.681119.

41. Furlan IS, Bridi EC, Amaral FLBD, França FMG, Turssi CP, Basting RT (2017) Effect of high- or low-concentration bleaching agents containing calcium and/or fluoride on enamel microhardness. Gen Dent 65:66–70.

List of Tables

Table 1. Mean values (±SD) of initial (SHi) and final (SHf) surface hardness, percentage of loss surface hardness (%SH), integrated area (KHN × μm), and trans-amelodentinal penetration of H_2O_2, according to experimental gels (n = 10). Distinct superscript lowercase letters indicate statistical difference among bleaching gels in each variable (Student–Newman–Keuls test, $p < 0.001$).

Figure Legends

Figure 1: Mean values (±SD) of total color alteration (ΔE) (A) and whitening index in dentistry (ΔWI_D) (B) by the International Commission of I'Eclairage (CIE) according to bleaching gels and time of analysis (n=10). Distinct superscript lowercase letters indicate statistical difference among the bleaching gels at each moment of analysis and among the moments of analysis for each bleaching gel (Student-Newman-Keuls; $p < 0.05$).

Figure 2. Cross-sectional hardness profiles at different depths in the enamel (n=10) according to the bleaching gels

Table 1. Mean values (SD) of initial (SHi) and final (SHf) surface hardness, percentage of loss surface hardness (%SH), integrated area (KHN × μm) and trans-amelodentinal diffusion of H_2O_2, according experimental gels (n = 10).

Bleaching Gels	Variables				
	SHi	SHf	%SH	KHN × μm	H_2O_2 (μg/mL)
H_2O_2	363.2[a] (7.4)	290.1[a] (9.2)	-20.1[a] (1.6)	18,019.1[a] (1,174.8)	6.56[a] (0.33)
H_2O_2/NaF	363.4[a] (7.8)	307.9[b] (7.5)	-15.3[b] (0.8)	18,621.9[a] (633.1)	6.99[b] (0.22)
H_2O_2/HMP	363.4[a] (7.7)	294.8[c] (7.8)	-18.9[c] (1.1)	18,425.6[a] (438.8)	6.54[a] (0.58)
H_2O_2/NaF/HMP	363.3[a] (7.9)	331.6[d] (8.5)	-8.7[d] (1.2)	20,461.8[b] (485.4)	6.03[c] (0.57)
H_2O_2/CaGlu	363.8[a] (7.8)	286.0[a] (7.3)	-21.4[a] (1.5)	17,062.8[c] (784.9)	6.75[a] (0.33)

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

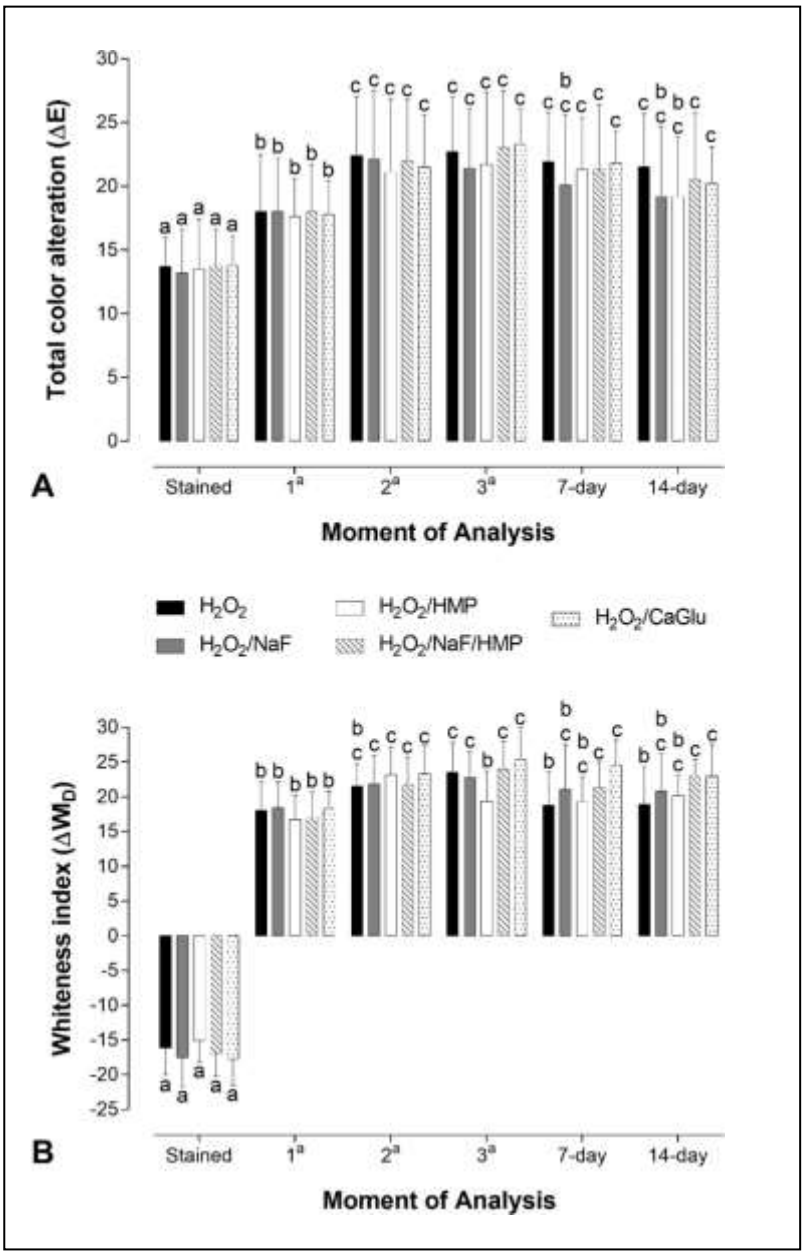

Figure 1. Mean values (±SD) of total color alteration (ΔE) (A) and whitening index in dentistry (ΔWI_D) (B) by the International Commission of I'Eclairage (CIE) according to bleaching gels and time of analysis (n=10). Distinct superscript lowercase letters indicate statistical difference among the bleaching gels at each moment of analysis and among the moments of analysis for each bleaching gel (Student-Newman-Keuls; $p < 0.05$).

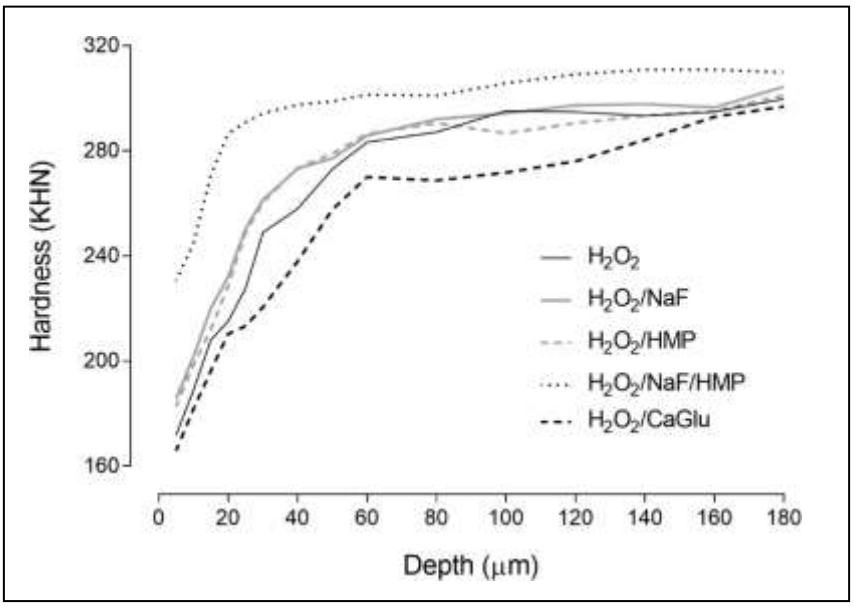

Figure 2. Cross-sectional hardness profiles at different depths in the enamel (n=10) according to the bleaching gels.

7. Anexo

Author Guidelines for Publishing in the Clinical Oral Investigations

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Objectives (stating the main purposes and research question)
- Materials and Methods
- Results
- Conclusions
- Clinical Relevance

These headings must appear in the abstract.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The entries in the list should be numbered consecutively.

If available, please always include DOIs as full DOI links in your reference list (e.g. “https://doi.org/abc”).

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibliography style file sn-basic.bst which is included in the Springer Nature Article Template.

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.

- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

- Electronic Figure Submission
- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1