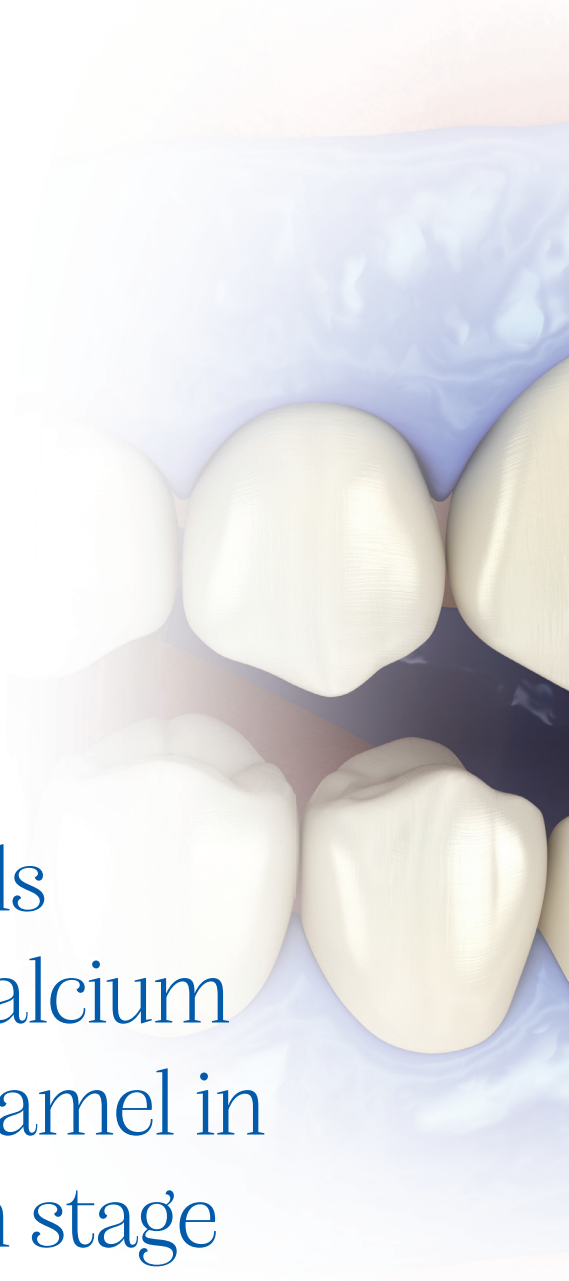


Influence of bleaching gels containing calcium on dental enamel in early erosion stage

Maria Júlia Valentini Francisco[I]

Bruna Guerra Silva[I]

Laura Nobre Ferraz[I]

Renata Pereira[I]

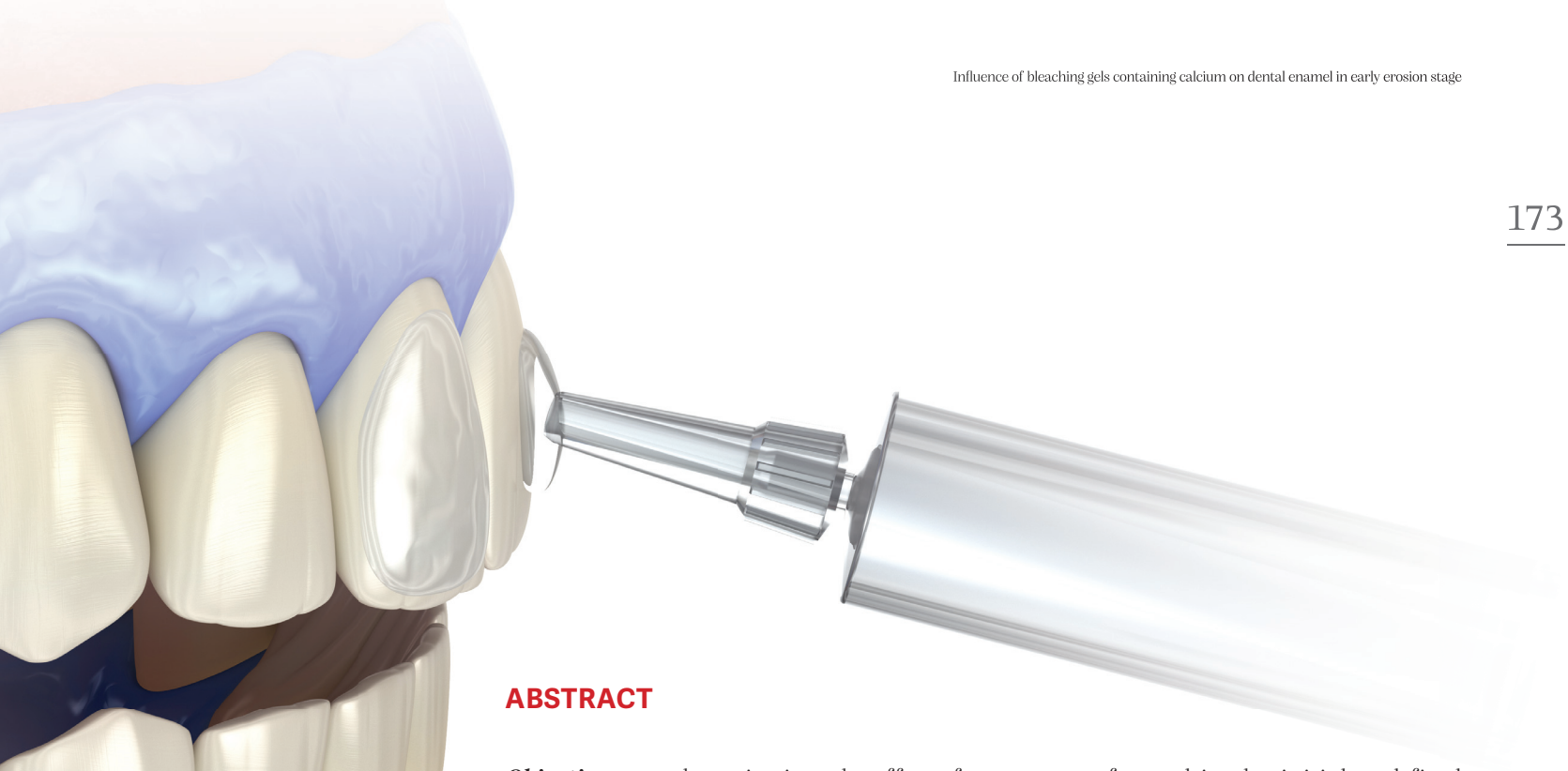
Flávio Henrique Baggio Aguiar[I]

Débora Alves Nunes Leite Lima[I]

https://doi.org/10.14436/2447-911x.17.3.172-185.aor • Submitted: July 20, 2020 • Revised and accepted: August 27, 2020

(I) Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba, Department of Restorative Dentistry, Area of Dentistry (Piracicaba/SP, Brazil).

How to cite: Francisco MJV, Silva BG, Ferraz LN, Pereira R, Aguiar FHB, Lima DANL. Influence of bleaching gels containing calcium on dental enamel in early erosion stage. J Clin Dent Res. 2020 Sept-Dec;17(3):172-85. **Contact address:** Maria Júlia Valentini Francisco. Av. Limeira, 901, CEP: 13.414-903, Piracicaba/SP, Brazil. E-mail: maria_juliavf@hotmail.com. *The authors report no commercial, proprietary or financial interest in the products or companies described in this article.*



ABSTRACT

Objective: to evaluate, in vitro, the effect of calcium-supplemented bleaching gels on enamel at initial stage of erosion regarding the bleaching efficacy and surface microhardness. ***Materials and Methods:*** Bovine specimens (4x4mm) were randomized into 5 groups (n=12): G1 - without bleaching (control group), G2 - 35% hydrogen peroxide (HP) without calcium, G3 - 35% HP with calcium, G4 - 7.5% HP without calcium and G5 - 7.5% HP with calcium. The specimens were stained with black tea solution for 6 days and eroded in 0.01M HCl solution, pH 2.3 under continuous shaking (50 rpm) for 30 seconds. Bleaching of groups G2 and G3 was performed in two sessions with 7 days of time interval. Groups G4 and G5 were daily bleached during 14 days for 1 hour. Color analyses (ΔL, Δb and ΔE*ab) and surface microhardness (KHN) were performed in the initial and final times. Data were submitted to ANOVA and Tukey's test (α=0.05). ***Results:*** For ΔL, Δb and ΔE*ab no statistical differences were found between the bleaching agents. The 7.5% HP with calcium showed less microhardness after bleaching and differed statistically between the initial and final times. The 35% HP with calcium showed higher microhardness among the bleaching gels. ***Conclusions:*** Calcium supplementation of bleaching gel did not interfere with the bleaching efficacy. The 35% HP bleaching gel with calcium resulted in less change of surface microhardness of enamel at initial stage of erosion.

KEYWORDS

Erosion. Tooth Bleaching, Calcium.

INTRODUCTION

Bleaching of vital teeth is a minimally invasive, safe and effective procedure[1,2] and may be performed through in-office bleaching technique, using high concentrations of hydrogen peroxide; through at-home bleaching technique, with low concentrations of hydrogen peroxide or by associating in-office and at-home bleaching techniques.[3] Regardless of the technique selected, the bleaching gel should be applied only on the enamel surface of vital teeth. Some patients, however, may present tooth enamel loss due to demineralizing processes, such as tooth erosion.

Tooth erosion is a process of structure loss through chemical dissolution of enamel caused by acids of non-bacterial origin.[4,5] In enamel, erosive demineralization is characterized by progressive softening, layer by layer, of the surface.[6] This altered surface is susceptible to wear and can be easily removed with mechanical effort,[6,7] resulting in permanent volume loss of tooth tissue.[6,8] At initial stages of erosion, the softening of enamel surface reaches a range depth of 1 to 10μm.[9] If there is no treatment, there is a progression of demineralization and, at advanced stages, the loss of enamel may result in exposure of dentin tissue.[6,8]

At advanced stages of tooth erosion, it is contraindicated to perform bleaching treatment due to exposure of dentin, which may increase hypersensitivity to bleaching.[3] Nevertheless, at early stages of erosion, as there is no exposure of dentin tissue, bleaching treatment may still be performed. Yet, the alterations of eroded enamel should be taken into consideration before bleaching.

Tooth erosion promotes changes in the enamel physical properties, such as decreased microhardness and increased surface roughness.[10] Accordingly, besides the type of technique, it is important to evaluate and select the most suitable bleaching gel for enamel with initial erosion lesions, so that the bleaching treatment does not enhance the changes in

physical properties of eroded enamel. Calcium supplementation of bleaching gels is intended to minimize possible morphological changes in the enamel structure[11-13], since, theoretically, the addition of calcium can saturate the bleaching agent and allow remineralization of enamel through ion exchange. This enable the restoration of enamel strength, thus minimizing adverse effects of tooth bleaching.[14]

The aim of this in vitro study was to evaluate the effect of tooth bleaching using different concentrations of hydrogen peroxide-based gels with or without calcium applied for at-home or in-office bleaching techniques in the bleaching efficacy - color change - and surface microhardness of tooth enamel at early stage of erosion. The hypotheses tested were: (1) bleaching gels with calcium would not interfere with the bleaching effectiveness and (2) bleaching gels with calcium would result in lower microhardness loss after bleaching of eroded enamel.

MATERIALS AND METHODS

Preparation of specimens

Bovine incisors were selected, cleaned and stored in 0.1% thymol. A total of 120 teeth blocks measuring 4x4mm and 3mm thickness (1mm enamel and 2mm dentin) were obtained using a metallographic cutter (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA). The blocks were then planed and polished with successive silicon carbide sandpapers (#1200/2500/p4000 Buehler Ltd, Lake Bluff, IL, USA) and felt discs (TCT, TWI - Arotec, Cotia, SP, Brazil) associated with diamond metallographic pastes (1 and ¼ μm - Arotec, Cotia, SP, Brazil) and specific lubricant (Arotec, Cotia, São Paulo, SP, Brazil).

The specimens were then immersed in black tea (Leão Junior S.A., Curitiba, PR, Brazil) for 6 days. The solution was replaced every 24 hours. Afterwards, a period of 14 days was awaited in order to

reach color stabilization. Specimens' surfaces, except of enamel's, were then protected with resistant acid varnish (Risqué Colorless, Taboão da Serra, SP, Brazil).

Sixty specimens were selected for color analysis and further 60 specimens were selected for surface microhardness analysis. These specimens were randomly divided into 5 groups (n=12), as follows: G1 - without bleaching (control group), G2 - 35% hydrogen peroxide (HP) without calcium, G3 - 35% HP with calcium, G4 - 7.5% HP without calcium and G5 - 7.5% HP with calcium.

Twenty-four hours before and during the experiment, all specimens were stored at 37º C and immersed in artificial saliva (1.5 mM of Ca; 0.9 mM of PO_4 and KCl 150 mM in Tris 20 mM buffer solution, pH 7.0), which was daily replaced.

Erosion of specimens

Erosion of specimens was performed using 17.6ml of hydrochloric acid per sample at 0.01M, pH 2.3 (adjusted with sodium hydroxide) under continuous shaking (50 rpm) at room temperature, for 30 seconds. Immediately after removal of specimens from the erosive solution, they were gently rinsed in running water to remove any acid remnants[15].

Bleaching of specimens

Handling and application of bleaching gels were performed according to manufacturer's recommendations, as follows.

- ***Bleaching with gels of 35% hydrogen peroxide:*** The tooth bleaching was performed according to the groups using gels of 35% hydrogen

peroxide with calcium (HP Blue Calcium, FGM, Joinville, SC, Brazil) or calcium-free (HP MAXX, FGM). Two whitening sessions were held with an interval of 7 days between them. In each session, the calcium-free bleaching gel (HP MAXX) was applied 3 times on the enamel surface during 15 minutes, totaling 45 minutes of application. The bleaching gel with calcium (HP Blue Calcium) was applied once for 40 minutes. Gels were then removed from the tooth surface with flexible cotton swabs, rinsed in running water and dried with absorbent paper.

- ***Bleaching with gels of 7.5% hydrogen peroxide:*** The tooth bleaching was performed according to the groups using gels of 7.5% hydrogen peroxide with calcium (White Class, FGM) or calcium-free (Pola Day, SDI, São Paulo, SP, Brazil). For this purpose, cylinder-shaped polystyrene resin bases were confectioned for each specimen. In the center of the base was embedded an addition silicon-based cube of same specimen dimensions. In order to simulate at-home dental bleaching, individual bleaching trays were confectioned in a vacuum former. The silicon-based cubes were removed from the resin base and the specimens were fixed in the empty space with aid of sticky wax. Tooth bleaching was performed for 14 days. The bleaching gel was daily applied on the specimens' surfaces and remained in contact with it for 1 hour, covered by the individual tray. Gels were then removed from the surface with flexible swabs, rinsed in running water and dried with absorbent paper.

Color analysis

To perform the color analysis, the specimens were placed in a polytetrafluoroethylene sample holder device inside a light chamber (GTI MiniMatcher MM-1, GTI Technology, Newburgh, NY, USA). The measurements were performed by a spectrophotometer (CM 700D, Konica Minolta, Osaka, Japan) initially and after the end of the bleaching treatment. The values were quantified through CIE L* b* system. The differences in the values of L* and b* coordinates between the initial and final measurements were expressed at ΔL and Δb. The general color change was calculated according to the formula: $\Delta E^*_{ab} = [(L1- L0)^2 + (a1- a0)^2 + (b1-b0)^2]^{½}$.

Surface microhardness analysis

The enamel surface microhardness analysis was performed using a Knoop diamond indenter (Shimadzu HMV-2000, Shimadzu Corp., Kyoto, Japan), under 50 grams load for 5 seconds. Five equidistant measurements were performed on the central region of the specimens' surfaces. The measurements were performed initially and after the end of the bleaching treatment. The mean of the measurements was recorded as the mean Knoop Microhardness (KHN) of each sample surface.

Statistical analyses

Data's normality and equality of variances were first confirmed. Afterwards, data were submitted two-way repeated-measures ANOVA with Tukey post-hoc test, with significance level at 5%.

RESULTS

Color

The results of color changes are presented in Table 1. The groups submitted to bleaching presented higher color change than the group without bleaching (control), in terms of brightness (ΔL) and chrome (Δb) variations, as well as general color (ΔE^*_{ab}) ($p \leq 0.05$). Groups submitted to bleaching did not differ statistically from each other ($p \geq 0.05$).

Table 1: Means (standard deviation) of color change (ΔL, Δb and ΔE*ab).

BLEACHING TREATMENT	ΔL	ΔB	ΔE^*_{AB}
Without bleaching	-0.85 (1.18)[b]	0.46 (2.28)[a]	1.76 (2.36)[b]
PH 35%	7.62 (0.70)[a]	-2.61 (1.61)[b]	8.38 (0.98)[a]
PH 35% with calcium	6.77 (0.61)[a]	-2.62 (1.39)[b]	8.33 (2.41)[a]
PH 7,5%	7.04 (1.45)[a]	-4.17 (2.35)[b]	8.78 (1.39)[a]
PH 7,5% with calcium	6.95 (1.26)[a]	-3.66 (1.06)[b]	8.11 (1.25)[a]

Different letters (lowercase vertically) indicate significant differences ($p \leq 0.05$).

Surface microhardness

The results of surface microhardness are shown in Table 2. A statistically significant difference was found between the initial and final times only for group of 7.5% HP with calcium. In the final time this group showed lower microhardness than in the initial time ($p \leq 0.05$).

In the final time, the group of 7.5% HP with calcium showed the lowest values among all the groups submitted to bleaching and was the only group that differed statistically from the group without bleaching (control) ($p \leq 0.05$). The group of 35% HP with calcium showed the highest values among the groups submitted to bleaching and did not differ statistically from the group without bleaching (control). The groups of 35% HP and 7.5% HP without calcium did not differ from the group without bleaching (control), nor from the group of 35% HP with calcium.

Table 2: Means (standard deviation) of surface microhardness (**KHN**) according to time.

BLEACHING TREATMENT	SURFACE MICROHARDNESS	
	Initial	Final
Without bleaching	347.6 (±26.5)^{Aa}	339.2 (±47.6)^{Aa}
PH 35%	340.9 (±24.1)^{Aa}	338.2 (±53.8)^{Aab}
PH 35% with calcium	341.1 (±26.6)^{Aa}	343.5 (±26.7)^{Aa}
PH 7,5%	341.9 (±24.2)^{Aa}	332.9 (±37.5)^{Aab}
PH 7,5% with calcium	348.6 (±26.5)^{Aa}	311.7 (±37.9)^{Bb}

Means followed by different letters indicate a statistically significant difference ($p \leq 0.05$). Uppercase compare different evaluation times for the same bleaching treatment; lowercase letters compare different treatments for the same evaluation time.

DISCUSSION

The first hypothesis tested in this study, that bleaching gels with calcium would not interfere with the bleaching effectiveness, was rejected, once calcium supplementation of bleaching gels did not interfere in the enamel bleaching efficacy. In this study, the specimens were stained with black tea in order to standardize their initial color.[1,16] Before bleaching, the solution stained the specimens in brown shades. Accordingly, any changes of the specimens' color would prove the bleaching gels effectivenesses. All bleaching gels, regardless of the addition of calcium, showed significant changes in the color of the specimens in terms of brightness (ΔL) and chrome (Δb) variations, as well as general color (ΔE^*_{ab}) after bleaching. The values of Δb describe the chrome of an object and tend to have negative values in the three-dimensional color system. All bleaching gels of this study showed decrease of b* values. The changes that occurred from the b*+ axis (yellow) to the b*- axis (blue) show that the specimens have become less yellowish. The values of L*, on the other hand, represent the degree of brightness that varies from 0 (black) to 100 (white)[17]. In this study, all the groups submitted to bleaching also showed increase of brightness, regardless of addition of calcium.

ΔE^*_{ab} is a parameter that provides information about the general color change of an object. Studies state that a perception threshold in color variation occurs when ΔE^*_{ab} is greater than 4.2.[18] Namely, values of ΔE^*_{ab} greater than this threshold represent clinically perceptible color changes. In the findings of this study, all groups submitted to bleaching presented values of ΔE^*_{ab} greater than 8.10, which point out remarkable color change. Such values differ from the control group, whose variation was 1.76. Furthermore, the groups submitted to bleaching did not differ statistically between each other. Thus, based on the color results, this study ensure the use

"Calcium in the bleaching gel does not interfere in the bleaching efficacy".

of a calcium supplemented bleaching gel on teeth with initial erosion regarding bleaching efficacy. The findings are in accordance with other studies from literature, which state that the presence of calcium in the bleaching gel does not interfere in the bleaching efficacy.[13,19,20]

The results of the color analysis may be explained by the active principle of the bleaching gels evaluated in this study. Regardless of the presence of calcium, all bleaching gels have hydrogen peroxide in their formulation. Hydrogen peroxide dissociates into free radicals, which interact with the long chain organic molecules responsible for the color of dental tissue and, through an oxirreduction reaction, promote tooth bleaching.[21,22]

The second hypothesis evaluated in this study, that bleaching gels with calcium would result in lower microhardness loss after bleaching of eroded enamel, was partially accepted. After bleaching, the 35% HP with calcium group was the bleaching group with the highest microhardness and did not differ from the group without bleaching. As expected, this result may be associated with the calcium present in its formulation. Studies report that the addition of calcium ions promotes saturation of bleaching gels, allowing their incorporation in the enamel hydroxyapatite crystals. This should increase resistance to demineralization and thus reduce possible harmful effects caused by hydrogen peroxide.[11,23,24] In addition, the calcium

present in the bleaching gel may act favoring remineralization after bleaching. The demineralization of enamel by hydrogen peroxide affects the ionic balance, which allows the deposition of calcium on the enamel surface[24] for remineralization to occur.

Despite the good performance of the 35% HP with calcium group, the 7.5% HP with calcium group showed the lowest microhardness among all the groups submitted to bleaching and differed from the group without bleaching. This result can be associated to the thickener present in its formulation. The 7.5% HP with calcium is the only bleaching gel evaluated in this study that presents Carbopol thickener in its composition. Carbopol is a carboxipolymethylene, ionic polymer of acid nature, derived from carboxylic acid.[25] Studies have shown that due to its acid pH, Carbopol may contribute to dental demineralization during bleaching.[25,26] Ávila et al.[27] show that Carbopol may interact with enamel surface due to its bioadhesive capacity. The bioadhesive capacity is related to ionic bonds between the polymers and tooth structure. The bioadhesivity of Carbopol and its interaction with enamel surface results in the formation of a very strong and thick layer of polymers,[27] which causes inhibition of hydroxyapatite crystals incorporation during the remineralization process by saliva,[28] thus justifying the results found in this study.

In addition, 7.5% HP with calcium was the only group which presented differences between the initial and final Microhardness values. Final time presented lower microhardness than the initial one. This result can also be associated with the characteristics of Carbopol thickener present in its composition. For the other bleaching gels, no statistical differences were found in the surface microhardness between initial and final times, pointing out enamel remineralization. This result may be associated with the action of artificial saliva. Remineralization is the process

of replacing the lost of minerals[29] due to bleaching procedure through saliva. In this study the specimens were immersed in artificial saliva during the whole experimental period. Zeczskowski and colleagues[30] showed that in vitro artificial saliva presents the same remineralization capability compared to natural saliva in surface microhardness analysis, thus justifying the results found in this study.

CONCLUSION

The bleaching efficacy was not affected by calcium supplementation of bleaching gels. The use of a bleaching gel based on 35% hydrogen peroxide with calcium resulted in less alteration of microhardness of dental enamel at initial stage of erosion.

ACKNOWLEDGMENTS

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001, and by Institutional Program for Scientific Initiation (Pibic).

REFERENCES

1. Joiner A. The bleaching of teeth: a review of the literature. J Dent. 2006 Aug;34(7):412-9.
2. Marson FC, Gonçalves RS, Silva CO, Cintra LTA, Pascotto RC, dos Santos PH, et al. Penetration of hydrogen peroxide and degradation rate of different bleaching products. Oper Dent. 2015 Jan-Feb;40(1):72-9.
3. Li Y. Safety controversies in tooth bleaching. Dent Clin North Am. 2011 Apr;55(2):255-63, viii.
4. Lussi A, Jaeggi T. Chemical factors. Monogr Oral Sci. 2006;20:77-87.
5. Pini NIP, Lima DANL, Lovadino JR, Ganss C, Schlueter N. In vitro efficacy of experimental chitosan-containing solutions as anti-erosive agents in enamel. Caries Res. 2016;50(3):337-45.
6. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion - an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45 Suppl 1:2-12.
7. Ganss C, Klimek J, Schlueter N. Erosion/abrasion-preventing potential of NaF and F/Sn/chitosan toothpastes in dentine and impact of the organic matrix. Caries Res. 2014;48(2):163-9.
8. Ganss C, von Hinckeldey J, Tolle A, Schulze K, Klimek J, Schlueter N. Efficacy of the stannous ion and a biopolymer in toothpastes on enamel erosion/abrasion. J Dent. 2012 Dec;40(12):1036-43.
9. Lippert F, Parker DM, Jandt KD. In vitro demineralization/remineralization cycles at human tooth enamel surfaces investigated by AFM and nanoindentation. J Colloid Interface Sci. 2004 Dec 15;280(2):442-8.
10. Ferraz LN, Pini NIP, Ambrosano GMB, Aguiar FHB, Lima DANL. Influence of cigarette smoke combined with different toothpastes on enamel erosion. Braz Oral Res. 2019 Dec 2;33:e114.
11. Giannini M, Silva AP, Cavalli V, Paes Leme AF. Effect of carbamide peroxide-based bleaching agents containing fluoride or calcium on tensile strength of human enamel. J Appl Oral Sci. 2006 Apr;14(2):82-7.
12. Borges AB, Yui KCK, D'Avila TC, Takahashi CL, Torres CRG, Borges ALS. Influence of remineralizing gels on bleached enamel microhardness in different time intervals. Oper Dent. 2010 Mar-Apr;35(2):180-6.
13. Alexandrino L, Gomes Y, Alves E, Costi H, Rogez H, Silva C. Effects of a bleaching agent with calcium on bovine enamel. Eur J Dent. 2014 July;8(3):320-5.
14. Bistey T, Nagy IP, Simó A, Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J Dent. 2007 Apr;35(4):325-30.
15. Oliveira GC, Tereza GPG, Boteon AP, Ferrairo BM, Gonçalves PSP, da Silva TC, et al. Susceptibility of bovine dental enamel with initial erosion lesion to new erosive challenges. PLoS One. 2017 Aug 17;12(8):e0182347.
16. Khashayar G, Bain PA, Salari S, Dozic A, Kleverlaan CJ, Feilzer AJ. Perceptibility and acceptability thresholds for colour differences in dentistry. J Dent. 2014 June;42(6):637-44.
17. Borges AB, Zanatta RF, Barros ACSM, Silva LC, Pucci CR, Torres CRG. Effect of hydrogen peroxide concentration on enamel color and microhardness. Oper Dent. 2015 Jan-Feb;40(1):96-101.
18. Alghazali N, Burnside G, Moallem M, Smith P, Preston A, Jarad FD. Assessment of perceptibility and acceptability of color difference of denture teeth. J Dent. 2012 July;40 Suppl 1:e10-7.
19. Torres CRG, Zanatta RF, Silva TJ, Borges AB. Effect of calcium and fluoride addition to hydrogen peroxide bleaching gel on tooth diffusion, color, and microhardness. Oper Dent. 2019 July-Aug;44(4):424-32.
20. Vieira I, Vieira Junior WF, Pauli MC, Theobaldo JD, Aguiar FHB, Lima DANL, et al. Effect of in-office bleaching gels with calcium or fluoride on color, roughness, and enamel microhardness. J Clin Exp Dent. 2020 Feb 1;12(2):e116-22.
21. Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: a review. Clin Oral Investig. 2010 Feb;14(1):1-10.
22. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. J Esthet Restor Dent. 2015 Sept-Oct;27(5):240-57.
23. Borges AB, Samezima LY, Fonseca LP, Yui KCK, Borges ALS, Torres CRG. Influence of potentially remineralizing agents on bleached enamel microhardness. Oper Dent. 2009 Sept-Oct;34(5):593-7.
24. Borges BCD, Pinheiro MHM, Feitosa DAS, Correia TC, Braz R, Montes MAJR, et al. Preliminary study of a novel in-office bleaching therapy modified with a casein phosphopeptide-amorphous calcium phosphate. Microsc Res Tech. 2012 Nov;75(11):1571-5.
25. Gouveia THN, de Souza DFS, Aguiar FHB, Ambrosano GMB, Lima DANL. Effect of ammonium acryloyldimethyltaurate copolymer on the physical and chemical properties of bleached dental enamel. Clin Oral Investig. 2020 Aug;24(8):2701-11.
26. Silva BG, Gouveia THN, da Silva MAP, Ambrosano GMB, Aguiar FHB, Lima DANL. Evaluation of home bleaching gel modified by different thickeners on the physical properties of enamel: an in situ study. Eur J Dent. 2018 Oct-Dec;12(4):523-7.
27. Ávila DMS, Zanatta RF, Scaramucci T, Aoki IV, Torres CRG, Borges AB. Influence of bioadhesive polymers on the protective effect of fluoride against erosion. J Dent. 2017 Jan;56:45-52.
28. van der Reijden WA, Buijs MJ, Damen JJ, Veerman EC, ten Cate JM, Nieuw Amerongen AV. Influence of polymers for use in saliva substitutes on de- and remineralization of enamel in vitro. Caries Res. 1997;31(3):216-23.
29. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001 Feb;85(2):162-9.
30. Zeczkowski M, Tenuta LMA, Ambrosano GMB, Aguiar FHB, Lima DANL. Effect of different storage conditions on the physical properties of bleached enamel: an in vitro vs. in situ study. J Dent. 2015 Sept;43(9):1154-61.