10.5005/jp-journals-10024-1514

ORIGINAL RESEARCH

Effect of Incorporation of Remineralizing Agents into Bleaching Gels on the Microhardness of Bovine Enamel *in situ*

[1]Alessandra Bühler Borges, [2]Carolina Anne Guimarães, [3]Eduardo Bresciani, [4]Carolina Judica Ramos
[5]Alexandre Luiz Souto Borges, [6]Carlos Rocha Gomes Torres

ABSTRACT

Aim: This study evaluated the effect of adding calcium or fluoride to 35% hydrogen peroxide (HP) bleaching gel and the effect of human saliva on the microhardness of sound and demineralized enamel, using an *in situ* model.

Materials and methods: Cylindrical bovine enamel specimens (3 × 2 mm) were divided into two groups (n = 30): sound enamel (SE) and demineralized enamel (DE). Each group was divided into three subgroups, according to the bleaching gel: 35% HP; 35% HP + calcium; 35% HP + fluoride. After bleaching therapy, the specimens were fixed to intraoral devices worn by 10 volunteers for 7 days. Surface enamel microhardness (SMH) was measured before and after bleaching procedures, and after 1 and 7 days of saliva exposure. Data were analyzed by Repeated Measures ANOVA (5%).

Results: The variable time resulted in significant differences for SE and DE groups (p = 0.001). For SE, significantly lower SMH was detected for control at post-bleaching period in comparison to the baseline and after 7 days. For DE, the lowest mean values were obtained before bleaching, and the addition of calcium to the peroxide significantly increased enamel SMH. The exposure to human saliva resulted in increased SMH.

Conclusion: The addition of potential remineralizing agents into bleaching gels might play an important role in maintaining the microhardness of sound enamel and in inducing remineralization of artificially demineralized enamel right after bleaching, and the remineralizing action of human saliva might minimize the deleterious effects of bleaching gels on enamel.

[1,3,5]Assistant Professor, [2]Graduate Student, [4]Adjunct Assistant Professor, [6]Associate Professor

[1-3,6]Department of Restorative Dentistry, UNESP-Univ Estadual Paulista, Institute of Science and Technology, São José dos Campos, São Paulo, Brazil

[4]Department of Social Dentistry and Pediatric Clinics, UNESP-Univ Estadual Paulista, Institute of Science and Technology São José dos Campos, São Paulo, Brazil

[5]Department of Dental Materials and Prosthodontics, UNESP-Univ Estadual Paulista, Institute of Science and Technology São José dos Campos, São Paulo, Brazil

Corresponding Author: Alessandra Bühler Borges, Assistant Professor, Department of Restorative Dentistry, UNESP-Univ Estadual Paulista, Institute of Science and Technology, São José dos Campos, São Paulo, Brazil, Phone: +55 12 3947-9374 e-mail: alessandra@fosjc.unesp.br

Clinical significance: The incorporation of calcium into HP bleaching gel might be beneficial for the initial phases of the bleaching procedure.

Keywords: Fluoride, Calcium, Enamel, Hydrogen peroxide, Microhardness.

How to cite this article: Borges AB, Guimarães CA, Bresciani E, Ramos CJ, Borges ALS, Torres CRG. Effect of Incorporation of Remineralizing Agents into Bleaching Gels on the Microhardness of Bovine Enamel *in situ*. J Contemp Dent Pract 2014;15(2):195-201.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

The development of high-concentrations of hydrogen peroxide (HP) gel formulations and the use of gingival barriers to simultaneously protect the gingival tissue of both arches have made in-office bleaching procedure easier and faster. This technique is especially indicated for scenarios of severe discolorations, discoloration of an individual tooth, absence of patient compliance, and to fulfill fast outcome expectations by patients.[1]

Although in-office bleaching has been overall reported as a safe and effective procedure, high-concentrated agents are potentially aggressive to dental tissues.[2,3] Several studies have investigated the effects of bleaching agents on enamel and controversial results have been obtained. While some authors observed a decrease in enamel microhardness after bleaching procedures, and its association with the low pH of bleaching gels,[4,5] this negative effect was not reported by others.[3,6]

Attempts to prevent mineral loss or to promote remineralization of bleached enamel have been made with the use of potential remineralizing agents. Calcium and fluoride agents were applied after the bleaching procedures or added to the bleaching gels resulting in positive outcomes.[4,7-13]

As initial demineralization might not be clinically detected at early stages, demineralized enamel resulted from initial decalcification of possible primary or secondary caries or from post-orthodontic treatment, might eventually undergo bleaching procedures. Thus, the investigation of deleterious effects of bleaching agents on demineralized substrate is

important. Moreover, the presence of remineralizing agents in bleaching gels might reduce the eventual demineralizing potential of bleaching agents on sound enamel or might promote remineralization to demineralized enamel.

Some manufacturers have added remineralizing agents to their bleaching gel formulations aiming to maintain or re-establish the microhardness of bleached enamel, and to reduce tooth sensitivity.[9,14,15]

The effect of human saliva on bleached enamel is an important consideration when evaluating the real potential of these remineralizing agents. Although the remineralizing potential of artificial saliva on bleached enamel has been observed *in vitro*,[16] no significant effect on the remineralization process of bleached enamel immersed into artificial saliva or remineralizing solutions was detected by others.[7,13] In a previous study, the deleterious effect of tooth whitening on enamel microhardness was observed *in vitro*, however these effects were not found *in situ*.[17] This suggests that human saliva presents improved remineralizing capability in comparison to artificial saliva.[18]

The aim of this *in situ* study was to evaluate the effect of adding fluoride or calcium to 35% HP gel on the microhardness of sound and demineralized enamel and the effect of human saliva on bleached enamel. The null hypotheses investigated were: (a) the addition of fluoride or calcium to the bleaching gel does not interfere with the microhardness of sound and demineralized bleached enamel; (b) the *in situ* exposure to saliva after 1 or 7 days does not interfere with the recovery of microhardness of bleached sound and demineralized enamel.

MATERIALS AND METHODS

Volunteer Selection

Ten volunteers were selected according to the inclusion criteria (absence of caries lesion and periodontal disease, normal salivary flow) and not violating the exclusion criteria (presence of fixed or removable dentures, use of orthodontic appliances, pregnant or nursing women). The participants signed an informed consent and the study was approved by the local IRB under protocol #050/2009-PH/CEP.

Specimen Preparation

Cylindrical specimens (3 mm in diameter) were obtained by cutting the buccal surface of bovine incisors with a trephine bur. The specimens were attached to a metal holder, previously adapted to 2.1 mm height. The enamel surface was placed facing the bottom of the device and the opposite surface was abraded with a 600 grit (FEPA-P; Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra, São Paulo, SP, Brazil). The specimens were then put back in the metal holder adjusted to 2 mm height, with the enamel at the surface to allow this tissue to be polished with sequential aluminum oxide abrasive papers (1200, 2400 and 4000 grit – FEPA-P). The resulting polished specimens presented 3 mm in diameter and 2 mm in thickness. The prepared specimens were examined under a stereomicroscope (Stemi 2000C – Carl Zeiss, Jena, Germany – 20x) to certify the absence of cracks or other surface defects. They were immersed into containers with distilled water at 121 °C for steam sterilization for 20 minutes.[19]

The baseline surface microhardness (SMH) of all specimens was measured using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan) with a Knoop diamond indenter under a 50 g load for 10 seconds. The average of three indentations spaced 100 μm apart, made at the center of the specimen was used for SMH determination. For baseline specimens' standardization, after initial SMH measurements, 60 enamel specimens presenting Knoop Hardness Number (KHN) ranging from 264.26 to 346.76 (mean 303.85, SD 21.5) were selected.

The specimens were divided into two groups (n = 30) according to the enamel treatment prior to the bleaching procedure: group SE (sound enamel) with specimens immersed into distilled water, and group DE (demineralized enamel). The DE specimens were protected with wax, except for enamel surface, and artificial enamel subsurface lesions were created by individual immersion and storage of the specimens in a buffer solution for 16 hours, following a previously reported protocol.[20,21] The demineralizing solution comprised of 50 mM acetate buffer solution containing 1.28 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.74 mM $NaH_2PO_4 \cdot 2H_2O$, and 0.03 ppm F at pH 5.0. The specimens were individually immersed into an unstirred solution at 37°C. The total volume of solution used was calculated using 2 ml/mm^2 of the enamel area.

Surface microhardness of demineralized specimens was measured and new KHN values were obtained, allowing the calculation of the percentage of surface microhardness loss ($\%SMR_L$), by using the equation: $\%SMH_L = 100 (SMH_{after\ demineralization} - SMH_{initial}/SMH_{initial})$. Specimens within each group (SE and DE) were further divided into three subgroups (n = 10), according to the experimental bleaching gels manipulated by manufacturer (FGM, Joinvile, SC, Brazil): 35% HP (control), 35% HP with addition of 0.6% sodium fluoride, and 35% HP with addition of 2% calcium gluconate. The pH of bleaching gels was measured using a calibrated pH Meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, Brazil) with an electrode (Digimed DME-CV8). The measured pH values were: 6.35 for the 35% HP gel with no additives; 8.11 for the fluoride-modified gel; and 7.99 for the calcium-modified gel.

The bleaching agents, presented in two bottles, were weighed according to the manufacturer's instructions, following the peroxide rate of 70% and 30% for the thickening agent, and then dispensed in two separate syringes. These syringes were connected and the two components were mixed by ten strokes. The obtained mixed gel was then applied on enamel surface for 50 minutes at room temperature. The bleaching procedure was performed *ex-vivo* in one session.

Post-bleaching SMH was measured for all specimens and the percentage of surface microhardness alteration was calculated for both SE and DE groups, using the following equation: $\%SMHa_{alteration} = 100 (SMH_{after\ bleaching} - SMH_{before\ bleaching} / KHN_{before\ bleaching})$. In SE group, the $SMH_{before\ bleaching}$ was the same as $SMH_{initial}$ and in DE group, the value of $SMH_{before\ bleaching}$ was the $SMH_{after\ demineralization}$ value.

In situ Stage

Ten intraoral devices were made of acrylic resin, containing six cavities of 3 mm in diameter and 3 mm in depth. In each device, six enamel specimens (one of each experimental group) were fixed with sticky wax in order to obtain an even surface between the specimen and the surface of the acrylic resin, aiming to reduce discomfort that might occur by wearing the device during the experimental period.[22] The specimens of SE and DE groups were fixed in the right and left sides of the device, respectively.

Volunteers were advised to wear the device 24 hours a day, for seven consecutive days, in order to allow the action of saliva on specimens throughout the study to simulate the natural conditions of oral environment.[22] During this period, the volunteers brushed their teeth with non-fluoride toothpaste. The devices were removed at mealtimes, rinsed and immediately repositioned after oral hygiene. SMH were measured after 1 and 7 days post-bleaching and the percentage of surface microhardness alteration was determined [$\%SMHa_{alteration} = 100 (SMH_{after}$ 1 and 7 days – $SMH_{post\text{-}beaching}/SMH_{post\text{-}bleaching}$)]. The experimental design of the study is illustrated in Figure 1.

Statistical Analysis

Surface microhardness data of SE and DE groups were separately analyzed. The data obtained after different periods of time (baseline immediately after bleaching and 1 and 7 days after bleaching) were statistically analyzed using Repeated Measures (RM) ANOVA (time as repeated variable) and Tukey's test, both with a significance level of $p < 0.05$.

RESULTS

Sound Enamel Groups

The percentage of SMH data obtained in different time intervals are presented in Table 1 (negative values mean reduction in SMH and positive values mean increase in SMH).

Repeated measures ANOVA revealed significant difference for time factor ($p = 0.001$). For groups factor ($p = 0.33$) and for interaction between factors ($p = 0.24$), there were no significant differences.

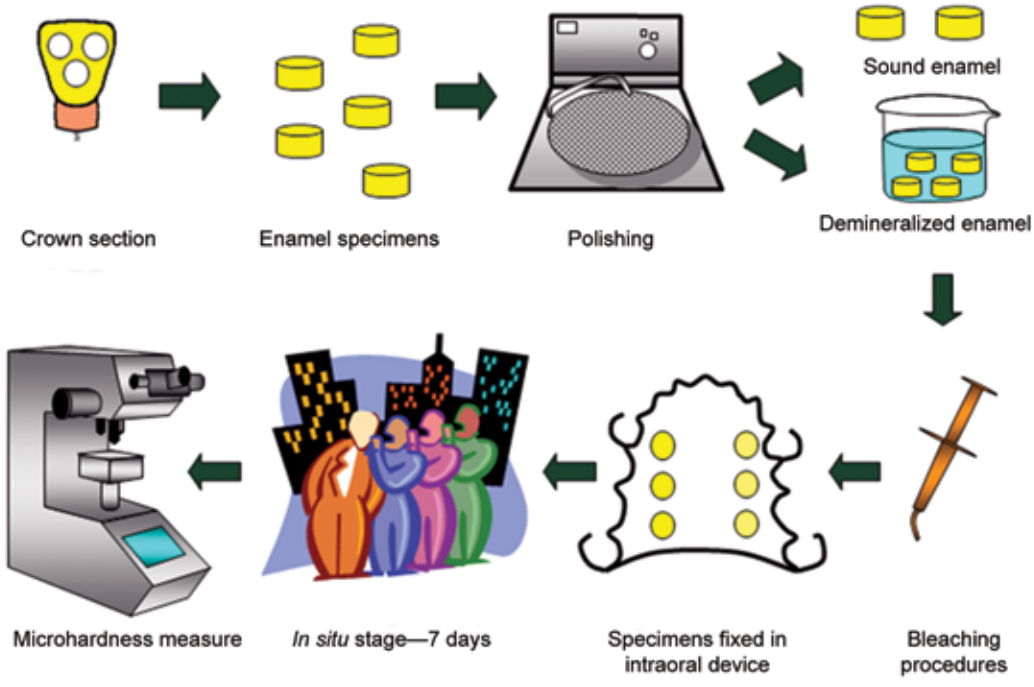

Fig. 1: Schematic drawing of the methodology

Table 1 also shows the mean KHN results and standard deviation (SD) as well as the Tukey Simple Effect test for the different groups and periods tested. Only the control group presented lower SMH mean values after bleaching, comparing the different periods of time evaluated within each group receiving different bleaching protocol. When the tested groups were compared within each evaluation period, significant lower SMH mean values for control group were observed compared to HP + Calcium group at the immediately post-bleaching evaluation.

Demineralized Enamel Groups

The percentage of SMH alterations obtained after the demineralization phase are close to 50% (data not shown).

The RM ANOVA revealed significant difference for time factor ($p = 0.001$). No significant differences were observed for the group factor ($p = 0.75$), and for the interaction between factors ($p = 0.11$).

Table 2 shows the mean KHN results and standard deviation (SD), as well as the Tukey Simple Effect test for different groups and periods tested. Group HP + Calcium showed higher SMH mean values in post-bleaching period than in baseline. When different groups were compared within each evaluated period, the group HP + Calcium exhibited significantly higher SMH mean values than control group immediately after bleaching. In general, all groups presented significantly increase of SMH when exposed to human saliva.

DISCUSSION

Although microhardness test does not provide direct information about the alterations of enamel and dentin contents,[11,23] it is a common method to measure changes of the mechanical properties of materials and hard tooth tissues, especially in demineralization and remineralization experiments. Since major interactions of enamel with the oral environment occur on the surface layer of the specimens, the evaluation of changes at this region is relevant. Thus, surface microhardness test seems to be a feasible choice to estimate mineral changes, as it might reflect the alterations of the surface layer, such as porosities and erosion of enamel.[11,24,25]

The bleaching treatment for the sound enamel group led to significant enamel SMH reduction. This SMH alteration has been documented previously[4,5,7,9,12,13,26] and can be related to the low pH of gels.[3] In the present study, the marketed tested HP gel showed a pH value slightly lower than neutral (6.25). It can be suggested therefore that the reduction in enamel microhardness might also be due to other factors, such as the oxidative effect of bleaching agents over organic contents of enamel, resulting in subsequent mineral loss;[27,28] and additionally to the ionic (Ca and P) undersaturation of bleaching gel, which is capable of interfering with the demineralization/remineralization enamel process even at pH values higher than 5.5.[13]

The first null hypothesis was accepted, as the group factor was not significant for both sound and demineralized enamel groups. On the other hand, for the multiple comparison analysis, the sound and demineralized enamel bleached with HP gel plus calcium showed significant higher SMH values than control group, and similar values as non-bleached enamel (baseline values) for the sound groups. It is supposed that the addition of remineralizing agents will saturate bleaching gel with ions and those can be either taken by enamel by means of ionic exchange, increasing its resistance to demineralization,[13] or preventing mineral loss from enamel.

Table 1: Mean KHN (SD), percentage of SMH alterations, and Tukey simple effects test results for sound enamel (SE) subgroups (n = 10)

Groups	*Baseline*	*Post-bleaching*	*After 1 day*	*After 7 days*
HP Control	300.58 (20.12) aA	256.77 (42.78) aB	296.72 (49.77) aAB	319.22 (46.32) aA
		–14.28%	–0.46%	7.04%
HP + Fluoride	303.87 (22.72) aA	273.80 (39.64) abA	290.93 (43.54) aA	298.62 (38.70) aA
		–9.96%	–3.97%	–1.24%
HP + Calcium	307.10 (23.35) aA	305.34 (39.64) bA	297.51 (25.07) aA	320.96 (54.87) aA
		–0.23%	–2.69%	4.5%

Mean values followed by different letters denote statistic difference (Tukey's test, $p < 0.05$).
Lower case letters should be considered within columns, while capital letters within rows.

Table 2: Mean KHN (SD) and results of Tukey Simple Effects test for demineralized enamel (DE) subgroups (n = 10) in different evaluation periods

Groups	*Baseline*	*Post-bleaching*	*After 1 day*	*After 7 days*
HP Control	150.37 (16.63) aA	162.78 (18.06) aAB	186.34 (43.29) aB	228.07 (30.66) aC
HP + Fluoride	152.56 (15.20) aA	174.67 (42.91) abA	210.97 (49.76) aB	215.82 (40.75) aB
HP + Calcium	148.44 (28.04) aA	197.31 (49.51) bB	207.99 (53.01) aB	210.34 (45.86) aB

Mean values followed by different letters denote statistic difference (Tukey's test, $p < 0.05$).
Lower case letters should be considered within columns, while capital letters within rows.

In addition to the reduction of deleterious effects induced by bleaching gels to enamel, the advantageous effects of potential remineralizing agents on bleaching gels may include the reduction of enamel solubility and decreased sensitivity due to mineral deposition to enamel crystallites.[15]

The second null hypothesis was rejected as demonstrated by the remineralizing activity of human saliva detected with demineralized groups at one day after the bleaching treatment. This observation is also valid for the 7-day period, when significant differences were detected due to total regression of the demineralizing effect of bleaching agents on sound enamel control group. Thus, the results of this study can reinforce the fundamental remineralizing role of saliva, minimizing the deleterious effects of bleaching agents,[17,22] and corroborate to the statement that when intraoral conditions are simulated as close as possible, the risk of decrease in microhardness of bleached enamel tends to be reduced.[18]

The use of non-fluoride toothpaste by the volunteers enabled the evaluation of the remineralizing action of saliva itself, and was done to eliminate fluoride as potential confounding factor. However, the use of fluoride products is widespread and might still represent an additional clinical factor for the recovery of lost mineral content, contributing to accelerate the action of saliva on the bleached enamel.[4,10,29]

Since bleaching products may eventually be used in patients presenting initial white spot lesions, the action of high-concentrated bleaching agents on demineralized enamel was investigated. The addition of potential remineralizing agents into bleaching gels due to their possible preventive effect[14] was also assessed.

Bleaching with 35% HP caused no exacerbation of enamel demineralization, as well as observed previously with less concentrated agents.[26] When calcium was added to bleaching agent, a positive effect on the remineralization process was observed within the DE group due to a significant increase in SMH. Supplements of calcium added in beverages, chewing gums, and dentifrices have been proposed to prevent dental caries and erosion and to attempt remineralization of initial caries lesions.[30-32] The availability of high levels of calcium as a soluble salt leads to precipitation of mineral phases of calcium-phosphate on enamel surface.[33]

On the other hand, the addition of fluoride to the bleaching gel was not capable of increasing SMH of demineralized enamel, probably due to the lower concentration of this agent in the final mixture of bleaching gel or the limited amount of calcium available in the surrounding oral environment. The results of previous studies about the effect of remineralizing agents added to bleaching gels on demineralized enamel are controversial. Tschoppe et al[14] observed no influence of fluoride-added bleaching gel on remineralization behavior of previously demineralized enamel, although a significant increase in microhardness was obtained when amorphous calcium phosphate was added to the bleaching gel. On the other hand, Gladwell et al[15] reported significant remineralizing effect of fluoride-enriched bleaching gel over demineralized enamel.

Additionally, the remineralizing potential of saliva on demineralized enamel was demonstrated in this *in situ* study, regardless of the type of bleaching treatment. The protective role of saliva involves the remineralizing potential by providing calcium and phosphorus to tooth structures, the salivary clearance potential, and also the buffer capacity, which prevents pH decrease and further loss of minerals.[34] Nevertheless, the present results might not be extrapolated to deeper parts of enamel lesions, once the remineralization of outer surface layers might occur more efficiently.[35]

Thus, the results of the present study suggest that, although the addition of potential remineralizing agents in bleaching gels may assist in the reduction of enamel demineralization induced by bleaching treatment at an immediate post-bleaching procedure, the fundamental action is played by saliva, since after 7 days all groups exhibited similar microhardness values regardless of the bleaching gel tested. Besides, when demineralized enamel specimens were analyzed immediately after bleaching, a significant remineralizing effect was observed only when calcium was added to bleaching agent.

Nevertheless, the results of this study may be viewed with caution. The protocol used for artificial caries production may influence the effects of subsequent demineralization or remineralization, as different protocols are reported to produce lesions with distinct surface layer, depth and mineral loss.[21,36] Although the analysis was performed by repeated measures and each volunteer wore the appliance with samples from each group, the possibility of loss of enamel surface during the *in situ* phase was not measured and this fact might be another limitation of the study. It also should be pointed out that microhardness testing demands polished specimens, in which removal of the original superficial layer of enamel may also influence the demineralization process.[37]

CONCLUSION

As a conclusion, the addition of potential remineralizing agents into bleaching gels should be encouraged as they might play an important role in maintaining the enamel microhardness or inducing remineralization of artificially demineralized bovine enamel right after bleaching procedures with 35% HP gel.

ACKNOWLEDGMENTS

This study was supported by São Paulo State Research Foundation – FAPESP (grants # 09/52212-6 and 09/51821-9).

The authors wish to thank FGM for manipulating the bleaching gels.

The study was approved by the local Institutional Review Board, under protocol #050/2009-PH/CEP.

REFERENCES

1. Buchalla W, Attin T. External bleaching therapy with activation by heat, light or laser-a systematic review. Dent Mater 2007; 23:586-596.
2. Papathanasiou A, Kastali S, Perry RD, Kugel G. Clinical evaluation of a 35% hydrogen peroxide in-office whitening system. Compend Contin Educ Dent 2002;23:335-8, 40, 43-4 passim; quiz 48.
3. Sulieman M, Addy M, Macdonald E, Rees JS. A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentine. J Dent 2004;32:581-590.
4. Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. J Prosthet Dent 2004; 92:337-342.
5. Attin T, Vollmer D, Wiegand A, Attin R, Betke H. Subsurface microhardness of enamel and dentin after different external bleaching procedures. Am J Dent 2005;18:8-12.
6. Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. Dent Mater J 2004;23:517-521.
7. Borges AB, Yui KC, D'Avila TC, Takahashi CL, Torres CR, Borges AL. Influence of remineralizing gels on bleached enamel microhardness in different time intervals. Oper Dent. 2010; 35:180-186.
8. Bizhang M, Seemann R, Duve G, Romhild G, Altenburger JM, Jahn KR, et al. Demineralization effects of 2 bleaching procedures on enamel surfaces with and without post-treatment fluoride application. Oper Dent 2006;31:705-709.
9. Attin T, Betke H, Schippan F, Wiegand A. Potential of fluoridated carbamide peroxide gels to support post-bleaching enamel re-hardening. J Dent 2007;35:755-759.
10. da Costa JB, Mazur RF. Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: an in vitro study. Oper Dent 2007;32:589-594.
11. Chen HP, Chang CH, Liu JK, Chuang SF, Yang JY. Effect of fluoride containing bleaching agents on enamel surface properties. J Dent 2008;36:718-725.
12. Borges AB, Samezima LY, Fonseca LP, Yui KC, Borges AL, Torres CR. Influence of potentially remineralizing agents on bleached enamel microhardness. Oper Dent 2009;34:593-597.
13. Cavalli V, Rodrigues LK, Paes-Leme AF, Brancalion ML, Arruda MA, Berger SB, et al. Effects of bleaching agents containing fluoride and calcium on human enamel. Quintessence Int 2010; 41:e157-165.
14. Tschoppe P, Neumann K, Mueller J, Kielbassa AM. Effect of fluoridated bleaching gels on the remineralization of predemineralized bovine enamel in vitro. J Dent 2009;37: 156-162.
15. Gladwell J, Simmons D, Wright JT. Remineralization potential of a fluoridated carbamide peroxide whitening gel. J Esthet Restor Dent 2006;18:206-212; discussion 12-13.
16. Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. Caries Res 2000;34:48-52.
17. Justino LM, Tames DR, Demarco FF. In situ and in vitro effects of bleaching with carbamide peroxide on human enamel. Oper Dent 2004;29:219-225.
18. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. Dent Mater 2009;25:143-157.
19. Pantera EA Jr., Schuster GS. Sterilization of extracted human teeth. J Dent Educ 1990;54:283-285.
20. Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent J 2008;19:21-27.
21. Magalhaes AC, Moron BM, Comar LP, Wiegand A, Buchalla W, Buzalaf MA. Comparison of cross-sectional hardness and transverse microradiography of artificial carious enamel lesions induced by different demineralising solutions and gels. Caries Res 2009;43:474-483.
22. Maia E, Baratieri LN, Caldeira de Andrada MA, Monteiro S, Jr., Vieira LC. The influence of two home-applied bleaching agents on enamel microhardness: an in situ study. J Dent 2008;36:2-7.
23. Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983;17:385-391.
24. Rehder Neto FC, Maeda FA, Turssi CP, Serra MC. Potential agents to control enamel caries-like lesions. J Dent 2009;37: 786-790.
25. Argenta RM, Tabchoury CP, Cury JA. A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. Pesqui Odontol Bras 2003;17:241-246.
26. Pinto CF, Paes Leme AF, Cavalli V, Giannini M. Effect of 10% carbamide peroxide bleaching on sound and artificial enamel carious lesions. Braz Dent J 2009;20:48-53.
27. Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. J Dent 1999;27:509-515.
28. Basting RT, Rodrigues AL Jr., Serra MC. The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness. Oper Dent 2005;30:608-616.
29. Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. J Oral Rehabil 1997;24:282-286.
30. Sullivan RJ, Charig A, Blake-Haskins J, Zhang YP, Miller SM, Strannick M, et al. In vivo detection of calcium from dicalcium phosphate dihydrate dentifrices in demineralized human enamel and plaque. Adv Dent Res 1997;11:380-387.
31. Itthagarun A, King NM, Yiu C, Dawes C. The effect of chewing gums containing calcium phosphates on the remineralization of artificial caries-like lesions in situ. Caries Res 2005;39:251-254.
32. Magalhaes AC, Moraes SM, Rios D, Buzalaf MA. Effect of ion supplementation of a commercial soft drink on tooth enamel erosion. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2009;26:152-156.
33. Garcia-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: the role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc. 2008;139 Suppl:25S-34S.
34. Edgar WM, Higham SM, Manning RH. Saliva stimulation and caries prevention. Adv Dent Res 1994;8:239-245.
35. Larsen MJ, Fejerskov O. Chemical and structural challenges in remineralization of dental enamel lesions. Scand J Dent Res 1989;97:285-296.

36. Lynch RJ, Mony U, ten Cate JM. Effect of lesion characteristics and mineralizing solution type on enamel remineralization in vitro. Caries Res 2007;41:257-262.

37. Xue J, Li W, Swain MV. In vitro demineralization of human enamel natural and abraded surfaces: a micromechanical and SEM investigation. J Dent 2009;37:264-272.