ORIGINAL RESEARCH

Light-activated Bleaching: Effects on Surface Mineral change on Enamel

[1]Adriana Fernandes da Silva, [2]Flávio Fernando Demarco, [3]Carine Tais Welter Meereis, [4]Maximiliano Sérgio Cenci, [5]Evandro Piva

ABSTRACT

Aim: This study evaluated the *in vitro* effect of 35% hydrogen peroxide (HP) on surface enamel change when activated with different light curing units (LCUs).

Materials and methods: Enamel blocks (4 × 4 × 2 mm) were obtained from bovine incisors. The initial microhardness of the enamel was determined for each specimen. After this enamel blocks were randomly divided into four groups (n = 10) and treated as follows: Control, no bleaching procedure performed; HP – LCU, application of 35% HP gel without light activation; HP + QTH, application of 35% HP gel and light activation with a Quartz Tungsten-Halogen (QTH); and HP + Light Emitting Diode, application of 35% HP gel and light-activation with a LED. New microhardness measurements were obtained, immediately, 7 and 14 days after treatment. The percentage of surface mineral change was calculated according to the baseline and post-treatment microhardness values. Additionally, six samples from each group were randomly selected and prepared for scanning electron microscopy (SEM) characterization. The data were analyzed using an analysis of variance (ANOVA) to detect differences between the three time periods, and an ANOVA and Tukey's test with a confidence level of 95%.

Results: There was no significant difference between the initial hardness values and hardness values after treatment in any of the groups or time periods (p > 0.05). No major surface alterations were detected with SEM when comparing control groups to those undergoing bleaching treatments.

Conclusion: The use of 35% HP in combination to QTH or LED light curing units LCU does not have detrimental effect on the enamel surface topography or in the mineral content, when compared with unbleached enamel or enamel submitted to 35% HP treatment alone.

Keywords: Tooth bleaching, Dental enamel, *in vitro*, Light source, Hydrogen peroxide, Hardness, Enamel morphology.

How to cite this article: da Silva AF, Demarco FF, Meereis CTW, Cenci MS, Piva E. Light-activated Bleaching: Effects on Surface Mineral change on Enamel. J Contemp Dent Pract 2014;15(5):567-572.

INTRODUCTION

The most popular vital bleaching technique is the at-home treatment with trays using 10% carbamide peroxide, because this treatment is the only treatment that has received acceptance from the American Dental Association (ADA), and this technique can provide results for up to 2 years.[1] However, in some situations, patients are unwilling or unable to be enrolled in long-term treatment (2-3 weeks for mouthguard bleaching) or they are not willing to wear a tray.[2] In these situations, a high-concentration bleaching agent, such as 35% HP or carbamide peroxide can be used in the dental office. This in-office treatment has been suggested to provide a faster and stronger bleaching effect.[3,4]

In addition, this high concentration of bleaching agents may be associated with light sources. Activation of the bleaching gel may be performed with different light-curing units: Quartz Tungsten-Halogen (QTH), Light emitting diode (LED) and lasers, and the combination of bleaching agents plus a light source has been indicated to improve the effect of treatment and produce faster results.[3] Despite the alleged advantage, clinical studies have questioned the improvement in bleaching effects.[5,6] In addition, other adverse effects have been related to bleaching technique, it independent of the concentration used, such as mineral tissue removal, causing increased porosity and permeability.[7,8] However, such alterations were hardly observed, when the dental structure was stored in artificial saliva.[9,10] The increase in tooth permeability can also produce deeper HP penetration that could reach the dental pulp, leading to adverse pulpal responses, because such responses are more prone to occur when in-office treatments are conducted, especially with an additional light source as adjunctive treatment.[11]

A recent *in situ* study analyzed the influence of different light activation sources and 35% HP on the microhardness of human enamel subjected to in-office dental bleaching. The authors observed that the light sources did not significantly alter the microhardness of human enamel after 14 days, but the use of HP together with a halogen light reduced microhardness after 1 and 7 days.[12] Another *in vitro* study observed a significant decrease in hardness when combining

[1,4]Associate Professor, [2,5]Full Professor, [3]Postgraduate Student

[1-5]Department of Restorative Dentistry, Federal University of Pelotas, Rio Grande do Sul, Brazil

Corresponding Author: Adriana Fernandes da Silva Associate Professor, Department of Restorative Dentistry Federal University of Pelotas, Rio Grande do Sul, Brazil, Phone: 555332256741, e-mail: adrisilvapiva@gmail.com

the application of a high-concentration peroxide agent with a light source,[13] but the authors used distilled water as the storage condition during the experiment. It is already known that the storage solution and study design will influence the effect of bleaching on dental structures.[10] Because, there is a lack of information in the scientific literature regarding the safety of bleaching using light sources,[4] and some of the results observed are contradictory, new studies are needed to investigate the potential adverse effects associated with this treatment.

Therefore, the purpose of the present study was to investigate the effects of 35% HP activated using different curing units on surface mineral change (SMC).

MATERIALS AND METHODS

Preparation of the Enamel Blocks

This study used 64 recently extracted bovine incisors. The pulp tissue was removed, and the crowns were sectioned longitudinally to obtain $4 \times 4 \times 2$ mm enamel blocks. The blocks underwent steam sterilization to avoid bacterial contamination. Each slab was included in a polyvinyl chloride (PVC) with only one side unsealed: buccal enamel. The samples were sequentially polished in decreasing order of disk granulation (400, 600, 800 and 1,200) with a polishing solution MetaDi Supreme® (Buehler, Dusseldorf, Germany) to obtain flat. About forty blocks were used for the microhardness tests, and 24 were used for the SEM evaluation. The specimens were stored in an artificial saliva solution at 37ºC according to previously reported methods.[14] The artificial saliva was changed daily.

Bleaching Treatment Protocol

The samples were treated with a 35% HP gel Whiteness HP Maxx® (FGM, Joinville, Brazil) and these were randomly allocated into four different groups (n = 10), as shown in Table 1. In the experimental groups, the bleaching agent was mixed at a rate of three drops of peroxide to one drop of thickening agent. Soon after enamel drying, the prepared gel was applied with a microbrush to form a 1 mm layer, corres-ponding to approximately 0.05 ml of the bleaching agent on each specimen. The gel remained on the enamel buccal surface for 15 minutes. When light activation was used, the light unit remained parallel to the surface and was applied twice for 20 seconds, with 2 minutes intervals between each light application and a standard distance of 5 mm. A detailed description of the light-curing units, including the light type, energy power and wavelength, (Table 1).

Following the bleaching treatment, the gel was washed off with an air-water spray. The bleaching procedures were repeated three times in each treatment session, resulting

in 45 minutes of bleaching agent contact with the enamel surface. To simulate the manufacturer's clinical recommendations, three bleaching treatments were performed: initially, after 7 and 14 days, using the same bleaching protocols. Throughout the experiment, the specimens were maintained in artificial saliva[14] (changed daily) at 37ºC.

Percentage of Surface Mineral Change

To determine the percentage of surface mineral change (SMC), the initial microhardness (baseline) was measured using a Knoop hardness ind-enter in a microhardness tester (FM-700, Future Tech Corp., Tokyo, Japan). Following a pilot study, a 25 gm load for 5 seconds was set as the parameter for producing indentations. In each specimen, 10 hardness readings were conducted at distances of 100 μm from each other in the center of the enamel blocks before treatment (baseline) (Fig. 1). After each bleaching protocol, new indentations were conducted, with 10 readings per sample: 5 indentations were located 100 μm above and 5 were located 100 μm below the baseline indentations (Fig. 1). The mean values for the experimental groups at different time periods were obtained using these 10 measurements, from which the percentage of SMC was determined in relation to the baseline measurement using the following equation: percentage of SMC = SMH after the treatment – baseline $\times$ 100/baseline.[15] The SMC was determined at three time periods: initially, at 7 and 14 days.

Statistical Analysis

The data within each group were subjected to statistical analysis using repeated measured one-way ANOVA to detect

Fig. 1: Schematic representation of microhardness measurements performed on enamel blocks before treatment: baseline (white diamond), after the first treatment session: 0 days (light gray diamond), after the second session: 7 days (dark gray diamond), and after the third session = 14 days (black diamond)

differences between the three time periods. Comparisons between the treatments independent of time were conducted with one-way ANOVA and Tukey's test, with a confidence level of 95%.

Surface Morphology Analysis using Scanning Electron Microscopy

Six enamel blocks ($2 \times 2 \times 2$ mm) from bovine incisors were obtained from each group (control and experimental), with two specimens allocated for each time period. The specimens were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and drystored in lightproof containers at 37ºC. The samples were ultrasonically cleaned using distilled water and then dried at 37ºC for 2 hours. Thereafter, the specimens were coated with gold and examined by SEM (JSM-5600 LV; Jeol Inc., Tokyo, Japan) at 18 kV to analyze the etching morphology provided by the different treatments. The analyses focused on the integrity, homogeneity, and continuity of the enamel surface using different magnifications.

RESULTS

The percentage of SMC values for the different groups during the evaluation periods are shown in Graph 1. Despite some differences, there were no statistically significant differences among the groups ($p > 0.05$), indicating that neither HP usage nor the application of a light source significantly altered the mineral content of the enamel blocks at any period (Fig. 2).

No significant surface alterations in surface morphology under SEM were observed when comparing the baseline values of the groups with the values after the bleaching protocols (Figs 3A to D). The enamel surfaces treated with
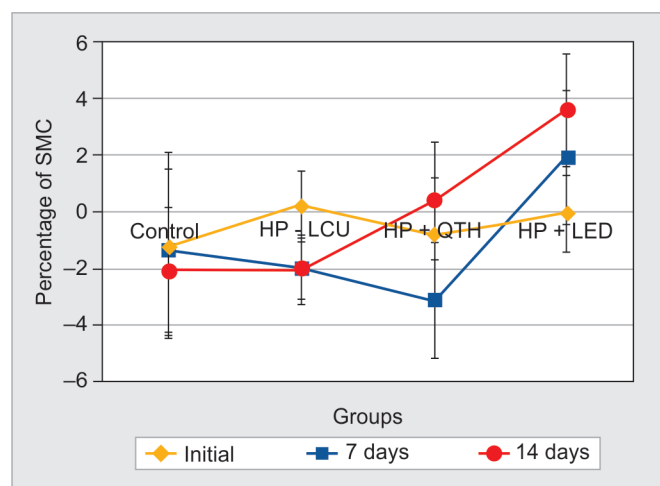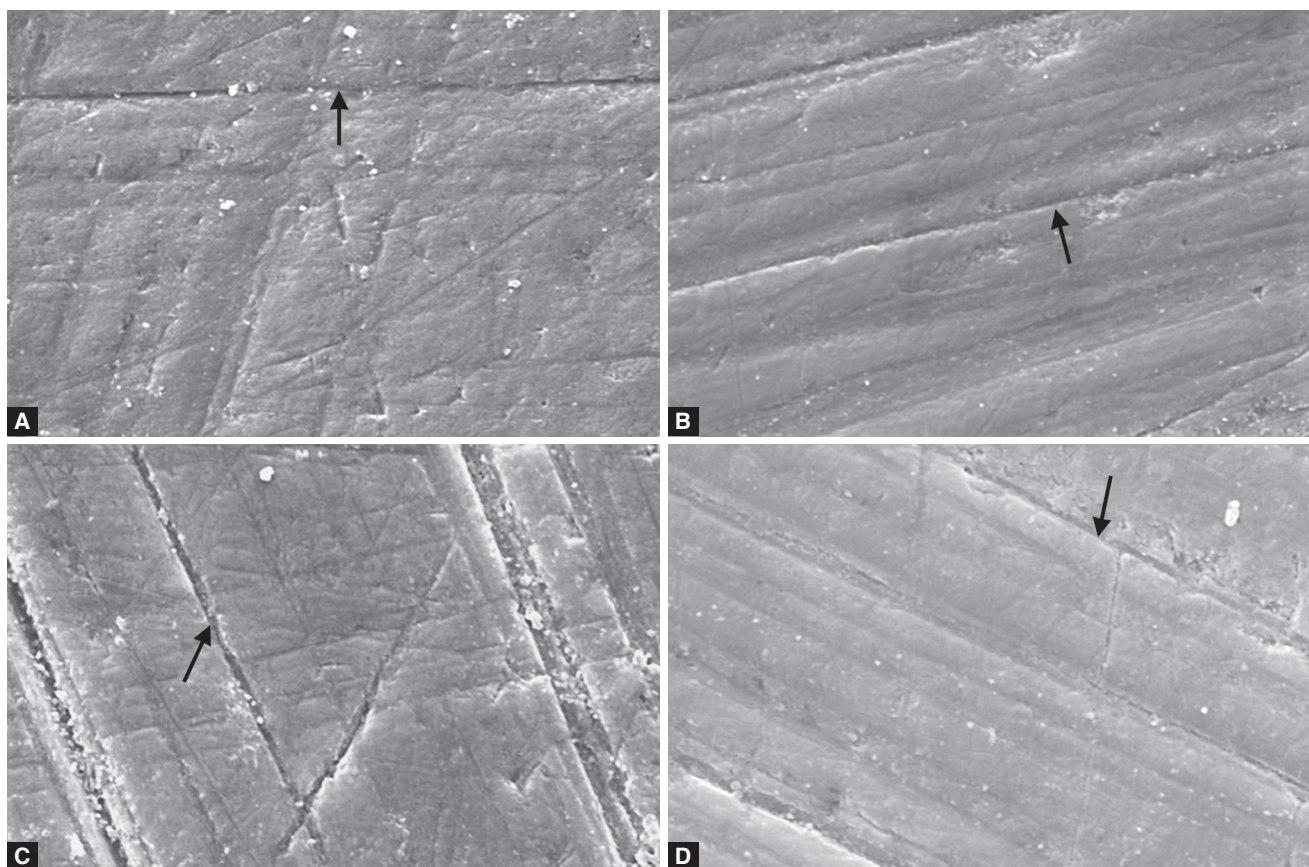

Fig. 2: Graphic presentation of the percentage of surface mineral change for different groups in different periods of time investigated. Despite some numeric differences observed, there was none statistical significant difference detected

a bleaching agent, with (Figs 3C and D) or without (Fig. 3B) a catalyst source, were very similar in appearance to the control group (Fig. 3A); the enamel surface did not show any evidence of porosities, but intact enamel crystallites were present.

DISCUSSION

The overall results of our study have demonstrated that neither the application of 35% HP nor the application of this bleaching agent coupled with a light source had a significant detrimental effect on the enamel surface. Studies have investigated the enamel microhardness before and after bleaching.[6,17-20] The results of these studies are controversial; some have observed a decrease in microhardness,[9,16,17,19] while these changes were not observed in other studies.[6,18,20] These conflicting results are due to a wide variety of differing factors, including different methods of investigation, bleaching agents, gel concentrations, application times, the use of catalyst sources and storage models. Therefore, the results of our study are more appropriate to the clinical situation, and in the presence of artificial saliva, the light-curing units combined with the 35% HP agent were not harmful to the enamel surface.

In our study, we used artificial saliva as the storage solution throughout the study, and no significant alterations in enamel topography and mineral content were observed. Artificial saliva closely resembles the composition and viscosity of natural human saliva,[14] and it is able to prevent the demineralization effect of bleaching agents, as previously reported.[10,21] Artificial saliva contains calcium and phosphate ions, which ensures ionic changes on tooth surfaces,[15] increasing surface hardness, decreasing permeability and increasing enamel resistance.[17,22] Consequently, it is extremely important to use artificial saliva as the storage solution when performing studies on the effect of bleaching on enamel; otherwise, the detrimental effects observed could be more related to the experimental design than the bleaching agent.[20]

The use of a light source for vital bleaching follows the same process, because the light is transformed into heat, and this heat could accelerate the process of color recovery.[4] The use of light sources to catalyze the immediate bleaching treatment is a relatively new approach, and few studies have investigated this technique. Some studies have reported tooth sensitivity during the in-office bleaching treatment with light,[23-25] and these observations were closely associated with the type of source used[23,24] and the time of enamel application.[25] Thus, patients who undergo bleaching with high concentrations of peroxide (35%) with application of a light

Figs 3A to D: Scanning electron microscopy images of enamel in the four study groups: with no bleaching treatment and no catalyst source (A) Control Group: without bleaching treatment and without light curing unit, (B) After bleaching treatment without light curing unit, (C) Bleaching treatment with halogen light, (D) After bleaching treatment with light emitting diode. The specimens under observation disclosed a normal enamel surface, without increased porosities in the bleached specimens, indicating none erosive effect produced by the treatments. The surface from experimental groups closely resembles that of the control group (sound nontreated bovine enamel). Noteworthy, we can observe the grooves produced by the 1,200 grit sandpaper polishing in all samples (arrow) magnification 5000×

Table 1: Experimental conditions carried out in the study

	Groups			
	Control	HP – LCU	HP + QTH	HP + LED
Bleaching treatment Performed	No	HP Maxx*	HP Maxx*	HP Maxx*
Light curing unit	No	No	Halogen light**	LED***

*Whitening whiteness hydrogen peroxide 35% (Whiteness HPMaxx FGM, Joinville, Brazil) Lot 120608, **Quartz-Tungsten halogen (XL3000; 3M ESPE, St Paul, MN, USA, 450 mW/cm²) and wavelength between 400 and 510 nm, ***Light emitting diode (LED RADII; SDI, Bayswater, Australia, 1400 mW/cm² and wavelength between 440 and 480 nm

source should not their undergo the prolonged application time of an in-office bleaching gel.[26]

We used two light sources as catalyst agents: QTH and LED. Both units have different energy outputs and wavelengths (Table 1), and LED is considered to produce a lower level of temperature increase compared with QTH.[3] If an increase in temperature accelerates the bleaching reaction, the QTH unit should provide an improved bleaching effect compared with the LED unit. However, this assumption has not been proven to occur in clinical trials, where neither QTH nor LED units were able to improve the bleaching effect.[5] It is also noteworthy that the increase in temperature produced by the light curing units during bleaching procedures could be harmful to the dental pulp, because high intensity light sources, such as plasma arc and lasers, may be able to significantly increase the intrapulpal temperature.[3] The increase in intrapulpal temperature and penetration of HP throughout the dental pulp may lead to serious pulp damage.[11] Therefore, high-concentration peroxide agents and light sources should be used with caution to prevent potential detrimental effects.[4]

Another mechanism to explain the pain experienced during bleaching treatment is the increase in enamel permeability with mineral loss and production of microcracks, which could facilitate the penetration of peroxide inside the tubules, causing fluid movement (hydrodynamic theory).[27] However, the SEM images from our study did not show significant surface alterations with the use of 35% HP: HP-plus light sources. In all samples, the enamel surface was essentially unchanged, and this finding could be the result of using artificial saliva, which prevented the potential erosive effect of bleaching agents.

Bovine teeth were used in this study due to their advantages over human teeth; they are easily obtained in a frigorific and have large surfaces,[28] unlike, human teeth are becoming seldom available for research purposes. Moreover, bovine enamel and dentin also have similar microstructures and hardness to human teeth.[29]

We used the percent of SMC instead of microhardness values. This methodology has been used in different studies for different purposes.[15,30] The main advantage of this method is that it provides a graph displaying the losses and gains in mineral structure over time. The percentage of SMC indicates some changes in the mineral content between groups at the different time periods; however, as stated in the statistical analysis, these alterations were not statistically significant. In addition, the graph produced for percentage of SMC with the SEM images suggests that the surface alterations were not clinically relevant.

CONCLUSION

Within the limitations of this study, we conclude that the use of 35% HP in combination to QTH and LED does not have detrimental effect on the enamel surface topography or in the mineral content, when compared with unbleached enamel or enamel submitted to 35% HP treatment alone.

CLINICAL SIGNIFICANCE

The use of 35% HP in combination with a light source does not have detrimental effect on the enamel surface.

ACKNOWLEDGMENTS

REFERENCES

1. Demarco FF, Meireles SS, Masotti AS. Over-the-counter whitening agents: a concise review. Braz Oral Res 2009;23 Suppl 1:64-70.
2. Meireles SS, Demarco FF, Tarquinio SB. Nonvital tooth bleaching with halogen light-activated agents: case reports and discussion. Gen Dent 2009 Jul-Aug;57(4):398-401.
3. Buchalla W, Attin T. External bleaching therapy with activation by heat, light or laser—a systematic review. Dent Mater 2007 May;23(5):586-596.
4. Ontiveros JC. In-office vital bleaching with adjunct light. Dent Clin North Am 2011 Apr;55(2):241-253.
5. Marson FC, Sensi LG, Vieira LC, Araujo E. Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. Oper Dent 2008 Jan-Feb;33(1):15-22.
6. Polydorou O, Hellwig E, Hahn P. The efficacy of three different in-office bleaching systems and their effect on enamel microhardness. Oper Dent 2008 Sep-Oct;33(5):579-586.
7. Ben-Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. Am J Dent 1995 Feb;8(1):29-32.
8. Josey AL, Meyers IA, Romaniuk K, Symons AL. The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel. J Oral Rehabil 1996 Apr;23(4):244-250.
9. Basting RT, Rodrigues AL Jr, Serra MC. The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time. J Am Dent Assoc 2003 Oct;134(10):1335-1342.
10. Justino LM, Tames DR, Demarco FF. In situ and in vitro effects of bleaching with carbamide peroxide on human enamel. Oper Dent 2004 Mar-Apr;29(2):219-225.
11. Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J. Human pulp responses to in-office tooth bleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010 Apr;109(4):59-64.
12. Araujo Fde O, Baratieri LN, Araújo E. In situ study of in-office bleaching procedures using light sources on human enamel microhardness. Oper Dent 2010 Mar-Apr;35(2):139-146.
13. Cervantes A, Valera M, Araújo Md. Microhardness study of bovine enamel to bleaching treatment activated for different sources of light. Braz Dent Sci 2006;9(3):78-86.
14. Serra MC, Cury JA. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quintessence Int 1992 Feb;23(2):143-147.
15. Tenuta LM, Lima JE, Cardoso CL, Tabchoury CP, Cury JA. Effect of plaque accumulation and salivary factors on enamel demineralization and plaque composition in situ. Pesqui Odontol Bras 2003 Oct-Dec;17(4):326-331.
16. Akal N, Over H, Olmez A, Bodur H. Effects of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel. J Clin Pediatr Dent 2001 Summer;25(4):293-296.
17. Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. J Oral Rehabil 1997 Apr;24(4):282-286.
18. Ren YF, Amin A, Malmstrom H. Effects of tooth whitening and orange juice on surface properties of dental enamel. J Dent 2009 Jun;37(6):424-431.
19. Ulukapi H. Effect of different bleaching techniques on enamel surface microhardness. Quintessence Int 2007 Apr;38(4):e201- e205.
20. Unlu N, Cobankara FK, Altinoz C, Ozer F. Effect of home bleaching agents on the microhardness of human enamel and dentin. J Oral Rehabil 2004 Jan;31(1):57-61.
21. Joiner A, Thakker G, Cooper Y. Evaluation of a 6% hydrogen peroxide tooth whitening gel on enamel and dentine microhardness in vitro. J Dent 2004;32(Suppl 1):27-34.
22. Wiegand A, Schreier M, Attin T. Effect of different fluoridation regimes on the microhardness of bleached enamel. Oper Dent 2007 Nov-Dec;32(6):610-615.
23. Amengual J, Forner L. Dentine hypersensitivity in dental bleaching: case report. Minerva Stomatol 2009 Apr;58(4):181-185.
24. Kossatz S, Dalanhol AP, Cunha T, Loguercio A, Reis A. Effect of light activation on tooth sensitivity after in-office bleaching. Oper Dent 2011 May-jun;36(3):251-257.

25. Reis A, Tay L, Herrera D, Kossatz S, Loguercio AD. Clinical effects of prolonged application time of an in-office bleaching gel. Oper Dent 2011 Nov-Dec;36(6):590-596.

26. Reis A, Dalanhol AP, Cunha TS, Kossatz S, Loguercio AD. Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. Oper Dent 2011 Jan-Feb;36(1):12-17.

27. Demarco FF, Meireles SS, Sarmento HR, Dantas RV, Botero T, Tarquinio SB. Erosion and abrasion on dental structures undergoing at-home bleaching. Clin Cosm Invest Dent 2011 Jul 18;(3):45-52.

28. Mota CS, Demarco FF, Camacho GB, Powers JM. Tensile bond strength of four resin luting agents bonded to bovine enamel and dentin. J Prosthet Dent 2003 Jun;89(6):558-564.

29. Muench A, da Silva EM, Ballester RY. Influence of different dentinal substrates on the tensile bond strength of three adhesive systems. J Adhes Dent 2000;2(3):209-212.

30. Paes Leme AF, Dalcico R, Tabchoury CP, Del Bel Cury AA, Rosalen PL, Cury JA. In situ effect of frequent sucrose exposure on enamel demineralization and on plaque composition after APF application and F dentifrice use. J Dent Res 2004;83(1):71-75.