Original Article

International Journal of Dental and Health Sciences
Volume 04,Issue 04

INFLUENCE OF VARIOUS LOCALLY PREPARED HYDROGEN PEROXIDE GEL CONCENTRATIONS ON ENAMEL COLOR AND ITS MICROHARDNESS: AN IN VITRO STUDY

Warade Roopali A.[1], Daokar sadashiv G.[2]
1. Department Of Conservative Dentistry & Endodontics, CSMSS Dental College and Hospital, Aurangabad
2. Department Of Conservative Dentistry & Endodontics, CSMSS Dental College and Hospital, Aurangabad

ABSTRACT:

Aim: To evaluate the effect of locally prepared hydrogen peroxide gels with different concentrations (20%, 30% and 35%) on enamel microhardness and on changes in enamel color

Material and method: 3 mm ×2 mm specimens were prepared from the buccal surface of the 50 freshly extracted human incisors tooth using a diamond disc. Specimens were randomly divided into five groups (n=10): group A (20% H_2O_2), group B (30% H_2O_2), group C (35% H_2O_2), group D: control and group E: thickner (gel without peroxide). After polishing, initial values of VHN_0 and color measurement, assessed by microhardness tester machine and spectrophotometer respectively. The gels were applied on the enamel surface for 30 minutes. Immediate values of VHN_i and color reading was taken. The specimen were then stored in artificial saliva for 7 days. After 7 days, new measurements of microhardness and color were made.

Results: Microhardness of surface enamel was not influenced by different gel concentration. Regarding color changes, ΔE data showed that the 35% gel presented a higher color alteration than the 20% gel.

Conclusion: Enamel microhardness was not influenced by different concentrations of hydrogen peroxide gels.All experimental gel preparation produced marked color change. Among those, 35% hydrogen peroxide gel exhibited higher whitening potential than the 20% gel, without intensifying the side effects on the enamel surface.

Keywords: Hydrogen peroxide gel, Microhardness, VHN.

INTRODUCTION:

Dental bleaching is a common procedure in general dentistry, as it is conservative and can lead to satisfactory results for changing dental color.[1] Desire for attractive smile has stimulated the search for effective treatments in the dentistry.[2]

The bleaching technique was first described in 1877 but became popular in 1989 with the introduction of the nightguard vital bleaching (NGVB) technique.[3] Hydrogen peroxide (H_2O_2) is an important agent used in bleaching procedure. H_2O_2 releases free radicals which penetrates into tooth structure causes oxidation of chromophore molecules, by means of redox reaction.[4] Oxidative agent converts chromophore molecules into less complex structure, gives brighter appearance to the tooth.[5]

Dental bleaching procedures can be performed either by dentist in a dental office, or at home by the patient with professional supervision. Both techniques are shown to be effective.[1] There is inverse relation between the concentrations of hydrogen peroxide in the bleaching gel with the application time needed to achieve satisfactory results. Therefore for faster results, with

*Corresponding Author Address: Dr Roopali A. Warade. E-mail: roopaliwarade@gmail.com

fewer applications, higher concentrations of hydrogen peroxide are required.[6]

Higher concentration of hydrogen peroxide can adversely affects to dental tissues when is used for dental bleaching procedure. The results are controversial. Alterations in enamel surface morphology,[7,8,9] as well as significant changes in microhardness values were observed by some author.[10,11] Slightly erosion of bleached enamel surface was observed.[7] Bistey and others showed that significant structural alterations, with loss of phosphate ions, occurred in the enamel surface when greater than 20% concentrations of hydrogen peroxide were used.[12]

Various bleaching agents are available in market. **In this study, whitening gels used were experimental and manipulated in laboratory, in the department of biochemistry in our institution**. Ingredients used for manipulation of bleaching agent are easily available and economical. Once prepared, bleaching agent is active for 7 days provided that it kept refrigerated.

Hence, the purpose of this in vitro study was to evaluate the effect of locally prepared hydrogen peroxide gels prepared **in our laboratory** in different concentrations (20%, 30% and 35%) on enamel microhardness and changes in enamel color immediately after application and after seven days.

MATERIALS AND METHODS

Sample Preparation:

50 freshly extracted undamaged and intact human incisors were used in this study and stored in normal saline solution until required. Enamel-dentin specimens 3 mm in diameter and 2 mm in height (1 mm of enamel and 1 mm of dentin) were prepared from the buccal surface of the tooth using a diamond disc. Enamel and dentin thickness were standardized, ground flat, and polished with sequential water cooled silicon carbide paper discs. The specimens were immersed in deionized water, placed in an ultrasonic bath for 10 minutes and then stored in distilled water for rehydration.

Specimens were randomly divided into five experimental groups (n=10) according to the concentration of the whitening gel.

Group A: 20% H_2O_2

Group B: 30% H_2O_2

Group C: 35% H_2O_2

Group D: control (distilled water)

Group E: thickener (gel without peroxide)

Color measurement

Prior to each bleaching treatment, the initial color of all specimens was taken. The baseline color coordinates were assessed in standard conditions using a

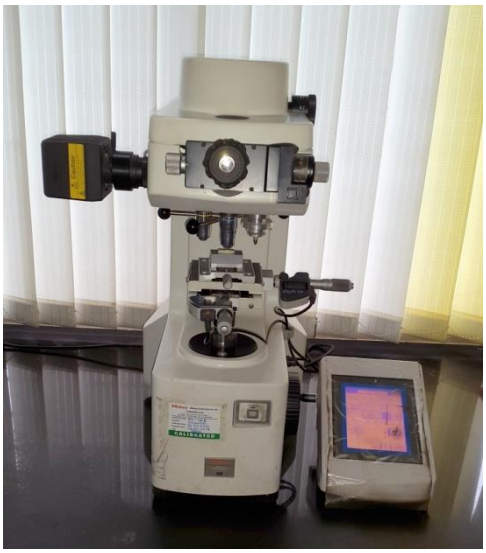
spectrophotometer (Ocean Optics HR4000).The spectrophotometer (Fig. 1) was adjusted for three consecutive measures, which were later averaged. The results of the color measurement were quantified in terms of the L*, a*, b* coordinate values established by the Commission Internationale de l'Eclariage (CIE) in which the L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white).The a* plane represents the degree of green/red color, and the b* plane represents the degree of blue/yellow color.

The measurement of color change after the bleaching procedures was made by calculating the variation of L* (ΔL), a* (Δa), and b* (Δb). The total color change (ΔE) was calculated according to the following formula:[13]

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{½}$$

Spectrophotometer

Microhardness Measurement: The initial surface microhardness (VHN_0) of all specimens was obtained before the bleaching procedures using a microhardness tester machine (Mitutoyo [HM – 100], Fig. 2) using a Vickers diamond indenter, under 25 g load for 10 seconds. Three hardness values for each specimen were averaged and reported as a single value VHN_0



Microhardness tester machine

Bleaching Procedures

The whitening gels used in this study were experimental and manipulated in our laboratory, resulting from the mixture of 50% hydrogen peroxide solution, alovera gel and glycerin.[14]Ingredients were manually mixed immediately before application in the proportion as shown in table 1

50% H_2O_2	Alovera gel	Glycerin	H_2O_2 gel
8 ml	12 gm	12 ml	20%
12 ml	8 gm	8 ml	30%
14 ml	6 gm	6 ml	35%
-	6 gm	6 ml	Thickener

Table 1

Immediately after mixing, the pH of all gels was calculated using a pH meter. The pH of thickener gel and 20%, 30% and 35% gels was respectively, 6.07, 4.5, 5.5, and 5.3.

A 1 mm layer of whitening gel was applied over the enamel surface of each specimen for 10 minutes and repeated three times, totaling 30 minutes of application. This protocol of application was chosen to simulate the clinical application. An aspiration cannula was used to remove the gel in between each application.

After application, the specimens were washed with deionized water and submitted to an immediate measurement of microhardness (VHNi) and color following the same procedure used for initial measurements. The specimens were then stored in artificial saliva with daily changes for 7 days. After the storage period, specimens were tested again to measure microhardness (VHN_7) and color as before.

Statistical analysis

The obtained data was statistically analyzed by using ANOVA using statistical package IBM SPSS-20.

RESULTS:

Microhardness mean values are shown in Table 2. The ANOVA showed no significant difference for all group for the time factor ($p>0.05$). For the color data (Table 3), the one-way ANOVA test showed significant differences among groups for the values of ΔL, Δb and ΔE ($p<0.05$).

Group	VHN_0	VHN_i	VHN_7
A	327.72(+/-8.61)	329.29(+/-8.72)	326.32(+/-4.90)
B	335.41(+/-13.2)	332.83(+/-10.7)	335.62(+/-13.0)
C	337.72(+/-8.2)	330.49(+/-10.5)	329.96(+/-12.2)
D	332.61(+/-13.4)	332.65(+/-10.0)	335.96(+/-17.5)
E	337.80(+/-11.7)	332.81(+/-13.99)	335.51(+/-10.0)

Table 2

Group	Δ L	Δ b	Δ E
A	1.24 (+/-1.07)	2.06 (+/-1.73)	3.23 (+/-1.25)
B	2.19 (+/-0.88)	3.35 (+/-0.81)	4.09(+/-1.00)
C	2.55 (+/-1.17)	3.49(+/-1.28)	4.69 (+/-1.52)
D	0.40(+/-1.96)	0.28 (+/-0.82)	1.09 (+/-0.83)
E	0.54 (+/-2.04)	0.46 (+/-0.81)	1.14 (+/-0.98)

Table 3

DISCUSSION:

Results showed that higher concentration of hydrogen peroxide in the bleaching gel was resulted in better improvement in color of a tooth. The gel concentration did not cause significant changes in the enamel surface microhardness, either immediately after application or after seven days.Among various studies demineralization of enamel have been observed after bleaching procedures [4,7,15] and those structural modifications have been assigned to the gel pH (less than 5.2).[15,16,17] Potential adverse effects on enamel and/or dentin after bleaching do not reflect the bleach itself but they mostly due to the pH of the formulation used.[2] Thus, gels with neutral pH are recommended for tooth bleaching to reduce deleterious effects on tooth enamel.[16]

The pH of gels tested in the present study was between 4.5 and 5.5. It showed insignificant changes for the time in contact with the enamel, as exposure time to peroxide may be insufficient to promote enough mineral modification which will affect the enamel microhardness. Apart from hydrogen peroxide concentration, structural changes of the enamel surface are time dependent. Bistey and others observed considerable changes at greater than 60 minutes of exposure to peroxide.[12]

The mechanism by which bleaching agents cause this reduction in microhardness is still unknown, but it is postulated that these agent are of acidic nature. In the present study thicker gel without the peroxide (pH 6.07) with the same basic composition was tested so as to verify that the thickener was unable to promote mineral dissolution by itself.

The strength of the carbon bonds present in the chromophore molecules is inversely related to the dental color. Higher the light absorption by complex molecules, the lower is the reflection, giving the sensation of a darker tooth. Dark tooth requires a higher application time of the bleaching gel or a higher concentration of the hydrogen peroxide.[18] Hydrogen peroxide solutions

with higher concentrations have more number of free radicals which increases the whitening potential.[19] As the hydrogen peroxide which penetrates enamel prisms can remain active for several days until it is completely neutralized,[20] the color measurement was made after this time period to allow enamel rehydration and attain color stability.[21] Color differences corroborated by the spectrophotometer might not be clinically relevant. In present study, the use of a spectrophotometer is justified by the improvement in the standardization of shade assessment, allowing accuracy and reproducible results compared with the human eye.[22, 23,24]

The adopted classification of ΔE values was determined by the National Bureau of Standards (NBS) that considers: 0.0 to 0.5 values: extremely slight change; 0.5 to 1.5: slight change; 1.5 to 3.0: perceivable change; 3.0 to 6.0: marked change; 6.0 to 12.0: extremely marked change; 12.0 or more: change to another color.In present study calculated ΔE values for group A, B and C were in the range of 3.0 to 6.0, which indicated marked change in color.[25]

Karpinia KA et al showed that bleached tooth presented a significant decrease of the Δb (reduction in the yellow color) and an increase of ΔL (brightness).[26] Bleaching gel with 35% hydrogen peroxide showed a better whitening effect compared with 10% carbamide peroxide gel and 20% hydrogen peroxide gel, with same treatment times respectively.[27] Based on these findings, it can be suggested that the whitening gels with 35% hydrogen peroxide can be used for patients who desire a faster whitening effect with minor influence on enamel surface properties.

Results obtained in this study were in agreement with Borges AB et al. They evaluated whitening potential of different hydrogen peroxide gel preparation (20%, 25%, 30% and 35%) and their effect on microhardness.[1] Amaral et al evaluated the calcium and phosphorus concentration in human enamel in vivo and found no differences between in-office (35% and 38%) and home use (10% and 20%) bleaching gels.[28] Metz et al performed in vivo study on teeth extracted for orthodontic reasons and found no differences in enamel microhardness.[29]

As this is an in vitro study, it was not possible to predict the adverse effects of high-concentration gels on tooth sensitivity and pulp cells.[30] also the effect the of pulp pressure on interfere in the penetration of the gel in vital teeth was unable to simulate.[31]

CONCLUSION:

1. Results of prepared experimental bleaching gel were found to be equally effective to that of commercially available bleaching products evident from literature.
2. Enamel microhardness was not influenced by different concentrations of hydrogen peroxide gels.

3. All experimental bleaching gel showed marked color change.
4. The 35% hydrogen peroxide gel exhibited higher whitening potential than the 20% gel, without intensifying the side effects on the enamel surface.
5. Prepared experimental bleaching gel is economical than other commercial available bleaching products.

REFERENCES:

1. Borges AB, Zanatta RF, Barros ACSM, Silva LC, Pucci CR and Torres CRG. Effect of Hydrogen Peroxide Concentration on Enamel Color and Microhardness. Operative Dentistry 2015;40-1:96-101
2. Sulieman M, Macdonald E, Rees JS, Newcombe RG and Addy M. Tooth Bleaching by Different Concentrations of Carbamide Peroxide and Hydrogen Peroxide Whitening Strips: An In Vitro Study. J Esthet Restor Dent 2006;18:93–101.
3. Maiti N and Das UP. Vital tooth bleaching: A Case report. American Journal of Advances in Medical Science 2014;2:1-6.
4. Borges AB, Torres CR, de Souza PA, Caneppele TM, Santos LF and Magalhaes AC. Bleaching gels containing calcium and fluoride: effect on enamel erosion susceptibility. International Journal of Dentistry 2012;347848.
5. Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC and Baratieri LN. Clinical performance of vital bleaching techniques. Operative Dentistry 2010;35(1):3-10.
6. Reis A, Kossatz S, Martins G and Loguercio A. Efficacy of and effect on tooth sensitivity of in-office bleaching gel concentrations: a randomized clinical trial. Operative Dentistry 2013;38(4):386-393.
7. Jiang T, Ma X, Wang Y, Tong H, Shen X, Hu Y and Hu J. Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence. Journal of Biomedical Optics 2008;13(1):014-019.
8. Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G and Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. Journal of Dentistry1999;27(7):509-515.
9. Chen HP, Chang CH, Liu JK, Chuang SF and Yang JY. Effect of fluoride containing bleaching agents on enamel surface properties. Journal of Dentistry 2008;36(9):718-725.
10. Lewinstein I, Fuhrer N, Churaru N and Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and

dentin. Journal of Prosthetic Dentistry 2004;92(4):337-342.

11. Borges AB, Samezima LY, Fonseca LP, Yui KC, Borges AL and Torres CR. Influence of potentially remineralizing agents on bleached enamel microhardness. Operative Dentistry 2009;34(5):593-597.
12. Bistey T, Nagy IP, Simo A and Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. Journal of Dentistry 2007;35(4):325-330.
13. Dietschi D, Rossier S and Krejci I. In vitro colorimetric evaluation of the efficacy of various bleaching methods and products. Quintessence International 2006;37(7):515-526.
14. Hydrogen Peroxide Gel. Available from: http://www.using-hydrogen-peroxide-gel.html
15. Magalhaes JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC and Barcellos DC. Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity. Acta Odontologica Scandinavia 2012;70(2):122-126.
16. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T and Wang Y. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. Journal of Dentistry 2011;39(10):686-692.
17. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T and Wang Y. Effects of two in-office bleaching agents with different pH on the structure of human enamel: an in situ and in vitro study. Operative Dentistry 2013;38(1):100-110.
18. Kwon YH, Huo MS, Kim KH, Kim SK and Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. Journal of Oral Rehabilitation 2002;29(5):473-477.
19. Kawamoto K and Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. Journal of Endodontics 2004;30(1):45-50.
20. Gerlach RW, Barker ML and Sagel PA. Objective and subjective whitening response of two self-directed bleaching systems. American Journal of Dentistry 2002; 15(Special Issue):7A-12A.
21. Barbosa CM, Sasaki RT, Florio FM and Basting RT. Influence of time on bond strength after bleaching with 35% hydrogen peroxide. Journal of Contemporary Dental Practice2008;9(2):81-88.
22. Douglas RD, Steinhauer TJ and Wee AG. Intraoral determination of the tolerance of dentists for perceptibility and acceptability of shade mismatch. Journal of Prosthetic Dentistry 2007;97(4):200-208.
23. Kielbassa AM, Beheim-Schwarzbach NJ, Neumann K, Nat R and Zantner C. In vitro comparison of visual and computer-aided pre- and post-tooth shade determination using various home bleaching procedures. Journal of Prosthetic Dentistry 2009; 101(2):92-100.

24. Khashayar G, Bain PA, Salari S, Dozic A, Kleverlaan CJ and Feilzer AJ. Perceptibility and acceptability thresholds for colour differences in dentistry. Journal of Dentistry 2014;42(6):637-644.
25. Adriana Postiglione Bührer Samra, Stella Kossatz Pereira, Leyla Cotrina Delgado and Christiane Phillipini Borges. Color stability evaluation of aesthetic restorative materials. Braz Oral Res 2008; 22(3):205-10.
26. Karpinia KA, Magnusson I, Sagel PA, Zhou X and Gerlach RW. Vital bleaching with two at-home professional systems. American Journal of Dentistry 2002;15 (Special Issue):13A-18A.
27. Caneppele TM, Borges AB and Torres CR. Effects of dental bleaching on the color, translucency and fluorescence properties of enamel and dentin. European Journal of Esthetic Dentistry 2013;8(2):200-212.
28. do Amaral FL, Sasaki RT, da Silva TC, Franca FM, Florio FM and Basting RT. The effects of home-use and in office bleaching treatments on calcium and phosphorus concentrations in tooth enamel: an in vivo study. Journal of the American Dental Association 2012;143(6):580-586.
29. Metz MJ, Cochran MA, Matis BA, Gonzalez C, Platt JA and Lund MR. Clinical evaluation of 15% carbamide peroxide on the surface microhardness and shear bond strength of human enamel. Operative Dentistry 2007;32(5):427-436.
30. Soares DG, Ribeiro AP, da Silveira Vargas F, Hebling J and de Souza Costa CA. Efficacy and cytotoxicity of a bleaching gel after short application times on dental enamel. Clinical Oral Investigation 2012;17(8):1901-1909.
31. Bowles WH and Ugwuneri Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. Journal of Endodontics 1987;13(8):375-377.