ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 03 | March - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN VITRO ANALYSIS OF EFFECT OF AT-HOME AND IN-OFFICE BLEACHING TREATMENTS AT PERIODIC INTERVALS ON HUMAN ENAMEL MICROHARDNESS AND PERMEABILITY

Dentistry

Dr. Sampurna Dutta Gupta* Asst. Professor Haldia Institute of Dental Sciences and Research. *Corresponding Author

Dr. Paromita Mazumdar Prof and HOD Guru Nanak Institute of Dental Sciences and Research.

Dr. Ipsita Maity Asso. Professor Guru Nanak Institute of Dental Sciences and Research.

ABSTRACT

Aim: To evaluate the effect of in-office and at-home bleaching agents on the microhardness and permeability of human enamel. **Methodology:** Teeth samples so obtained were divided into groups , Group 1 – Control , Group 2 – At Home bleaching group , Group 3 - In-office bleaching group with incorporated Sodium Fluoride. All the bleaching treatments were done according to manufacturer's instructions at prescribed intervals. **Microhardness** analysis was performed as Pre-treatment microhardness analysis to obtain base-line values. After bleaching treatments, microhardness test was done post-treatment at periodic intervals and was compared with the baseline values to observe the changes. **Permeability** test was done after completing bleaching treatment, the samples treated for dye penetration test using 50 % Silver Nitrate as the tracer agent , the samples were dipped in it for 16 hrs and 2hrs in developer solution. **Results:** Microhardness test of the groups revealed significant decrease in microhardness in in-office bleaching agent. However remineralisation using artificial saliva and further testing after 7 and 14 days revealed increase in microhardness. Permeability testing on the other hand post-treatment revealed significant increase in dye-penetration in at-home as well as in- office bleaching agents.

KEYWORDS

At-home bleaching agent, in-office bleaching agent, microhardness analysis, permeability testing, tracer agent.

INTRODUCTION

Esthetics play an important role in the oral health of a population. The Constitution of the World Health Organization (WHO) in 1946 defined health as "A state of complete physical, mental, and social well-being not merely the absence of disease." [1] Tooth discolorations can cause significant cosmetic problem especially when it affects the anterior teeth, which is usually exposed when a patient smiles. Anterior tooth discoloration is one of the most frequent reasons for seeking dental treatment. [2] Although interdisciplinary evaluation and treatment are crucial in achieving optimum smile, understanding the patient's wish isessential to the success of therapy. Therefore, to cater to the patient requirement for improved dental esthetic results in the shortest possible time, non-restorative treatments for discoloured teeth i.e. bleaching treatments are preferred. The common and desired treatment to enhance dental esthetics was found to be whitening of teeth. This has led to the widespread use of bleaching agents.

Bleaching techniques for vital teeth can be classified into two categories: in-office bleaching and at-home bleaching. Many studies have reported that bleaching products may cause enamel mineral loss at different levels and alterations in microhardness and other mechanical properties over a period of time.[3] These alterations may vary depending on the product concentration, time duration of application, pH of the product and temperature change due to bleaching activation methods. The present study is designed to find out the changes in enamel properties specifically enamel permeability and micro-mechanical properties after exposure to different concentrations of carbamide peroxide bleaching gel at different time periods.

Significant scientific research till date that have evaluated the microhardness and permeability of dental enamel to bleaching procedures, have been mainly conducted in bovine enamel. Moreover , very less comparison have been drawn till date to compare the differences produced by lower and higher concentrations of bleaching agents with difference in pH . In the proposed study , it will be seen if there is any difference in enamel microhardness and permeability with the different bleaching treatments.

MATERIALS AND METHOD

The present study was carried out using thirty freshly extracted anonymous human maxillary permanent first premolars with fully formed apices extracted due to orthodontic reason were selected for the study. Following extraction, the teeth were washed, cleaned of calculus and soft tissue remnants using a hand curette and disinfected in 0.1% thymol solution over 24 hours and kept in normal saline at 37 0 C till the time of use (within 10 days of extraction).

Inclusion Criteria

- Recently extracted first premolars (for orthodontic purpose)
- Age group of 15-30 years
- Without any caries
- Without any abrasion, attrition or erosion.
- Without any visible coronal cracks (visually inspected for crack on enamel surface)
- Without any previous restorations

Exclusion Criteria

- Grossly carious premolars
- Structural deformities (amelogenesis imperfecta, dentinogenesis imperfecta, enamel hypoplasia, dentine dysplasia, regional odontodysplasia, fluorosis)

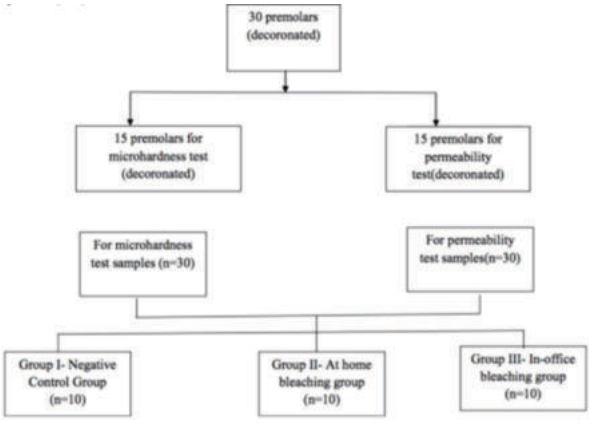

Table 1: Study Model Design For Microhardness As Well As Permeability Testing

Fig 1: Thirty extracted maxillary premolar teeth specimens

PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr


Fig 2: Decoronation of all the teeth samples

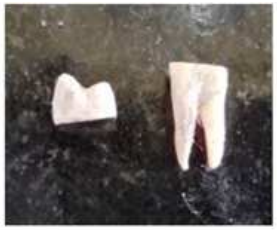
Fig 3: A Decoronated tooth specimen

Group I – Untreated samples were kept in this group which served as the control group for the study.

The teeth samples were decoronated using double-faced diamond disks, using a low-speed hand piece and deionized water was used as a coolant during sectioning. at the level of cement-enamel junction (CEJ).

Group II- The decoronated teeth samples prepared according to the test performed were treated using Pola Night (16% carbamide peroxide) which is an at-home bleaching agent. The samples were treated according to manufacturer's instructions, i.e. 90 minutes a day for 15 days. The samples were rinsed with water spray for 30 seconds and air dried after each treatment.

Group III- The decoronated teeth samples prepared according to the test performed were treated using Opalescence Boost (40% Hydrogen Peroxide) which is an in-office bleaching agent. The samples were treated according to manufacturer's instructions, i.e. 15 minutes three times a day, every alternate day for four days. The samples were rinsed with water spray for 30 seconds and air dried after each treatment.

All the specimens were stored in artificial saliva solution in individual containers (ice-cube trays) at 37°C (± 1°C). The artificial saliva solution was changed every two days. for the entire duration of the study (before, after and in-between bleaching procedure.

Microhardness Determination


Fig 4: Mesio-distal sectioning of decoronated tooth sample for microhardness analysis


Fig 5: Sectioned buccal and palatal halves of the specimen


Fig 6: Polypropylene moulds

Fig 7: Representative sample of each group after embedment in cold cure acrylic resin


Fig 8: Application of bleaching agent


Fig 9: Microharness testing of the teeth specimens

- For microhardness analysis the samples were divided mesio-distally into two halves.
- Thirty teeth samples were produced from fifteen teeth.
- The teeth samples were embedded individually with the buccal/ labial surface facing outwards and the cut surface facing towards the self-curing resin (DPI-RR cold cure, acrylic resin material) in a polyvinyl chloride ring mold with approximately 2.0 cm indiameter, allowing the external surface of the enamel (midlle third portion of the sample) to be exposed.
- The samples were polished using #600, 1000 and 1200 grit aluminium oxide abrasive papers.
- Pre-treatment microhardness analysis was done prior to bleaching treatment to obtain base-line values
- All the samples were randomly allotted to the three groups and bleached accordingly.
- After bleaching treatment, microhardness tests was done after 24 hours and at seven and fourteen days post treatment. The samples were stored in artificial saliva for the entire duration.
- At every time period previously described, three microhardness indentations were made , using a Vickers microhardness tester with a 50g, 100-g and 200g load for 15 seconds dwell time to make the enamel indentations. The distance between two consecutive indentations was 50mm.

Permeability Analysis


Fig 10: Bucco-lingual sectioning of decoronated tooth sample for permeability analysis


Fig 11: Mesial and distal halves of the tooth specimen


Fig 12: Thinning of the samples

Fig 13: Representative sample of each group with a thickness of 0.5 mm

- For permeability tests, the fifteen decoronated tooth samples were taken.
- All surfaces except the buccal surface was sealed using one coat of cyanoacrylate resin and three coats of nail varnish.
- All the samples were randomly allotted to the three groups and bleached with 16 % carbamide peroxide, 40% hydrogen peroxide orkept as a negative control (unbleached).
- After bleaching protocol the nail varnish and cyanoacrylate resin were removed using abrasive papers.
- The samples were sectioned using a diamond disc, bucco-lingually , so as to obtain two samples from a single tooth. Hence thirty samples were obtained from fifteen teeth.
- The samples so obtained were thinned down to almost 0.5mm thickness using bench lathe motor fitted with carborundum stones.
- After completing bleaching treatment, the samples were treated for dye penetration test immediately (within one hour).
- 50 % Silver Nitrate was used as the tracer agent , the samples were dipped in it for 16 hrs and 2hrs in developer solution.
- The samples so obtained for enamel permeability were placed on glass slides and photographed under an optical microscope with 100 X magnification. The images of penetration of dye were taken with an inbuilt software.
- The dye penetration degree was analyzed by one evaluator, using a 0 to 4 score system: score 0 - no dye penetration;

score 1 - less than half the enamel thickness
score 2 – half the enamel thickness.
score 3 - full extent of enamel without reaching the dentin
score 4 - tracer agent reaching dentin

Fig 14. Permeability assessment in control group (score 0). Control group shows the least permeability.

Fig 15. Permeability assessment in 16% carbamide peroxide group (score 1). Shows increased permeability in comparison to control group.

Fig 16. Permeability assessment in 40% hydrogen peroxide group (score 2). This group shows the most permeability.

The data so obtained was recorded and subjected to statistical analysis

Statistical Analysis

Statistical Analysis was performed with help of Epi Info (TM) 7.2.2.2. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC). Data has been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Descriptive statistical analysis was performed to calculate the means with corresponding standard deviation (s.d.). Also One Way Analysis of variance (ANOVA) followed by post hoc Tukey's Test was performed with the help of Critical Difference (CD) at 5% and 1% level of significance to compare the mean values.

Table 1: One-way ANOVA Of Pre-operative Microhardness (VHN) Values Of All The Samples :

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	4,374.0667	2	2,187.0333	1.1261	0.3391
Error	52,438.9000	27	1,942.1815		
Total	56,812.9667	29			

Table 2: One-way ANOVA Of Post Treatment Microhardness (VHN) Values Compared With – Ve Control:

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	2,137.8667	2	1,068.9333	0.5799	0.5668
Error	49,770.8000	27	1,843.3630		
Total	51,908.6667	29			

Table 3: One-way ANOVA Of Microhardness (VHN) 7 Days Post-bleaching:

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	3,070.8667	2	1,535.4333	0.7740	0.4711
Error	53,560.6000	27	1,983.7259		
Total	56,631.4667	29			

Table 4: One-way ANOVA Of Microhardness (VHN) 14 Days Post-bleaching

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	2,333.0667	2	1,166.5333	0.5280	0.5958
Error	59,657.9000	27	2,209.5519		
Total	61,990.9667	29			

Table 5: One-way ANOVA Of Microhardness (VHN) Of –ve Control At Initial, 7 Days And 14 Days

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	124.8667	2	62.4333	0.0313	0.9692
Error	53,891.0000	27	1,995.9630		
Total	54,015.8667	29			

Table 6: One-way ANOVA Of Microhardness (VHN) Of 16% CP At Pre-treatment, Post-Treatment, 7 Days And 14 Days:

Source	sum of squares SS	degrees of freedom vv	mean square MS	F statistic	p-value
Treatment	3,253.4750	3	1,084.4917	0.2781	0.8408
Error	140,369.9000	36	3,899.1639		
Total	143,623.3750	39			

Table 7: One-way ANOVA Of Microhardness (VHN) Of 40% HP At Pre-treatment, Post-Treatment, 7 Days And 14 Days

Source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
Treatment	2,132.0750	3	710.6917	3.5147	0.0247
Error	7,279.3000	36	202.2028		
Total	9,411.3750	39			

Table 8: Tukey HSD Results Of Microhardness (VHN) Of 40% HP At Pre-treatment, Post-Treatment, 7 Days And 14 Days

Treatment pairs	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Pre vs Post	3.8856	0.0003744	significant
Pre vs 16% CP	3.6471	0.0647755	insignificant
Pre vs 40% HP	1.0007	0.8891322	Insignificant
Post vs 16% CP	0.0445	0.8999947	Insignificant
Post vs 40% HP	2.6909	0.2449510	Insignificant
16% CP vs 40% HP	2.6464	0.2582700	Insignificant

Table 9: One-way ANOVA Of Pre Dye Penetration Of Different Groups

Source	sum of squares SS	degrees of freedom	mean square MS	F statistic	p-value
Treatment	10.8667	2	5.4333	22.2921	0.0000
Error	6.5808	27	0.2437		
Total	17.4475	29			

Table 32: Tukey HSD Results Of Pre Dye Penetration Of Different Groups

Treatment pairs	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
-ve control vs 16% CP	1.1000	0.0001	Significant
-ve control vs 40% HP	1.4000	0.0000	Significant
16% CP vs 40% HP	0.3000	0.3762	Insignificant

RESULTS:

Microhardness testing revealed that there was no significant difference between baseline microhardness values and post treatment microhardness values of all the samples except for the samples treated with 40% hydrogen peroxide, which had decreased the microhardness values to a greater degree than the 16% carbamide peroxide group and the negative control group.

On remineralisation using artificial saliva for 16 % carbamide peroxide group at 7 days and 14 days there was a slight increase in microhardness values but they were not statistically significant, however slight increase in values was observed.

For the 40% hydrogen peroxide group there was a significant increase in microhardness at both 7 and 14 days remineralisation treatment.

Permeability analysis demonstrated significant differences between the groups with 40% hydrogen peroxide showing greatest permeability followed by 16% carbamide peroxide group and least permeability was demonstrated by the negative control group.

DISCUSSION

Tooth bleaching has been recommended to include the entire smile zone. The smile zone varies from patient to patient, but generally it extends till the maxillary second premolar region. Hence, in the present study, maxillary firstpremolars extracted due to orthodontic reason was selected.. They are easily available and the buccal and the palatal cusps are of almost equal sizes which was an important factor as longitudinal sectioning was done to obtain buccal and palatal halves from a single tooth sample in this study.

The procedure and treatment duration differ according to the category but the basic bleaching material is either hydrogen peroxide or carbamide peroxide. In general, the efficacy of hydrogen peroxide containing products are approximately the same when compared with carbamide peroxide containing products with equivalent or similar hydrogen peroxide content and delivered using similar format and formulations, either tested in vitro or in vivo.[4]

In the current study an at- home bleaching agent comprising of 16% carbamide peroxide and an in-office bleaching agent comprising of 40% hydrogen peroxide was used because these agents are most commonly utilised for bleaching and also to compare difference in results if any, due to the varying concentration and duration of application.

Several factors such as pH, application time and concentration [5,6,7] of the bleaching agent may predict the influence of these substances on dental structures. It has been reported that the greater the peroxide concentration, the more acidic pH of the bleaching product, affecting the chemical and physical structure of enamel, [8] especially below the pH of 5.2. [9]. The products should have a relatively neutral pH to minimize the potential for damage that could be caused by highly acidic or highly basic solutions. In the current study, the bleaching agents used had a neutral pH (6.8-7.2), to avoid this problem. The samples were treated according to manufacturer's instructions, i.e. 90 minutes a day for 15 days for the Pola Night group (16% carbamide peroxide) which is an at-home bleaching agent. Manufacturer's instructions were followed accordingly for the Opalescence Boost group (40% Hydrogen Peroxide) which is an in-office bleaching agent, i.e. 15 minutes three times a day, every alternate day for four days. This was done to mimic the recommended time and number of applications in-vivo.

Samples were immersed in artificial saliva at all times for the 21 days the study was conducted (excepting the bleaching treatment time) , also the solution was replaced every two days. Some in vitro studies use remineralizing solutions to store the specimens.[91] Like in the present study, artificial saliva was used because it simulates accurately intraoral conditions, compared to storage in distilled water. Also, due to its high mineral content, artificial saliva [92] may increase remineralization of bleached enamel, different from distilled water, which has no remineralizing effect. Studies also confirmed that the saliva was able to increase themineral content of bleached enamel. In this study, the in-office product containing 40% Hydrogen peroxide had in addition, 0.11% sodium fluoride incorporated in it. To overcome the adverse effects of bleaching treatment, such as mineral loss, [10] different carbamide peroxide formulations have been developed, and the addition of fluoride to agents has been studied.[11] Fluoride ions are able to enhance crystal growth and retard dissolution of enamel minerals because of their participation in the solution phase, which increases supersaturation or decreases undersaturation.[12] they largely affect its dissolution rate.

Changes in organic and inorganic content after bleaching treatment may be evaluated by microhardness tests. Although microindentation hardness tests do not provide information about the elementary changes, they are commonly used for the material property changes in the enamel and dentin, especially following demineralization and remineralization experiments.

In the current study the microhardness of enamel from maxillary first premolars was found to be within a range of 343-523 VHN. The pre-treatment microhardness values of the 16% carbamide peroxide group were in the range of 441.10±64.04 VHN., and post- treatment values were in the range of 416.60±61.29 VHN, although numerical changes in the values were observed, the decrease was statistically insignificant. In the 40% hydrogen peroxide group , the pre-treatment values were in the range of 415.00±13.50 VHN and the post- treatment values were in the range of 398.40±15.17 VHN and the change was statistically significant. . According to the results of the present study, higher concentration of the bleaching gel results in higher decrease of dental enamel microhardness. This alteration has been correlated to the oxidative action of hydrogen peroxide on the organic phase of enamel. Disruption of the enamel organic matrix results in loss of the crystalline material sketched out of this matrix, leaving zones of erosion intercalated with areas of intact enamel[106,110]. Also, presence of nitrogen and oxygen molecules have been reported on bleached enamel surface, probably due to the interaction of peroxide with the enamel proteins, suggests that hydrogen peroxide is able of dissolving the enamel organic matrix.

After 7 and 14 days storage in artificial saliva, an increase in microhardness values was noticeable although the baseline values was not restored. At 7 days post-treatment storage in artificial saliva the 16% carbamide peroxide group had a microhardness value ranging from 423.30±61.03 VHN, the values had increased from the post-treatment values but the increase was statistically insignificant. At 7 days post-treatment storage in artificial saliva the 40% hydrogen peroxide group had slightly increased microhardness values ranging from 398.60±16.47, compared to post-treatment values, athough the values were not statistically significant. At 14 days post-treatment storage in artificial saliva, the 16% carbamide peroxide group had a microhardness value ranging from 429.50±63.36 VHN, which indicated increase in microhardness values which was not statistically significant. At 14 days post-treatment storage in artificial saliva, the 40% hydrogen peroxide group had a microhardness value ranging from 410.50±11.18 VHN, which indicated increase in microhardness values which was not statistically significant.

Enamel permeability test using 50% silver nitrate solution was found to be highest for 40% hydrogen and that of control group was found to be the lowest of all. Human enamel comprise of porosities,[13,14] that allows penetration of bleaching agent. It is likely that bleaching products and its by-products penetrate into the enamel through these porosities by a diffusion process, similar to what occurs after fluoride application. In the present research, all bleached groups showed a higher permeability compared to the control group.

CONCLUSION

Within the limitations of the current study, it can be concluded,

- The microhardness of enamel from maxillary first premolars was found to be within a range of 343-523 VHN
- Among the bleaching agents tested, 40% hydrogen peroxide showed maximum decrease in microhardness values, and the minimum change was noted in the negative control group.
- Maximum decrease in microhardness values according to the time period was observed in 40% hydrogen peroxide immediately post-bleaching, the minimum change in microhardness values were recorded in control group at all time periods.
- Storage in artificial saliva had a positive effect on the decreased microhardness values. Both the 40% hydrogen peroxide and 16% carbamide peroxide had increased microhardness values at 7 days and 14 days post bleaching.
- Among the bleaching agents tested 40% hydrogen peroxide demonstrated maximum enamel permeability, the negative control group demonstrated minimum enamel permeability. A correlation was seen between the concentration of bleaching agent and enamel permeability.

REFERENCES

1. World Health Organization. (2006). Constitution of the World Health Organization – Basic Documents, October 2006;45.
2. Glick M, Monteiro da Silva M, Seeberger GK, et al. FDI Vision 2020: shaping the future of oral health. Int Dent J. 2012;62:278-291
3. Amarlal D, Rayen R, Muthu MS. Macroabrasion in Pediatric Dentistry. Journal of Clinical Pediatric Dentistry. 2006;31(1):9–13.
4. Dominguez JA, Bittencourt B, Michel M, Sabino N, Gomes JC, Gomes OM. Ultrastructural evaluation of enamel after dental bleaching associated with fluoride. Microsc Res Tech. 2012; 75: 1093-98.
5. Featherstone JDB, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries like lesions hy quantitative microradiography and microhardness profiles. *Caries Res.* 1983; 17(5):385-391.
6. Featherstone JDB, O'Really MM, Shariati M, Brugler S. Enhancement of remineralization in vitro and in vivo. In: Leach AS (ed). Factors relating to demineralization and remineralization of the teeth. Oxford: IRL, 1986: 23-34.
7. Ben-Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. *Am. J. Dent* 1995; 8(l):29-32
8. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations—a systematic review. Dent Mater 2004. 20:852-61
9. 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; 35: 325- 30.

10. Smidt A, Weiler D, Roman I, Gedalia I. Effect of bleaching agents on microhardness and surface morphology of tooth enamel. *Am. J. Dent.* 1998; 11: 83-85.
11. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. J Endod 1996;22:23?5.
12. Ben?Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. Am J Dent 1995;8:29?32.
13. Cross KJ, Huq NL, Stanton DP, Sum M, Reynolds EC. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle? alpha(S1)?casein(59?79) by stabilized amorphous calcium fluoride phosphate nanocomplexes. Biomaterials 2004;25:5061?9.
14. Spalding M, Taveira LAA, Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: alone, with saliva, and with 10% carbamide peroxide. J Esthet Restor Dent 2003;15:154-165.