

An *In vitro* Study on Post Bleaching Pigmentation Susceptibility of Teeth and Scanning Electron Microscopy Analysis

S Pushpa Latha¹, Vani Hegde², Syed Ahmed Raheel³, Bassel Tarakji⁴, Saleh Nasser Azzeghaiby⁵, Mohammad Zakaria Nassani⁶

Contributors:

¹Senior Lecturer, Department of Conservative Endodontics, The Oxford Dental College & Hospital, Bengaluru, Karnataka, India; ²Professor and Head, Department of Conservative Dentistry & Endodontics, AME's Dental College and Hospital, Raichur, Karnataka, India; ³Lecturer, Department of Oral Medicine, Al-Farabi College of Dentistry and Nursing, Riyadh, Saudi Arabia; ⁴Professor, Department of Oral & Maxillofacial Sciences, Al-Farabi College of Dentistry and Nursing, Riyadh, Saudi Arabia; ⁵Assistant Professor, Department of Oral Surgery, Al-Farabi College of Dentistry and Nursing Riyadh, Saudi Arabia; ⁶Associate Professor, Department of Restorative Dentistry, Al-Farabi College of Dentistry and Nursing, Riyadh, Saudi Arabia.

Correspondence:

Dr. Raheel SA. Department of Oral & Maxillofacial Sciences, Al-Farabi College of Dentistry and Nursing, Riyadh, Saudi Arabia. Phone: +966-541543979, Email: rahil1484@gmail.com

How to cite the article:

Latha SP, Hegde V, Raheel SA, Tarakji B, Azzeghaiby SN, Nassani MZ. An *in vitro* study on post bleaching pigmentation susceptibility of teeth and scanning electron microscopy analysis. J Int Oral Health 2014;6(5):84-8.

Abstract:

Background: To determine the susceptibility of teeth for repigmentation after bleaching.

Materials and Methods: Forty premolars were assigned to three groups ($n = 12$). Group 1 was bleached using 30% w/v hydrogen peroxide 15 min 3 times a day every other day for 4 days. In Group 2 was bleached using 16% carbamide peroxide (Polanight), 90 min a day for 15 days. 2 days later, the shades of the bleached teeth were recorded. Remaining 4 teeth were bleached according to Group 1 and 2 and were subjected to atomic force microscopy, scanning electron microscopy analysis.

Results: Specimens of at home bleaching were lighter than the specimens of inoffice bleaching.

Conclusion: The susceptibility of enamel to pigmentation can be increased after bleaching, and pigmentation is greater if bleaching is performed with H_2O_2 . The percentage change (lighter) was more for at home bleaching specimens as compared to inoffice bleaching specimens.

Key Words: Atomic force microscopy, at home bleaching, in office bleaching, repigmentation

Introduction

With the desire for a whiter, a more attractive smile, tooth bleaching in the dentist's office or at home is rapidly gaining

popularity.¹ Many agents have been used in the past, and a number of new methods have continued to be introduced. It was oxalic acid first by Chappel in 1877 which was used as bleaching the agent it was followed by various forms of chlorine until H_2O_2 was first used by Harlan in 1884. The first paper discussing the concept of night guard bleaching was published in 1986 by Haywood and Heymann, after which the night guard bleaching has been gaining popularity. Inoffice procedure mainly utilizes hydrogen peroxide usually in concentrations ranging from 35% to 50%. Carbamide peroxide in the concentration ranging from 10% to 22% is the most common for at home treatment/night guard vital bleaching/matrix bleaching/dentist prescribed/home applied bleaching. "Power bleaching" uses 30-35% H_2O_2 solution in conjunction with heat or light to increase the kinetics of stain removal.²

These bleaching solutions have been reported to cause a wide variety of changes in enamel and dentin. Carbamide peroxide although has been related to cause demineralization of enamel,³ it actually reduces the susceptibility to dental caries.⁴ When carbamide peroxide bleaching agent was applied with a tooth brush, the enamel roughness has been noted to be increased.⁵ Fracture resistance of the treated samples has been noted to be reduced.⁶ Bond strength of resin to bleached enamel is known to be affected.⁷

Hydrogen peroxide can result in a decrease in microhardness of enamel.⁸ Hydrogen peroxide may cause significantly more loss of calcium from the enamel surface than carbamide peroxide.³ Following H_2O_2 application, a decline in mechanical properties⁹ and fracture toughness¹⁰ and an increase in enamel surface dissolution by phosphoric acid have been reported.¹¹

One study reported that bonding of resin to carbamide peroxide bleached enamel returned to values closer of those of non-bleached enamel in 24 h, but it took a week to return to those of controls in 35% hydrogen peroxide.⁷ No conclusive data are available on the changes in surface morphology. Some researchers have reported that structural and morphological changes in superficial enamel following bleaching with hydrogen peroxide or carbamide peroxide are insignificant.¹² One scanning electron microscopy (SEM) evaluation revealed a significant morphological change

on enamel using both carbamide peroxide and hydrogen peroxide. It concludes that both the bleaching agents alter the microhardness, roughness, and morphology of dental enamel surface.⁸ These changes are more pronounced in *in vitro* conditions, because in *in vivo* conditions, saliva could reverse these changes.¹³ These irregularities could result in more susceptibility to repigmentation, hence adversely affecting the whitening treatment.

In a recent clinical study, home bleaching treatment with 10% carbamide peroxide was found more effective, more acceptable to patients and required less chair time to inoffice bleaching with 35% H₂O₂ (Zekonis *et al.*).¹⁴ Carbamide peroxide is suggested as a safer alternative to hydrogen peroxide-based systems.¹⁵⁻¹⁷

To disclose microleakage¹⁸ and nanoleakage,¹⁹ silver nitrate has been used as a stain due to its high contrast with tooth structure and stability after precipitation. It has been used in the pigmentation susceptibility study.²⁰ Small particle size allows silver nitrate to penetrate minimal and clinically undetectable imperfections and make them readily visible. More recently, it has been used as a disclosing dye in a confocal scanning laser microscope study of carbamide peroxide bleaching.²¹

In atomic force microscopy (AFM), a probe sweeps the surface and collects the information about its structural features. A computer then generates an image of the surface using the collected data.^{22,23} It has been used to study the surface topography²¹ also in a study confirming a decrease in physical properties of enamel and dentin after bleaching with H₂O₂.⁹ SEM also gives an idea of the surface morphology and is been used before for morphology analysis.

The purpose of this study is to determine which bleaching system is more susceptibility to pigmentation after bleaching, also is giving an insight about the efficacy of the two systems. Silver nitrate staining was used to determine *in vitro* changes in surface characteristics. AFM and SEM observation was performed to provide some reference to changes that occurred on surface enamel.

Materials and Methods

Forty extracted premolars of 16-22 years old patients undergoing orthodontic treatment were used in this study. Apical foramina were sealed with glass ionomer cement (Fuji Type 1, GC Corporation, Tokyo, Japan). All samples were coated with a water resistant transparent varnish. The mesial half of their buccal surface was ground with 250 grit sandpaper to expose flat enamel surface without dentin. Afterward, the flat surface were metallurgically polished with 400, 600, 1000, 2000 grit sand paper and polished using pumice (Safe plus) and Soflex polishing strips. Specimens

were viewed under stereomicroscope with ×10 magnification to verify the absence of dentin.

Thirty-six samples were randomly assigned to three groups, and each sample was scanned and paired with a matching shade from vita shade guide (Vitapan). The Vita shade guide was used for value disposition, assigning an incremental number to each shade (Table 1). The shade comparisons were performed by 3 investigators. When there was a disagreement among the investigators, the mean of the 3 values was recorded.

In the control group, 12 specimens were immersed in distilled water for 15 days. In Group 1, 12 specimens were bleached using 30% w/v hydrogen peroxide 15 min 3 times a day every other day for 4 days. In Group 2, 12 samples were bleached using 16% carbamide peroxide (Polanight), 90 min a day for 15 days. All the samples in Group 1 and 2 were stored in distilled water in between the bleaching procedures and at the end of the treatment. At the end of 15 days, samples from Group 1 and 2 were compared with the Vita shade guide and their new shade was registered.

Two days later, all samples were immersed in silver nitrate at 50% by weight for 4 h and fixed for 24 h using a radiographic fixing solution. Each specimen was then washed with running water and wiped for 15 s with a cloth impregnated in 0.04 µm alumina suspension to remove loosely precipitated silver nitrate from the surface. The correction number for each matching shade guide was recorded and used for the statistical analysis. Tukey test was used to determine differences between the groups and one-way analysis of variance at α = 0.05 was used to determine the significant of statistical differences among groups.

For additional microscopic comparison of the samples, 4 enamel dentin sections were obtained from the facial surface of the other 2 premolars. 2 specimens were bleached according to Group 1 regimen and 2 were bleached according to Group 2 regimen and subjected to AFM and SEM.

Results

A summary of the results can be seen in Table 2 and Graph 1. At baseline, there was no statistical difference among the three groups.

Table 1: Numbers assigned to the Vita shade guide from the highest to the lowest value.

Shade	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Table 2: Mean shade value pre and post bleaching and after repigmentation.

Control group	8.0	8.0	7.9
Hydrogen peroxide group	7.0	4.0	9.0
Carbamide peroxide group	9.0	4.0	8.5

After bleaching was performed, two study group samples became lighter in shade and the percentage of change was slightly more with carbamide peroxide group.

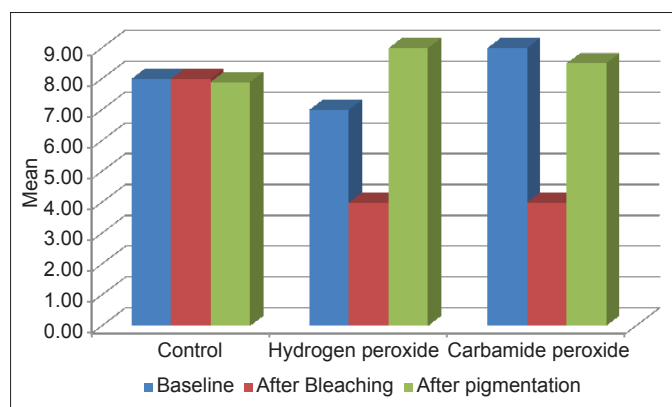
When silver nitrate was introduced, the teeth became darker. The amount of change in the shade value of samples was different for two groups; this change was greater for Group 1 samples than for other group of samples.

AFM study showed increased surface roughness of enamel following bleaching treatments. Samples bleached with 30% hydrogen peroxide showed slightly more small grooves on their surface than did samples bleached with 16% carbamide peroxide (Figure 1).

SEM study showed increased surface roughness of enamel following bleaching treatment with 30% hydrogen peroxide supporting the AFM analysis (Figure 2).

Discussion

There is a general belief among the general population and anecdotal evidence among dentist practitioners that in the office bleaching is superior to at home bleaching. Haywood and Berry (2001) have stated that the efficacy of in office bleaching treatment.



Graph 1: Mean (and standard deviation) shade values before and after bleaching and after pigmentation.

This was an *in vitro* study aimed at determining whether the pigmentation capability of enamel is altered by the 2 most common bleaching treatments. Also giving an idea of efficacy of the two systems used in the study.

The initial polish of the specimens surface provided a flat section of teeth where any excess or loose pigment could easily be washed away, therefore, not affecting the results. The silver nitrate dye created a measurable difference in shade value, making it possible to verify and compare the results in each group.

This study revealed that 16% carbamide peroxide applied at home can be as and slightly more effective than 30% hydrogen peroxide applied in office. Based on this revelation, more patients may opt for the at home treatment. Patients' preferences whether they find visiting the dentist or wearing the bleaching tray more convenient will play a more significant role in treatment selection. This may in return affect the current trends and the industry toward the preferred route of administration for bleaching materials.

The repigmentation effects were also observed among the test group samples. The shade value in the hydrogen peroxide group became darker than the carbamide peroxide; these findings prove that the hydrogen peroxide renders the tooth surface with more irregularities, which are more prone to being restained. This can have a negative effect on clinical treatment with hydrogen peroxide. Based on these results alone, once the teeth are whitened, the procedure needs to be performed routinely to sustain the results for extended periods of time.

These results may not necessarily correlate with the same clinical outcome. The enamel that was evaluated in this study is not the external enamel that is believed to be more mineralized and dense.²⁴ Therefore, the marked repigmentation effect could be of less significance in a clinical setting. Additionally in a clinical setting, repigmentation occurs at a lower degree if at all because of proven remineralizing effects of saliva on exposed enamel.²⁵ It is still essential that further clinical

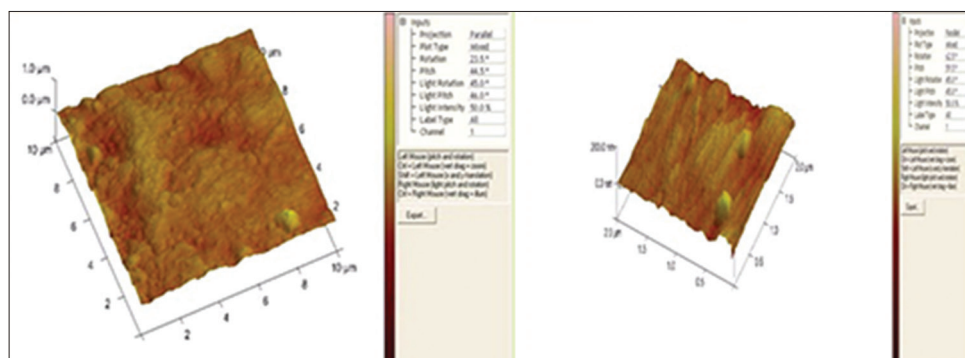


Figure 1: Three-dimensional atomic force microphotograph of enamel surfaces bleached with 30% hydrogen peroxide (left), three-dimensional atomic force microphotograph of enamel surfaces bleached with 16% carbamide peroxide (right).

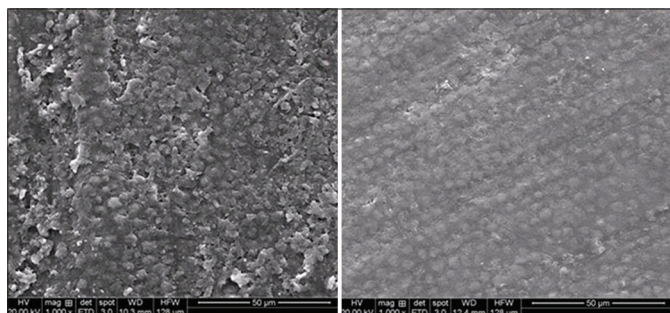


Figure 2: Scanning electron microscopic evaluation of the enamel surfaces bleached with 30% hydrogen peroxide (left) scanning electron microscopic evaluation of the enamel surfaces bleached with 16% carbamide peroxide (right).

studies be performed to determine if *in vivo* environment is efficient enough in suspending the surface altering effects of bleaching and if so how long it takes for enamel to return to its previous condition and not have a higher susceptibility to being stained. It has been reported that treatment effects are clinically stake up to 6 months with carbamide peroxide bleaching.

AFM topographic analysis provided some insight to surface morphological changes. The two bleaching systems used in this study increased the surface roughness and increased concentration of H_2O_2 created numerous grooves on the enamel surface. This supports the macroscopic findings of this study and other reported AFM studies on bleached enamel.

Conclusion

Under the conditions of this *in vitro* study, it was concluded that susceptibility of enamel to pigmentation can be increased after bleaching, and pigmentation is greater if bleaching is performed with H_2O_2 .

References

1. da Costa JB, McPharlin R, Paravina RD, Ferracane JL. Comparison of at-home and in-office tooth whitening using a novel shade guide. *Oper Dent* 2010;35(4):381-8.
2. Zantner C, Derdilopoulou F, Martus P, Kielbassa AM. Randomized clinical trial on the efficacy of a new bleaching lacquer for self-application. *Oper Dent* 2006;31(3):308-16.
3. Tezel H, Ertas OS, Ozata F, Dalgat H, Korkut ZO. Effect of bleaching agents on calcium loss from the enamel surface. *Quintessence Int* 2007;38(4):339-47.
4. Alves EA, Alves FK, Campos Ede J, Mathias P. Susceptibility to carieslike lesions after dental bleaching with different techniques. *Quintessence Int* 2007;38(7):e404-9.
5. de Freitas PM, Turssi CP, Hara AT, Serra MC. Monitoring of demineralized dentin microhardness throughout and after bleaching. *Am J Dent* 2004;17(5):342-6.
6. Attin T, Müller T, Patyk A, Lennon AM. Influence of

- different bleaching systems on fracture toughness and hardness of enamel. *Oper Dent* 2004;29(2):188-95.
7. Unlu N, Cobankara FK, Ozer F. Effect of elapsed time following bleaching on the shear bond strength of composite resin to enamel. *J Biomed Mater Res B Appl Biomater* 2008;84(2):363-8.
8. Pinto CF, Oliveira Rd, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. *Braz Oral Res* 2004;18(4):306-11.
9. Hairul Nizam BR, Lim CT, Chng HK, Yap AU. Nanoindentation study of human premolars subjected to bleaching agent. *J Biomech* 2005;38(11):2204-11.
10. Lewinstein I, Hirschfeld Z, Stabholz A, Rotstein I. Effect of hydrogen peroxide and sodium perborate on the microhardness of human enamel and dentin. *J Endod* 1994;20(2):61-3.
11. Yeh ST, Su Y, Lu YC, Lee SY. Surface changes and acid dissolution of enamel after carbamide peroxide bleach treatment. *Oper Dent* 2005;30(4):507-15.
12. Nucci C, Marchionni S, Piana G, Mazzoni A, Prati C. Morphological evaluation of enamel surface after application of two 'home' whitening products. *Oral Health Prev Dent* 2004;2(3):221-9.
13. Chow L, Wefel JS. The dynamics of de and re-mineralisation. *J Prof Excell* 2009;7(2):42-6.
14. Zekonis R, Matis BA, Cochran MA, Al Shetri SE, Eckert GJ, Carlson TJ. Clinical evaluation of in-office and at-home bleaching treatments. *Oper Dent* 2003;28(2):114-21.
15. Bishara SE, Suleiman AH. Effect of enamel bleaching on the bonding strength of orthodontic brackets. *Am J Orthod Dentofac Orthop* 1993;104(5):444-7.
16. Stokes AN, Hood JA, Dhariwal D, Patel K. Effect of peroxide bleaches on resin-enamel bonds. *Quintessence Int* 1992;23(11):769-71.
17. García-Godoy F, Dodge WW, Donohue M, O'Quinn JA. Composite resin bond strength after enamel bleaching. *Oper Dent* 1993;18(4):144-7.
18. Setien VJ, Cobb DS, Denehy GE, Vargas MA. Cavity preparation devices: Effect on microleakage of Class V resin-based composite restorations. *Am J Dent* 2001;14(3):157-62.
19. Li H, Burrow MF, Tyas MJ. The effect of concentration and pH of silver nitrate solution on nanoleakage. *J Adhes Dent* 2003;5(1):19-25.
20. Iwamoto N, Shimada Y, Tagami J. Penetration of silver nitrate into bleached enamel, dentin, and cementum. *Quintessence Int* 2007;38(3):e183-8.
21. Setien V, Roshan S, Cala C, Ramirez R. Pigmentation susceptibility of teeth after bleaching with 2 systems: An *in vitro* study. *Quintessence Int* 2009;40(1):47-52.
22. Mahmoud SH, Elembaby Ael S, Zaher AR, Grawish Mel-A, Elsabaa HM, El-Negoly SA, et al. Effect of 16% carbamide peroxide bleaching gel on enamel and dentin

- surface micromorphology and roughness of uremic patients: An atomic force microscopic study. *Eur J Dent* 2010;4(2):175-82.
23. Feng HP. The power of atomic force. *Nat Struct Mol Biol* 2000;7:354-5.
24. Mann AB, Dickinson ME. Nanomechanics, chemistry and structure at the enamel surface. *Monogr Oral Sci* 2006;19:105-31.
25. Ingram GS, Agalamanyi EA, Higham SM. Caries and fluoride processes. *J Dent* 2005;33(3):187-91.