Original Article

In vitro evaluation of the effect of addition of biomaterials to carbamide peroxide on the bleaching efficacy and microhardness of enamel

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Abstract

Background and Aim: Teeth bleaching, although considered safe and conservative, cause microscopic changes in the tooth structure. The aim of this study is to evaluate the bleaching efficacy of carbamide peroxide (CP) bleaching gel when modified with the incorporation of bioactive glass (BG) and hydroxyapatite (HA) and its effect on enamel microhardness.

Materials and Methods: Forty-five maxillary incisors were decoronated, artificially stained and mounted in acrylic. The samples were divided into three groups of 15 each and subjected to the following bleaching protocol for 8 h/day at 37°C for 2 weeks: Group 1 – 16% CP, Group 2 – CP modified with BG, and Group 3 – CP modified with hydroxyapatite (HA). Spectrophotometric color assessment using CIE L*a*b* system and Vickers microhardness were assessed before and after bleaching. Data were analyzed using Student's paired *t*-test and one-way ANOVA followed by Tukey's *post hoc* analysis.

Results: There was a significant change in color (L*a*b*) in all the three groups when compared to the baseline values. However, no significant difference in the total color change (ΔE) was observed between the three groups. Enamel microhardness reduced significantly in the CP group, whereas it increased in the BG and HA group after bleaching. Scanning electron microscopy images of BG and HA groups showed crystalline deposits suggesting mineral deposition.

Conclusion: Addition of biomaterials can be a beneficial alternative to bleaching with CP alone, considering the increase in microhardness without hindering the bleaching action.

Keywords: Bioactive glass; biomaterials; bleaching; carbamide peroxide; hydroxyapatite; microhardness; scanning electron microscopy

INTRODUCTION

Bleaching is considered the most conservative, safe, and economical treatment option for tooth discoloration. Hydrogen peroxide (HP) and carbamide peroxide (CP) are the commonly used agents for in-office and at-home bleaching, respectively. CP bleaching is popular as its different concentrations are milder and less caustic than HP making it convenient to use.[1]

Tooth whitening is mainly based on the action of free radicals that penetrate tooth structure and oxidize the organic chromophore molecules. This action may also alter the organic and inorganic components of enamel and dentin resulting in loss of calcium (Ca) and phosphate (P) ions, morphological modifications on the surface and subsurface, reduction in surface microhardness and abrasion resistance, increase in surface roughness, susceptibility to caries, erosion and staining.[2-7] Several

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factors including the pH of the gel, by-products such as urea, vehicles such as glycerin and carbopol are also found to cause demineralization.[8]

Several measures have been undertaken to prevent this demineralization and promote remineralization of the bleached enamel. Addition of remineralizing agents such as fluoride has shown some improvement in solubility of enamel by the deposition of minerals in enamel crystallites. However, some studies have shown that fluoridated bleaching gels could not produce significant remineralization of bleached enamel.[9] Furthermore, an ideal remineralizing agent should replace calcium and phosphate ions in addition to fluoride.[10]

To achieve this, attempts have been made to add biomimetic materials such as hydroxyapatite (HA) and bioactive glass (BG) to HP.[11,12] These are alkaline salts that buffer the acidity of HP and reduce the demineralization effects thus preventing microhardness loss. While the benefits of addition of these biomaterials to HP have been reported in the literature, addition of these agents to CP, commonly advocated in night guard bleaching has not been explored much.[11-13]

Hence, this study was undertaken to evaluate the bleaching efficacy of CP bleaching gel when modified with the incorporation of BG and HA and its effect on the enamel microhardness. The alterations in the enamel surface were evaluated using the scanning electron microscopy (SEM). The null hypothesis tested was that addition of biomaterials to CP does not affect: (1) its bleaching efficacy and (2) microhardness of enamel.

MATERIALS AND METHODS

Forty-five freshly extracted human maxillary incisors devoid of caries, restorations, hypoplastic defects or visible cracks were selected and placed in 0.2% thymol. The teeth were cleaned of debris and calculus using an ultrasonic scaler, decoronated 1–2 mm apical to the cemento-enamel junction with diamond discs and the entire crown was used for the study.

Staining procedure

A 1 L mixture of black tea and red wine (1:1) was made. Ten grams of tea powder were boiled for 5 min in 500 ml of distilled water to produce the tea solution. The samples were stained before testing by immersing it in this solution for 7 days at 37°C. After staining, the specimens were placed in a wax mold and mounted in self-cure polymethyl methacrylate resin (DPI-RR Cold cure). The enamel surfaces were grounded flat using aluminum oxide polishing papers.

Baseline color

The color of the specimens was determined by a spectrophotometer (Premier Colorscan SS 5100A) after calibration, by placing the probe at the junction of the incisal and middle third of each specimen. Color measurements were obtained with regard to the three coordinate values (CIE $L^*a^*b^*$).

Baseline microhardness

Vickers microhardness values were obtained for all the samples using a nano indenter (Nanatom) under a load of 100 g and an indentation time of 20 s. Each specimen had three indentations made on its surface, spaced 100 μm apart, and the values were averaged to determine the baseline microhardness value.

Bleaching procedure

For every specimen, a tray was fabricated with low-density polyethylene plates of 1 mm thickness in a vacuum plasticizer (Dunaform). The samples were randomly divided into three groups of 15 each and the following bleaching protocol was carried out for 8 h every day at 37°C in an incubator for 2 weeks.

- Group 1 ($n = 15$): 16% CP gel (Ultrawhite, Ammdent)
- Group 2 ($n = 15$): 1 g BG (Perioglas, Novabone) +1 ml distilled water mixed with 1 ml of 16% CP gel
- Group 3 ($n = 15$): 1 g HA (Sybograf, Eucare) +1 ml distilled water mixed with 1 ml of 16% CP gel.

A small amount of bleaching gel was applied onto the tray and placed over the tooth surface. After 8 h, the bleaching gel was thoroughly rinsed off with distilled water and kept immersed in artificial saliva at 37°C.

Final color and microhardness assessment

After 2 weeks, the final color and microhardness assessment was done in the same way as baseline and the values were compared. The ΔL^*, Δa^*, and Δb^* values were obtained by comparing the L^*, a^* and b^* values before and after bleaching. These values were used in the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ to calculate total color difference (ΔE).

Scanning electron microscopic analysis

Three samples were selected from all the three groups and evaluated for the physical alterations in the enamel. For the SEM analysis, the surface of the sample was dried, mounted in aluminum stubs and sputter-coated with gold for 4 min. The surface morphology of enamel was examined at ×3000 magnification.

Statistical analysis

Student's paired *t*-test was used to compare the mean L^*, a^* and b^* values and micro hardness values before and after bleaching. One-way ANOVA test followed by Tukey's HSD

post hoc analysis was used to compare the mean ΔL^*, Δa^*, Δb^*, and ΔE values and microhardness values between the three groups. The level of significance was set at $P < 0.05$.

RESULTS

Bleaching efficacy

The mean ± standard deviation of L^*, a^*, and b^* values is shown in Table 1. There was a significant difference in L^*, a^* and b^* color parameters before and after the bleaching protocol in all three groups ($P < 0.001$). Table 2 shows the comparison of ΔL^*, Δa^*, and Δb^* parameters among the different groups and this was statistically significant ($P < 0.001$). The total color change (ΔE) was highest for the CP group (3.869) followed by BG group (3.708) and HP group (3.601), however, this difference was not statistically significant ($P > 0.05$).

Microhardness

Table 3 shows that the change in Vickers microhardness value after bleaching was statistically significant in all three groups. The CP group showed a reduction in mean microhardness ($P < 0.001$), whereas it increased in the BG ($P < 0.001$) and HA ($P = 0.02$) groups after bleaching and this was statistically significant. On comparing between the different groups, the CP group showed a significantly lower microhardness than BG and HA groups ($P < 0.001$). BG group showed a higher microhardness than HA group; however, this was not significant ($P = 0.45$).

Scanning electron microscopic analysis

Figure 1a and b shows surface porosities, craters, depressions, and irregular surface of enamel after bleaching with 16% CP gel. Figure 1c-f show a relatively smooth enamel surface with minimal surface irregularities and crystalline deposits over the enamel surface suggesting mineral deposition by BG and HA.

Table 1: Comparison of mean L*, a*, and b* values before and after bleaching

Variables	Time	Mean±SD		
		Group - CP	Group - BG	Group - HA
L*	Before	69.631±3.120[a]	71.105±4.294[a]	77.722±4.450[a]
	After	70.596±3.105[b]	71.705±4.287[b]	78.617±4.441[b]
a*	Before	−0.447±0.066[a]	0.253±0.337[a]	−1.237±0.078[a]
	After	−1.383±0.083[b]	−1.666±0.550[b]	−3.700±0.170[b]
b*	Before	7.712±0.384[a]	7.818±0.419[a]	3.342±0.387[a]
	After	4.085±0.384[b]	4.733±0.548[b]	0.890±0.141[b]

Different superscript letters (a, b) denote a statistically significant difference in the L*, a*and b* values before and after bleaching within each group (Student's paired *t*-test). CP: Carbamide peroxide, BG: Bioactive glass, HA: Hydroxyapatite, SD: Standard deviation

Table 2: Comparison of mean ΔL*, Δa*, Δb*, and ΔE values between the three groups

Variables	Mean±SD		
	Group - CP	Group - BG	Group - HA
ΔL*	0.965±0.051[a]	0.600±0.030[b]	0.895±0.050[c]
Δa*	−0.936±0.043[a]	−1.919±0.338[b]	−2.463±0.167[c]
Δb*	−3.627±0.182[a]	−3.085±0.479[b]	−2.453±0.427[c]
ΔE	3.869±0.178[a]	3.708±0.379[a]	3.601±0.354[a]

Different superscript letters (a, b, c) indicate statistically significant difference for ΔL*, Δa*, Δb* and ΔE between different groups (one-way ANOVA with Tukey's *post hoc* analysis). CP: Carbamide peroxide, BG: Bioactive glass, HA: Hydroxyapatite, SD: Standard deviation

Table 3: Mean microhardness values before and after bleaching and the change in microhardness

Groups	Mean±SD		
	Before	After	Change
CP	202.34±11.21	149.92±17.87	−52.41±16.36*,[a]
BG	205.26±15.86	217.31±12.34	12.05±10.26*,[b]
HA	205.22±19.12	211.74±19.47	6.52±9.61*,[b]

*Statistical significance in the mean change in microhardness within each group (Student's paired *t*-test). Different superscript letters (a, b) indicate the mean change in microhardness values between different groups are statistically different (one-way ANOVA with Tukey's *post hoc* analysis). CP: Carbamide peroxide, BG: Bioactive glass, HA: Hydroxyapatite, SD: Standard deviation

DISCUSSION

The bleaching agents break down to form free radicals that diffuse through the interprismatic spaces of enamel and dentinal tubules and interact with the organic chromophores. This action was found to attack the organic and inorganic portions of the tooth causing mineral loss and decrease in physical properties such as microhardness and fracture resistance of enamel.[2,14] The acidic pH of the bleaching agents and by-products such as urea that could remove enamel proteins and related mineral elements also contributes to this.[8]

Türkun *et al*. have suggested that the bleached enamel takes about 3 months to revert to normal.[15] Even though the remineralization and protective effects of saliva may overcome the detrimental bleaching effects *in vivo*, it is still necessary to minimize the risk of even minor damage caused by these agents.[16] For this purpose, biomaterials such as BG and HA have been added in a ratio of 1:1 to CP in this study as higher concentrations did not provide superior benefits in an earlier study done by de Vasconcelos *et al*.[17]

The samples were stained with a mixture of tea solution and red wine for evaluating bleaching efficacy as proposed by Kielbassa *et al*.[18] This provided matchable baseline values for comparison. The color analysis before and after bleaching has been done with a spectrophotometer using CIE $L^*a^*b^*$ color system. Although several methods such as shade guides, photography, colorimeters, spectrophotometers, and computer digitization are available to assess color, CIE $L^*a^*b^*$ is preferred as it helps in quantifying the color properties of teeth and is considered the most complete color space.[19] The L^* axis represents the degree of lightness (0-black to 100-white). The a^* plane

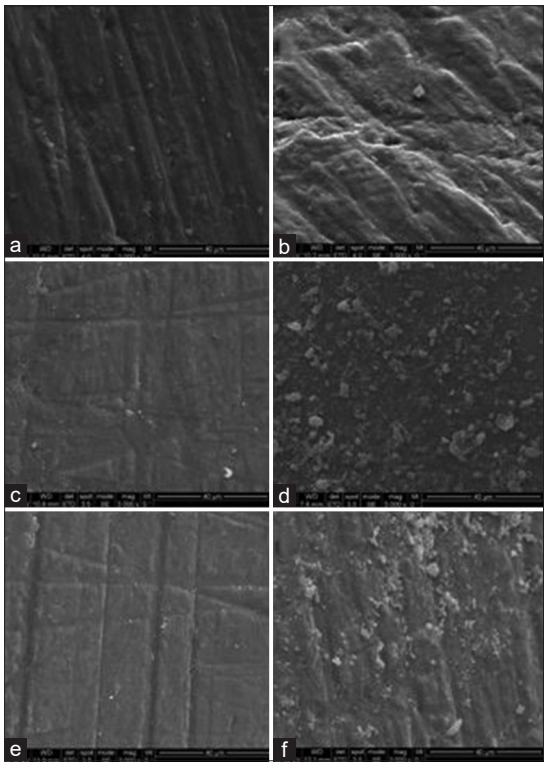

Figure 1: Scanning electron microscopy image (×3000) showing enamel surface of (a and b) Carbamide peroxide group, (c and d) Bioactive glass group and (e and f) Hydroxyapatite group

represents the degree of green/red color (−a* = green and +a* = red) whilst the b* plane represents the degree of blue/yellow color (−b* = blue and +b* = yellow).

Previous studies have reported that color changes are influenced predominantly by the changes in L* and b* than a* values. The reduction in b* value occurs more rapidly and consistently and hence is considered a more important indicator of tooth whitening.[20] In this study, after bleaching, all the groups showed change in color parameters (L*, a*, b*) that was statistically significant from the baseline. The ΔE value of at least 3.3 is considered visually perceptible.[21] Since ΔE values are obtained in the range of 3.6–3.9 in this study, all bleaching treatments can be considered effective and this validates that the addition of biomaterials did not hinder the bleaching action. Hence, the first part of the null hypothesis is not rejected.

Studies have shown bleaching to cause loss of Ca and *P* ions from the enamel. Microhardness of the tooth was evaluated as it reflects the mineral content of the tooth.[22] BG and HA are alkaline salts that can buffer the acidity of the bleaching agent, making it less acidic and limiting mineral loss. Furthermore, these particles also adhere to the enamel surface, creating a shield and minimizing the detrimental effects of the bleaching agent.[11-13]

The enamel surfaces were polished with abrasive papers as a uniform surface is necessary for better precision of the indentations for microhardness testing. It was evident from Vickers testing that there was a decrease in the microhardness value compared to the baseline for the CP group that was statistically significant. On the other hand, in the BG and HA groups, microhardness was higher than the CP group in addition to being higher than the baseline value. This indicates that the addition of biomaterials not only prevented the microhardness loss caused by bleaching with CP, but also further increased the hardness of the enamel. Hence, the second part of the null hypothesis is rejected.

45S5 BG contains 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 in weight and releases Ca^{2+}, Na^+, and PO_4^{3-} ions in aqueous environments.[23] While it is possible that BG deposits could hinder peroxide penetration and reduce the effectiveness of whitening, this theory was not verified, similar to previous study by Deng *et al*.[12] The rapid ionic exchange of Na^+ with H^+ or H_3O^+ at glass-liquid interface allows Ca^{2+} and PO_4^{3-} to be released to result in a supersaturated ionic reservoir for the enamel apatite. Progression of these reactions leads to crystallization of carbonate enriched HA in enamel.[12] Earlier studies have shown that BG can promote remineralization[24] through interfacial apatite precipitation.[25] It was also capable of inhibiting and reversing initial caries progression in enamel.[23,26]

HA is a biomaterial that has chemical and structural similarity with natural bone and tooth minerals. Studies have shown the ability of HA to repair early caries lesions.[27,28] The alkalinity of HA is said to facilitate the bleaching process by accelerating the formation of free radicals from HP.[11] This may be attributed to the fact that in a basic solution, the formation of free radicals requires a lower activation energy and that the reaction rate is higher.[29] Efficient teeth whitening has been reported when HA was incorporated in the toothpaste.[30] Addition of HA has shown better color stability post bleaching along with occlusion of enamel surface porosities.[31]

The SEM images corroborate the findings of this study. The CP group showed a highly irregular enamel surface with surface porosities, craters, and depressions [Figure 1a and b]. The BG and HA groups showed a relatively smoother enamel surface compared to the CP group, with fewer surface irregularities [Figure 1c and e]. Small disorderly fashions of crystalline deposits were seen on the enamel surface of BG and HA groups, suggesting mineral deposition by biomaterials [Figure 1d and f].

The addition of biomaterials may reduce the postoperative sensitivity,[32] staining, and caries susceptibility that is frequently associated with bleached enamel. However,

further studies are required to evaluate the long-term benefits of adding these biomaterials and their effect on restorative procedures. The time duration for which the increased microhardness is sustained in the oral environment also needs to be evaluated.

CONCLUSION

Addition of BG and HA to CP has shown to be more beneficial than bleaching with CP alone, considering the increase in microhardness without affecting its bleaching action.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Tezel H, Atalayin C, Erturk O, Karasulu E. Susceptibility of enamel treated with bleaching agents to mineral loss after cariogenic challenge. Int J Dent 2011;2011:953835.
2. Klaric E, Rakic M, Sever I, Milat O, Par M, Tarle Z. Enamel and dentin microhardness and chemical composition after experimental light-activated bleaching. Oper Dent 2015;40:E132-41.
3. Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: A preliminary report. J Prosthet Dent 1992;67:852-5.
4. Hosoya N, Honda K, Iino F, Arai T. Changes in enamel surface roughness and adhesion of *Streptococcus mutans* to enamel after vital bleaching. J Dent 2003;31:543-8.
5. Berger SB, Coelho AS, Oliveira VA, Cavalli V, Giannini M. Enamel susceptibility to red wine staining after 35% hydrogen peroxide bleaching. J Appl Oral Sci 2008;16:201-4.
6. Singh RD, Ram SM, Shetty O, Chand P, Yadav R. Efficacy of casein phosphopeptide-amorphous calcium phosphate to prevent stain absorption on freshly bleached enamel: An *in vitro* study. J Conserv Dent 2010;13:76-9.
7. Maleknejad F, Ameri H, Kianfar I. Effect of intracoronal bleaching agents on ultrastructure and mineral content of dentin. J Conserv Dent 2012;15:174-7.
8. Cavalli V, Arrais CA, Giannini M, Ambrosano GM. High-concentrated carbamide peroxide bleaching agents effects on enamel surface. J Oral Rehabil 2004;31:155-9.
9. 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;37:156-62.
10. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res 2010;89:1187-97.
11. Jiang T, Ma X, Wang Z, Tong H, Hu J, Wang Y. Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide. J Dent 2008;36:907-14.
12. Deng M, Wen HL, Dong XL, Li F, Xu X, Li H, *et al.* Effects of 45S5 bioglass on surface properties of dental enamel subjected to 35% hydrogen peroxide. Int J Oral Sci 2013;5:103-10.
13. Khoroushi M, Shirban F, Kaveh S, Doustfateme S. Effect of three nanobiomaterials on microhardness of bleached enamel. Restor Dent Endod 2016;41:196-201.
14. Da Cunha FB, Rodrigues E Silva BH, Freitas De Paula BL, Alencar CM, de Albuquerque Jassé FF, Silva CM. Effect of high concentrated fluoride-based dentifrice on the hardness, roughness, and color of the bleached enamel. J Conserv Dent 2018;21:433-7.
15. Türkun M, Sevgican F, Pehlivan Y, Aktener BO. Effects of 10% carbamide peroxide on the enamel surface morphology: A scanning electron microscopy study. J Esthet Restor Dent 2002;14:238-44.
16. Justino LM, Tames DR, Demarco FF. *In situ* and *in vitro* effects of bleaching with carbamide peroxide on human enamel. Oper Dent 2004;29:219-25.
17. de Vasconcelos AA, Cunha AG, Borges BC, Vitoriano Jde O, Alves-Júnior C, Machado CT, *et al.* Enamel properties after tooth bleaching with hydrogen/carbamide peroxides in association with a CPP-ACP paste. Acta Odontol Scand 2012;70:337-43.
18. Kielbassa AM, Beheim-Schwarzbach NJ, Neumann K, Nat R, Zantner C. *In vitro* comparison of visual and computer-aided pre- and post-tooth shade determination using various home bleaching procedures. J Prosthet Dent 2009;101:92-100.
19. Türkün M, Celik EU, Aladağ A, Gökay N. One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique. J Esthet Restor Dent 2010;22:139-46.
20. Gomes MN, Francci C, Medeiros IS, De Godoy Froes Salgado NR, Riehl H, Marasca JM, *et al.* Effect of light irradiation on tooth whitening: Enamel microhardness and color change. J Esthet Restor Dent 2009;21:387-94.
21. Vichi A, Ferrari M, Davidson CL. Color and opacity variations in three different resin-based composite products after water aging. Dent Mater 2004;20:530-4.
22. 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;25:143-57.
23. Burwell AK, Litkowski LJ, Greenspan DC. Calcium sodium phosphosilicate (NovaMin): Remineralization potential. Adv Dent Res 2009;21:35-9.
24. Costa JL, Besegato JF, Manzoli TM, Nogueira BR, de Almeida EN, Kuga MC. Influence of bleaching agent containing bioactive glass on color and microhardness of bovine enamel. Int J Odontostomatol 2022;6:497-503.
25. Curtis AR, West NX, Su B. Synthesis of nanobioglass and formation of apatite rods to occlude exposed dentine tubules and eliminate hypersensitivity. Acta Biomater 2010;6:3740-6.
26. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An *in vitro* pH-cycling study. J Conserv Dent 2014;17:3-7.
27. Huang SB, Gao SS, Yu HY. Effect of Nano-hydroxyapatite concentration on remineralization of initial enamel lesion *in vitro*. Biomed Mater 2009;4:034104.
28. Geeta RD, Vallabhaneni S, Fatima K. Comparative evaluation of remineralization potential of nanohydroxyapatite crystals, bioactive glass, casein phosphopeptide-amorphous calcium phosphate, and fluoride on initial enamel lesion (scanning electron microscope analysis) – An *in vitro* study. J Conserv Dent 2020;23:275-9.
29. Cotton FA, Wilkinson G. Oxygen. In: Cotton FA, Wilkinson G, editors. Advances in Inorganic Chemistry. A Comprehensive Text. New York: Interscience Publisher; 1972. p. 403-20.
30. Limeback H, Meyer F, Enax J. Tooth whitening with hydroxyapatite: A systematic review. Dent J (Basel) 2023;11:50.
31. Moosavi H, Darvishzadeh F. The influence of post bleaching treatments in stain absorption and microhardness. Open Dent J 2016;10:69-78.
32. Bizreh Y, Milly H. Effect of bioactive glass paste on efficacy and post-operative sensitivity associated with at-home bleaching using 20% carbamide peroxide: A randomized controlled clinical trial. Eur J Med Res 2022;27:194.