

Original Article

A comparative evaluation of the effect of three different concentrations of in-office bleaching agents on microhardness and surface roughness of enamel – An *in vitro* study

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ABSTRACT

Background: To evaluate the changes in the micro-hardness and surface roughness of enamel treated with three different concentrations of in-office bleaching agents.

Materials and Methods: In this *in vitro* study, 60 human incisors were divided into two groups (Group A and Group B). To obtain the baseline values, a Vickers tester was used to determine the surface microhardness in Group A, and a Surtronic tester was used for evaluation of surface roughness in Group B. Each group was then further subdivided into three subgroups and subjected to bleaching with Dash (Groups A1 and B1), Pola Office (Groups A2 and B2), and Opalescence Boost (Groups A3 and B3) containing 30%, 35%, and 40% hydrogen peroxide (HP), respectively. Samples were again subjected to testing to obtain the postbleaching values. Pre- and postbleaching data were analyzed by paired *t*-test. Intergroup comparison was carried out using one-way ANOVA ($P \leq 0.05$).

Results: A significant decrease in microhardness values was observed following bleaching in all the three groups, with Group A1 showing maximum percentage decrease (2.58%), followed by Group A2 (1.23%) and Group A3 (0.73%). Furthermore, an increase in surface roughness was observed following bleaching, with Group B1 showing maximum percentage increase (14.80%), followed by Group B2 (8.25%) and Group B3 (5.79%). However, there was no significant difference in either microhardness or surface roughness when comparing the postbleaching values among the three bleaching agents.

Conclusion: In-office bleaching agents may adversely affect the microhardness and roughness of enamel surface which are not related to the concentration of HP used.

Key Words: Bleaching agents, enamel, hardness, opalescence

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INTRODUCTION

Discoloration remains one of the most common clinical conditions that lead patients to seek esthetic treatment.^[1] Techniques that involve the conservative management of the discolored anterior teeth are

preferred so that there is a minimal alteration of the intact tooth structure. Bleaching is one such unobtrusive technique employed to improve the

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appearance of discolored teeth and is preferred overlaminates, veneers, or full-coverage crowns which require removal of sound dental tissue.^[2,3]

Hydrogen peroxide (HP) and carbamide peroxide (CP) at different concentrations are commonly used for bleaching purposes. In-office bleaching technique generally uses relatively high concentrations of bleaching agents (25%–40% HP or 35% CP) for shorter time periods to achieve satisfactory outcomes with fewer applications.^[4]

Bleaching treatment, however, may also simultaneously cause morphological alterations in mineralized structures, such as reduced surface microhardness (SMH) indicating the dissolution and degradation of enamel structure and an increased surface roughness. A rough surface may, in turn, predispose the teeth to extrinsic staining, plaque adhesion and maturation, bacterial adhesion, and consequent periodontal diseases and breakdown.^[5,6]

Although the efficacy of various bleaching agents in lightening the shade of teeth is established, the safety of some of these oxidizing agents is a subject of concern.^[7,8] Adverse effects of different concentrations of bleaching agents on dental tissues, therefore, need to be carefully evaluated to determine ideal protocols for better and safer outcomes while using the in-office technique.

Various in-office bleaching agents with different concentrations of HP have flooded the market today. Among these, the concentrations of 30% (Dash, Philips, USA), 35% (Pola Office, SDI, Victoria, Australia), and 40% HP (Opalescence Boost, Ultradent Products, Inc., South Jordan, UT, USA) have been used in this study.

In the comprehensive literature review, there has been no previous study comparing the effects of these three concentrations (30%, 35%, and 40%) of HP on SMH and surface roughness (Ra) of enamel, thus emphasizing the importance of present study.

MATERIALS AND METHODS

Sixty freshly extracted defect-free human incisors extracted for periodontal reasons not related to this *in vitro* study were collected. The age range of the individuals from which the teeth were collected was between 40 and 55 years. The teeth were then disinfected in a solution of 0.1% thymol for 24 h following the Centers for Disease Control guidelines and stored in saline until the samples were used.

Preparation of samples

The crowns of the teeth were separated from the roots at the level of the cemento-enamel junction using a water-cooled diamond disk (Shofu Dental Asia-Pacific Pte. Ltd., Singapore) in a low-speed handpiece (Marathon, Seyang, Japan). Following this, the crown samples were examined using a stereo-microscope at $\times 5$ magnification (Lawrence and Mayo, Pune, Maharashtra, India) for any surface structural damage, and those with cracks and defects were excluded. The 60 selected samples were then embedded in autopolymerizing self-cure acrylic resin (Pyrax Polymers, Roorkee, Uttarakhand, India) using plastic molds of diameter 20 mm with the labial surface facing upwards. The samples were kept in cold water until complete curing of the resin to avoid thermal effects generated by the resin during the curing process. After 24 h, the specimens were removed from the molds and were then flattened in a polishing machine (Central Scientific Instrument Corporation, Agra, India) using sequential silicon carbide abrasive papers (200, 400, 600, 800, 1000, and 1200) under water cooling, in order to form the parallel planar surfaces.

Prebleach sample testing

Samples were randomly divided into the following two groups according to the test performed:

- Group A – Microhardness testing ($n = 30$)
- Group B – Surface roughness testing ($n = 30$).

Determination of microhardness

The SMH of each sample was determined using a digital display Vickers microhardness tester (Innovatest, Europe) with a square-based diamond pyramid indenter at 200 g force for 10 s. For this, each sample was divided into nine equal parts using two vertical and two horizontal lines, and testing was performed in the most central part of the sample. After removal of the load, the two impression diagonals were measured, usually to the nearest 0.1 μm with a filar micrometer, and averaged. Microhardness measurements were taken before initial exposure to the bleaching agents (baseline) and were calculated as mean value of three measurements.

Determination of surface roughness

A Surtronic surface roughness tester (Taylor Hobson, Leicester, England) was used to measure the surface roughness before the bleaching procedure (baseline). The tip of the roughness tester touched the specimen

and explored 2.5 mm diagonally. Three linear measurements in different directions were recorded, and the average surface roughness was determined for each specimen. Surface roughness was indicated by Ra value for each sample.

Bleaching protocol

Both Groups A and B were, in turn, randomly divided into three equal subgroups of ten samples each based on the bleaching agents used. The bleaching procedure was done in each group as per the respective manufacturer's instructions.

- Groups A1 and B1 – Dash (Philips, USA) (30% H₂O₂)

Dash whitening gel is a patented formula formulated to be stable without refrigeration for storage and ensures superior ease of use. It uses a single-syringe technique requiring no premixing, thus saving time and energy.

Application procedure involved the placement of whitening accelerator to all the samples using a swab. The flocked tip provided in the kit was then firmly attached to Dash whitening gel syringe. A thick layer of 1–2 mm of gel was applied to the samples and left for 15 min. The gel was then wiped off with the help of gauze.

- Groups A2 and B2 – Pola Office (SDI, Victoria, Australia) (35% H₂O₂)

Pola Office is a single-use, simple in-office system. It is a neutral pH gel which contains built-in desensitizer, i.e., potassium nitrate.

For application, the tip was firmly attached to Pola Office syringe, and its plunger was carefully pulled back to release pressure. The contents of syringe were carefully extruded into the powder pot and mixed immediately using a brush applicator until a homogeneous gel was formed. A thick layer of gel was applied to the samples and left for 8 min. The gel was then wiped off with the help of gauze.

- Groups A3 and B3 – Opalescence Boost (Ultradent Products, Inc., South Jordan, UT, USA) (40% H₂O₂)

Opalescence Boost is a powerful 40% HP formula which does not require light for activation. It contains PF (potassium nitrate and fluoride) as a desensitizing agent.

Before application, the product was activated by syringe-to-syringe mixing. Red and clear syringes

were securely attached to each other, and their contents were mixed rapidly for about 50 times ending with the solution in the red syringe. Clear syringe was removed and disposed, and a Micro 20ga FX tip was attached onto the red syringe. A thick layer of 0.5–1.0 mm of gel was applied to samples and left for 20 min. The gel was then wiped off with the help of gauze.

Gel application was repeated three times for each sample. After the final application, the gel was removed using a gauze piece and the samples were rinsed with distilled water.

Postbleach sample testing

After completion of bleaching procedures, Group A and Group B samples were again evaluated for Vickers microhardness and surface roughness values, respectively, as per previous protocol. The data thus obtained were tabulated for statistical evaluation.

Statistical analysis

Data obtained were subjected to statistically analysis with SPSS software version 21 (SPSS Inc., Chicago, IL, USA). Paired *t*-test was used for comparing pre- and postbleach values for each group. $P \leq 0.05$ was considered as statistically significant. Furthermore, the data were subjected to one-way ANOVA for intergroup comparison.

RESULTS

Table 1 shows the mean microhardness values and standard deviations of pre- and postbleach values for each bleaching agent. On application of paired *t*-test, a highly significant reduction was observed in the mean microhardness values when the postbleach results were compared to the baseline values within a group for all the three different bleaching agents ($P < 0.05$).

Table 2 shows the mean surface roughness and standard deviations of pre- and postbleach values for each bleaching agent. On application of paired *t*-test, a highly significant increase was observed in the mean surface roughness values when the postbleach results were compared to the baseline values within a group for all the three different bleaching agents ($P < 0.05$).

Tables 3 and 4 show the intergroup comparison of the mean values of microhardness and surface roughness, respectively, following bleaching between the three different bleaching agents. One-way ANOVA test showed that there was no significant difference ($P > 0.05$) in the mean values of

Table 1: Means and P value of Vickers microhardness (pre- and posttest)

Group	Mean±SD		Percentage reduction	t	P
	Pretest	Posttest			
Dash (A1)	371.1±13.9	361.5±12.3	2.58	7.69	0.000*
Pola Office (A2)	363.3±16.3	358.8±16.3	1.23	10.14	0.000*
Opalescence Boost (A3)	368.7±15.9	366.0±16.6	0.73	4.36	0.001*

*Highly significant. SD: Standard deviation

Table 2: Means and P value of surface roughness in micrometer (pre- and posttest)

Group	Mean±SD		Percentage increase	t	P
	Pretest	Posttest			
Dash (B1)	0.329±0.082	0.378±0.082	14.80	8.10	0.000*
Pola Office (B2)	0.327±0.069	0.354±0.078	8.25	6.38	0.000*
Opalescence Boost (B3)	0.328±0.075	0.347±0.075	5.79	6.04	0.001*

*Highly significant. SD: Standard deviation

Table 3: Mean, range, standard deviation, and P value of microhardness (posttest)

Group	Mean±SD	Minimum-maximum	P
Dash (A1)	361.5±12.3	345.2-379.4	0.569*
Pola Office (A2)	358.8±16.3	331.3-382.5	
Opalescence Boost (A3)	366.0±16.6	338.1-391.5	

*Nonsignificant. SD: Standard deviation

Table 4: Mean, range, standard deviation, and P value of surface roughness in micrometer (posttest)

Group	Mean±SD	Minimum-Maximum	P
Dash (B1)	0.378±0.082	0.270-0.500	0.657*
Pola Office (B2)	0.354±0.078	0.240-0.470	
Opalescence Boost (B3)	0.347±0.075	0.230-0.450	

*Nonsignificant. SD: Standard deviation

microhardness and surface roughness among the three agents following bleaching.

DISCUSSION

Bleaching of vital teeth involves direct contact of a strong oxidizing bleaching gel with the enamel surface for an extensive period of time which differs depending on the product used, thus increasing the concern about the possible adverse effects on the enamel. Literature reveals that bleaching agents may have a negative influence on the integrity of organic enamel structures, such as proteins and collagen.^[9] Furthermore, there is evidence of mineral loss, increased porosity, loss of fluoride, modification in the calcium: phosphate ratio, organic matrix degradation, increased susceptibility to erosion or caries, increased surface roughness, reduced enamel microtensile strength, reduced fracture stability, or a decrease in abrasion resistance of bleached dental

hard tissues, thereby supporting the hypothesis that bleaching agents are chemically active components, potentially able to induce substantial structural alterations in human dental enamel.^[10]

Previous investigations have shown suitability and practicality of using Vickers microhardness test for evaluating the surface changes of enamel following treatment with various bleaching agents.^[11,12] However, it must be stated that any observed alterations in tooth structure and/or modification of its mechanical properties following bleaching treatment are influenced by the load applied, time of indentation during testing, and the position of indents.^[13] Considering that the demineralization change is limited to the surface enamel, a minimum load of 200 g was applied for 10 s.

Human enamel exhibits large regional variations in structure related to the differences in local chemistry and microstructure. Therefore, enamel microhardness may vary from one area of the tooth surface to the other.^[14] Hence, in order to standardize the procedure, indents in the present study were made in the most central part of the enamel surface after dividing it into nine equal parts using two vertical and two horizontal lines.

In the present study, all the three groups showed a highly significant decrease in the microhardness of enamel after bleaching compared to the baseline values which are consistent with previous investigations performed by Grazioli *et al.*^[15] and Jurema *et al.*^[16] Other studies reporting similar results are those by Azer *et al.*,^[17] de Arruda *et al.*,^[18] Attin *et al.*,^[19] and Basting *et al.*^[20]

The loss of mineral content and organic matrix decreases enamel microhardness. Enamel mineral loss after bleaching is explained by the oxidation mechanism of the bleaching agent. It is assumed that HP breaks down into free radicals, which act as strong oxidative agents and decompose the organic and inorganic enamel matrix, leading to alterations in the chemical and morphological structure of enamel.^[21] These alterations are reversible and may not be clinically significant; however, since they have potential adverse effects, it is important that bleaching procedures be carried out correctly, according to the manufacturer's instructions, to ensure the safety of the treatment.

Results also showed a highly significant increase in the surface roughness of enamel after bleaching compared to the baseline values for all the three bleaching agents. The results are in agreement with various previous studies.^[22-26] This increase may be attributed to the loss of interprismatic substance and sodium and magnesium ions. Other similar studies have also reported that the micromorphological observation of the bleached enamel leads to the exaggerated prism irregularities with high mean Ra values.^[27]

The outcomes of this study, however, are in contrast with some of the previous studies that revealed either no significant changes or changes that are of negligible quantity for clinical aspects in mechanical, morphological, or chemical properties of enamel following bleaching treatment.^[27-30] The variability in change of properties of enamel in different studies is attributed to multiple factors including the study design. Hence, there is a great need to develop a standardized protocol to evaluate the effects of tooth-bleaching products on microhardness and surface roughness of enamel.

Among the materials tested in the present study, a reduction in microhardness and an increase in surface roughness were seen to be relatively more with Dash (Groups A1 and B1), followed by Pola Office (Groups A2 and B2) with least changes observed with Opalescence Boost (Groups A3 and B3); the results, however, were not found to be statistically significant between them.

Maximum reduction in microhardness (2.58%) and maximum increase in surface roughness (14.8%) seen with Dash, as compared to other two groups, can be attributed mostly to the difference in gel pH.

pH of Dash is in the range of 4.8–5.2 in contrast to other two groups which have a neutral pH. It has been reported that enamel demineralization occurs when the pH falls below 5.2.^[31] Acidic H₂O₂-based whitening products induce superficial enamel alterations, including demineralization, loss of the aprismatic layer, calcium loss, and an increase in surface roughness.^[32] Lewinstein *et al.*^[11] claimed that this would, in fact, acid etch the enamel surface of teeth. However, because H₂O₂ is more stable in an acidic environment, the majority of commercial products have a lower pH to maintain their shelf life.

Therefore, it was hypothesized that the loss of enamel mineral content *in vitro* was mainly attributed to acidic erosion rather than the effect of peroxide *per se*. This finding was well consistent with previous studies by Sulieman *et al.*^[33] and Sun *et al.*^[34] Furthermore, Sa *et al.*^[30] demonstrated that in-office bleaching agents with low pH values could induce alterations in enamel morphology under *in vitro* conditions and that, *in vivo*, the presence of natural human saliva could abolish the demineralization effect caused by low pH.

Least reduction in microhardness (0.73%) and minimum increase in surface roughness (5.79%) were observed with Opalescence Boost despite the highest concentration of HP. This may be attributed to the composition of the gel containing PF (3% potassium nitrate and 1.1% fluoride) that helps to maintain the health of enamel throughout the whitening procedure. Cavalli *et al.*^[35] reported that mineral loss was minimized by addition of fluoride to bleaching agents. It is stated that the saturation of fluoride in the gel allows its incorporation into the enamel apatite, increasing the resistance to demineralization.^[36] As described in previous studies, opening of diffusion channels in enamel caused due to action of HP facilitates diffusion of fluoride into deeper enamel layers and enhances remineralization.^[37] In addition, Opalescence Boost has neutral pH, further reducing the adverse effects on enamel caused due to acid exposure.

The values obtained with Pola Office when testing for microhardness (1.23% reduction) and surface roughness (8.25% increase), though better than Dash, were found to be inferior to Opalescence Boost with nonsignificant difference among the three bleaching agents. These findings may be attributed to the fact that Pola Office has a neutral pH in contrast to Philips Dash that is acidic in nature, thus minimizing

the deleterious effects on enamel caused due to acidic exposure. Furthermore, Pola Office lacks fluoride which is an important remineralizing agent that reverses the effect of bleaching agent by its incorporation into enamel as fluorapatite.

Thus, from the results of present study, it may be concluded that the surface properties of enamel may be compromised with the use of high concentrations of HP in in-office bleaching agents, which is further detrimentally affected if the pH of the gel is acidic. Structural changes and superficial roughness occur at a microscopic level, leading to plaque accumulation and subsequently extrinsic staining, tooth decay, and periodontal disease. Although such alterations are not clinically appreciable, it is difficult to determine whether they are microscopically reversible. It is considered that saliva acts as a remineralizing agent and may increase the microhardness of dental enamel during and after bleaching in clinical conditions.^[38] Moreover, fluoride containing bleaching agents with neutral pH may offer the advantage of minimizing the adverse effects of bleaching agents on enamel. They may also improve the surface micromorphological characteristics of dental structures through the deposition of calcium fluoride crystals, in addition to maintaining the balance between the phenomena of demineralization and remineralization.^[35,39] The combination of these factors perpetuates dental rigidity and prevents clinically visible alterations that may change the dental structure subjected to dental bleaching.

Nevertheless, as it was conducted *in vitro*, this study presents some limitations, especially the absence of pulp tissue in the tooth samples, making it impossible to predict the side effects of high-concentration gels on tooth sensitivity and pulp cells, as well as the absence of pulp pressure, which can interfere in the penetration of the gel in vital tooth. Thus, further *in vivo* studies are required to extrapolate the results of the present study in clinical situations where saliva and remineralizing contents present in the dentifrices may effectively restore the altered surface topography of the enamel.

CONCLUSION

Within the limitations of the present study, the following conclusions can be drawn:

- From the results obtained from the evaluations of SMH and Ra values, it may be concluded that

there is a significant reduction in microhardness and increase in surface roughness of enamel following bleaching when compared to baseline values irrespective of the concentration of HP used

- The alteration of physical properties is likely due to the demineralization effects that are caused by the diffusion of HP and the acidic pH of the bleaching gel. In an effort to reduce the erosive effect of bleaching solution, preference should be given to those solutions that have a pH close to neutral.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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