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Comparative Evaluation of The Effect of Various Remineralizing Agents on Surface Microhardness of Enamel Following In-Office Bleaching: An in Vitro Study

[1]Dr Shreekrishna K, Post Graduate Student, Department of Conservative Dentistry and Endodontics, D A Pandu Memorial R V Dental College, Bengaluru

[2]Dr. Karanam Apoorva Prakash, Reader, Department of Conservative Dentistry and Endodontics, D A Pandu Memorial R V Dental College, Bengaluru

[3]Dr. B S Keshava Prasad, Professor and HOD, Department of Conservative Dentistry and Endodontics, D A Pandu Memorial R V Dental College, Bengaluru

Corresponding Author: Dr Shreekrishna K, Post Graduate Student, Department of Conservative Dentistry and Endodontics, D A Pandu Memorial R V Dental College, Bengaluru

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Abstract

Aim: To evaluate and compare the effect of FluoroDip Bioactive, Ultra EZ, and Dente 91 toothpaste on the surface microhardness of enamel following in-office bleaching with 35% hydrogen peroxide.

Materials and Methods: Forty extracted human incisor teeth were prepared and randomly divided into four groups (n=10). Baseline enamel surface microhardness was recorded using a Vickers hardness tester. All specimens underwent in-office bleaching with 35% hydrogen peroxide. Group 1 received no remineralizing agent, Group 2 was treated with FluoroDip Bioactive varnish, Group 3 with Ultra EZ gel, and Group 4 with Dente 91 toothpaste. After treatment, specimens were stored in distilled water for 14 days, following which post-treatment microhardness was measured. Statistical analysis was performed using appropriate tests with significance set at $p<0.05$.

Results: Baseline microhardness values showed no statistically significant differences among groups ($p=0.32$). Post-treatment results demonstrated a significant difference between groups ($p<0.001$). Group 1 showed a significant reduction in enamel microhardness. Group 2 showed minimal recovery, while Groups 3 and 4 demonstrated significant increases in microhardness, with Dente 91 exhibiting the highest values.

Conclusion: In-office bleaching with 35% hydrogen peroxide significantly reduces enamel microhardness. Post-bleaching application of remineralizing agents improves enamel hardness, with Dente 91 showing superior remineralization, followed by Ultra EZ and FluoroDip Bioactive

Keywords: In-office bleaching, remineralizing agents, surface micro-hardness test.

Introduction

Tooth bleaching has become one of the most frequently sought cosmetic dental procedures, primarily due to the increasing demand for an esthetic smile and minimally invasive treatment modalities.[1] Among bleaching techniques, in-office bleaching using high-concentration hydrogen peroxide (HP) remains the most popular because of its rapid and effective whitening results.[2] Hydrogen peroxide, typically in concentrations ranging from 25% to 40%, acts as a potent oxidizing agent that penetrates enamel and dentin, breaking down chromogenic molecules responsible for discoloration.[3]

However, despite its efficacy, bleaching with 35% hydrogen peroxide has been associated with undesirable side effects on enamel, including mineral loss, increased surface roughness, decreased microhardness, dentin hypersensitivity and alterations in surface morphology.[4,5,6] Bleaching agents such as hydrogen peroxide and carbamide peroxide diffuse through enamel and dentin, increasing dentinal permeability and fluid movement within dentinal tubules.[7] This hydrodynamic stimulation activates pulpal nociceptors, resulting in transient sharp pain.[8,9] The degree of hypersensitivity is influenced by peroxide concentration, exposure time, bleaching technique, and pre-existing enamel or dentin defects. The reduction in enamel microhardness is primarily attributed to demineralization caused by the acidic pH of bleaching agents and the release of reactive oxygen species that interfere with the organic and inorganic structure of enamel.[10] These structural changes may predispose teeth to hypersensitivity, erosion, and decreased resistance to caries.[11]

To counteract these adverse effects, the application of remineralizing agents post-bleaching has been recommended.[12] Remineralization involves the redeposition of calcium and phosphate ions into the enamel lattice, thereby restoring its hardness and surface integrity.[13] Several agents have been developed to promote this process, including fluoride-based formulations, bioactive glasses, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), and nano-hydroxyapatite.[14]

Fluorodip Bioactive is a fluoride-containing bioactive material designed to enhance remineralization by releasing calcium, phosphate, and fluoride ions that integrate into the enamel structure to form fluorapatite crystals.[15] Ultra EZ, a desensitizing gel containing potassium nitrate and fluoride, aids in reducing post-bleaching sensitivity while contributing to enamel rehardening.[16] Dente 91 toothpaste, enriched with biomimetic calcium phosphates and herbal remineralizing components, has recently gained attention for its potential to restore mineral content and improve enamel surface properties after bleaching.[17]

Thus, evaluating and comparing the remineralization potential of these agents on bleached enamel becomes essential to determine their relative effectiveness in reversing the loss of enamel microhardness induced by in-office bleaching. This in vitro study was aimed to compare and evaluate the effect of Fluorodip Bioactive, Ultra EZ, and Dente 91 toothpaste on the surface microhardness of enamel following in-office bleaching with 35% hydrogen peroxide.

Materials and Methods

This study was conducted at Department of Conservative Dentistry and Endodontics, DAPM RV Dental College, Bengaluru. Ethical clearance was obtained from the

Research Sustenance and Institutional Review Board Committee, DAPM RV Dental College, Bengaluru.

Sample preparation

A total of 40 recently extracted intact human incisor teeth were collected and stored in distilled water. (Fig. 1) Teeth without caries, without restoration and teeth extracted for periodontal problems were included in the study. Endodontically treated tooth, severely attritted and teeth with developmental defect were excluded.

Figure 1:

Diamond disc was used to remove the teeth's roots 2 mm apically to the cementoenamel junction. (Fig. 2 and 3) The crowns of the teeth were then inserted in a self-curing acrylic block. (Fig. 4)

Figure 2:

Figure 3:

Figure 4:

400-grit silicon carbide abrasive paper was used to flatten the enamel surfaces, 600 and 1200-grit silicon carbide abrasive paper was used to polish them, until a circular area of 10 mm diameter was exposed. (Fig. 5 and 6)

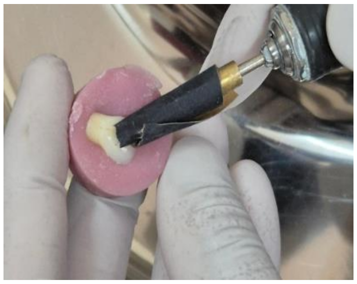


Figure 5:

Figure 6:

After that, the specimens were divided into four groups at random (n = 10) and subjected to surface microhardness examination to ascertain the pre-operative hardness by calculating the Vicker's hardness number. (Fig. 7)

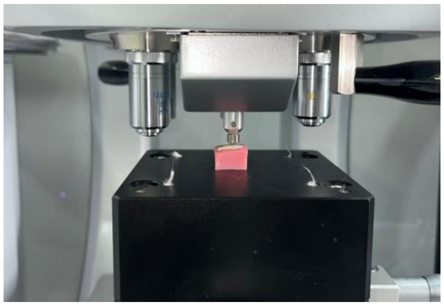


Figure 7:

Bleaching and remineralizing agent application:

Randomly allocated teeth samples were then subjected to in-office bleaching and remineralizing agent application as follows:

Group 1: One Pola Office syringe with firmly attached tip was taken and the back plunger was carefully pulled back to release pressure, then carefully extruded into the powder pot. Immediately it was mixed using a brush applicator until gel was homogenous. Then a thick layer of gel was applied onto the tooth surface. Three 8-minute applications were made on each specimen, consecutively. After first and second applications, the bleaching agent was removed with cotton rolls, and, at the end of the third application period, the agent was rinsed off the enamel surface with running water. (Fig. 8, 9, 10, 11)

Figure 8:

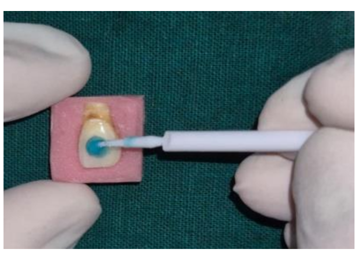


Figure 9:

Figure 10:

Figure 11:

Group 2: The bleaching agent was prepared and applied similar to Group 1. After bleaching procedure, 0.5ml of FluoroDip Bioactive varnish, was applied to enamel surfaces using micro brush as a thin film and air dried for 10 to 20 seconds and slightly moistened the area of application to ensure proper setting of the varnish. (Fig. 12)

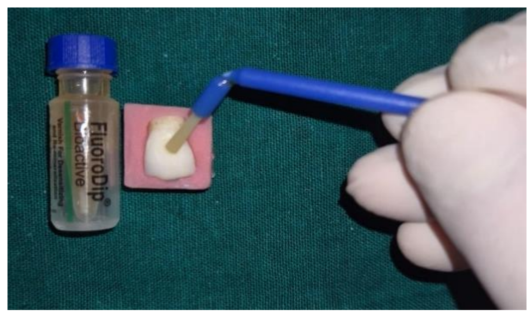


Figure 12:

Group 3: Following the bleaching process, enamel surfaces were treated for four minutes with UltraEZ (Ultradent Products, Inc., Utah, USA). Using running water, the agent was rinsed off. (Fig. 13)

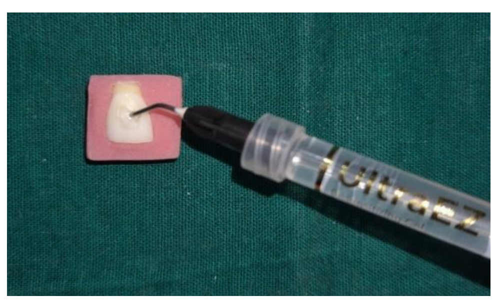


Figure 13:

Group 4: Dente 91 tooth paste was applied to enamel surfaces following bleaching as mentioned for Group 1 and allowed to sit undisturbed for two minutes. The tooth paste was then washed away by running water. (Fig.14)

Figure 14:

Following bleaching and remineralizing agent application, samples were kept in distilled water for 14 days. Surface microhardness measurements were repeated 14 days after bleaching and remineralizing agent application procedure.

Results

During the pre-bleaching and remineralization phase, Group 1 exhibited a mean Vickers Hardness number of 314.50 ± 59.33. Group 2 showed a slightly lower mean hardness of 288.10 ± 33.23. Group 3 recorded the lowest mean hardness among the four groups at 275.30 ± 50.91. Group 4 demonstrated a mean hardness of 312.00 ± 69.27. The observed differences in mean Vickers Hardness values across the four groups did not reach statistical significance (p=0.32). (Table. 1)

Table 1:

Comparison of Vickers Hardness number between 4 groups during Pre Bleaching and Remineralization using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p- value
Group 1	10	314.50	59.33	225.0	436.0	0.32
Group 2	10	288.10	33.23	245.0	331.0	
Group 3	10	275.30	50.91	217.0	349.0	
Group 4	10	312.00	69.27	202.0	392.0	

Following the bleaching and remineralization phase, Group 1 exhibited the lowest mean Vickers Hardness number at 217.60 ± 33.24. Group 2 showed a higher mean hardness of 289.80 ± 41.09. Group 3 recorded a mean hardness of 309.90 ± 45.43. Group 4 demonstrated the highest mean hardness among all groups at 376.70 ± 68.57. The differences in mean Vickers Hardness values across the four groups were statistically significant (p<0.001). (Table no.2)

Table 2:

Comparison of Vickers Hardness number between 4 groups during Post Bleaching and Remineralization using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	p- value
Group 1	10	217.60	33.24	177.0	287.0	<0.001*
Group 2	10	289.80	41.09	227.0	361.0	
Group 3	10	309.90	45.43	256.0	391.0	
Group 4	10	376.70	68.57	263.0	462.0	

* Statistically Significant

Group 1 demonstrated significantly lower mean Vickers Hardness values when compared to all other groups. When compared to Group 3, Group 1 showed a mean difference of -92.30 (p=0.001). The difference was most pronounced between Group 1 and Group 4. Group 2 did not differ significantly from Group 3, with a mean difference of -20.10 (p=0.80). However, Group 2 showed a statistically significant lower hardness compared to Group 4 (p=0.002). Group 3 also recorded significantly lower values than Group 4 (p=0.02). (Table no.3)

Table 3:

Multiple comparison of mean difference in the Vickers Hardness number between groups during Post Bleaching and Remineralization using Tukey's Post hoc Test

(I)Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-values
			Lower	Upper	
Group 1	Group 2	-72.20	-131.08	-13.32	0.01*
	Group 3	-92.30	-151.18	-33.42	0.001*
	Group 4	-159.10	-217.98	-100.22	<0.001*
Group 2	Group 3	-20.10	-78.98	38.78	0.80
	Group 4	-86.90	-145.78	-28.02	0.002*
Group 3	Group 4	-66.80	-125.68	-7.92	0.02*

* Statistically Significant

Group 1 showed a significant reduction in mean Vickers Hardness following the bleaching and remineralization period and was statistically significant (p=0.001). Group 2 exhibited minimal change which was not statistically significant (p=0.85). In contrast, Group 3 demonstrated a significant increase in hardness, Group 4 also showed a high statistically significant difference ($p < 0.001$). (Table no. 4)

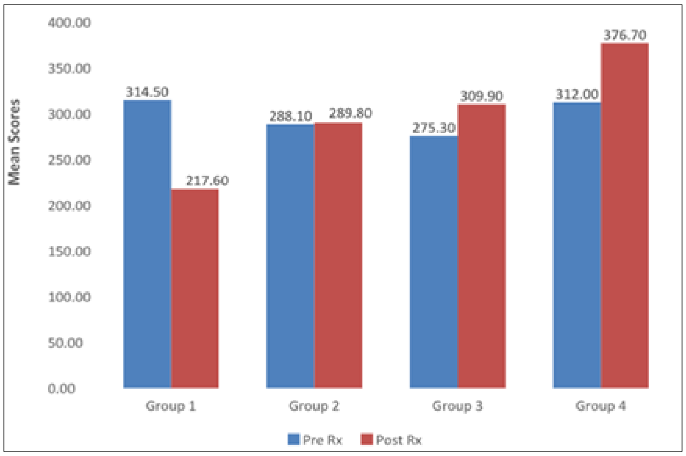
Table 4:

Comparison of mean Vickers Hardness Number between Pre and Post Bleaching and Remineralization period using Paired Sample t Test

Groups	Time	N	Mean	SD	Mean Diff	p-value
Group 1	Pre Rx	10	314.50	59.33	96.90	0.001*
	Post Rx	10	217.60	33.24		
Group 2	Pre Rx	10	288.10	33.23	-1.70	0.85
	Post Rx	10	289.80	41.09		
Group 3	Pre Rx	10	275.30	50.91	-34.60	0.04*
	Post Rx	10	309.90	45.43		
Group 4	Pre Rx	10	312.00	69.27	-64.70	<0.001*
	Post Rx	10	376.70	68.57		

* Statistically Significant

Graph 1:



Mean Vickers Hardness Number between Pre and Post Bleaching and Remineralization period

Discussion

Tooth sensitivity is the most frequent adverse effect of all peroxide-based whitening processes, and attempts have been made to mitigate the sensitivity brought on by bleaching techniques. Enamel morphology, microhardness, and hard tissue volume may all be affected by bleaching with 35–38% hydrogen peroxide. [18] To prevent or reduce tooth sensitivity and the possible negative effects of tooth whitening operations on enamel surfaces, remineralizing agents might be used. Following dental bleaching, remineralizing substances like calcium, fluoride, hydroxyapatite, and amorphous calcium phosphate (ACP) can be used to address negative effects on the enamel surface.[19] This study evaluated the effect of applying remineralizing agents — namely FluoroDip, Bioactive, Ultra EZ, and Dente 91 — after in-office bleaching regimen on the surface microhardness (SMH) of enamel.

Multiple studies have documented that high-concentration peroxide bleaching induces measurable reductions in enamel microhardness.[1] In a study using 35 % hydrogen peroxide gels, there was a statistically significant reduction in Vickers hardness of bleached enamel compared with baseline values.[20] The mechanism is thought to involve oxidative degradation of the small organic matrix within enamel and increased porosity due to mineral loss, which reduces mechanical integrity. [21] The extent of hardness loss is influenced by peroxide concentration, exposure time, gel pH, and whether remineralizing adjuncts are included.[22] The baseline reduction in SMH after bleaching in this study therefore underscores the importance of adjunctive remineralization to mitigate enamel compromise.[2] The Vickers hardness test offers precise, minimally invasive evaluation, providing reliable microhardness assessment for small, heterogeneous dental structures. Hence, was chosen for the present study.[18]

The use of remineralizing agents has been shown to enhance enamel surface hardness after demineralization or chemical challenge.[23] Toothpastes or pastes containing fluoride, bioactive glass, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), or nano-hydroxyapatite (nHA) have all demonstrated increases in microhardness relative to demineralized controls.[24] Remineralization restores the mineral content (calcium, phosphate, fluoride) and deposits minerals into porous enamel, effectively reinforcing the surface layer.[25] A recent report on a fluoride-incorporated bioactive glass material showed enhanced microhardness and volume restoration in enamel.[26] Another study found that the application of a remineralizing agent after bleaching improved microhardness values toward baseline. [27]

In the context of the present investigation, Dente 91 has been shown in in-vitro work to accelerate remineralization processes, reduce mineral loss, and enhance antimicrobial protection.[28] According to Kutuk ZB et al, its nanometric size enables penetration into dentinal tubules and enamel microcracks, sealing them effectively while restoring tooth microstructure and composition. Nano-hydroxyapatite, calcium and phosphate donors, and agents like xylitol reduce demineralization and support mineral deposition.[18]

Although data on Ultra EZ and FluoroDip Bioactive are less widely published in peer-reviewed literature, their formulations (bioactive glass and fluoride-based complexes) align with known mechanisms of enamel remineralization.[29] Bioactive glass and fluoridated bioactive glass have been associated with significant microhardness recovery when compared to conventional fluoride treatments.[30] Bioactive glass (BAG) promotes enamel remineralization by releasing calcium/phosphate ions to form a protective surface apatite layer. [31]

Therefore, the improvement in enamel microhardness observed after application of these agents in this study is consistent with previously established findings.[26] However, this dense surface layer can restrict ion diffusion, potentially limiting effective remineralization of deeper and subsurface areas. UltraEZ's fluoride component promotes remineralization by encouraging calcium and phosphate ion uptake in demineralized enamel. According to Peter Edward Ibrahim et al, UltraEZ may provide remineralizing efficacy lesser possibly due to lower fluoride content (0.11 w/w).[31]

Dente91 and UltraEZ showed better enamel microhardness improvement than FluoroDip Bioactive due to their distinct remineralization mechanisms.[23] Dente91 contains nano-hydroxyapatite, which closely mimics natural enamel crystals and directly deposits into interprismatic defects, producing effective and stable increases in microhardness.[24,29] UltraEZ, though primarily a desensitizing agent, provides fluoride that enhances enamel re-hardening and limits mineral loss during repeated application, resulting in measurable hardness gains.[2] In contrast, FluoroDip Bioactive depends on fluoride and bioactive glass ion release, which may require longer contact time and favorable conditions for optimal remineralization.[26] Thus, direct mineral replacement and fluoride-mediated re-hardening explain the superior performance of Dente91 and UltraEZ. Microhardness testing is performed 14 days after bleaching and remineralizing agent application to allow sufficient time for enamel mineral re-equilibration and ion uptake, as bleaching causes transient mineral loss and reduced hardness.[32] Remineralizing agents require time for sustained release and diffusion of calcium, phosphate, and fluoride ions into subsurface enamel, leading to stabilization of hardness values. [33, 34]

This study, being in-vitro, may not fully replicate the dynamic oral environment, including saliva flow, pellicle formation, and masticatory stress.[35] Previous reports have shown that in-vivo conditions, especially the presence of saliva, can mitigate hardness loss caused by bleaching. [22] The formulation details and contact times of the remineralizing agents may differ in clinical use, affecting real-world outcomes.[24] While surface microhardness is a reliable indicator of enamel integrity, it does not fully describe subsurface mineral alterations or microstructural changes; additional analyses such as SEM and EDX would strengthen the evidence.[29] Moreover, the timing between remineralization and bleaching (immediate versus delayed) might influence results and deserves further evaluation. [36]

Future research should include long-term clinical trials to validate whether remineralizing agents maintain enamel hardness after bleaching under real oral conditions. [2] Comparative studies evaluating synergistic effects of combining agents, such as fluoride with bioactive glass, may reveal enhanced and more stable remineralization. [23] Future investigations should also assess nanostructural changes in enamel using advanced imaging to better understand mineral deposition patterns.[26] Additionally, studies should address how saliva composition and pH fluctuations influence remineralization outcomes across individuals.[27]

Conclusion

Within the limitations of this in-vitro study, in-office bleaching with 35% hydrogen peroxide significantly reduced enamel surface microhardness. Application of remineralizing agents effectively reversed this loss to varying degrees. Dente 91 demonstrated the greatest enhancement in enamel microhardness, followed by Ultra EZ, while FluoroDip Bioactive showed comparatively lesser recovery than the other two remineralizing agents. These findings suggest that post-bleaching remineralization, particularly with nano-hydroxyapatite-based formulations, is beneficial for restoring enamel integrity after high-concentration bleaching procedures.

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