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Effect of Two Calcium-Based Materials on Dentin Microhardness and Composite Bond Strength after Internal Bleaching

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ABSTRACT

Introduction: The primary aim of this study was to evaluate and compare the effects of CPP-ACP (Casein phosphopeptide–amorphous calcium phosphate), calcium hydroxide, and their combination on dentin microhardness following internal bleaching with hydrogen peroxide, and to assess the influence of these interventions on the shear bond strength of composite resin to dentin.

Materials & Methods: In this study, 24 sound extracted human premolar teeth were collected and sectioned buccolingually, yielding a total of 48 dentin specimens. Baseline microhardness was recorded in the designated area. Subsequently, internal bleaching was performed in the same region, and the specimens were incubated for 5 days. Post-bleaching microhardness was recorded. The specimens were randomly divided into four groups: control, calcium hydroxide, CPP-ACP, and a combination of CPP-ACP and calcium hydroxide. The samples were incubated for one week, after which final microhardness measurements were obtained. Composite resin was then bonded to the dentin surface using a cylindrical plastic mold with a standardized cross-sectional dimension and a height of 3 mm. Shear bond strength and failure modes were recorded by observation under a stereomicroscope.

Results: Internal bleaching with 35% hydrogen peroxide significantly decreased dentin microhardness in all specimens. Following the application of the tested materials, statistically significant differences were observed among the groups, with the control group showing the lowest microhardness values. Significant increases in microhardness were found in the CPP-ACP and CPP-ACP + calcium hydroxide groups, with the highest values recorded in the combination group. Although the combination group had the highest mean shear bond strength, no statistically significant differences were found among the groups. All failures were adhesive in nature.

Conclusion: Within the limitations of this *in vitro* study, internal bleaching with 35% hydrogen peroxide reduces dentin microhardness. Application of CPP-ACP, either alone or combined with calcium hydroxide after bleaching, effectively restores dentin microhardness without adversely affecting the shear bond strength of composite resin to dentin.

Keywords: Calcium Phosphates, Caseins, Dentin, Hydrogen Peroxide, Calcium Hydroxide, Tooth Remineralization, Tooth Bleaching

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Introduction

Tooth discoloration in endodontically treated teeth remains a common esthetic concern, and internal bleaching is widely considered a conservative approach for improving the appearance of discolored non-vital teeth [1, 2]. Among the available bleaching agents, hydrogen peroxide and carbamide peroxide are the most commonly used materials due to their strong oxidizing potential and clinical effectiveness [3].

The bleaching mechanism involves the diffusion of peroxide into dental hard tissues and the generation of reactive oxygen species that oxidize chromogenic compounds responsible for discoloration [4]. Despite its effectiveness, peroxide-based bleaching agents may adversely affect dentin by altering its chemical composition and reducing microhardness [5, 6]. These structural changes may compromise dentin integrity and affect the performance of subsequent restorative procedures [7].

Residual oxygen species within dentin may interfere with resin polymerization and reduce bond strength when adhesive procedures are performed immediately after bleaching [8]. Delayed bonding has been shown to improve adhesion by allowing oxygen dissipation [9, 10]. Another concern with internal bleaching is peroxide diffusion toward cervical areas, which may contribute to undesirable biological effects. Therefore, proper intracoronal barriers and controlled bleaching protocols are essential [11, 12]. To minimize these adverse effects, remineralizing strategies have been proposed. CPP-ACP is a bioactive compound capable of stabilizing calcium and phosphate ions and promoting remineralization [13, 14]. Previous studies have demonstrated that CPP-ACP improves dentin microhardness and enhances substrate quality [15, 16]. Calcium hydroxide is also commonly used after internal bleaching due to its high alkalinity and ability to neutralize acidic conditions. It may also improve dentin conditions after bleaching, although its effect on bonding performance remains controversial [17, 18].

Despite the growing body of evidence, few studies have directly compared CPP-ACP and calcium hydroxide, particularly regarding their combined effect on dentin properties following internal bleaching.Therefore, the present study aimed to evaluate the effects of CPP-ACP and calcium hydroxide, individually and in combination, on dentin microhardness and composite bond strength after internal bleaching with 35% hydrogen peroxide.

Materials & Methods

This *in vitro* study was conducted on sound human premolars and approved by the Ethics Committee of Babol University of Medical Science, Babol, Iran (Approval No. 9910814). All procedures were performed in accordance with the Declaration of Helsinki.

The sample size was determined based on previous *in vitro* studies and standard statistical considerations, with a significance level of 0.05 and a statistical power of 80% [19]. A total of 48 specimens were required. For this purpose, 24 intact human premolars extracted for orthodontic reasons were collected, mounted, and sectioned buccolingually to obtain 48 dentin samples. The inclusion criterion was sound, caries-free human premolars. Teeth were collected by convenience sampling and stored in saline solution. One week before the experiment, the teeth were disinfected in 0.2% Chloramine-T solution [20]. The teeth, including the roots, were mounted in epoxy resin and sectioned buccolingually using a diamond disc (Edenta AG, Au, Switzerland) under water cooling. After polishing with 500 and 800 grit silicon carbide papers, a standardized circular area of sound dentin (3 mm in diameter) near the pulp was marked using stamp ink and a 3 mm serum tube. The materials used in this study are listed in Table 1.

Table 1. The materials used in this study

Material	Manufacture	Country
Calcium hydroxide paste (Multi cal)	Pulpdent	USA
CPP-ACP paste (Tooth Mousse)	GC Corporation	Japan
Hydrogen peroxide Gel35% (opalescence Endo)	Ultradent products Inc.	USA
Adhesive (single bond II)	3M ESPE	USA
Composite resin (Filtek Z250)	3M ESPE	USA
Etchant (37% phosphoric acid, Condac)	FGM	Brazil

Microhardness Assessment

The baseline microhardness of the specimens was measured using a Vickers microhardness tester (Koopa Co., Sari, Iran). A Vickers diamond indenter applied a load of 200 gf (0.2 kgf) for 10 seconds to the marked dentin area (Figure 1). The resulting diamond-shaped indentation was observed under a 10× microscope, and the diagonal lengths were measured (Figure 2). Microhardness values (HV0.2) were calculated according to the standard Vickers formula. Three indentations were made within the marked area, and the mean value was recorded as the baseline microhardness. A plastic mold with a diameter and height of 3 mm was then fixed onto the marked dentin area using an adhesive (Allplast; Razi Chemical Co., Tehran, Iran). Internal bleaching was performed by placing 35% hydrogen peroxide gel, Opalescence Endo (Ultradent, South Jordan, USA), inside the mold on the dentin surface, and the mold was secured in place with a piece of cellophane.

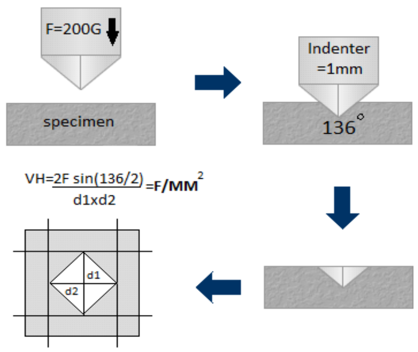

Figure 1: Vickers hardness analysis with a diamond tip

Figure 2: Hardness test sample and 200gf force

The bleaching procedure was carried out according to the manufacturer's instructions for 5 days, during this period, the specimens were stored in an incubator (IP series; LTE Scientific Ltd., Oldham, UK) at 37°C and 100% relative humidity. After bleaching, the samples were rinsed for one minute, the molds were removed, and post-bleaching microhardness was measured at three points using the same method, with a minimum distance of 200 µm between each indentation to avoid interaction between measurements. The mean value was recorded as the post-bleaching microhardness.

Experimental Grouping

The specimens were randomly assigned to four groups (n = 12) using a computer-generated randomization method:

Group 1 (Control): Specimens received no additional treatment after bleaching.

Group 2: Calcium hydroxide paste (Multi Cal; Pulpdent, Watertown, MA, USA) was applied to the dentin surface using a standardized plastic mold, then stabilized with cellophane.

Group 3: CPP-ACP paste (Tooth Mousse; GC Corp., Tokyo, Japan) was applied in an identical manner using the same standardized mold, then stabilized with cellophane.

Group 4: An equal volume mixture of calcium hydroxide and CPP-ACP was prepared and applied using the same mold, then similarly stabilized with cellophane.

All materials were stored at 37°C and 100% humidity for one week. Afterward, specimens were rinsed and final microhardness was measured. A schematic illustration summarizing the experimental procedure is presented in Figure 3.

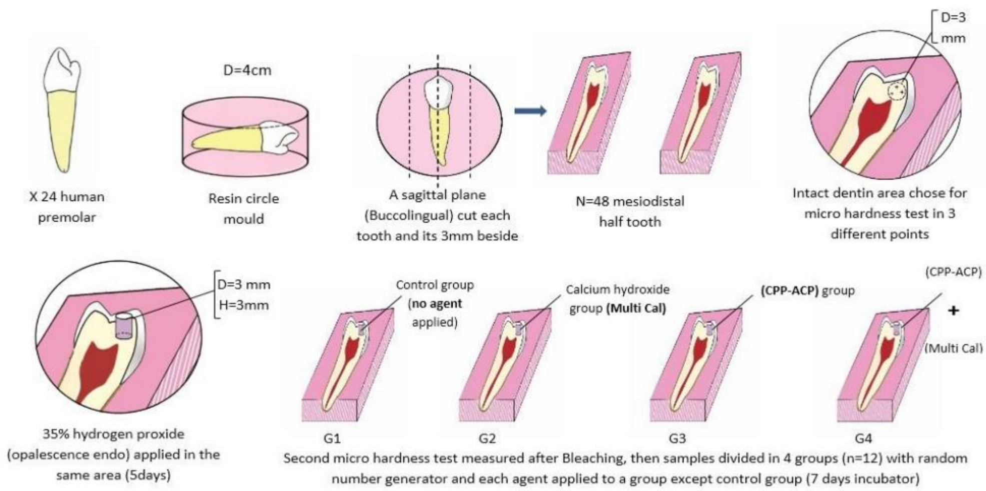

Figure 3: Schematic illustration of the experimental procedure.

Shear Bond Strength Evaluation

All specimens were etched with 37% phosphoric acid gel (Condac; FGM, Joinville, Brazil) for 15 seconds, rinsed, and gently air-dried for 5 seconds. Two layers of Single Bond II adhesive (3M, St.Paul, USA) were applied for 15 seconds, air-thinned, and light-cured using a Valo LED curing unit (Ultradent, South Jordan, USA) at 1000 mW/cm². A resin composite (Filtek Z250; 3M, St.Paul, USA) was placed in plastic cylinders (3 mm height, 3 mm internal diameter) positioned on the dentin surface and light cured for 20 seconds. Specimens were stored for 24 hours at 37°C and 100% humidity. The plastic cylinders were then carefully removed using a scalpel blade. The samples underwent 1000 thermocycles in a thermocycling machine (Nemo Sanat, Tehran, Iran) between 5°C and 55°C water baths, with a dwell time of 30 seconds and a transfer time of 30 seconds.

A custom alignment jig was used to position each specimen to ensure that the loading blade was parallel to the adhesive interface prior to testing. Shear bond strength was measured using a universal testing machine (Koopa TB; Koopa Co., Tehran, Iran) at a crosshead speed of 0.5 mm/min. Bond strength was calculated by dividing the failure load by the bonded area (mm²) and expressed in MPa. The mean shear bond strength for each group was calculated. Failure modes were examined under a stereomicroscope at 20× magnification and classified as: 1. adhesive (at the tooth–restoration interface), 2. cohesive (within dentin or composite), or 3. mixed.

Statistical analysis

Data were analyzed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Independent t-tests and one-way ANOVA were used for comparisons. The significance level was set at $P < 0.05$.

Results

The mean dentin microhardness significantly decreased after bleaching in all 48 specimens ($p < 0.001$). The mean initial dentin microhardness was 66.67 ± 7.93, while the mean post-bleaching microhardness was 61.63 ± 7.28. The relatively wide standard deviations observed in some groups may reflect biological variability and should be considered when interpreting the results.

No statistically significant difference was observed in baseline microhardness among the groups ($p > 0.05$). However, significant differences were found among the groups after bleaching ($p = 0.030$) and after the interventions ($p < 0.001$).

Pairwise comparisons showed significantly lower microhardness values in the control group compared to the other groups ($p < 0.001$). The mean dentin microhardness in the control group decreased by 20.10 units ($p < 0.001$).

An increase in dentin microhardness was observed in the calcium hydroxide, CPP-ACP, and combined groups; however, this increase was statistically significant only in the CPP-ACP group ($p = 0.008$) and the combined CPP-ACP and calcium hydroxide group ($p = 0.002$) (Table 2).

Table 2: Comparison of mean hardness scores after bleaching and intervention in the study groups

Group	Primary	After bleaching	Latest	Difference in average hardness between final and post-bleaching	p-value*
Control	63.4 ± 8.3	56.85 ± 6.22	36.75 ± 8.34	-20.10 ± 5.06	<0.001
Calcium hydroxide	66.4 ± 7.7	54.85 ± 7.67	65.95 ± 6.16	1.10 ± 5.35	0.48
CPP-ACP	66.9 ± 7.2	61.42 ± 7.15	65.99 ± 7.37	4.56 ± 4.93	<0.008
CPP-ACP + Calcium hydroxide	70.0 ± 8.5	63.41 ± 6.16	70.41 ± 4.70	7.00 ± 5.78	<0.002
p-value**	0.3	0.03	<0.001	<0.001	

*Paired t-test
**Analysis of variance test

No statistically significant difference was found among the groups in the change between baseline and post-bleaching microhardness values. In contrast, significant differences were observed in the changes between baseline and final microhardness, as well as between post-bleaching and final values ($p < 0.001$) (Table 3).

Table 3: Comparison of differences in mean hardness scores of the study groups (initial, after bleaching, and after intervention)

Group	Diff 1 (Mean ± SD)	Diff 2 (Mean ± SD)	Diff 3 (Mean ± SD)
Control	- 6.58 ± 7.44 ^{*A}	- 26.70 ± 17.30 ^{A}	-20.11 ± 14.44 ^{A}
Calcium hydroxide	- 1.51 ± 6.71 ^{A}	- 0.40 ± 7.29 ^{B}	1.10 ± 5.12 ^{B}
CPP-ACP	- 5.47 ± 4.62 ^{A}	- 0.90 ± 5.69 ^{B}	4.56 ± 4.71 ^{B}
CPP-ACP + Calcium hydroxide	- 6.59 ± 6.56 ^{A}	0.43 ± 6.91 ^{B}	7.01 ± 5.51 ^{B}
p-value	0.154	<0.001	<0.001

Diff 1: Difference in mean hardness score after bleaching compared to initial hardness
Diff 2: Difference in final hardness score compared to initial hardness
Diff 3: Difference in final hardness score compared to hardness after bleaching
*Capital letters in each column indicate statistically significant differences.

The mean shear bond strength was higher in the combined CPP-ACP and calcium hydroxide group compared to the other groups; however, one-way ANOVA showed no statistically significant difference among the groups (Table 4).

Table 4: Comparison of mean composite-to-dentin shear bond strength scores after internal bleaching treatment in the study groups

Group	Mean ± Standard deviation (Mpa (N/mm²))	Mean (Kg/f)	p-value*
Control	3.66±1.20	0.71	0.71
Calcium hydroxide	3.14±0.92	0.59	
CPP-ACP	3.16±2.01	0.65	
CPP-ACP + Calcium hydroxide	3.67±1.68	0.75	

Failure mode analysis under a stereomicroscope (20× magnification) showed that all specimens exhibited adhesive failure at the dentin–composite interface.

Discussion

The present study showed that internal bleaching with 35% hydrogen peroxide significantly reduced dentin microhardness. This result aligns with previous reports indicating that peroxide-based bleaching agents negatively affect dentin mechanical properties [21, 22]. These changes are mainly attributed to the oxidative degradation of dentin components. Free radicalsderived from hydrogen peroxide can disrupt the collagen matrix and alter mineral content, leading to decreased hardness and increased dentin permeability [6]. Previous studies have also reported chemical and morphological changes in dentin after bleaching procedures [23, 24].

In the present study, CPP-ACP significantly improved dentin microhardness. This effect can be explained by its ability to deliver calcium and phosphate ions and promote remineralization [14, 15]. Similar results have been reported in studies evaluating the effect of calcium hydroxide on dentin after bleaching [17, 25]. Calcium hydroxide also showed a positive effect on dentin microhardness, although the difference was not statistically significant. This may be due to its alkaline nature and calcium ion release [26]. Previous studies have similarly reported its influence on dentin properties after bleaching [20, 27]. The combined application of CPP-ACP and calcium hydroxide resulted in the highest microhardness values, suggesting a potential synergistic effect between these materials [9]. This may be explained by increased calcium ion availability in an alkaline environment, which can further promote remineralization processes and hydroxyapatite formation.

No statistically significant differences in bond strength were observed among the study groups. This finding is consistent with previous reports indicating that the adverse effects of bleaching on adhesion are often transient and may diminish over time. Delayed bonding protocols have been shown to improve bond strength by allowing dissipation of residual oxygen within dentin [10, 28]. In addition, the use of remineralizing agents does not appear to adversely affect adhesive performance and may contribute to stabilization of the bonding substrate [16, 29]. From a clinical perspective, the findings suggest that CPP-ACP, either alone or in combination with calcium hydroxide, may be considered as a post-bleaching treatment to improve dentin mechanical properties without compromising bonding performance. However, due to the *in vitro* nature of the present study, the results should be interpreted with caution, as intraoral conditions such as saliva, thermal stresses, and functional loading were not fully simulated [30].

A notable strength of this study is the simultaneous evaluation of dentin microhardness and bond strength, providing a more comprehensive assessment of post-bleaching effects. Nevertheless, limitations include the *in vitro* design and the use of a single bleaching protocol. Future studies are recommended to evaluate long-term outcomes and clinical performance.

Conclusion

Within the limitations of this *in vitro* study, the use of remineralizing agents after internal bleaching may help preserve dentin integrity. Specifically, CPP-ACP, especially when combined with calcium hydroxide, appears to enhance the recovery of dentin mechanical properties without adversely affecting adhesive performance. These findings highlight the potential of post-bleaching interventions to improve the clinical outcomes of restorative procedures. Further clinical and long-term investigations are needed to validate these results.

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Conflicts of Interest

The authors declare no potential conflict of interest with respect to the authorship and/or publication of this paper.

Author's Contribution

Mohammad Bagheri developed the original idea and protocol, and summarized the study. Niloufar Ghoreishi drafted the manuscript and edited the article. Faraneh Mokhtarpour analyzed results and developed final conclusion. Behnaz Esmaili reviewed the manuscript for important intellectual content. The study was supervised and edited by Ghazaleh Ahmadi and Elham Mahmoudi. All authors have read and approved the final manuscript.

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