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# Impact of Smear Layer Removal Protocols on Color Dynamics and Dentin Hardness Following Intracoronal Bleaching

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## Abstract:

**Objective:** This study evaluated the impact of smear layer removal protocols on intracoronal bleaching outcomes, focusing on color shifts and dentin hardness.

**Material and Methods:** Artificially stained human premolars underwent dentin surface treatments: distilled water, 17% EDTA, and 37% phosphoric acid (PA) before intracoronal bleaching with sodium perborate. Control teeth received no treatment. Tooth color and dentin hardness were measured at 7, 14, and 21 days post-bleaching with a VITA Easyshade spectrophotometer and Vickers hardness tests, respectively. Statistical analysis included Shapiro-Wilk and Levene's tests, and two-way repeated ANOVA with Tukey's HSD for shade and microhardness comparison ($\alpha$=0.05).

**Results:** All the bleaching treatments effectively lightened teeth, with the most pronounced color change ($\Delta E00$) observed after the first week. This was followed by a significant decrease in intensity during the second and third weeks. Color metrics showed reduced redness (a* values) and increased yellowness (b* values) across all the groups, with no significant differences in color change or dentin hardness between the treatments (p-value>0.05). However, a notable decrease in dentin hardness was observed in the cervical sections compared to the coronal sections in all the groups (p-value<0.05).

**Conclusion:** Smear layer removal with 37% PA or 17% EDTA did not significantly improve bleaching efficacy or affect dentin hardness. These findings support streamlined treatment protocols that omit smear layer removal without compromising clinical outcomes.

**Keywords:** CIEDE2000, CIELAB, dentin hardness, Intracoronal bleaching, smear layer, Sodium perborate

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## Introduction

The intracoronal bleaching technique has been widely used to treat discoloration in root canal treated teeth. Its origin dates back to Spasser in 1961[1], who introduced the temporary placement of a mixture containing sodium perborate ($NaBO_3 \bullet 4H_2O$) and water in the pulp chamber of anterior teeth to lighten the discoloration. Since then, efforts have been made to improve the effectiveness of intracoronal bleaching, including replacing water with hydrogen peroxide or utilizing heat-activated hydrogen peroxide. However, the use of hydrogen peroxide and heat has proven inadvisable due to undesirable side effects, particularly external cervical root resorption[2-4]. More importantly, a combination of sodium perborate and water has demonstrated equal efficacy to sodium perborate mixed with hydrogen peroxide[5-9]. Consequently, this technique is currently widely accepted and commonly employed for intracoronal bleaching of root-filled teeth.

The smear layer, formed during tooth preparation, is composed of enamel and dentin chips, tissue debris, and microorganism residuals. This layer, typically 2-5 μm in thickness, can infiltrate the dentinal tubules. The presence of a smear layer has been found to reduce dentin permeability[10,11], which may impede the diffusion of bleaching agents into the dentinal tubules, thereby reducing their effectiveness. The smear layer is typically removed during endodontic irrigation; however, it may re-form under certain conditions. For instance, it can be recreated when dentin is prepared with burs, such as during orifice barrier placement before intracoronal bleaching or when a filling is removed at a subsequent bleaching appointment. Some reports suggested using 37% phosphoric acid to etch the pulp chamber before applying a bleaching agent in order to remove the smear layer and potentially enhance the penetration of the bleaching agent into the dentin[12-16]. In addition to 37% phosphoric acid, 17% ethylenediaminetetraacetic acid (EDTA) irrigation has been shown to effectively remove the smear layer and is widely used in root canal treatment. Some studies have reported that 37% phosphoric acid is more effective than 17% EDTA in smear layer removal and in enhancing the penetration of bleaching agents into dentinal tubules[16,17]. However, other studies suggest that smear layer removal, whether by phosphoric acid[9,17,18] or EDTA[17], may not significantly improve bleaching efficacy compared to when the smear layer is left intact. This inconsistency may arise from variations in study protocols, particularly in the methods of color evaluation and the types of bleaching agents used. Previous research has demonstrated that the choice of bleaching agent significantly influences treatment outcomes[19]. While most prior studies employed hydrogen peroxide, only a limited number have investigated sodium perborate mixed with water. Notably, the effect of EDTA treatment prior to bleaching with sodium perborate mixed with water remains unexplored, highlighting an important gap in the literature and a compelling area for further investigation.

Although chemical agents used in dental bleaching demonstrate tooth-whitening effectiveness, they may also have adverse effects on dental hard tissues, including changes in chemical structure[20], increased surface porosity and irregularities[21], as well as alterations in surface microhardness of the dentin[22]. These concerns are particularly relevant when hydrogen peroxide is used in the treatment. Therefore, if removing the smear layer allows better penetration of bleaching agents into the dentin, it may also enhance the negative effects of bleaching on dentin quality.

Spectrophotometers have been widely employed in dentistry for the precise measurement of tooth shade, recognized for their superior accuracy and reproducibility compared to human visual evaluation[23]. This technology proves valuable in indicating the exact shade of teeth both before and after bleaching[24]. The spectrophotometer uses the CIELAB color system, also known as CIE 1976

(Lab*). This system represents color stimuli through 3 coordinates: an achromatic dimension (lightness, L*), 2 chromatic dimensions for red–green (a*), and yellow–blue (b*) channels[25]. Color alterations can be assessed using either the CIELAB (*ΔE*ab*) or the CIEDE2000 (ΔE00) formula. The latter has been reported to align more closely with human visual perception than the CIELAB[26].

By analyzing shifts in CIELAB values and assessing dentin hardness, we aimed to evaluate the impact of smear layer removal using either 37% phosphoric acid or 17% EDTA prior to intracoronal walking bleaching with sodium perborate mixed with water. The null hypothesis was that smear layer removal with 37% phosphoric acid would not result in any differences in color change or dentin hardness compared to 17% EDTA. Additionally, neither treatment would differ from applying the bleaching agent to a surface that retains the smear layer. These findings will help identify essential procedures, highlight those that should be avoided, and distinguish those that are unnecessary while ensuring optimal treatment outcomes.

## Material and Methods

This study was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Prince of Songkla University, Thailand (EC5902-07-P-LR). The sample size was calculated using G*Power version 3.1.9.5 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany)[27] with α=0.05 and power=0.8, incorporating mean and standard deviation values from a previous study[28]. Consequently, a sample size of 10 teeth per group for each time point was established, resulting in a total of 30 teeth per group.

### Specimen preparation

A total of 123 permanent upper and lower human premolars, extracted for orthodontic purposes from patients aged 18–28 years, were obtained from the oral surgery clinic at Prince of Songkla Dental Hospital. Written informed consent was obtained from all patients. The teeth were placed in 5.25% sodium hypochlorite solution (The Department of Pharmacy, Prince of Songkla Dental Hospital, Thailand) for one hour and subsequently cleaned using hand scalers, a rubber cup with pumice, and then stored in deionized water at room temperature until used. All teeth were examined under a microscope (smz1500, Nikon, Tokyo, Japan) at 20×magnification to exclude teeth with cavities, cracks, or restorations, and those with more than one canal, discoloration, incomplete root formation, resorption, or calcification. The teeth were radiographically examined in mesiodistal and buccolingual directions in order to standardize the pulp chamber size.

A traditional endodontic access preparation was performed on each tooth using high-speed round and cylinder diamond burs (Dentsply Maillefer, Tulsa, OH, USA) with water spray. Subsequently, the apical portion of the roots was trimmed to the level of 4 mm below the cementoenamel junction (CEJ) using an Isomet cutting machine. (Isomet 4000, Buehler, Lake Bluff, IL, USA). Canal enlargement was achieved using Gates-Glidden burs no. 3 and 4 (Dentsply Maillefer, Switzerland). During preparation, each tooth was irrigated with 10 mL of 2.5% sodium hypochlorite (The Department of Pharmacy, Prince of Songkla Dental Hospital, Thailand) using a 27-gauge needle and syringe for a total duration of 3 min. Final irrigation was performed with 5 mL of 17% EDTA (Merck, Darmstadt, Germany) for 60 seconds, followed by 10 mL of 2.5% sodium hypochlorite for 60 s. The canals were then dried using paper points (Dentsply-Maillefer, Switzerland).

### Artificial staining

All teeth were stained with human whole blood (modified method from Yui et al.[29] and Freccia and Peters[30]). Each tooth was placed individually in a 15-mL tube containing 4 mL of human whole blood, and then centrifuged at 10,000 rpm for 10 min (Velocity 18R, Dynamica Scientific, Livingston, United Kingdom). This process resulted in a

biphasic solution: a supernatant containing plasma and a precipitate. After removing the supernatant, an additional 10 min of centrifugation was applied to the tooth with the blood precipitate. Following this, the tubes containing the teeth underwent centrifugation at 10,000 rpm for 10 min at 37 °C twice daily for 3 consecutive days, and the tubes were stored at 37 °C and 100% humidity in an incubator (Memmert BE 500, Memmert GmbH, Schwabach, Germany). Subsequently, a hemolysate containing hemoglobin protein was prepared by adding 3 mL of distilled water to the blood samples, followed by centrifugation for 20 min to induce further hemolysis of the red blood cells. The resulting hemolysate was separated from the precipitate and transferred to the tubes containing the teeth. Afterward, the tubes were centrifuged for 3 consecutive days as described previously. Throughout this period, the teeth were irrigated with 5 mL of distilled water each day, re-inserted into the tubes with the blood, and then stored at 37 °C and 100% humidity. After 6 days, the blood was replaced, and all the previously mentioned procedures were repeated for an additional 6 consecutive days. Following this, tooth shades were measured (as described below), and teeth with similar shades were chosen. Each root canal was filled retrogradely using a temporary restoration material (Caviton, GC, Tokyo, Japan) until it was approximately 1 mm below the buccal CEJ. Subsequently, the teeth were then stored in 100% humidity at 37 ºC for 24 hr.

### Smear layer and root surface preparation

An intracoronal smear layer was created using a #4 steel round bur operated with a low-speed handpiece[9,18]. The dentin walls were circumferentially ground twice, with each grinding lasting 4 seconds, under a dental operating microscope (OMPI pico, Zeiss Microscopy, Germany) with 21.25x magnification. Subsequently, the cavities were irrigated with 5 mL of distilled water. The buccal wall thickness at the middle third of the access cavity was standardized at 2.5-3 mm, determined by measuring with a mesiodistal radiograph using computer software (Image-Pro 1 Plus image analysis software by Media Cybernetics). Nail polish was then applied to the entire root surface up to the CEJ.

### Dentin surface treatment

The teeth were randomly divided into 4 groups, including 1 negative control group and 3 experimental groups, based on the dentin surface treatment protocol as follows:

Negative control and distilled water group (DW): irrigated with 10 mL distilled water for 60 s.

Ethylenediaminetetraacetic acid group (EDTA): irrigated with 5 mL 17% EDTA for 60 s, followed by 10 mL distilled water for 60 s.

Phosphoric acid group (PA): treated with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15 s, then irrigated with 10 mL distilled water for 60 s.

All irrigations were conducted using a 27-gauge needle and syringe, with the needle positioned 1 mm coronal to the apical filling material, ensuring it did not touch the canal walls.

### Intracoronal bleaching

Intracoronal bleaching was conducted on all experimental groups using a mixture of sodium perborate (KemAus, Cherrybrook, Australia) with distilled water at a ratio of 2 g to 1 mL. The mixture, approximately 0.022 g, was applied to the pulp chamber using an amalgam carrier with a 2-mm diameter and 5-mm depth tip. For the control group, no bleaching agent was placed in the cavity. The access opening was filled with wet cotton pellets and sealed with a temporary restoration (Caviton, GC corporation, Tokyo, Japan) until a thickness of 3 mm was achieved. The teeth then were stored in 100% humidity at 37ºC. The bleaching agents were replaced with a fresh mixture on days 7, 14, and 21.

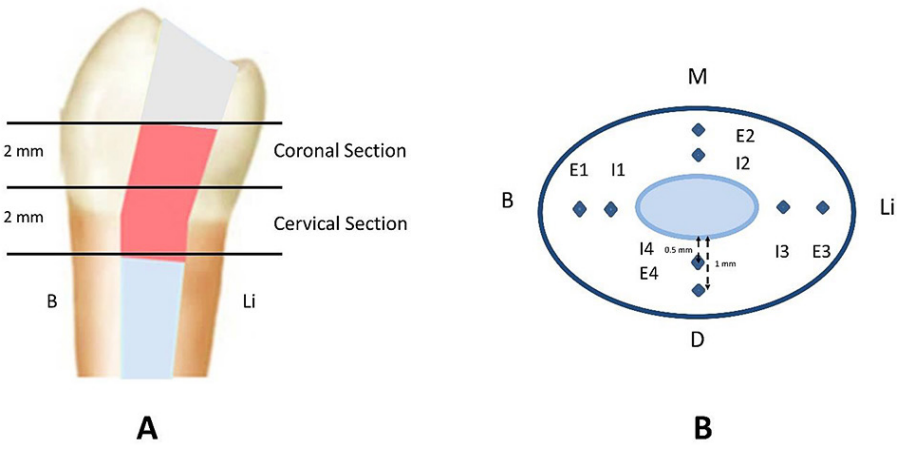
### Tooth color evaluation

Color measurements were performed before and after bleaching for 7, 14, and 21 days, using an intraoral spectrophotometer VITA Easyshade Advance 4.0 (VITA Zahnfabrik, Bad Sackingen, Germany). To ensure consistent tooth area measurements, 4 points on the buccal surface were marked with a permanent marker, creating a central area of 7 mm in diameter for spectrophotometer probe placement. Furthermore, for each measurement, we securely positioned each tooth in an individual jig attached to an apparatus, ensuring that the probe was placed perpendicular to and flush with the tooth surface[31]. These measurements were carried out in a dark room with consistent lighting from a daylight fluorescent bulb, providing a light intensity of 1,200 lux, positioned 40 centimeters from the test tooth.

Three color measurements were taken at 10-second intervals by a single researcher (K. S.), who was blinded to the tooth's group affiliation. The mean for each coordinate was then calculated. The intraclass correlation coefficient (ICC) for the 3 repeated measurements ranged from 0.97 to 0.99. The color difference, $\Delta$E00, was determined according to CIEDE2000[32]. Subsequently, the means of the $\Delta$E00 values were calculated.

### Microhardness testing

After completing the tooth bleaching for 7, 14, and 21 days, 10 teeth from each group were randomly selected. These teeth were then embedded in a clear acrylic resin cylinder (Takilon, Rodent srl, Milan, Italy) and horizontally sectioned into two 2-mm-thick sections (Figure 1). The first section (cervical section) was obtained from 1 mm below to 1 mm above the CEJ. The second section (coronal section) was from 1 mm to 3 mm above the CEJ (Figure 1A). The dentin sections were sequentially ground and polished using 320, 600, 1200, and 2400 grit silicon carbide paper. Vickers hardness test was conducted on the dentin sections using a microhardness tester (Buehler Micromet II, Buehler, Lake Bluff, IL) with a 50g load applied for 10s. Indentations were created at 8 locations on the coronal side of each specimen (Figure 1B), positioned at distances of 0.5 mm (inner dentin) and 1 mm (outer dentin) from the pulpal wall. A single researcher, uninformed of the tooth's group affiliation, measured the lengths of the 2 diagonals of these




B=buccal, Li=lingual, M=mesial, D=distal

**Figure 1** Diagram showing (A) the locations from which the dentin sections were taken for analysis. (B) The indentation positions for the Vickers hardness test.

indentations. Following this, Vickers hardness numbers were obtained. Mean microhardness values for the inner and outer dentin of each specimen were then calculated.

### Scanning electron microscopy

An additional tooth was included in each experimental group for SEM evaluation, specifically to confirm the removal of the smear layer. Each tooth was divided into mesial and distal halves. The tooth specimens were then dehydrated through a graded ethanol series ranging from 50% to 100% in 10% increments. The specimens were sputter-coated with gold using the SPI-ModuleTM Sputter coater (SPI, West Chester, USA) and examined under a SEM (JSM-5800LV, JEOL, Tokyo, Japan).

### Statistical analysis

The data were assessed for normality using the Shapiro-Wilk test and homogeneity with Levene's test. Mean values for shade and dentin microhardness were analyzed via two-way repeated ANOVA, and multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test (α=0.05).

## Results

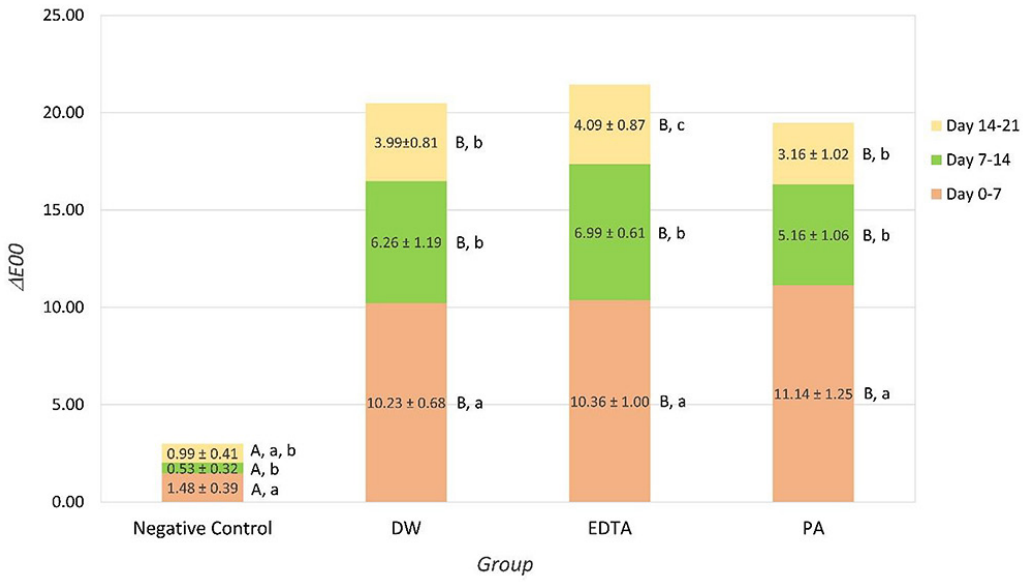
Data on color alteration before and after bleaching, categorized by the groups and studied time points, are presented in Table 1. Prior to bleaching, all teeth exhibited L* values ranging between 57.96 and 69.73, a* values between −0.13 and 2.98, and b* values between 13.14 and 22.80. Following the bleaching process, all 3 protocols significantly whitened the teeth, with the whitening effects intensifying over time. Within each group, a decrease in a* values and an increase in b* values were observed. However, no statistically significant differences in L*, a*, and b* values were detected among the experimental groups at each time point (p-value>.05). The mean $\Delta$E00 are shown in Figure 2.

Table 2 presents the mean microhardness values of dentin following intracoronal bleaching across various groups. The analysis reveals no significant differences

**Table 1** Mean (±S.D.) of L*, a*, and b* coordinates obtained before and after bleaching, using different smear layer removal protocols at different time points

| Group | Coordinate | Mean of coordinates ± S.D. at different times (day) | | | | |
|---|---|---|---|---|---|---|
| | | Before | Day 7 | Day 14 | Day 21 | |
| Negative | L* | 63.05±3.10 | 64.63±2.76 | 64.24±2.22 | 65.10±2.98 | |
| control | a* | 1.22±0.63 | 1.35±0.69 | 1.59±0.78 | 1.46±0.99 | |
| | b* | 16.67±2.36 | 15.63±2.58 | 15.32±2.40 | 14.83± 1.65 | |
| DW | L* | 63.27±5.31 | 75.06±6.97 | 83.71±5.59 | 89.10±3.94 | † |
| | a* | 1.68±1.30 | 1.23±0.78 | 0.59±1.53 | −0.71±1.21 | † |
| | b* | 17.97±4.83 | 26.14±4.12 | 30.68±3.16 | 28.75±3.15 | † |
| EDTA | L* | 63.37±3.31 | 74.93±4.57 | 84.40± 4.30 | 89.71±2.48 | † |
| | a* | 0.64±0.77 | 1.38±1.15 | 1.15±2.58 | −0.56±1.21 | † |
| | b* | 15.18±1.87 | 25.17±4.50 | 30.09±2.99 | 29.09±3.56 | † |
| PA | L* | 65.02±4.71 | 77.90±6.98 | 85.42±5.95 | 90.21±4.02 | † |
| | a* | 1.12±0.95 | 1.06±1.08 | 0.46± 1.14 | −0.17±1.37 | † |
| | b* | 17.38±2.37 | 28.27±4.41 | 29.36 ± 4.97 | 28.31±4.45 | † |

No statistically significant differences were observed in L*, a*, and b* values among the experimental groups at each time point (p-value>0.05). †Indicates statistically significant differences (p-value< 0.05) within each row for the respective coordinates over various time points DW=distilledwater, EDTA=17% ethylenediaminetetraacetic acid, PA= 37% phosphoric acid, S.D.=standard deviation




DW=distilledwater, EDTA=ethylenediaminetetraacetic acid, PA=phosphoric acid

**Figure 2** Mean of color alteration [CIEDE2000 (ΔE00)±standard deviation (S.D.)] after bleaching, using different smear layer removal protocols over 3 time periods (0–7 days, 7–14 days, and 14–21 days)

[A, B]Different uppercase letters indicate statistically significant differences (p-value<0.05) between groups within each time period. [a, b] Different lowercase letters indicate statistically significant differences (p-value< 0.05) within each group at various time periods.

in microhardness between the control and experimental groups. Within the experimental groups, no statistically significant differences were observed, irrespective of the time period or distance from the pulpal cavity, with a few exceptions. Specifically, on day 7, all experimental groups exhibited significantly lower microhardness of the inner dentin of the cervical sections compared to the coronal section (p-value<0.05). Within the DW group, the microhardness of the outer dentin in the coronal sections on day 21 was significantly greater than that observed on days 7 and 14 (p-value=0.006).

SEM analysis (Figure 3) demonstrated that the smear layer remained intact following treatment with distilled water. In contrast, the application of either 17% EDTA for 60 seconds or 37% phosphoric acid for 15 seconds effectively removed the smear layer and opened the dentinal tubules. The surface treated with phosphoric acid (Figure 3C) showed a more noticeable etching effect compared to the EDTA-treated surface (Figure 3B).

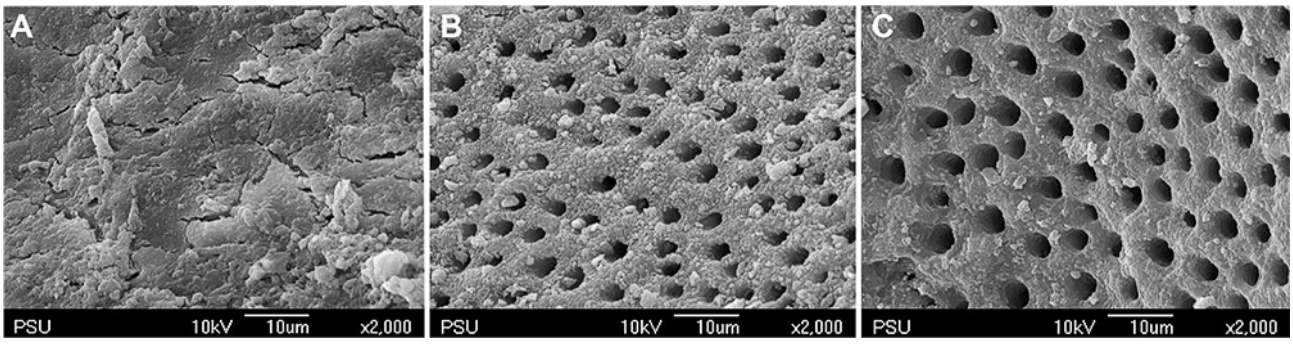
## Discussion

In this study, we evaluated the impact of smear layer removal using phosphoric acid or EDTA on bleaching outcomes and dentin hardness. Our findings supported the null hypothesis, as the analysis of ΔE00 revealed no statistically significant differences among the 3 groups (p-value>0.05) at any time point. These results are consistent with previous research[17], which found that smear layer removal did not enhance bleaching effectiveness compared to untreated dentin. However, the same study reported that 37% phosphoric acid achieved greater bleaching effectiveness than 17% EDTA. In

**Table 2** Mean (±S.D.) of Vickers hardness at different locations after bleaching, utilizing different smear layer removal protocols at three studied points

| Group | Dentin Location | Section level | Mean Vickers hardness±S.D. at different time points | | |
|---|---|---|---|---|---|
| | | | Day 7 | Day 14 | Day 21 |
| Negative control | Inner | Cervical | 63.26±2.08 | 63.82±2.60 | 64.57±2.85 |
| | | Coronal | 64.04±1.97 | 64.20±1.61 | 64.67±2.41 |
| | Outer | Cervical | 65.26±0.43 | 65.56±0.95 | 66.64±2.33 |
| | | Coronal | 65.94±2.53 | 65.38±3.68 | 66.45±1.49 |
| DW | Inner | Cervical | 61.85±2.95* | 62.99±2.93 | 63.45±4.51 |
| | | Coronal | 64.39±2.35* | 63.92±2.66 | 66.50±4.54 |
| | Outer | Cervical | 64.23±2.79 | 63.35±3.20 | 66.99±3.28 |
| | | Coronal | 64.28±3.20* | 62.45±2.94* | 67.84±3.46* |
| EDTA | Inner | Cervical | 59.79±3.68* | 63.45±3.44 | 63.49±4.50 |
| | | Coronal | 63.66±4.27* | 66.72±4.03 | 66.78±2.85 |
| | Outer | Cervical | 62.93±3.73 | 65.79±2.89 | 66.23±2.98 |
| | | Coronal | 64.14±4.43 | 65.91±4.24 | 67.95±2.98 |
| PA | Inner | Cervical | 60.29±2.68* | 61.90±5.80 | 62.50±6.76 |
| | | Coronal | 63.63±2.61* | 63.04±4.21 | 65.56±4.52 |
| | Outer | Cervical | 63.81±2.76 | 64.67±3.64 | 65.15±4.91 |
| | | Coronal | 64.23±2.53 | 63.69±3.20 | 66.03±4.59 |

No statistically significant differences in microhardness were noted among all groups across different time points and dentin locations, except where indicated by an asterisk (*), * Indicates a statistically significant difference (p-value<0.05). DW=distilledWater, EDTA=ethylenediaminetetraacetic acid, PA=phosphoric acid, S.D.=standard deviation




**FullText**

**Figure 3** SEM images of intrapulpal dentin surfaces at 2,000x magnification after different treatments: (A) Distilled water (10 mL, 60 s). (B) 17% EDTA (5 mL, 60s), followed by distilled water (10 mL, 60 s). (C) 37% phosphoric acid (15s), followed by distilled water (10 mL, 60 s)

contrast, our findings did not show similar differences. These discrepancies may be attributed to variations in the bleaching agents used and differences in follow-up periods, as the previous study employed 35% hydrogen peroxide as the bleaching agent and monitored color changes for only 2 weeks. Earlier studies[9,18] that examined phosphoric acid treatment followed by bleaching with sodium perborate also found that the presence or absence of the smear layer did not significantly impact bleaching outcomes. However, differences in bleaching protocols and color evaluation methods between our study and previous investigations should be considered. For example, Casey et al.[18] used a higher concentration of phosphoric acid (50%) for smear layer removal and a mixture of sodium perborate and 30% hydrogen peroxide as the bleaching agent. Tooth color was assessed visually using tab-type shade guides, a method known for its limitations in reliability[33]. Conversely, Horn et al.[9] evaluated tooth color and stability after 8 days of bleaching, reporting only L* values.

Since patients are typically advised to return within 3 to 10 days to assess color changes and determine the need for additional sessions[34], intracoronal bleaching generally requires 1 to 3 applications. Therefore, in this study, we monitored tooth color changes at 7-day intervals over a 21-day period, aligning with common clinical follow-up protocols. Upon evaluating color changes within each group across the 3 assessment periods, the highest ΔE00 values were observed during the first week for all bleaching protocols. These results indicate an immediate and significant reaction between the bleaching agents and pigmented organic molecules during the initial days. Following this period, a significant decrease in ΔE00 values was observed, even with the replacement of the bleaching agent with a fresh batch. Notably, the EDTA group exhibited a significant reduction in ΔE00 during the third week of bleaching, whereas the PA and DW groups maintained consistent ΔE00 values throughout the second and third weeks. These observations seem to contradict another study[16], which found that EDTA produced significantly greater dentin penetration during the second and third weeks compared to the first. Collectively, these findings, along with the observation that smear layer removal does not significantly improve bleaching outcomes, highlight the complexity of bleaching dynamics. As suggested by Casey et al.[18], hydrogen peroxide, regardless of the presence of the smear layer, may still diffuse through the dentin due to interconnections between dentinal tubules. Furthermore, hydrogen peroxide itself alters the dentin structure, increasing permeability[35], which may offset any potential advantage of the initial smear layer removal. Moreover, the effectiveness of the bleaching procedure may not rely solely on the agent's penetration into the dentin. Factors such as the chemical composition, concentration of the bleaching agent, and its interaction with the dentin's organic components and pigment molecules play a more significant role. As pigment molecules are progressively depleted, the whitening effect diminishes, and further lightening becomes limited, regardless of the bleaching agent's penetration.

A prospective multicenter study identified the 50:50% perceptibility and acceptability thresholds for color change in CIEDE2000 as 0.8 and 1.8, respectively[36]. In our study, all bleaching protocols yielded ΔE00 values above these thresholds, and the differences between the experimental groups fell below the acceptability threshold. This suggests that while all the protocols induced noticeable changes, the variations among treatments were not clinically distinguishable.

Within each group, over time, an increase in L* values was observed across all experimental groups, indicating a progression towards brighter tooth color. Furthermore, we noted a decrease in a* values, which signifies a reduction in reddish shades, alongside an increase in b* values, indicating a shift towards the yellow spectrum. These observations align with prior studies

that have reported increases in L* values and decreases in a* values[29, 37]. However, in contrast to our findings, these studies reported a decrease in b* values. This discrepancy could be due to the use of different bleaching agents, suggesting that various bleaching protocols may yield different color outcomes. The insights gained from changes in tooth color metrics are beneficial for clinicians. They enable the customization of bleaching protocols to closely match individual patient needs and establish realistic expectations, thereby ensuring patients are thoroughly informed about potential treatment outcomes.

In the negative control group, minor changes in L*, a*, and b* values were noted, but these changes were not statistically significant. The persistently lower L* values in the control group, compared to those in the experimental group, indicate a darker shade. This suggests that the observed color change in the experimental group was primarily a result of the bleaching treatment rather than natural stain fading.

In our comparison of Vickers microhardness, we found that most differences in values were not statistically significant, with some exceptions noted. Specifically, on day 7, we observed a lower microhardness of the inner dentin in cervical sections compared to coronal dentin across all experimental groups. Considering the proximity of these areas to the bleaching agent placed inside the pulp cavity, this could suggest localized effects of the bleaching process, irrespective of the smear layer's presence. Despite these observed differences, our results indicate that the presence or absence of the smear layer, along with the removal protocol, did not significantly affect dentin microhardness. This finding aligns with the observations of Chng et al.[22, 38], who demonstrated that intracoronal bleaching with sodium perborate mixed with distilled water over a 7-day period did not affect the Vickers[22] and Knoop[38] hardness of the dentin. However, it is important to note the differences between our study and theirs. Our research specifically investigated the impact of smear layer removal on the bleaching process, an aspect not addressed by Chng et al. Furthermore, our extended study duration of up to 21 days provides additional insights into the long-term effects of bleaching agents on dentin. Previous studies[20,22,38-40] have consistently shown that intracoronal bleaching with 30% hydrogen peroxide used either alone or in combination with sodium perborate, significantly weakens the dentin. This weakening effect may be attributed to the fact that bleaching agents containing hydrogen peroxide typically have a pH of approximately 1.7, leading to greater dissolution of minerals from the dentin. In contrast, the pH of sodium perborate mixed with distilled water is approximately 9.7 [22]. This alkaline pH level could potentially reduce dentin demineralization. As demonstrated in a study[20], immersing tooth specimens in sodium perborate mixed with distilled water for 7 days did not affect the calcium and phosphorus levels in the enamel, dentin, and cementum. This finding aligns with the observed stability in dentin hardness over the same period. However, a significant reduction in sulfur levels was noted, suggesting potential damage to the organic matrix components. In our study, dentin microhardness remained consistent after 21 days, further indicating the likelihood of stable calcium and phosphorus levels in dentin for this duration. Nevertheless, the extended exposure of up to 21 days could potentially exacerbate the decline in sulfur levels. This highlights the necessity of future research in order to explore the long-term effects of bleaching treatments on the composition of dental tissues and their physical properties.

From a clinical standpoint, while bleaching initially results in significant color changes, each subsequent session produces progressively less pronounced effects. Additionally, repeated bleaching can compromise dentin integrity by degrading the collagen matrix and potentially reducing microhardness, especially in the cervical regions. This underscores the importance of controlled, well-monitored bleaching protocols that balance aesthetic outcomes with long-term tooth structure preservation.

Clinicians should educate patients about the risks of excessive bleaching and set realistic expectations regarding the diminished whitening effect in subsequent sessions.

This study was designed to meticulously control variables that could impact experimental results. The concentration and amount of the bleaching agent were carefully regulated. To further reduce variability, only teeth with uniform dentin thickness on the buccal side were selected. Additionally, the locations and positions for color and microhardness assessments were standardized. Despite these precautions, certain limitations remain. Our laboratory conditions may not perfectly mimic the actual conditions of the oral cavity, as we did not simulate factors such as saliva presence, water exposure, and temperature fluctuations. Moreover, although anterior teeth are more commonly treated with intracoronal bleaching in clinical practice, our study utilized premolars due to the challenge of acquiring anterior teeth. This selection could affect the generalizability of our findings. Future studies should consider including anterior teeth to enhance the clinical applicability of the results. Additionally, to provide a more comprehensive evaluation of bleaching protocols, future research should examine additional parameters, such as patient-reported outcomes, including aesthetic satisfaction. Furthermore, assessing the integrity of the tooth post-bleaching could offer valuable insights into the long-term effects of different bleaching agents on dental hard tissues.

## Conclusion

Within the limitations of this study, it can be concluded that removing the smear layer with 37% phosphoric acid or 17% EDTA did not significantly improve the efficacy of sodium perborate intracoronal bleaching, in terms of color change or dentin hardness. The color shifts were consistent across all experimental groups, suggesting that smear layer removal is not essential for optimizing bleaching outcomes. These findings support the adoption of simplified protocols that exclude smear layer removal without compromising clinical efficacy.

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### Author contributions

Kwunklao Saichuea: Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Project Administration; Boonrat Sattapan: Methodology, Writing – Review & Editing, Supervision; Boonlert Kukiattrakoon: Software, Formal analysis, Data Curation, Writing – Original Draft; Kewalin Thammasitboon: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Funding acquisition

### Ethical approval

The research study obtained ethical approval (EC5902-07-P-LR) from the Faculty of Dentistry, Prince of Songkla University, Thailand.

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## Conflict of interest

The authors do not have any financial interest in the companies whose materials are included in this article.

## References

1. Spasser HF. A simple bleaching technique using sodium perborate. NY State Dent J 1961;27:332–4.
2. Harrington GW, Natkin E. External resorption associated with bleaching of pulpless teeth. J Endod 1979;5:344–8.

3. Heller D, Skriber J, Lin LM. Effect of intracoronal bleaching on external cervical root resorption. J Endod 1992;18:145–8.
4. Montgomery S. External cervical resorption after bleaching a pulpless tooth. Oral Surg 1984;57:203–6.
5. Ho S, Goerig AC. An in vitro comparison of different bleaching agents in the discolored tooth. J Endod 1989;15:106–11.
6. Rotstein I, Mot C, Friedman S. Prognosis of intracoronal bleaching with sodium perborate preparations in vitro: 1-year study. J Endod 1993;19:10–2.
7. Ari H, Ungor M. In vitro comparison of different types of sodium perborate used for intracoronal bleaching of discoloured teeth. Int Endod J 2002;35:433–6.
8. Weiger R, Kuhn A, Lost C. Radicular penetration of hydrogen peroxide during intra-coronal bleaching with various forms of sodium perborate. Int Endod J 1994;27:313–7.
9. Horn DJ, Hicks ML, Bulan-Brady J. Effect of smear layer removal on bleaching of human teeth in vitro. J Endod 1998;24:791–5.
10. Pashley DH. Smear layer: physiological considerations. Oper Dent Suppl 1984;3:13–29.
11. Fogel HM, Pashley DH. Dentin permeability: effects of endodontic procedures on root slabs. J Endod 1990;16:442–5.
12. Rodrigues LM, Vansan LP, Pécora JD, Marchesan MA. Permeability of different groups of maxillary teeth after 38% hydrogen peroxide internal bleaching. Braz Dent J 2009;20:303–6.
13. Fuss Z, Szajkis S, Tagger M. Tubular permeability to calcium hydroxide and to bleaching agents. J Endod 1989;15:362–4.
14. Hulsmann M. Endodontie. Stuttgart, New York: Georg ThiemeVerlag; 1993.
15. Cannabrava VP, Fernandes SL, Calabria MP, et al. Bleaching technique effect on dentin permeability. Am J Dent 2014;27:145–8.
16. Elbahary S, Gitit Z, Flaisher-Salem N, et al. Influence of irrigation protocol on peroxide penetration into dentinal tubules following internal bleaching: a confocal laser scanning microscopy study. J Clin Pediatr Dent 2021;45:253–8.
17. Bengoa F, Dufey N, Buchheister G. Evaluation of internal bleaching with 35% hydrogen peroxide in dentin conditioned with 37% phosphoric acid and 17% EDTA. J Oral Res 2020;9:490–9.
18. Casey L J, Schindler WG, Murata SM, et al. The use of dentinal etching with endodontic bleaching procedures. J Endod 1989;15:535–8.
19. Gaidarji B, Perez BG, Durand LB. Effectiveness of three bleaching techniques for endodontically treated teeth: a randomized clinical trial. Oper Dent 2024;49:519–30.
20. Rotstein I, Dankner E, Goldman A, et al. Histochemical analysis of dental hard tissues following bleaching. J Endod 1996;22:23–5.
21. Zalkind M, Arwaz JR, Goldman A, et al. Surface morphology changes in human enamel, dentin and cementum following bleaching: a scanning electron microscopy study. Endod Dent Traumatol 1996;12:82–8.
22. Chng HK, Palamara JEA, Messer HH. Effect of hydrogen peroxide and sodium perborate on biomechanical properties of human dentin. J Endod 2002;28:62–7.
23. Paul S, Peter A, Pietrobon N, Hämmerle CH. Visual and spectrophotometric shade analysis of human teeth. J Dent Res 2002;81:578-82.
24. Chu SJ. Use of a reflectance spectrophotometer in evaluating shade change resulting from tooth-whitening products. J Esthet Restor Dent 2003;15:542–8.
25. Westland S. Review of the CIE system of colorimetry and its use in dentistry. J Esthet Restor Dent 2003;15:S5–12.
26. Gómez-Polo C, Portillo Muñoz M, Lorenzo Luengo MC, Vicente P, Galindo P, Martín Casado AM. Comparison of the CIELab and CIEDE2000 color difference formulas. J Prosthet Dent 2016;115:65-70.
27. Faul F, Erdfelder E, Lang AG, et al. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91.
28. Cavalli V, Shinohara MS, Ambrose W, Malafaia FM, Pereira PN, Giannini M. Influence of intracoronal bleaching agents on the ultimate strength and ultrastructure morphology of dentine. Int Endod J 2009;42:568-75.
29. Yui KC, Rodrigues JR, Mancini MN, Balducci I, Gonçalves SE. Ex vivo evaluation of the effectiveness of bleaching agents on the shade alteration of blood-stained teeth. Int Endod J 2008;41:485-92.
30. Freccia WF, Peters DD. A technique for staining extracted teeth: a research and teaching aid for bleaching. J Endod 1982;8:67–9.
31. VITA Easyshade Advance 4.0: Operating Instructions. Bad Zackingen: Germany; 2013.
32. Sharma G, Wu W, Dalal E. The CIEDE2000 color-difference formula: implementation notes, supplementary test data, and mathematical observations. Color Res Appl 2005;30:21–30.

33. Culpepper WD. A comparative study of shade-matching procedures. J Prosthet Dent 1970;24:166–73.
34. Berman LH, Hargreaves KM. Cohen's Pathways of the Pulp. 12th ed. St. Louis: Elsevier; 2020;p.e10.
35. Rodrigues LM, Vansan LP, Pécora JD, Marchesan MA. Permeability of different groups of maxillary teeth after 38% hydrogen peroxide internal bleaching. Braz Dent J. 2009;20:303–6.
36. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, et al. Color difference thresholds in dentistry. J Esthet Restor Dent 2015;27(Suppl1):S1-9.
37. Ishikawa-Nagai S, Terui T, Ishibashi K, et al. Comparison of effectiveness of two 10% carbamide peroxide tooth-bleaching systems using spectrophotometric measurements. J Esthet Restor Dent 2004;16:368–75.
38. Chng HK, Yap AUJ, Wattanapayungkul P, et al. Effect of traditional and alternative intracoronal bleaching agents on microhardness of human dentine J Oral Rehabil 2004;31:811–6.
39. Rotstein I, Lehr Z, Gedalia I. Effect of bleaching agents on inorganic components of human dentin and cementum. J Endod 1992;18:290–3.
40. Lewinstein I, Hirschfeld Z, Stabholz A, et al. Effect of hydrogen peroxide and sodium perborate on the microhardness of human enamel and dentin. J Endod 1994;20:61–3.