ORIGINAL ARTICLE

Turk Endod J 2025;10(3):195–202
doi: 10.14744/TEJ.2025.49379

Effects of bleaching agents on the surface microhardness and surface morphology of calcium silicate-based cements: An in vitro study

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Purpose: To assess the effects of exposure to sodium perborate, carbamide peroxide, and hydrogen peroxide on the surface microhardness and surface morphology of ProRoot MTA and Biodentine.

Methods: A total of 50 specimens of each cement, ProRoot MTA and Biodentine, were prepared. The specimens were assigned into 5 groups based on the bleaching agent applied (n=10): A mixture of sodium perborate and distilled water (SP-DW), a mixture of sodium perborate and 3% hydrogen peroxide (SP-HP), carbamide peroxide (CP), hydrogen peroxide (HP), and control group. Bleaching agents were changed four times on every fourth day. Surface microhardness and surface morphology were assessed using a microhardness tester and scanning electron microscope, respectively. Data were analysed using two-way ANOVA and Tukey's test.

Results: All bleaching agents caused morphological differences on the surfaces of the both cements. All bleaching agents, except SP-DW, caused a decrease in the microhardness of both cements, with HP causing the greatest decrease, followed by CP and SP-HP, respectively. Biodentine showed higher surface microhardness values than ProRoot MTA in all groups.

Conclusion: Biodentine may be recommended as a cervical barrier and a mixture of sodium perborate and distilled water may be the safest bleaching agent to preserve the surface properties of CSCs.

Keywords: Bleaching agents; calcium silicate-based cements; microhardness; surface morphology.

Introduction

Tooth color plays a crucial role in creating an esthetically pleasing smile, significantly affects esthetic self-perception, and has a psycho-social effect on individuals (1–3). When a single tooth becomes discolored, its negative impact on appearance can be particularly noticeable, as it disrupts the overall harmony of the smile (4). Intracoronal bleaching offers a conservative approach to improving the esthetics of discolored root-filled teeth (5). In most cases, multiple appointments are needed to achieve the desired shade and match the color of the darkened tooth to the surrounding teeth (6).

Intracoronal bleaching can be performed using different

Cite this article as: Naiboglu P, Kosar T, Yucel AC. Effects of bleaching agents on the surface microhardness and surface morphology of calcium silicate-based cements: An in vitro study. Turk Endod J 2025;10:195-202.
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Submitted: July 07, 2025 Revised: July 11, 2025 Accepted: July 21, 2025 Published: December 09, 2025

bleaching agents, including hydrogen peroxide, carbamide peroxide, a mixture of sodium perborate and hydrogen peroxide or distilled water (6,7). Bleaching is usually achieved by the release of hydrogen peroxide from these agents followed by diffusion of reactive oxygen species along the dentinal tubules, oxidizing pigments and restoring tooth color (5). Due to their acidity, bleaching agents may etch the dentin surface, disturb the smear layer covering the dentinal tubules, and lead to greater permeability. As a result, greater amounts of hydrogen peroxide can penetrate the dentinal tubules and external cervical resorption may occur if the level exceeds the critical limit (7). To reduce this risk, it is recommended to place a cervical barrier prior to intracoronal bleaching (6,7).

Mineral trioxide aggregate (MTA), the first introduced calcium silicate-based cement (CSC) (8), can be used in vital pulp treatment, apexification, root-end filling, pulpotomy, repair of perforations, and endodontic surgery (9,10). Despite its beneficial properties such as bioactivity, biocompatibility, antimicrobial efficacy, and sealing ability (11,12), MTA has been criticized for its disadvantages such as difficult handling properties, tooth discolouration, and long setting time (13).

Biodentine™ (Septodont, St. Maur-des-Fossés, France) entered the market in 2009 as a "dentin replacement" with the claim of eliminating some of the disadvantages of MTA. The cement is presented in both powder and liquid components, with the liquid containing $CaCl_2$ to accelerate and regulate the setting process. A water-soluble polymer was added to reduce the quantity of water. This helped in reducing the viscosity of the cement and improving its handling properties (8,14–16). With its superior physical properties such as higher density, compressive strength, surface hardness, flexural strength and elastic modulus, faster setting time, reduced porosity and improved handling properties, Biodentine™ offers a promising alternative to MTA for use in endodontics, restorative dentistry, pediatric dentistry, and dental traumatology (9,17).

In recent years, ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) and Biodentine have been investigated for use as a cervical barrier in intracoronal bleaching (18–20). Despite its clinical importance, only a few studies have examined the impact of bleaching agents on the surface microhardness and surface morphology of CSCs (21,22). Therefore, this study focused on evaluating the effects of sodium perborate, carbamide peroxide, and hydrogen peroxide on the surface microhardness and surface morphology of ProRoot MTA and Biodentine using the walking bleach technique. The null hypotheses tested were as follows: (1) bleaching agents would not affect the surface microhardness of both CSCs and (2) bleaching agents would not alter the surface morphology of both CSCs.

Materials and Methods

The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (23) (Fig. 1).

Specimen Preparation

A total of 100 cylindric acrylic blocks, each containing a hole 4 mm in depth and 6 mm in diameter were prepared using a self-curing acrylic resin (Imicryl, Konya, Türkiye).

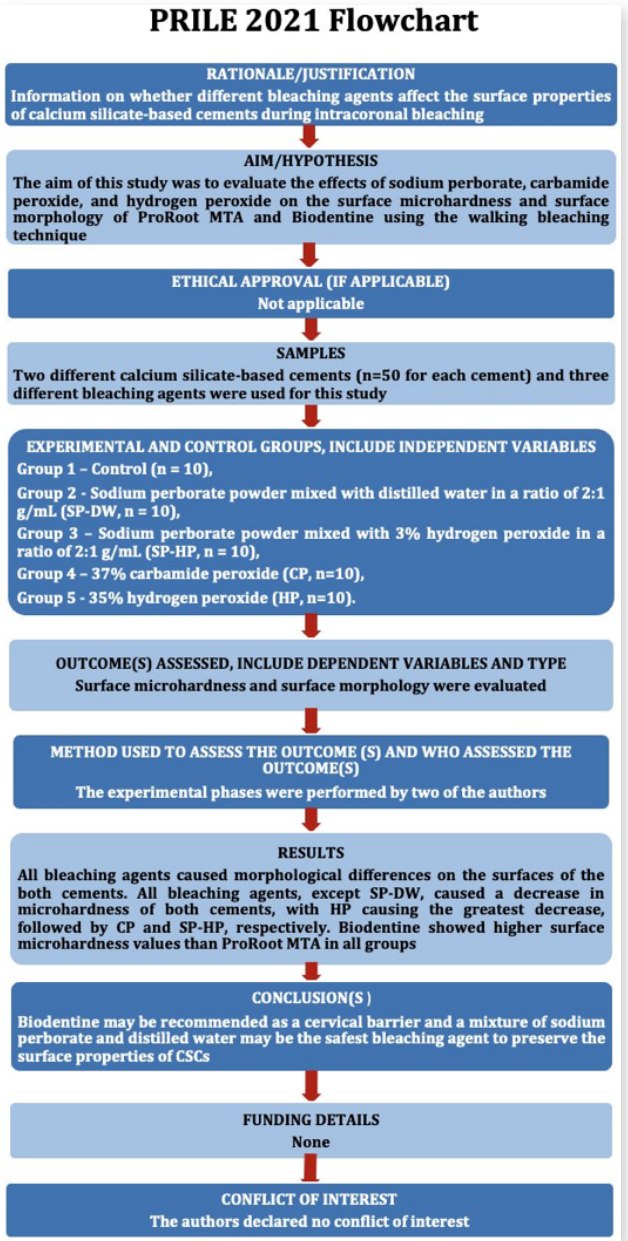

Fig. 1. PRILE 2021 flowchart.

Table 1. Composition, steps of application, manufacturer and lot number details of the CSCs used in the study

CSC	Composition	Steps of application	Manufacturer	Lot number
ProRoot MTA	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, calcium sulphate dehydrate or gypsum Liquid: Water	Mix 1 pouch of powder with 1 micro-dose ampule of liquid for about 1 minute	Dentsply Tulsa Dental, Johnson City, TN, USA	0000304442
Biodentine	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, iron oxide, and zirconium oxide. Liquid: Calcium chloride and hydrosoluble polymer	Pour 5 drops of liquid into the capsule, place the capsule on a mixing device and mix for 30 s	Septodont, Saint-Maur-des-Fossés, France	B28762

CSC: Calcium silicate-based cement.

Table 2. Bleaching agents used in the study

Bleaching agent	Component	Manufacturer
Sodium perborate	Sodium perborate tetrahydrate	Merck KGaA, Darmstadt, Germany
Whiteness Super-Endo	37% carbamide peroxide	FGM Products, Joinville, SC, Brazil
Opalescence Endo	35% hydrogen peroxide	Ultradent Products, Inc., South Jordan, UT, USA

The blocks were allocated into two groups (n=50) according to the type of CSC used: Biodentine (Septodont, Saint-Maur-des-Fosses, France) and ProRoot MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) (Table 1). Each cement, prepared in accordance with the manufacturer's instructions, was placed into the cavities, covered with moist cotton pellets and kept at 37°C with 100% humidity for 96 h. Then, the surfaces of the specimens were polished under constant water irrigation using silicon carbide papers with grit sizes of 600 and 1000 each applied for 30 s. After polishing, the specimens were rinsed using distilled water for 1 min and air-dried for 5s.

50 specimens of each CSC were randomly allocated into 5 groups (n=10) based on the bleaching agent used (Table 2).

Control group: The surfaces of the specimens did not receive any treatment

Sodium perborate-distilled water (SP-DW) group: Sodium perborate powder mixed with distilled water in a ratio of 2:1 g/mL

Sodium perborate-hydrogen peroxide (SP-HP) group: Sodium perborate powder mixed with 3% hydrogen peroxide in a ratio of 2:1 g/mL

Carbamide peroxide (CP) group: 37% Carbamide peroxide

Hydrogen peroxide (HP) group: 35% Hydrogen peroxide

The surfaces of the specimens were treated with bleaching agent, covered with damp cotton pellets and sealed with temporary filling material until the next session four days later (Cavit, 3M ESPE, Seefeld, Germany). The bleaching agent was changed every 4 days and this protocol was repeated four times (6). The temporary material was then removed, the surfaces of CSCs were rinsed using distilled water and dried with air.

Microhardness Measurement

Vickers microhardness measurements were performed on each specimen utilizing a microhardness tester (Duramin Microhardness Tester, Struers, Cleveland, USA) and a diamond indenter applying a 50 g for 10 s. Four indentations were randomly placed on the surface, each at least 1 mm apart from adjacent indentations and the specimen edges. The diagonal diameter of each indentation was evaluated under a microscope and the Vickers microhardness value was recorded. The mean microhardness value was calculated for each specimen.

Scanning Electron Microscope Analysis

For morphological evaluation, 5 additional specimens for each material were prepared as previously described and stored under identical storage conditions. After 96 h, the specimens were allocated into five groups: Control group, SP-DW group, SP-HP group, CP group, and HP group. The relevant bleaching agent was applied to the surfaces of the specimens in the test groups as described earlier.

Table 3. Mean ± standard deviation of surface microhardness (VHN) of each group

	ProRoot MTA Mean ± SD	Biodentine Mean ± SD
Control	45.76±3.97^{Aa}	59.42±4.03^{Ba}
SP-DW	43.57±2.12^{Aab}	56.83±2.34^{Ba}
SP-HP	42.34±3.63^{Ab}	53.36±3.07^{Bb}
CP	35.21±2.64^{Ac}	42.35±2.00^{Bc}
HP	25.44±2.41^{Ad}	35.97±2.61^{Bd}

SP-DW: Sodium perborate-distilled water; SP-HP: Sodium perborate-hydrogen peroxide; CP: Carbamide peroxide; HP: Hydrogen peroxide. Different superscript uppercase letters (A,B) in each column and lowercase letters (a,b,c,d) in each row indicate statistically significant differences ($p<0.05$). SD: standard deviation; VHN: Vickers microhardness.

No treatment was applied to the specimens in the control group. The specimens were sputter-coated with gold using an SPI-Module Sputter Coater (Structure Probe Inc., PA, USA) then examined under a scanning electron microscope (SEM) (EVO LS10, Zeiss, Oberkochen, Germany) to evaluate their surface topography. SEM images of the specimens were obtained at different magnifications (1000x, 2500x, 3000x, and 5000x).

Statistical Analysis

Data were analysed using SPSS 25.0 (SPSS, Chicago, IL, USA) for Windows. Parametric statistical tests were applied after the data were evaluated for normal distribution (Shapiro-Wilk) and homogeneity of variance (Levene test). Two-way analysis of variance (ANOVA) was used to analyse the Vickers microhardness (VHN) data, followed by Tukey's test for post hoc multiple comparisons ($p<0.05$).

Results

Surface Microhardness

Two-way ANOVA indicated that both factors (bleaching agent and CSC) significantly impacted the surface microhardness values ($p<0.001$). The interaction of these two independent variables was also significant ($p<0.05$).

The mean and standard deviation values of the Vickers surface microhardness for each group are listed in Table 3. In the control group and all bleaching agents groups, Biodentine had significantly higher microhardness values compared to ProRoot MTA ($p<0.001$). For both CSCs, the lowest microhardness was observed in the HP group, and the HP group demonstrated significantly lower microhardness values than all other groups ($p<0.001$). Similarly, for both CSCs, the CP group exhibited significantly lower microhardness values compared to the SP-HP, SP-DW, and control groups ($p<0.001$). For both CSCs, significantly lower microhardness values were found in the SP-HP group compared to the control group ($p<0.05$). For ProRoot MTA, the difference between the SP-HP and SP-DW groups was not statistically significant ($p>0.05$). However, for Biodentine, the SP-HP group demonstrated significantly lower microhardness value than the SP-DW group ($p<0.05$). For both cements, the difference between the control and SP-DW groups was not statistically significant ($p>0.05$).

SEM Analysis

The SEM images of ProRoot MTA and Biodentine in the control, SP-DW, SP-HP, CP, and HP groups are illustrated in Fig. 2.

The SEM image of ProRoot MTA in the control group showed globular aggregate particles, rod-like, plate-shaped, and cubic crystals, and microchannels (Fig. 2A). In the SP-DW group, amorphous globular aggregate particles, microchannels, rod-like and plate-shaped crystals were evident (Fig. 2C). In the SP-HP group, selective erosion surrounding crystalline structures on the ProRoot MTA surface was evident by the presence of globular crystals and microchannels (Fig. 2E). The selective removal of matrix from the periphery of crystals resulted in a spongy appearance in the CP group. Cubic crystals, microchannels, and porosity were also observed in this group (Fig. 2G). In the HP group, extensive erosion, cracked surfaces, reticular pattern matrix structure, irregular needle-like crystals, and extensive porosity were formed on the ProRoot MTA surface due to superficial dissolution caused by hydrogen peroxide (Fig. 2I).

The SEM image of Biodentine in the control group showed cubic crystals as a common finding. Additionally, microchannels and plate-shaped crystals were also evident (Fig. 2B). In the SP-DW group, the specimen surface consisted of cubic crystals of irregular shape and size and microchannels. Lightly stratified structures were also noticed on the surfaces of the cubic crystals (Fig. 2D). In the SP-HP group, selective matrix loss around the crystalline structures, cubic and globular crystals, and microchannels were evident on the Biodentine surface (Fig. 2F). In the CP group, the Biodentine structure was characterized predominantly by cubic crystals, with rod-like crystals less frequently and mostly located between the cubic crystals. Furthermore, erosive surfaces over the cubic crystals and the presence of microchannels were observed (Fig. 2H). In the HP group, all cubic crystals dissolved due to the impact of hydrogen peroxide and the Biodentine surface consisted of amorphous globular aggregate particles, microchannels, and void depressions (Fig. 2J).

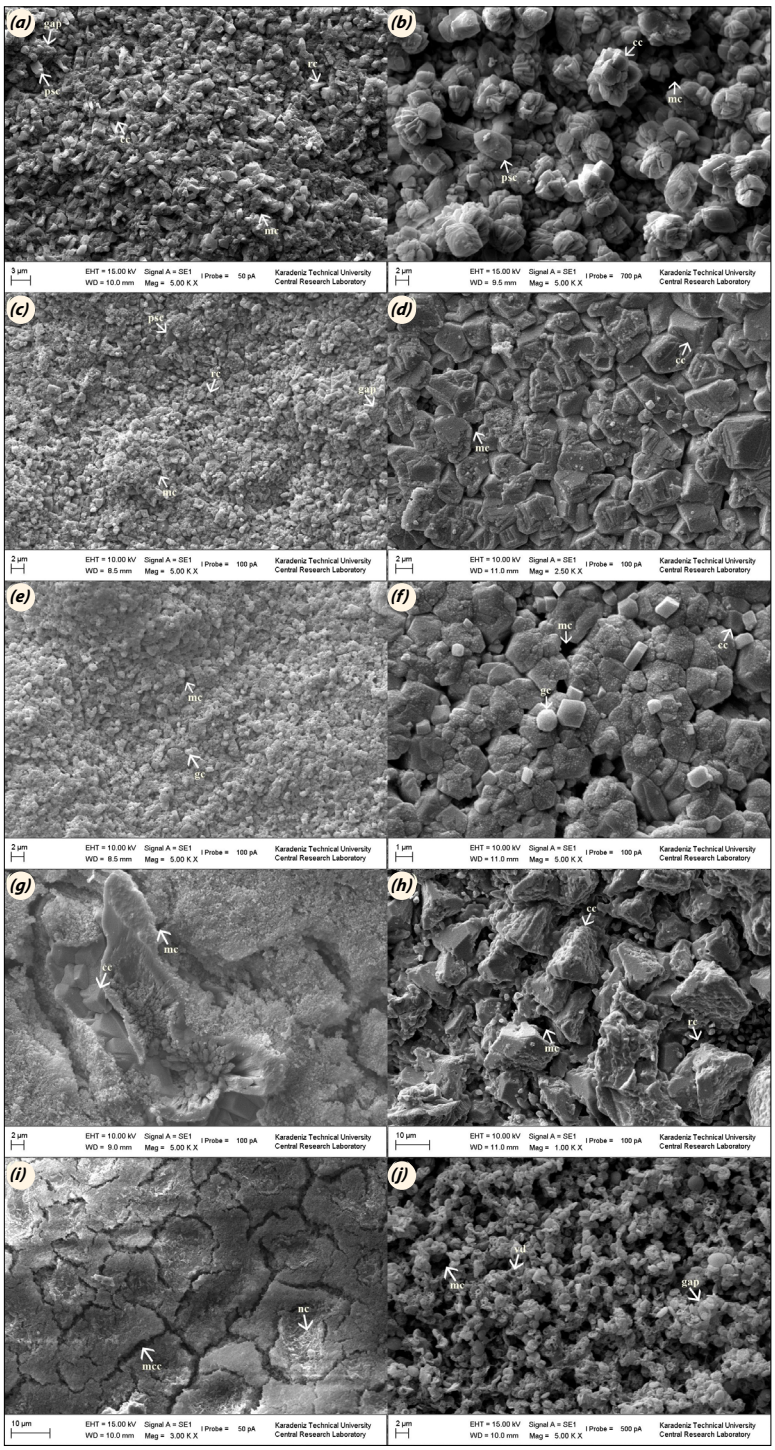

Fig. 2. SEM images at different magnifications (A, C, E, and G, 5000x; I, 3000x) of ProRoot MTA and (B, F, and J, 5000x, D, 2500x, and H, 1000x) of Biodentine. **(a)** globular aggregate particles (gap), plate-shaped crystals (psc), rod-like crystals (rc), cubic crystals (cc), and microchannels (mc) can be seen on the ProRoot MTA surface in the control group. **(c)** globular aggregate particles (gap), plate-shaped crystals (psc), rod-like crystals (rc), and microchannels (mc) can be seen on the ProRoot MTA surface in the SP-DW group. **(e)** globular crystals (gc) and microchannels (mc) can be seen on the ProRoot MTA surface in the SP-HP group. **(g)** cubic crystals (cc) and microchannels (mc) can be seen on the ProRoot MTA surface in the CP group. **(i)** microcracks (mcc) and needle-like crystals (nc) can be seen on the ProRoot MTA surface in the HP group. **(b)** cubic crystals (cc), plate-shaped crystals (psc), and microchannels (mc) can be seen on the Biodentine surface in the control group. **(d)** cubic crystals (cc) and microchannels (mc) can be seen on the Biodentine surface in the SP-DW group. **(f)** cubic crystals (cc), globular crystals (gc), and microchannels (mc) can be seen on the Biodentine surface in the SP-HP group. (H): cubic crystals (cc), rod-like crystals (rc), and microchannels (mc) can be seen on the Biodentine surface in the CP group. **(j)** globular aggregate particles (gap), microchannels (mc), and void depressions (vd) can be seen on the Biodentine surface in the HP group.

Discussion

In the present study, two different CSCs (ProRoot MTA and Biodentine) were exposed to a mixture of sodium perborate and distilled water, a mixture of sodium perborate and 3% hydrogen peroxide, carbamide peroxide, and hydrogen peroxide in order to mimic a clinical situation. According to our results, all bleaching agents, except for the mixture of sodium perborate and distilled water, decreased surface microhardness of both CSCs. Therefore, the first null hypothesis was partially rejected. In this study, the highest decrease in surface microhardness was determined for HP group for both CSCs, followed by CP and SP-HP groups, respectively. The number of studies examining the impact of bleaching agents on the surface microhardness of CSCs is quite limited (21,22). Consistent with the present study, Serin Kalay (2019) investigated the impact of hydrogen peroxide, carbamide peroxide, and a mixture of sodium perborate and distilled water on the surface hardness of ProRoot MTA and reported that both hydrogen peroxide and carbamide peroxide reduced the microhardness of MTA (21). However, Ahmadi and Meraji, in their study examining the effects of sodium perborate-distilled water mixture and hydrogen peroxide on the surface microhardness of MTA Repair HP, MTA Angelus, and Biodentine, stated that both bleaching agents reduced the surface microhardness of all cements, but a more pronounced effect was obtained with hydrogen peroxide (22).

It has been reported that the surface microhardness of CSCs decreases as a result of exposure to acidic pH (24–26). In the present study, the pH of 35% hydrogen peroxide tested was reported to be 5, the pH of 37% carbamide peroxide was 6-7 (21), and the pH of a mixture of sodium perborate and distilled water or hydrogen peroxide prepared at a ratio of 2:1 g/mL was greater than 7.4 (27). In the study, it was determined that surface microhardness decreased as the pH value decreased for both cements. Although the mixture of sodium perborate and hydrogen peroxide did not have an acidic pH, it caused a decrease in surface microhardness. Hydrogen peroxide may cause bubbling when it comes into contact with the surfaces of both CSCs (28), this oxygen bubbling may have changed the surface morphology of the cements. The low microhardness obtained in the SP-HP group may be due to the negative impact of hydrogen peroxide on the surface microstructure.

In this study, consistent with previous studies (29,30), it was determined that Biodentine had higher microhardness than ProRoot MTA in all groups. The increased microhardness observed in Biodentine can be explained by the low water-to-powder ratio facilitated by the presence of a plasticizing hydrosoluble polymer in the mixing liquid, thereby improving workability (9,15,16,31). In addition, one study has shown that a low water-to-powder ratio increases the mechanical strength of the material and leads to minimum porosity (32). In the present study, the higher microhardness values obtained with Biodentine may be attributed to the low water/powder in the structure of Biodentine.

Consistent with previous studies (21,28,33–35) the current study findings demonstrated that all bleaching agents altered the surface morphology of both ProRoot MTA and Biodentine. Therefore, the second null hypothesis was rejected. SEM images showed that more pronounced surface changes in the HP group than in the CP group, and in the CP group than in the SP-HP and SP-DW groups. It was observed that the crystalline structures in ProRoot MTA and Biodentine, which were evident in the control group, dissolved in the CP group, while the dissolution gradually intensified in the HP group. It has been reported that crystal clusters on the MTA surface dissolve in acidic pH environment, indicating structural weakening (24,25) and leading to a least stable cohesive structure (25). In this study, the observed decrease in surface microhardness in both HP and CP groups may be attributed to the dissolution of crystalline structures on the surfaces of the ProRoot MTA and Biodentine. It has been stated that the hardness value of ProRoot MTA decreases in acidic environment, resulting in a more porous microstructure of this material (24). It was reported that higher acidity in the solution resulted in more pronounced porosity in the ProRoot MTA samples (26). Additionally, it is well established that porosity generally shows a negative correlation with surface hardness; that is, as porosity decreases, surface hardness tends to increase (36). In the present study, SEM examination revealed greater porosity in specimens exposed to carbamide peroxide and hydrogen peroxide, but within the limitations of SEM, it was not possible to determine a precise and objective degree of porosity (26). The low microhardness values obtained in the HP and CP groups may be due to the negative effect of the porosity observed on the surfaces of the cements in these groups on the surface microhardness.

In the present study, SEM images revealed that the lower pH (pH 5 for HP and pH 6-7 for CP) associated with higher concentrations of hydrogen peroxide had a stronger impact on the surface structure of ProRoot MTA and Biodentine. Consistent with previous studies (28,35), these findings show that the effects of bleaching agents on the surface microstructure of CSCs depends on the hydrogen peroxide concentration. When exposed to moisture, 10% carbamide peroxide dissociates into 6.65% urea and 3.35% hydrogen peroxide (5). According to the manufacturer, 13.36% hydrogen peroxide was released from the

37% carbamide peroxide used in the current study. With the increase in hydrogen peroxide release, the negative effects of bleaching agents on the surfaces of CSCs have also increased.

In this study, more surface morphology changes were detected in the SP-HP group than in the SP-DW group. It is thought that hydrogen peroxide may have formed bubbling when it came into contact with the surfaces of both CSCs (28); this oxygen bubbling may explain the more pronounced surface changes observed in the SP-HP group compared to the SP-DW and control groups. Additionally, SP-DW group altered the surface morphology of both CSCs. Sodium perborate, a powdered oxidizing and whitening agent, remains stable under dry conditions but decomposes in the presence of water, warm air, or acid, producing sodium metaborate, nascent oxygen, and hydrogen peroxide (7,37). In this study, it was thought that the changes in surface morphology observed in the SP-DW group may have been caused by hydrogen peroxide released from sodium perborate.

The main limitation of this study is that it was conducted under in vitro conditions; however, the ideal approach is to evaluate restorative materials in clinical environments. Findings are limited to the brand and type of materials used in this study. Furthermore, this in vitro study did not include ageing procedures such as thermal and mechanical load cycling, and long-term effects of the materials were not assessed. Therefore, further studies are needed to determine the effects of bleaching agents and aging conditions on the surface properties of various CSCs.

Conclusion

All bleaching agents disturbed the surface morphology of ProRoot MTA and Biodentine. A substantial decrease in surface microhardness was found in all groups, except the mixture of sodium perborate and distilled water. Hydrogen peroxide induced the greatest decrease in surface microhardness, followed by carbamide peroxide and the mixture of sodium perborate and 3% hydrogen peroxide, respectively. Biodentine showed higher surface microhardness than ProRoot MTA in all groups. The present findings suggest that Biodentine may be preferred as a cervical barrier and a mixture of sodium perborate and distilled water may be the most conservative bleaching agent on CSC surfaces.

Authorship Contributions: Concept: P.N., T.K., A.Ç.Y.; Design: P.N., T.K., A.Ç.Y.; Supervision: P.N., T.K., A.Ç.Y.; Fundings: P.N., T.K.; Data Collection and/or Processing: P.N., T.K.; Analysis and/or Interpretation: P.N., A.Ç.Y.; Literature Review: P.N.; Writer: P.N., T.K.; Critical Review: A.Ç.Y.

Use of AI for Writing Assistance: Not declared

Source of Funding: None declared.

Conflict of Interest: None declared.

Ethical Approval: Ethical approval was not required for this study.

Informed consent: Not applicable.

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