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The occluding effects of layered calcium phosphate and cyanoacrylate on dentinal tubules: a SEM study

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Abstract

Background Dentin hypersensitivity (DH) is characterized by short and sharp pain that impacts quality of life. Effective management focuses on occluding exposed dentinal tubules. This study compared the effectiveness of intermittent and simultaneous calcium phosphate (CP) application with cyanoacrylate (CA) in tubule occlusion.

Methods Eighty extracted human premolars without periodontal disease were used to prepare standardized root dentin specimens. After removal of the crowns and apical thirds, disk-shaped fragments were obtained and treated with EDTA to remove the smear layer. The specimens were then randomly allocated to eight groups and treated with CP, CA, or their combination using different protocols (single, multiple, intermittent, or simultaneous layers). All samples were stored in artificial saliva until examination. Dentin tubule occlusion was evaluated by scanning electron microscopy (SEM), and quantitative measurements of tubule exposure and diameter were performed. Statistical analyses were conducted using nonparametric tests, with the significance level set at $p < .05$.

Results The CA and CP + CA groups were most effective in occluding dentinal tubules ($p < .01$). Intermittent CP application showed similar results to these groups and was more effective than simultaneous application ($p < .01$). In addition, a positive correlation was found between the number and diameter of exposed tubules across all groups ($p < .01$).

Conclusion Intermittent CP application enhanced tubule occlusion compared with simultaneous use. Under the limitations of this in vitro study, the addition of CA to CP produced outcomes comparable to those of intermittently applied CP.

Keywords Scanning electron microscopy, Dentin hypersensitivity, Treatment, Calcium phosphate, Cyanoacrylate

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Introduction

Dentin hypersensitivity (DH) occurs when exposed dentin tubules cause sharp pain in response to stimuli such as acidic or sweet foods, temperature changes, pressure, tooth brushing, or airflow [1]. Factors like abrasion, attrition, gingival recession, improper brushing, bruxism, and periodontal disease can lead to DH by exposing dentin and opening the dentin tubules to the oral environment [2–4].

The prevalence of DH ranges owing to varying research methodologies and diagnostic criteria, and its prevalence varies from 3.8 to 40.7% [5, 6]. Moreover, the buccal cervical areas of the canines and premolars, where gingival recession is most common, are the areas most affected by DH [5]. The impact of DH on patients' daily activities and quality of life is a significant concern, as it can hinder essential oral hygiene practices and interfere with normal dietary and speech functions [7]. Prolonged DH, defined as more than six months, has been linked to the development of psychological distress and emotional disturbances in affected individuals [4, 8].

The primary goal of DH therapy is to block dentinal tubules, reduce fluid movement, or decrease nerve sensitivity to alleviate pain [9]. Various agents have been investigated to achieve these effects including ions/salts [10], oxalates [11], calcium compounds [12], bioactive glasses [13], strontium chloride [14], potassium nitrate [15], glutaraldehyde [16], silver nitrate, and pro-argin [17]. Although many of these agents can partially reduce DH symptoms, their clinical use has been limited due to drawbacks such as cytotoxicity, allergic reactions, or insufficient durability [16, 18, 19] and the excessive surface roughness associated with lasers [3]. These limitations have encouraged the search for more biocompatible and long-lasting alternatives.

Calcium phosphate (CP) has attracted growing attention because of its biocompatibility, chemical similarity to tooth mineral, and ability to transform into hydroxyapatite [3, 20]. CP desensitizers, containing tetra calcium phosphate and anhydrous dicalcium phosphate, effectively reduce dentin permeability while protecting both dentin and enamel [21]. In vitro studies have shown that CP can precipitate on the dentin surface, occlude exposed tubules by crystallization, and reduce dentin permeability [3, 22–24]. However, CP is acid-labile, and its efficacy may depend on application strategy, with intermittent or layered applications reported to enhance tubule occlusion [22, 24].Cyanoacrylate (CA), on the other hand, is a biocompatible tissue adhesive that rapidly polymerizes upon contact with moisture to form a sealing film. Clinical and experimental studies have demonstrated its ability to reduce DH by covering dentinal tubules and sealing root surfaces [25–27]. Compared with other methods such as lasers, CA has shown favorable results, offering faster and simpler application [23, 27]. However, the variability of application activities due to the limited number of studies on the layered or combined application of these substances necessitated conducting research in this area. Given CP's biomimetic mineralization potential and CA's strong sealing ability, their combined or layered use may provide complementary benefits. However, limited evidence is available on such protocols, highlighting the need for further in vitro investigation.

Despite the extensive range of agents and application methods that have been utilized in the treatment of DH, no single approach has yet achieved complete elimination of sensitivity. While there are encouraging results reported in the literature concerning CP applications, no studies have been identified that examine the effectiveness of new methods or materials that would enhance its efficacy [22]. In light of the existing literature, this study hypothesizes that the intermittent application of CP, or its combination with CA, may result in an enhancement of dentinal tubule occlusion. The present study, we hypothesized that intermittent CP application and the CP + CA combination would result in superior tubule occlusion compared with single or simultaneous CP application.

Materials and methods

The study protocol was approved by the Clinical Research Ethics Committee of the Faculty of Dentistry, Gazi University (Ethics Committee approval date: 24/09/2020 Decision No: GUDHKAEK. 2020.20/3). Informed consent was obtained from patients for the collection of isolated teeth. The experimental design was figured as a graphical abstract in (Fig. 1).

Eighty premolar teeth without periodontal disease or attachment loss and with an indication of orthodontic extraction were used to obtain the dentin fragments [11, 28]. The extracted teeth were first examined in detail with a Lercher loupe (2.8 × 420) to exclude cavities, cracks, or fractures on the tooth surface and were kept in 10% formalin solution until the teeth were prepared.

The cementoenamel junction of the extracted teeth was taken as the starting point, and specimens were prepared by taking 3-mm-long pieces from the root area [3, 29, 30]. Following the removal of the crowns of the teeth and the apical third of the root, the disk-shaped dentin fragments were obtained using a double-sided diamond saw on rotating hand tools attached to the dental unit under water cooling. The dentin fragments were then divided into two parts. $3 \times 3 \times 1$ mm^3 cavities were drilled with a diamond fissure drill to limit the application area in the center of the dentin specimens to be used for SEM examination, to remove the cement layer on the root surface, and to eliminate the margin of error during the examination.

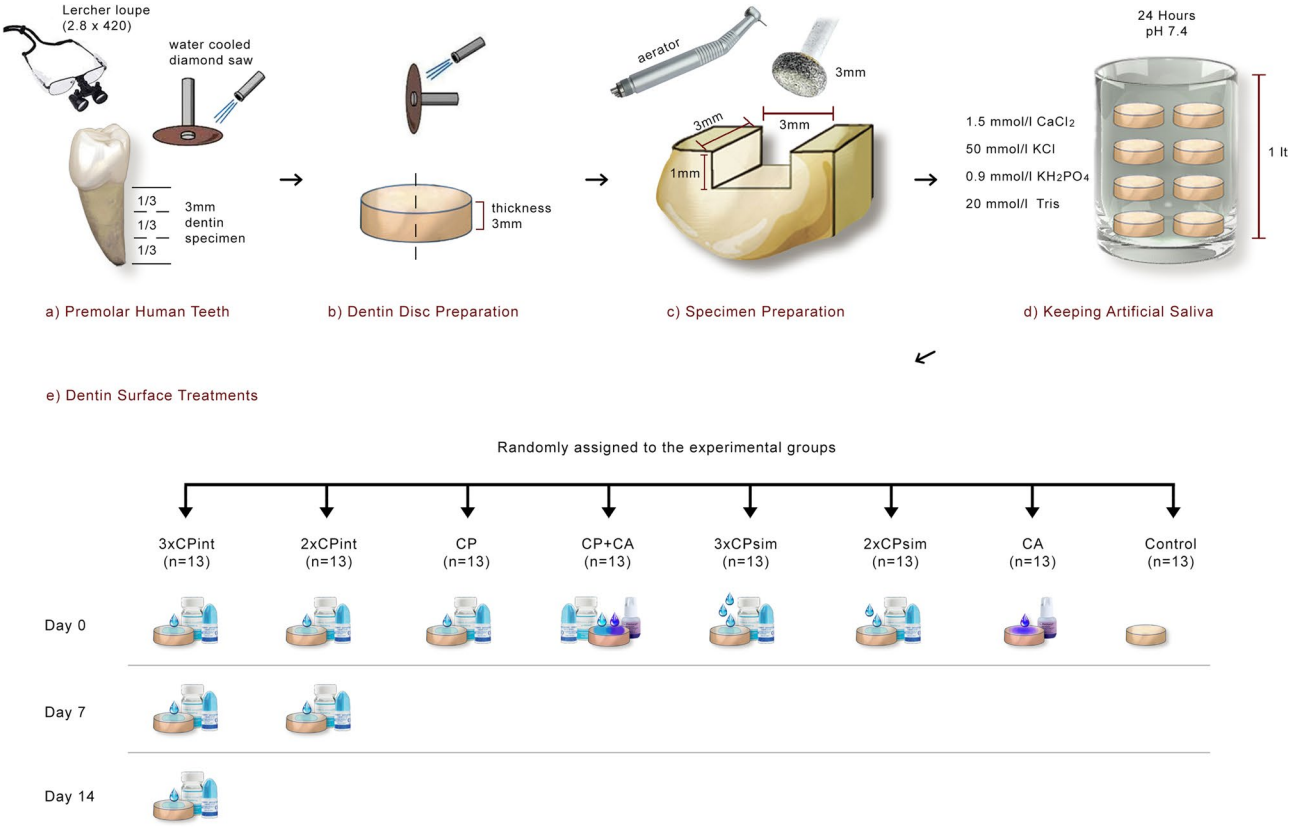


Fig. 1 Flowchart of the study: (**a**) The extracted teeth were observed in detail with a Lercher loupe (2.8 x 420) to exclude cavities, cracks, or fractures on the tooth surface. The crowns of teeth and apical third of the root were cut and removed; (**b**) The disk-shaped dentin fragments were obtained using a double-sided diamond saw under water cooling. The dentin fragments were divided into two parts; (**c**) 3x3x1 mm cavities were drilled with a diamond fissure drill to limit the application area in the center of the dentin specimens; (**d**) All groups were kept on hold in artificial saliva for 24 hours after the last administration until the examination and during the period between applications; (**e**) Specimens were randomly divided into eight groups of 13 each

The specimens were kept in saline solution until use. Subsequently, the dentin pieces were immersed in a 17% ethylenediaminetetraacetic acid (EDTA) (pH:7.4) solution (Ultradent Products, USA) for a duration of one minute to remove the smear layer on the surfaces. Following EDTA immersion, the specimens were rinsed with distilled water for ten seconds and subsequently air-dried for five seconds. The specimens were then randomly allocated into eight groups, with each group comprising 13 specimens. All groups were kept on hold in artificial saliva (1.5 mmol/l $CaCl_2$, 50 mmol/l KCl, 0.9 mmol/l KH_2PO_4 and 20 mmol/l, pH 7.4) [31] at room temperature for 24 h after the last administration until the examination. Additionally, in groups with intermittent application, the samples were stored in artificial saliva during the intervals between applications.

CP (Teethmate; Kuraray et al., Tokyo, Japan) was mixed with tetracalcium phosphate [Ca4(PO4)2O] and dicalcium phosphate anhydrous (CaHPO4) powder and water in 30 s and applied to the dry dentin surface for 30 s using a micro brush according to the manufacturer's instructions. The air-drying process was conducted with a distance of 5 cm for 10 s after each layer application from the same dental unit in simultaneously CP application groups (2xCPsim and 3xCPsim) and CP with CA group [32]. The intermittently CP application groups (3xCPint and 2xCPint), in which the layering applications were spaced one week apart, were preserved in artificial saliva during the period between applications. Groups containing CA, a single coat of CA (Peri Acryl; Glustitch et al.) were also treated with a micro brush.

The groups were as follows (Table 1);

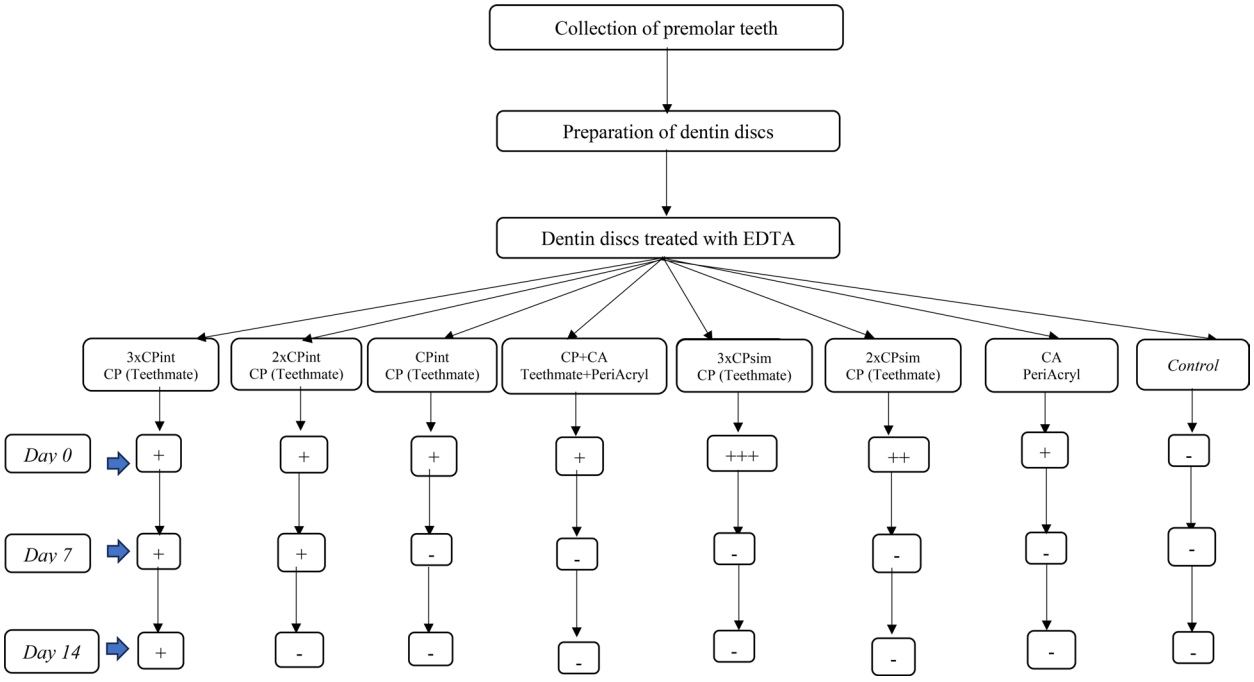
3xCPint ($n = 13$): Specimens were finished as a three-layer CP by applying a single layer of CP to the dentin surface on days 0, 7, and 14.

2xCPint ($n = 13$): Specimens were finished as a two-layer CP by applying a single layer of CP to the dentin surface on days 0 and 7.

CP ($n = 13$): A single layer of CP was applied to the dentin surface on day 0.

CP + CA ($n = 13$): A single layer of CP was applied to the dentin surface on day 0. Following air-drying of CP, CA was applied to the surface.

Table 1 Flow chart of the groups



3xCPsim ($n = 13$): Three layers of CP were applied to the dentin surface simultaneously on day 0.

2xCPsim ($n = 13$): Two layers of CP were applied to the dentin surface simultaneously on day 0.

CA ($n = 13$): A single layer of CA was applied to the dentin surface on day 0.

Control ($n = 13$): No materials were performed to the dentin surface.

Semi-quantitative measurements with SEM

After the application of desensitizers, the dentin samples were allowed to dry for 24 h at room temperature. The specimens were individually taped to the specimen holders using double-sided adhesive tape and placed in an ion sprayer (Polaron, SC502 Sputter Coater, Italy) to coat the dentin surfaces. They were then coated with a thin gold layer at a pressure of 2 mbr/Pa and a power of 10 mA for 120 s. The coated samples were examined in SEM (JSM-6060LV, JEOL, Japan) at 15 kV and 10 kV accelerating voltage, zero-degree tilt angle, and 11 mm working distance. SEM images were obtained at two magnifications (1000x and 2000x) from a total of 104 specimens.

All morphometric measurements and categorizations were performed by a single researcher, who was blinded to the treatment approach, based on the analysis of SEM images [33, 34]. The number of completely exposed dentinal tubules (CET), the number of semi-exposed dentinal tubules (SET) and the number of completely exposed tubules (SET + CET) were determined by marking the calibrated 1000x micrographs using the gray pixel intensity difference between the dentinal tubules and the surrounding area in the Image J program (Fig. 2a-b). Since the number of completely occluded dentinal tubules (COT) could not be counted, the count of COT was calculated for each sample by subtracting the total of the numbers CET and SET obtained from the specimens in the relevant group from the mean of the total count of CET in the control group. The mean diameter measurements of completely exposed dentinal tubules (CET_R) were made using the measurement scale of the Image J program (Fig. 2c) [35, 36]. Percentage values of the number of all exposed tubules (CET or SET);

$$\textit{The number of CET or SET} / \textit{The number of SET in the control group } x ; 100$$

was calculated using the formula . [31], [37]

To determine the pattern of surface deposition of the applied agents, the 2000x micrographs were categorized according to the following descriptive characteristics [31, 38].

A: Dentinal tubules are partially occluded and smaller, with little or no debris on the application surface.

B: Dentinal tubules are mostly occluded, with no film or debris on the application surface.

C: Dentinal tubules are mostly occluded; a film layer or debris partially covers the application surface or has some surface changes.

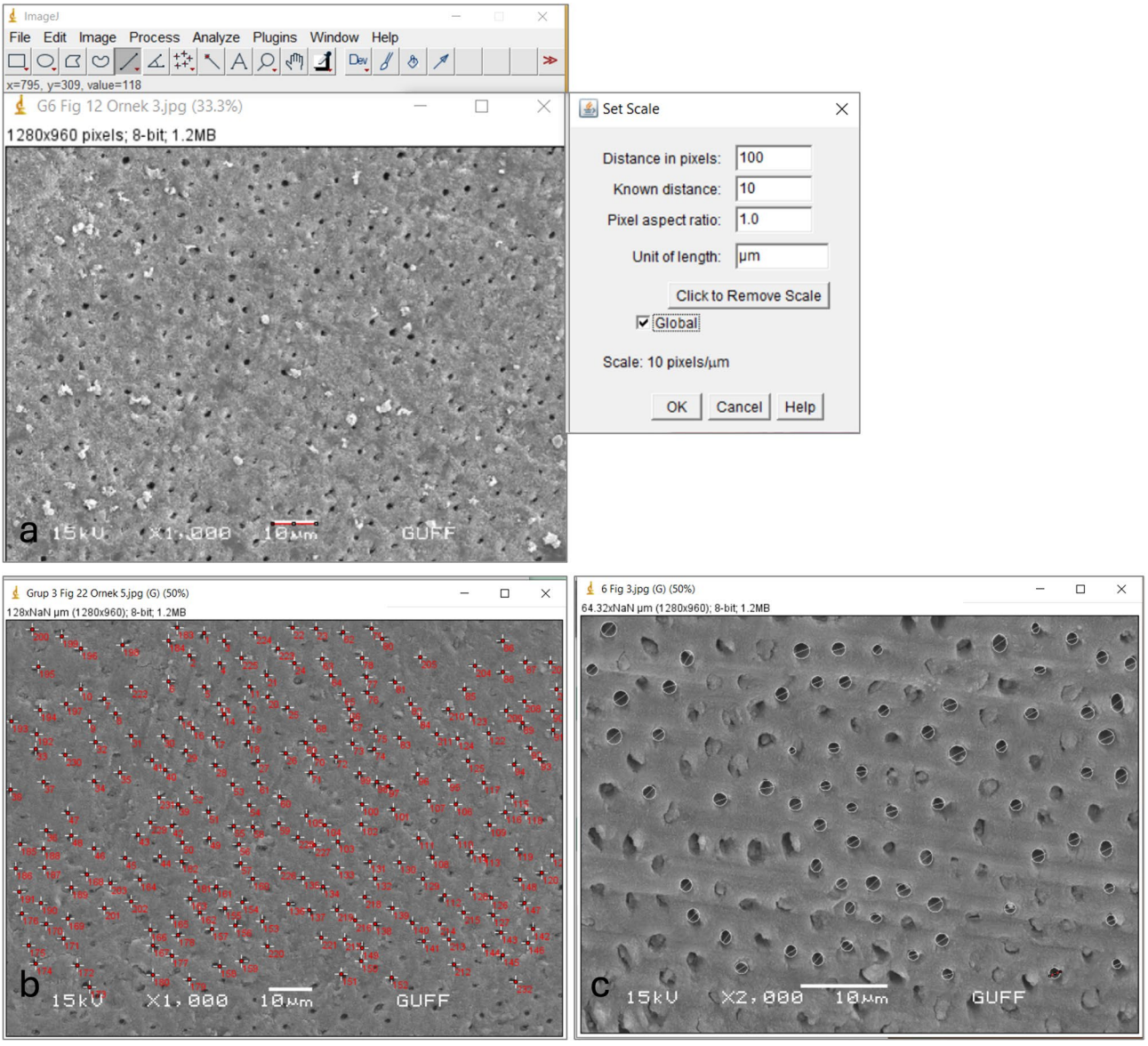


Fig. 2 **a** Loading SEM micrographs into the ImageJ software and calibrating the micrographs using the reference scale bar; **b**) Counting exposed and partially occluded dentinal tubules using the ImageJ image analysis software; **c**) Calculating the area and mean diameter of open dentinal tubules using the ImageJ software

D: All dentinal tubules are occluded, and the application surface is covered with a precipitate film and/or a resin.

Statistical analysis

The G-POWER 3.1 program was used in the study to determine the sufficient sample volume. The effect size was calculated as = 0.3905411. The sample size was set to 104 samples to obtain the test power (1-β) = 0.80, with a minimum of 13 samples in each group.

Data were analyzed using IBM SPSS V23. Quantitative values of tubules examined at 1000x magnification of SEM micrographs of eight different groups were evaluated using the Shapiro-Wilk test for normal distribution. The Kruskal-Wallis H test was used for group comparison of these data that did not show normal distribution, and multiple comparisons were examined with the Dunn test. Since the data did not show normal distribution, Spearman's rho coefficient was used to analyze the relationship between the number and diameter of all exposed tubules. Quantitative data were expressed as medians with interquartile ranges (IQRs), while categorical variables obtained from ×2000 magnification of SEM micrographs were presented as percentages. Significance level was taken as $p < .05$.

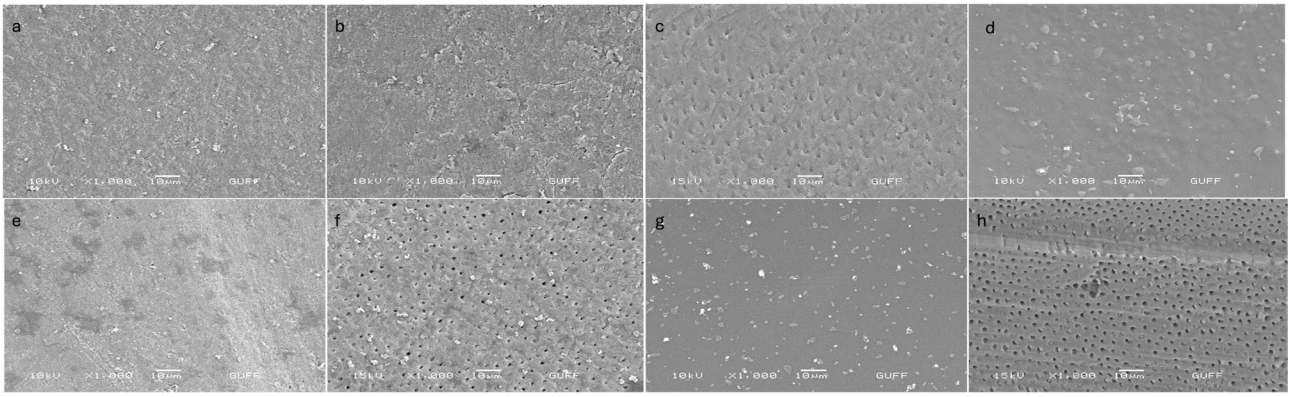


Fig. 3 SEM images (x1000) illustrate the degree of dentinal tubule occlusion after applying different treatment agents and methods: (**a**) 3xCPint (three-layer calcium phosphate a significant material accumulation closes most tubules and narrows their diameters; (**b**) 2xCPint (two-layer calcium phosphate), most tubules are sealed, with remaining ones showing reduced diameters; (**c**) CP (immediate single-layer calcium phosphate), a thinner particulate layer penetrates and narrows tubules but is less extensive than multilayer groups; (**d**) CP+CA (one layer calcium phosphate + cyanoacrylate), nearly all tubules are sealed, with the rest showing diameter reduction.; (**e**) 3xCPsim (three layers calcium phosphate), most tubules are sealed, with the remaining ones slightly narrowed; (**f**) 2xCPsim (two layers calcium phosphate), a thinner particulate structure narrows tubules compared to other multilayer groups; (**g**) CA (cyanoacrylate), nearly all tubules are sealed, with a surface film layer due to cyanoacrylate content and (**h**) control, open tubules of varying diameters remain aligned parallel to each other

Table 2 Comparison of the diameter of completely exposed tubules and number of completely or semi-exposed dentinal tubules

	3xCPint ***n*** **= 13**	**2xCPint** ***n*** **= 13**	**CP** ***n*** **= 13**	**CP + CA** ***n*** **= 13**	**3xCPsim** ***n*** **= 13**	**2xCP-sim** ***n*** **= 13**	**CA** ***n*** **= 13**	**Control** ***n*** **= 13**	***p***
Completely exposed dentinal tubules diameters (CET_R) (µm)	0.75 (0–0.87)^{ab}	0.77 (0–1)^{abc}	0.83 (0.7–0.93)^{bc}	0 (0–2)^{ab}	0.2 (0.59–0.98)^{bc}	0.81 (0–1)^{bc}	0 (0–0.74)^{a}	1 (0.5–2)^{c}	**< 0.001***
Number of completely or semi-exposed dentinal tubules (CET + SET) (n)	3 (0–12)^{abcd}	2 (0–5)^{abc}	6,5 (1–89)^{cde}	0 (0–4)^{ab}	5 (1–9)^{bcd}	20 (0–140)^{de}	0 (0–6)^{a}	187 (149–266)^{e}	**< 0.001***

a-e There is no difference between groups with the same letter

* Kruskal Wallis H test, Median (Min. – Max)

Results

The number of exposed tubules was counted for each group on SEM micrographs. According to the data obtained, all treatment groups achieved successful results in terms of narrowing of dentinal tubule diameters compared to the control group (p <.001) (Fig. 3).

Evaluation of the number of semi or completely exposed tubules

The number of semis or completely exposed tubules (SET + CET) was statistically lower in all test groups than in the control group (p <.001). Among the test groups, the number of SET + CET was significantly lower in both CA groups (CP + CA and CA) compared to the other groups (p <.001). There was no significant difference in the number of SET + CET between the 3xCPint and 2xCPint groups and both CA groups (CP + CA and CA). The number of SET + CET was found statistically lower in the 2xCPint group than in the 2xCPsim group (p <.001). When comparing the CP groups with different applications (3xCPint, 2xCPint, CP, 3xCPsim, 2xCPsim) with each other, the number of SET + CET was shown statistically higher in the 2xCPsim group than in the other groups (p <.001) (Table 2).

Evaluation of mean diameters of completely exposed dentin tubules

There was no statistically significant difference in CET _R between the 3xCPint and 2xCPint groups and both CA (CP + CA and CA) groups. When the CP groups with different applications (3xCPint, 2xCPint, CP, 3xCPsim, 2xCPsim) were evaluated against each other, the 3xCPint group was found statistically lowest; in contrast, the CP group had the superior (p <.001) (Table 2).

Comparisons of the number of completely exposed, semi-exposed, and completely occluded tubules among the groups

Among the groups, CA groups (CP + CA and CA) had more COT and less SET than the other groups (p <.001). Among the CP groups (3xCPint, 2xCPint, 1xCP, 3xCP-sim, 2xCPsim), the 3xCPint and 2xCPint groups, where the shift application was one week apart, showed similar

results to the CP + CA and CA groups in terms of the number of COT and SET. Among the CP groups with different applications, the CP group with single application and the 3xCPsim group with simultaneously triple application showed a similar effect regarding the number of SETs. The 2xCPsim group with simultaneous double application statistically had more SET and less COT than the 2xCPint group with intermittent double application ($p < .001$). The 2xCPsim group was statistically lower than the 2xCPint group in terms of dentinal tubule occlusion ($p < .001$). The comparison of the values of CET, SET, and COT between the groups is shown in Table 3.

Correlation analysis between the number and diameter of all exposed tubules

As a result of all groups, it was found that there was a statistically significant positive correlation between the number of CET + SET and CET _R ($r = .719$; $p < .001$).

Determination of surface properties

The half-images of the surface properties of the agents applied to each group were categorized, and the frequency values (percentage) of these categories belonging to the groups were calculated. Accordingly, category C was the most frequent category with 70.6%, 60%, 58.3%, 92.3% and 46.2% in 3xCPint, 2xCPint, CP, 3xCPsim and 2xCPsim groups, respectively, while CP + CA and CA category of group D was 69.2% (Fig. 4a-b).

Discussion

The hypothesis of this study was that the combined application of two agents with different effect mechanisms would increase the efficiency of tubule occlusion. To test this, the effect of a CP ($Ca_3(PO_4)_2$)-containing desensitizer applied in multiple layers to the root surfaces, at one-week intervals and simultaneously, a CA agent applied to the root surfaces and the combined application of both agents on occluding the dentinal tubules was evaluated.

DH is closely associated with the exposure of root dentinal tubules to the oral environment, which occurs following removal of the smear layer [38]. To replicate this clinical condition in vitro and allow accurate evaluation of the test agents, specimens in the present study were pretreated with 17% EDTA. EDTA is a chelating agent that effectively demineralizes dentin and removes the smear layer, thereby exposing tubules and creating a standardized substrate for analysis [3, 22, 39, 40]. The extent of DH is known to be directly related to both the number and diameter of patent dentinal tubules, which determine the transmission of external stimuli to the pulp [41, 42]. For this reason, quantitative assessment of tubule occlusion is essential when investigating desensitizing treatments. Several microscopic techniques, including SEM [22], stereomicroscopy [43], atomic force microscopy [3], and confocal laser scanning microscopy [44] have been employed for this purpose. Among these, SEM remains the most widely used due to its ability to provide high-resolution, three-dimensional surface images and detailed visualization of dentinal tubule morphology [3, 22, 45]. Consequently, to evaluate the efficacy of the test agents employed in our study for DH treatment in vitro, the diameter and area of exposed dentinal tubules, the number of open tubules, and the number of semi-occluded tubules were evaluated using SEM, in accordance with the studies in the literature [3, 22, 46, 47]. This approach facilitates effective comparison of our groups.

The Image J program, widely regarded as one of the most effective methods, was utilized to standardize the evaluation of dentinal tubules in numerous in vitro studies [48–51]. In the present study, this program was employed to analyze the micrographs taken for each

Table 3 Comparison of the completely exposed tubules, completely occluded tubules and semi-exposed tubules between groups

Variables	3xCPint *n* = 13	2xCPint *n* = 13	CP *n* = 13	CP + CA *n* = 13	3xCPsim *n* = 13	2xCPsim *n* = 13	CA *n* = 13	Control *n* = 13	*p**
Completely exposed tubules (CET, n)	0 (0–2)^{a}	0 (0–0)^{a}	0 (0–29)^{a}	0 (0–1)^{a}	0 (0–3)^{a}	0 (0–21)^{a}	0 (0–0)^{a}	187 (149–266)^{b}	< 0.001
% Completely exposed tubules (%CET)	0 (0–1.01)^{a}	0 (0–0)^{a}	0 (0–14.57)^{a}	0 (0–0.5)^{a}	0 (0–1.51)^{a}	0 (0–10.55)^{a}	0 (0–0)^{a}	100 (100–100)^{b}	< 0.001
Completely occluded tubules (COT, n)	196 (187–199)^{bcde}	197.5 (194–199)^{cde}	192.5 (110–198)^{abc}	199 (195–199)^{de}	194 (190–198)^{bcd}	179 (59–199)^{ab}	199 (193–199)^{e}	0 (0–0)^{a}	< 0.001
Semi-exposed tubules (SET, n)	3 (0–12)^{bcd}	2 (0–5)^{abc}	6.5 (1–60)^{cd}	0 (0–4)^{ab}	5 (1–9)^{cd}	20 (0–125)^{d}	0 (0–6)^{ab}	0 (0–0)^{a}	< 0.001
%Semi-exposed Tubules (%SET)	1.51 (0–6.03)^{bcd}	1.01 (0–2.51)^{abc}	3.26 (0.5–30.15)^{cd}	0 (0–2.01)^{ab}	2.51 (0.5–4.52)^{cd}	10.05 (0–62.81)^{d}	0 (0–3.01)^{ab}	0 (0–0)^{a}	< 0.001

Median (Min. – Max.)

a-e There is no difference between groups with the same letter

*Kruskal Wallis H test

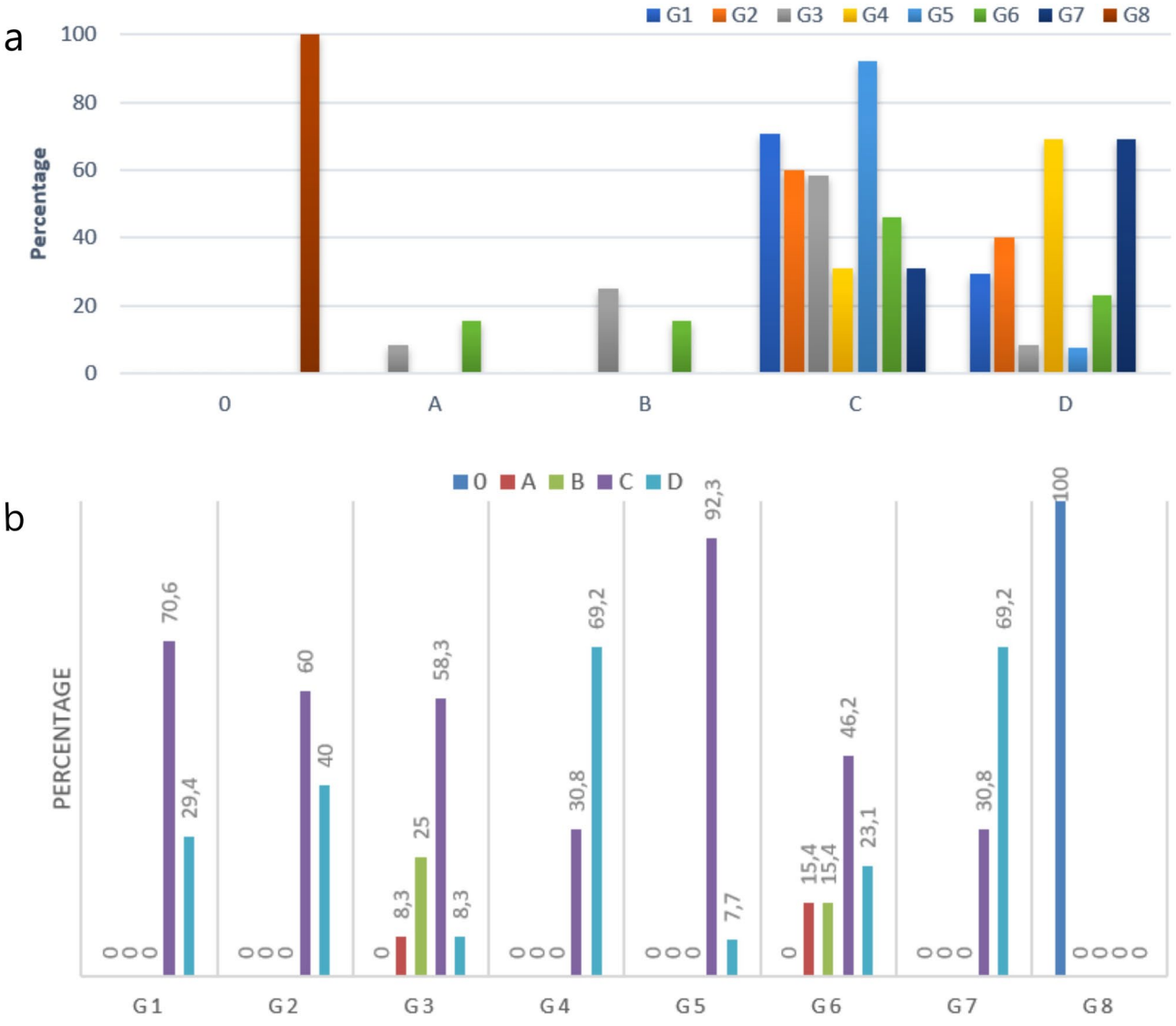


Fig. 4 **a** Distribution of groups by categories in terms of surface properties (**b**) Distribution of surface property categories by groups

sample to extract numerical data, thereby enabling the assessment of the morphological details of the dentin surface. Following the loading of the micrographs into the program, the CET and SET were marked, and the SET + CET and CET_R were calculated. The present study evaluated the correlation between CET_R and SET + CET, and a statistically significant and positive correlation was found. In other words, it was found that the CET_R value was high even in the samples with a high number of SET + CET. This finding provides evidence that the degree of tubule occlusion caused by the agents applied to the test samples can be determined.

The hypothesis that the layered or combined application of desensitizing agents enhances the effectiveness of dentinal tubule occlusion has been postulated. Zhou et al. reported that the three-layer application of CP occluded the dentinal tubules more effectively than the single-layer application of CP [22]. The CP-containing agent utilized in this study penetrates dentinal tubules in an aqueous environment, converting into hydroxyapatite, which is the primary component of both dentin and enamel, thereby exerting its effects [21]. Research indicates that the post-application interval allows the CP to reach the necessary saturation levels of hydroxyapatite, calcium, and phosphate for effectively occluding the dentinal tubules [22, 24, 52]. Additionally, the intermittent application of the CP has been postulated to facilitate the hardening of the agent within the tubules, which may result in a more rigid structure during subsequent application sessions [24]. Furthermore, Nazım et al. found that the mean open dentinal tubule diameter in the combined application group (CP + CA) (median: 0.25 µm) was statistically significantly smaller than that in the CP-alone application group (median: 1.24 µm) [46]. The findings

of our study are consistent with these reports in the literature. Specifically, the intermittent application of two or three CPs was found to be more effective in occluding dentinal tubules than the simultaneous application of CPs, suggesting that this method may be a promising approach for reducing DH. However, there is a paucity of studies comparing different layering techniques in the literature, which limits direct comparisons with other methods. This highlights the need for future studies to investigate the efficacy of various application intervals and explore new strategies for optimizing the layering process.

CA provides a physical barrier by sealing dentinal tubules, thereby preventing external stimuli from reaching the pulp [53]. In a clinical study, Barroso et al. reported that CA demonstrated good results in the treatment of DH and was 7% more effective compared to the control group [26]. In a related study, Perez et al. reported that the effect of CA on DH was similar to that of certain techniques (laser + fluoride varnish, laser + propolis). However, they stated that the faster and simpler application of CA could be considered an advantage over other techniques [27]. In contrast calcium phosphate compounds have been shown to penetrate the tubules and promote dentin remineralization through the formation of hydroxyapatite-like precipitates [21]. To date, however, no specific study has investigated the synergistic effects of CA and CP agents in the treatment of DH [54]. Nevertheless, both agents have been reported to effectively reduce hypersensitivity by occluding dentinal tubules [46]. Based on our in vitro findings, we hypothesize that while CA may facilitate immediate tubule closure, CP could contribute to the longer-term stability of the barrier through sustained mineralization. Moreover, the polymeric structure formed by CA might enhance the retention of CP precipitates, while calcium phosphate deposition may support a more biocompatible healing process by forming hydroxyapatite-like structures resembling dentin.

One of the major concerns regarding the long-term effectiveness of desensitizing agents is their susceptibility to chemical and mechanical stress, which can lead to gradual degradation and reduced efficacy over time. Chemical stress factors are caused by external factors such as acidic drinks and dietary acids. Studies have shown that low pH environments can partially dissolve the precipitates formed by calcium phosphate-based agents, leading to reopening of dentinal tubules and subsequently a decrease in the effectiveness of the desensitizing agent [55, 56]. Other studies have reported that CP is an acid-sensitive material and that this problem can be reduced by applying a thicker layer [10, 20]. In order to enhance the durability of the material in relation to mechanical and chemical factors, the present study aimed to enhance the effectiveness of CP with the same number of layers by applying them at different intervals. In addition, Nazım et al. reported that CA can be a material resistant to oral fluids and acids [46]. In line with these studies, the present study demonstrated that CA application on CP and intermittent layered CP application increased the degree of tubular occlusion.

Several studies have demonstrated that air-drying parameters—such as distance, pressure, and duration—significantly influence dentin surface characteristics, including the thickness of the applied layer and the bonding strength [22, 32, 57]. As reported in previous studies El-Askary et al. [58, 59] reported that shorter air-drying distances improved bond strength, whereas increasing the distance resulted in thicker surface layers, while Chiba et al. [57] and Lee et al. [32] emphasized that prolonged drying times could reduce bond strength and alter layer thickness. Collectively, these findings highlight the importance of standardizing air-drying conditions when evaluating desensitizing agents. In the present study, to avoid variability, air-drying was performed at a fixed distance of 5 cm for 10 s in all groups. Despite this standardization, our results showed that intermittently applied CP at 7-day intervals produced significantly better tubule occlusion compared with simultaneous CP application. This suggests that the air-drying step, when performed during simultaneous layering, may compromise the efficacy of CP in sealing dentinal tubules.

In many in vitro studies evaluating DH using different materials and methods, it was observed that the materials used had different deposition patterns on the sample surfaces [31, 37, 60]. In the study by Al-Saud et al., the surface categories on SEM images proposed by West et al. [60] were modified, and the dentin surface characteristics of the specimens were categorized [31]. The results indicated that the potassium fluoride-containing agent in the study formed a film layer on the dentin surface that occluded all dentinal tubules, similar to the agent containing CA used in the present study. In accordance with the study by Al-Saud et al. [31], the samples were categorized to qualitatively evaluate the dentin surface properties after the application of the test substances. It was found that CA completely occluded all dentinal tubules, while CP mostly occluded the dentinal tubules and partially covered the application surface with a film layer or residue.

Despite the prominence of the findings of this study, there are some limitations of this study. First, the implementation of a study that includes a method to examine the synergistic mechanisms between CA and CP in the management of DH is crucial to determine their long-term effectiveness. However, evaluating the resistance and durability of CA with CP or intermittent applications of CP under chemical and mechanical stress conditions,

which we have obtained promising results, may provide information closer to the clinical need. Consequently, further research is necessary to develop strategies aimed at enhancing the stability of CP and CA in conjunction with CP.

Conclusion

In conclusion, layered CP applications (two or three layers) applied intermittently appeared more effective than single or simultaneously layered applications. Under the conditions of this in vitro study, CA-containing agent showed the highest level of tubule occlusion, either alone or in combination with CP. While CA demonstrated effectiveness in vitro, layered CP applications at one-week intervals may represent a more clinically applicable approach due to CA's short retention time and susceptibility to mechanical removal. Nevertheless, further in vitro and clinical studies are needed to evaluate resistance to acid exposure, brushing abrasion, and artificial saliva, as well as to clarify synergistic mechanisms and long-term efficacy.

Abbreviations

CA	N-butyl cyanoacrylate
CET	Completely exposed dentinal tubules
CET_R	Diameter measurements of completely exposed dentinal tubules
CP	Calcium phosphate desensitizer
CPsim	Application of calcium phosphate desensitizer simultaneously
CPint	Application of calcium phosphate desensitizer intermittently
COT	Completely occluded dentinal tubules
DH	Dentin hypersensitivity
EDTA	Ethylenediaminetetraacetic acid
SEM	Scanning electron microscopy
SET	Semi exposed dentinal tubules

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Authors' contributions

Özge Uzuner Bilgiç: Methodology; project administration; writing – original draft; investigation; formal analysis. Sühan Gürbüz: Methodology, writing – review and editing; conceptualization; investigation; writing – original draft; supervision. Altan Doğan: Project administration, writing – original draft; supervision.

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Data availability

Data sets from the present study are available and can be forwarded to the corresponding author's e-mail address upon reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Clinical Research Ethics Committee of the Faculty of Dentistry, Gazi University (Ethics Committee approval date: 24/09/2020 Decision No: GUDHKAEK 2020.20/3). The authors stated that all experiments were performed in accordance with relevant guidelines and regulations. Informed consent was obtained from patients for the collection of isolated teeth.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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