RDE Restorative Dentistry & Endodontics

Research Article

eISSN 2234-7666
Restor Dent Endod. 2026 February;51(1):e8
https://doi.org/10.5395/rde.2026.51.e8

Comparative evaluation of dentinal tubule occlusion by desensitizing agents after tooth bleaching: an *in vitro* study

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ABSTRACT

Objectives: This study aimed to evaluate the efficacy of three commercially available desensitizing agents in occluding dentinal tubules, which may help reduce tooth sensitivity following a bleaching treatment.

Methods: Twenty healthy human third molars were utilized in this investigation. The samples were prepared by transversely sectioning 2.5 mm of the crowns to expose the dentin. They were initially treated with 15% ethylenediaminetetraacetic acid gel for 4 minutes, followed by application of Perfect Bleach (VOCO GmbH) bleaching agent (16% carbamide peroxide) for 2 hours. The samples were randomly allocated into four groups ($n = 5$), each receiving one of the following treatments: group 1: No treatment (control), group 2: treated with UltraEZ (Ultradent Products Inc.,), containing potassium nitrate and sodium fluoride, group 3: treated with Perfect Protect (VOCO GmbH), also containing potassium nitrate and sodium fluoride and group 4: treated with TheraSol Whitening & Sensitive (ABC Kinitron IKE), containing strontium acetate and sodium monofluorophosphate. Subsequently, the specimens were examined using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy to evaluate dentin tubule occlusion.

Results: SEM observations showed no occlusion of dentin tubules in the control group, whereas groups 2 to 4 exhibited significant occlusion. The most effective treatment was Perfect Protect ($p < 0.05$), while UltraEZ and TheraSol Whitening & Sensitive demonstrated similar effectiveness, with no statistically significant difference between them ($p > 0.05$).

Conclusions: The tested desensitizing agents effectively occluded dentin tubules to a considerable extent. Differences in their effectiveness were attributed to variations in their formulations.

Keywords: Protect dentin desensitizer; Scanning electron microscopy; Tooth bleaching; X-ray emission spectrometry

INTRODUCTION

Tooth sensitivity (TS) after tooth bleaching is a common, temporary side effect resulting from the strong bleaching agents typically used during the procedure, including hydrogen peroxide (H_2O_2) or carbamide per-

Received: July 16, 2025 **Revised:** October 6, 2025 **Accepted:** October 27, 2025

Citation
Dionysopoulos D, Mourouzis P, Papageorgiou S, Tolidis K. Comparative evaluation of dentinal tubule occlusion by desensitizing agents after tooth bleaching: an *in vitro* study. Restor Dent Endod 2026;51(1):e8.

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oxide ($CH_6N_2O_3$) in various concentrations [1]. These substances penetrate through the enamel and extend into the dentin, irritating the nerve endings inside the teeth. Patients may experience sharp, transient pain or increased sensitivity to hot, cold, or sweet foods and drinks, usually for a few days. Sensitivity usually subsides within 1 to 4 days after treatment, but it can be managed with desensitizing gels, fluoride treatments, and avoiding extreme temperature changes [2].

This sensitivity is thought to arise when H_2O_2 molecules pass through the enamel and dentin, ultimately reaching the pulp and inducing an inflammatory response [2]. It is important to note that this mechanism differs from the typical causes of dentin hypersensitivity (DH). The most common theory proposes that DH results from stimulus-induced fluid movement within the dentin tubules, which subsequently activates nociceptors at the pulp-dentin interface [3]. It is postulated that this fluid shift stimulates intradental myelinated A-fibers, along with some unmyelinated A-fibers, resulting in the characteristic short, sharp pain associated with DH [4]. While bleaching-induced sensitivity is usually temporary, individuals with pre-existing DH may experience more intense and prolonged discomfort following the procedure [5].

TS has been encountered in 49%–100% of patients undergoing tooth bleaching in previous clinical studies [6]. The severity of TS is influenced by various parameters, including the type and composition of the bleaching agent, the duration of the procedure, the precision of the technique, and any pre-existing TS [7]. While the exact mechanism behind bleaching-induced TS remains unclear, it is believed to result from the penetration of H_2O_2 into the dental pulp [8]. Because of its low molecular weight (34.01 g/mol), H_2O_2 and its byproducts, including reactive oxygen species, can pass through the permeable enamel and dentin, and they reach the pulp tissue and initiate the release of inflammatory mediators, like interleukin-1β and receptor activator of nuclear factor kappa B ligand, which initiate an inflammatory response [9]. In this case, pulpal pressure increases, resulting in an increased outflow of fluid. This mechanism amplifies the responsiveness of the pulp nerves, making them more sensitive than they normally are.

To manage TS at home, patients are recommended to use specially formulated toothpastes, gels, and mouthwashes designed to effectively alleviate discomfort [1]. Such products may contain potassium salts [10], sodium fluoride [11], stannous fluoride [12], arginine [13], nano-hydroxyapatite [14], bioactive glass [15], casein phosphopeptide-amorphous calcium phosphate [16], or strontium salts [17]. These active agents work by either blocking dentin tubules or inhibiting nerve impulse transmission [18].

Fluoride-based agents are commonly used for this purpose, acting by promoting remineralization of the tooth hard tissues and by occluding dentinal tubules with calcium fluoride (CaF_2) crystals [11]. Moreover, potassium nitrate-containing agents are often used after tooth bleaching treatments. Potassium nitrate diffuses along dentinal tubules to depolarize nerve endings, reducing nerve excitability [19]. Recently, strontium-based agents have been suggested as a means to alleviate symptoms of TS. Strontium acetate works mainly by occluding dentinal tubules, thereby reducing the transmission of stimuli to the tooth pulp. In particular, strontium ions (Sr^{2+}) precipitate as strontium carbonate or strontium phosphate within open dentinal tubules, creating a physical barrier that blocks fluid movement inside the tubules [20].

Additionally, various techniques have been proposed for managing TS in dental practice, with studies producing divergent results. Dentist-applied treatments that inhibit nerve impulse transmission include gels containing potassium salt [21] and low-level laser therapy [6]. Meanwhile, in-office approaches aimed at occluding dentin tubules involve the application of fluoride-containing varnishes or gels [22], silver diamine fluoride [23,24], oxalate salts [25], air-abrasion with bioactive glasses [26], adhesive agents [27], and high-intensity laser irradiation [28].

This laboratory investigation aimed to assess the efficacy of three contemporary commercially available desensitizing agents in occluding dentin tubules to reduce TS following a bleaching procedure. While various desensitizing agents have been previously studied, there is limited evidence on the performance of recently introduced commercial formulations. Two of the tested products were recently introduced to the market and contained a combination of potassium nitrate (KNO_3)

https://doi.org/10.5395/rde.2026.51.e8

and sodium fluoride (NaF), as well as strontium acetate ($C_4H_6O_4Sr$) and sodium monofluorophosphate (MFP). An older product served as a positive control. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were used to evaluate the extent of dentin tubule occlusion and analyze the composition of the resulting precipitates. Two null hypotheses were proposed:

H_{01}: The tested desensitizing treatments would not result in the same degree of dentinal tubule occlusion as observed in the untreated control group.

H_{02}: The tested treatments would not exhibit equivalent effectiveness in occluding dentin tubules.

METHODS

Samples preparation

This study was approved by the Ethics and Research Committee of Aristotle University of Thessaloniki (No. 224/21-03-2024) and conducted in accordance with the ethical principles outlined in the 1964 Declaration of Helsinki and its subsequent revisions, as well as the regulations of Aristotle University of Thessaloniki. Informed consent was obtained from all patients for the use of their extracted teeth in research.

A total of twenty healthy human third molars, extracted for periodontal reasons at the Clinic of Oral Surgery, Aristotle University of Thessaloniki, were collected and preserved in a 0.5% chloramine T solution at 6°C for a maximum of 2 months.

To approach the dentin surface, the enamel was carefully sectioned at the midpoint of the crown's occlusal-cervical dimension (approximately 2.5 mm below the occlusal surface) utilizing a low-speed precision cutting device (Isomet 11-1180, Buehler, Lake Bluff, IL, USA) under continuous water rinsing. Additionally, 1 mm-thick sections were also obtained from each tooth to examine the penetration depth of dentin tubule occlusion. The sectioned surfaces were then polished using a grinding device (Jean Wirtz TG 250, Jean Wirtz GmbH, Düsseldorf, Germany) operating at 200 rpm under constant water flow (50 mL/min). Sequential polishing was performed using silicon carbide abrasive papers (Apex S system, Buehler) in grits of 600, 800, 1,000, and 1,200, each applied for 20 seconds.

Next, the dentin surfaces were conditioned with 15% ethylenediaminetetraacetic acid (EDTA; Endo-Prep Cream, CERKAMED, Stalowa Wola, Poland) for 4 minutes to remove the smear layer from the dentinal tubule openings. This was followed by thorough rinsing with distilled water and subsequent air-drying. To ensure complete removal of any residual smear layer, the samples were then immersed in an ultrasonic bath (Euronda Spa, Montecchio Precalcino, Vicenza, Italy) for 5 minutes. After treatment, all specimens were stored in artificial saliva at 37°C. The artificial saliva was composed of 0.103 g/L $CaCl_2$, 0.019 g/L $MgCl_2 \bullet 6H_2O$, 0.544 g/L KH_2PO_4, 2.24 g/L KCl, and buffer (TCP-KOH) was added to adjust the pH to 7 [29].

Bleaching procedure

The cut surfaces of the tooth specimens were subjected to a whitening procedure using a 16% carbamide peroxide bleaching agent (Perfect Bleach, VOCO GmbH, Cuxhaven, Germany). The gel was applied according to the manufacturer's instructions, forming a consistent 1 mm layer evenly distributed over the entire surface of each specimen. The bleaching agent was applied to the dentin surfaces for 2 hours, simulating the first day of a 7–14 day at-home bleaching protocol. The gel remained in place for 2 hours before the samples were carefully rinsed with distilled water and gently brushed with a toothbrush to remove any residual gel. Eventually, all samples were placed in artificial saliva and maintained at 37°C.

Experimental groups

After the bleaching process, the tooth specimens were randomly assigned to four groups (n = 5) and subjected to different desensitizing protocols as follows:

Group 1 (negative control): No desensitizing treatment was applied.

Group 2: UltraEZ (Ultradent Products Inc., South Jordan, UT, USA) desensitizing gel, which contains 3% KNO_3 and 0.25% NaF, was applied evenly and left on the dentin for 60 minutes. The samples were then rinsed with distilled water, brushed with a toothbrush, and stored in artificial saliva at 37°C. This product served as the positive control in the study, as it has been extensively investigated and is the older one.

Group 3: the specimens were smeared with Perfect Protect (VOCO GmbH) desensitizing gel, which contains 3% KNO_3 and 0.11% NaF, and left for 60 minutes on the dentin. Next, the specimens were rinsed with distilled water, brushed with a toothbrush, and stored in artificial saliva at 37°C.

Group 4: TheraSol Whitening & Sensitive (ABC Kinitron IKE, Athens, Greece) desensitizing gel, containing 8% strontium acetate, was applied evenly and left on the dentin for 60 minutes. The specimens were then rinsed with distilled water, brushed with a toothbrush, and stored in artificial saliva at 37°C. The application of the tested materials was performed according to the manufacturer's instructions. The technical information of the commercial products used in the current study is shown in Table 1.

Scanning electron microscopy observations and energy-dispersive X-ray spectroscopy analysis

To evaluate the impact of the tested treatments on dentinal tubule occlusion, SEM (JSM-840, JEOL Ltd., Tokyo, Japan) was employed. Observations were focused on the central region of the sectioned dentin surface, specifically within a defined square area measuring 4 mm by 4 mm (Figure 1). Before observations, all specimens were dried in a desiccator. The specimens were subsequently affixed to aluminum stubs and coated with a thin carbon layer approximately 200 Å thick using a low-vacuum evaporator. They were then examined under a SEM at an accelerating voltage of 20 kV.

A total of ten SEM images were captured at ×500 magnification from various regions of the dentin surface to assess alterations in surface morphology. Additionally,

Table 1. The active agents of the tested commercial products of the study, according to the manufacturers

Product	Manufacturer	Form	Application	Active agents
UltraEZ	Ultradent Products Inc., South Jordan, UT, USA	Gel	Once for 60 min after bleaching	3% KNO_3 (11,600 ppmK^+) 0.11% NaF (1,130 ppmF^-)
Perfect Protect	VOCO GmbH, Cuxhaven, Germany	Gel	Once for 60 min after bleaching	3% KNO_3 (11,600 ppmK^+) 0.11% NaF (1,130 ppmF^-)
TheraSol Whitening & Sensitive	ABC Kinitron IKE, Athens, Greece	Paste	Once for 60 min after bleaching	8% strontium acetate ($C_4H_6O_4Sr$) 0.76% MFP (1,000 ppmF^-)

KNO_3, potassium nitrate; MFP, sodium monofluorophosphate; NaF, sodium fluoride.

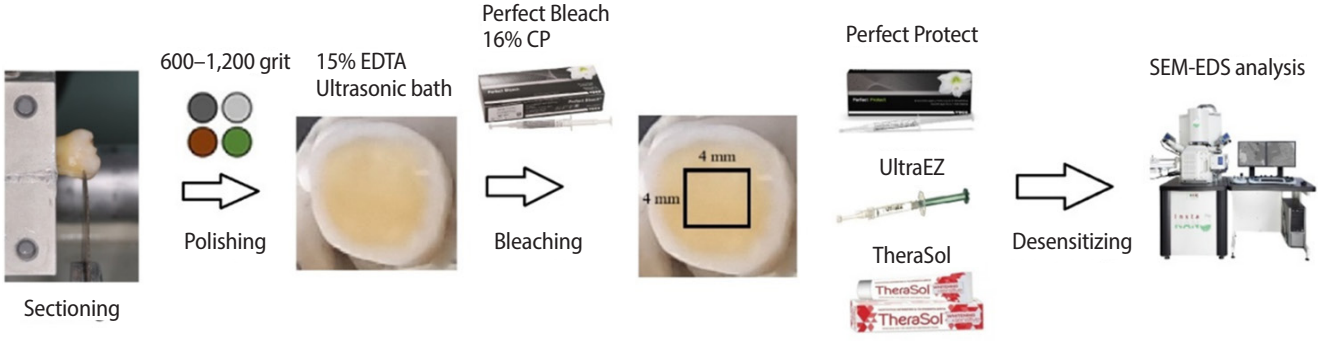

Figure 1. The workflow of the experimental part of the study. EDTA, ethylenediaminetetraacetic acid; CP, carbamide peroxide; SEM, scanning electron microscopy; EDS, energy-dispersive X-ray spectroscopy.

https://doi.org/10.5395/rde.2026.51.e8

ten more images were acquired at ×1,000 magnification to determine the percentage of dentinal tubules that were occluded, and at ×3,000 magnification to measure the degree of tubule occlusion and the posttreatment tubule diameters. For this purpose, a scale (0–2) was adopted, where "0" indicated no reduction of the diameter of the tubules (0%), "1" indicated a 50% reduction, and "2" a 100% reduction. Two experienced evaluators, blinded to the treatment groups, independently analyzed the images to assess the extent of tubule occlusion, and the average of their evaluations was recorded. Prior to SEM observations, the two examiners participated in a calibration session using a set of representative SEM images to standardize the assessment criteria for dentin tubule occlusion. Inter-examiner reliability was assessed using Cohen's kappa coefficient, which demonstrated good agreement ($\kappa = 0.82$). Additionally, EDS was employed to identify the composition of the particles responsible for occluding the dentinal tubules. The methodology of evaluating the dentin tubule occlusion was based mainly on Peyro Mousavi *et al.* [30] and Doğan *et al.* [31].

Statistical analysis

Statistical analysis was conducted using IBM SPSS ver. 25.0 (IBM Corp, Armonk, NY, USA). The sample size was determined to ensure 80% statistical power at a 0.05 significance level, with the expected effect size estimated from preliminary pilot study data, resulting in a required sample size of approximately five specimens per group. The Shapiro-Wilk test was used to assess data normality, while the Levene test evaluated homogeneity of variances. To compare the diameter of open dentinal tubules (in μm) and the number of open tubules per 0.01 mm^2, a one-way analysis of variance was performed. Tukey's *post hoc* test was then used to identify statistically significant differences among the groups at the 0.05 level. Since tubule occlusion was evaluated using a categorical scoring system, nonparametric tests were applied. The Kruskal-Wallis test was used for group comparisons, followed by the Mann-Whitney *U* test for pairwise analysis. A *p*-value below 0.05 was considered statistically significant.

RESULTS

Table 2 displays the mean values and standard deviations for the diameter of open dentinal tubules (μm), the degree of tubule occlusion (rated on a 0–2 scale), and the number of open tubules per 0.01 mm^2 of dentin surface across the experimental groups.

All desensitizing treatments significantly enhanced dentin tubule occlusion compared to the negative control ($p < 0.05$). The most effective treatment was the use of Perfect Protect gel, which achieved the highest tubule occlusion rate (79.2%), followed by the other two treatments with comparable effectiveness (56.3% UltraEZ and 58.6% TheraSol). The degree of tubule occlusion correlated with the number of occluded tubules, as shown in Table 2. Additionally, all treatments resulted in a significant decrease in the average diameter of dentin tubules compared to the untreated specimens ($p < 0.05$). Figure 2 displays representative SEM images of the treated dentin surfaces after the bleaching procedure

Table 2. Diameter of open dentinal tubules, degree of tubule occlusion, and number of open tubules in the experimental groups after treatment

Group (active agent)	Diameter of open tubules (μm)	Level of tubule occlusion (scale 0–2)	Number of open tubules per 0.01 mm^2	Percentage of occluded tubules (%)
Group 1 (control)	3.03 ± 0.53^{A}	0.00 ± 0.00^{A}	295.6 ± 45.4^{A}	3.5
Group 2 (UltraEZ)	1.73 ± 1.09^{B}	0.80 ± 0.43^{C}	133.8 ± 122.2^{C}	56.3
Group 3 (Perfect Protect)	1.14 ± 0.74^{B}	1.40 ± 0.54^{B}	63.4 ± 72.0^{B}	79.2
Group 4 (TheraSol Whitening & Sensitive)	1.49 ± 0.90^{B}	1.20 ± 0.44^{B}	126.6 ± 85.1^{C}	58.6

Values are presented as mean ± standard deviation.
The percentage of occluded tubules (%) was calculated relative to the total number of tubules visible at ×1,000 magnification.
Same uppercase superscripts in columns indicate no significant differences among the treatments ($p > 0.05$).
UltraEZ : Ultradent Products Inc., South Jordan, UT, USA; Perfect Protect: VOCO GmbH, Cuxhaven, Germany; TheraSol Whitening & Sensitive: ABC Kinitron IKE, Athens, Greece.

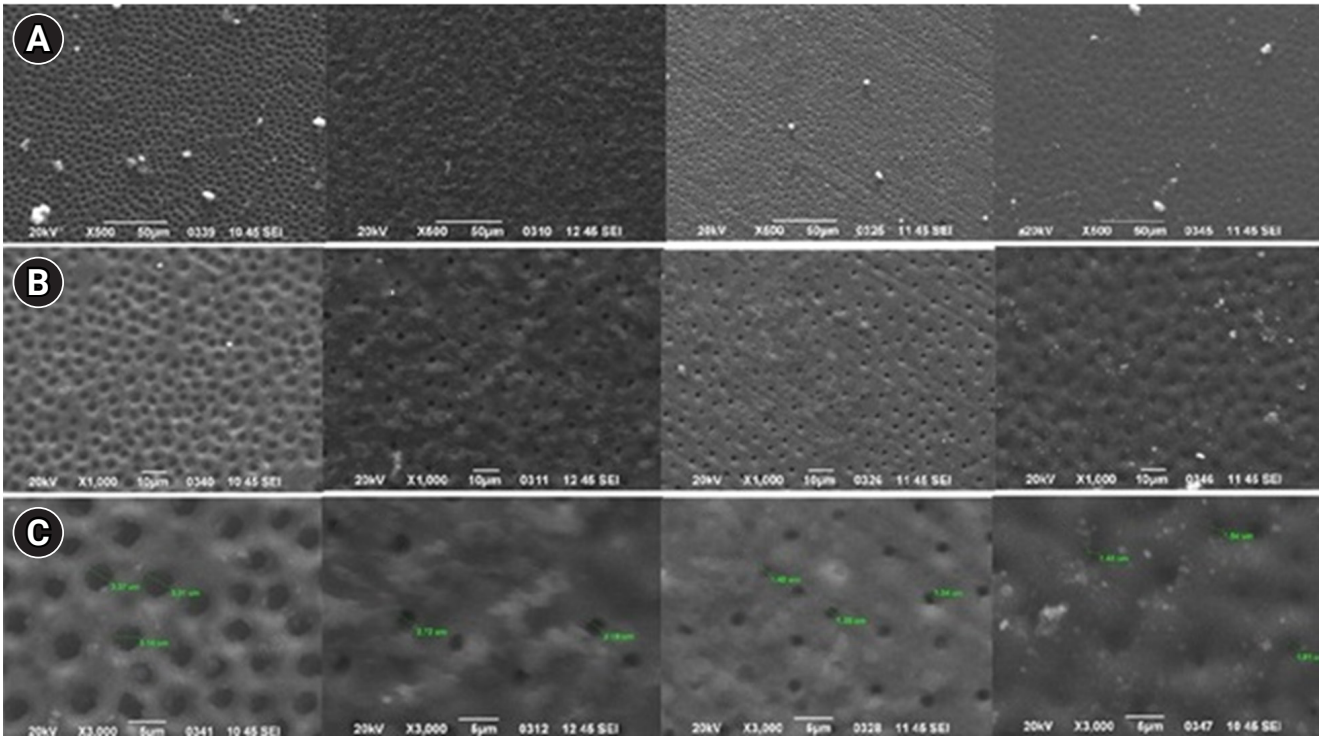

Figure 2. Representative scanning electron microscopy photomicrographs showing the dentin surface after the treatments of each experimental group at three magnifications: ×500 (A), ×1,000 (B), and ×3,000 (C). 1: control, 2: UltraEZ (Ultradent Products Inc., South Jordan, UT, USA), 3: Perfect Protect (VOCO GmbH, Cuxhaven, Germany), and 4: TheraSol Whitening & Sensitive (ABC Kinitron IKE, Athens, Greece).

for each experimental group at magnifications of ×500, ×1,000, and ×3,000, respectively.

EDS analysis identified the elemental content of the particles or precipitates responsible for tubule occlusion. In groups 2 and 3, traces of potassium and fluorine were detected, originating from the gels containing KNO_3 and NaF. In group 4, traces of strontium and fluorine were detected, originating from the paste containing strontium acetate and MFP (Figure 3). Elemental composition for each group is shown in Table 3.

DISCUSSION

According to the findings of this study, the first null hypothesis (H_{01})—which proposed that the treatments tested would not produce the same level of dentinal tubule occlusion as the untreated control group—was accepted. This means that the tested treatments were effective in occluding dentinal tubules. Moreover, the second null hypothesis (H_{02}), which proposed that the tested treatments would differ in their effectiveness at occluding dentinal tubules, was also supported. The discrepancies in effectiveness were probably attributed to different mechanisms of dentin tubule occlusion, compositions, and concentrations of the active agents, depending on the treatment applied, as reported in various previous studies [19,20,30,32,33].

The first two desensitizing gels contained KNO_3 and NaF as active agents. The effectiveness of this combination against TS has been previously documented [34]. Although the contents of these compounds were similar (3% KNO_3 and 0.11% NaF), the effectiveness on occluding dentin tubules was significantly higher for the latter product. The advantageous results for Perfect Protect (a percentage of occluded tubules of 79.2% compared to 56.3% for UltraEZ) may, however, be related to the special insertion processes used, leading to an improved microrheological profile. The optimized shear viscosity results in improved wetting of the tooth surface. Furthermore, Fick's second law indicates that increased

https://doi.org/10.5395/rde.2026.51.e8

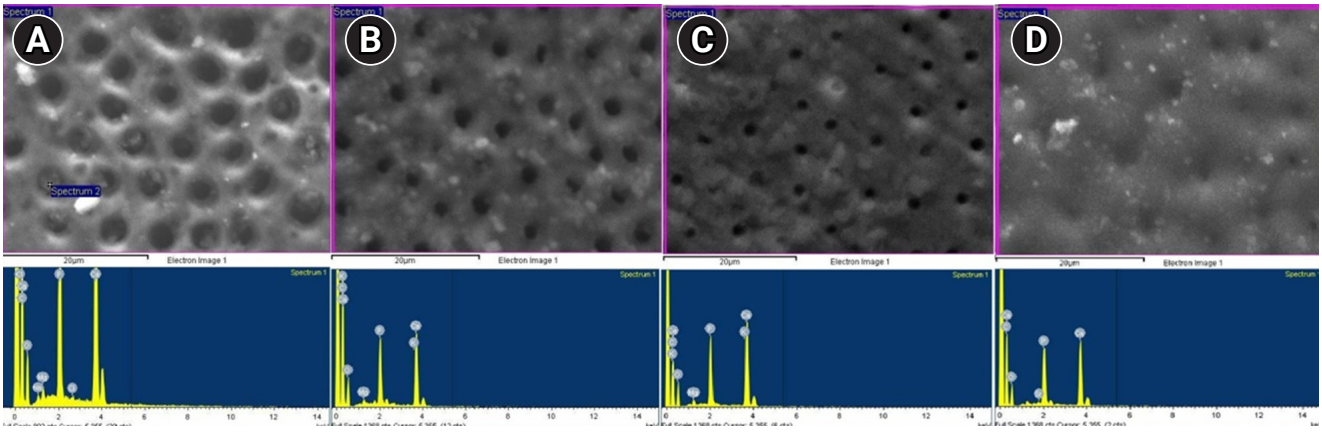

Figure 3. Representative scanning electron microscopy photomicrographs showing the dentin surface after the treatments of each experimental group at three magnifications: ×500 (A), ×1,000 (B), and ×3,000 (C).
1: control, 2: UltraEZ (Ultradent Products Inc., South Jordan, UT, USA), 3: Perfect Protect (VOCO GmbH, Cuxhaven, Germany), and 4: TheraSol Whitening & Sensitive (ABC Kinitron IKE, Athens, Greece).

Table 3. Elemental content (wt%) of the enamel surface after the desensitizing treatments for each experimental group

Element	Group 1 (control)	Group 2 (UltraEZ)	Group 3 (Perfect Protect)	Group 4 (TheraSol Whitening & Sensitive)
Ca	34.74 ± 3.59[a]	33.67 ± 3.77[a]	35.49 ± 3.91[a]	28.81 ± 3.71[a]
P	21.08 ± 2.78[a]	21.77 ± 2.42[a]	20.47 ± 2.37[a]	21.87 ± 2.58[a]
Na	0.71 ± 0.10[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Cl	0.41 ± 0.08[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Mg	1.04 ± 0.11[a]	1.59 ± 0.25[a]	1.80 ± 0.31[a]	0.00 ± 0.00[a]
Si	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	2.78 ± 0.42[b]
K	0.00 ± 0.00[a]	0.31 ± 0.09[b]	0.37 ± 0.09[c]	0.00 ± 0.19[b]
Sr	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[a]	3.07 ± 0.53[a]
F	0.00 ± 0.00[a]	0.88 ± 0.22[a]	1.16 ± 0.31[a]	0.65 ± 0.19[a]
O	42.02 ± 4.83[a]	41.78 ± 3.40[a]	40.70 ± 3.52[a]	42.82 ± 4.10[a]

Values are presented as mean ± standard deviation.
Different lowercase superscripts in the rows denote statistically significant differences ($p < 0.05$).
UltraEZ : Ultradent Products Inc., South Jordan, UT, USA; Perfect Protect: VOCO GmbH, Cuxhaven, Germany; TheraSol Whitening & Sensitive: ABC Kinitron IKE, Athens, Greece.

gel volume leads to enhanced diffusion. Consequently, using a thinner layer of desensitizing gel limits the penetration of active ingredients into the dentin surface, potentially reducing the effectiveness of dentinal tubule occlusion [35].

Potassium salt-based products function by raising the potassium ions (K^+) concentration in nerve endings, which diminishes the nerve's capacity to send sensory signals and modifies its action potential [36]. When applied to exposed dentin, these potassium salts increase K^+ levels within the dentinal tubules and adjacent tissues, disrupting the usual processes of nerve cell depolarization and repolarization [37]. Nerve cells generate and transmit electrical signals, or action potentials, through ion movement, particularly Na^+ and K^+. Upon stimulation, Na^+ ions enter the cell, causing depolarization and triggering nerve impulse transmission. Potassium ions then exit the cell to restore its resting state. However, in high concentrations—such as those found in potassium-based desensitizing agents—the movement of K+ is disrupted. The excess potassium outside nerve cells interferes with efficient repolarization, reducing the nerve's responsiveness to stimuli. As a result, the transmission of pain signals triggered by temperature fluctuations or mechanical stimuli is reduced. This process helps alleviate TS by inhibiting nerve impulse

transmission associated with pain [1,13]. In the current study, the effectiveness of this mechanism was not investigated, as the alleviation of TS symptoms can only be evaluated clinically. Nevertheless, EDS analysis revealed traces of potassium on the dentin surface, confirming the existence of the products that included KNO_3.

The active ingredient responsible for dentin tubule occlusion in the two previously mentioned products was NaF. Fluoride aids in depositing minerals, such as calcium and phosphate, onto the dentin surface, thereby enhancing its strength and hardness, while promoting dentin remineralization [38]. Fluoride ions, upon contact with the tooth surface, aid in sealing exposed dentinal tubules [39]. The role of fluorine compounds in reducing dentin permeability has been widely studied [40,41]. These compounds contribute to an increase in the presence of CaF_2 crystals within the tubules, leading to lower dentin permeability [22]. Since these crystals are nearly insoluble in saliva, they provide a temporary protective effect. Sodium fluoride-based dental products are commonly used to reduce calcium salt content on the surface of dentin; however, these salts can be removed by saliva and tooth brushing. If fluorapatite forms posttreatment, it provides greater stability against the effects of saliva, mechanical abrasion, and dietary influences [42]. However, SEM observations in the current study did not detect such deposits on the dentin, although traces of fluorine were found by elemental analysis.

The third desensitizing product (TheraSol Whitening & Sensitive) investigated in the present study contained 8% strontium acetate and 0.76% MFP. The effectiveness of strontium acetate for TS has been affirmed in previous studies [43–45]. Strontium acetate may contribute to the occlusion of dentinal tubules by substituting calcium ions within the hydroxyapatite crystal lattice with strontium ions. In addition to this mineral interaction, it also plays a role in reducing TS by interfering with nerve depolarization processes [46]. Strontium behaves in a manner similar to calcium within the human body [47] and is capable of replacing it during the process of apatite biomineralization [48]. In the oral environment, Sr^{2+} ions—like Ca^{2+} and other divalent cations with a similar charge-to-size ratio—can readily incorporate into the hydroxyapatite matrix [20]. Studies have shown that strontium encourages dentin formation by enhancing the proliferation, differentiation, and mineralization of human dental pulp stem cells, potentially supporting the development of tertiary dentin [49].

A key characteristic of strontium salts is their ability to form complex strontium phosphate compounds, thereby chemically influencing dentin [47]. Past studies have confirmed the formation of a Ca-Sr apatite, specifically $Ca_6Sr_4(PO_4)_6(OH)_2$, which results from the substitution of intracrystalline calcium in apatite with strontium [50]. Additionally, strontium salts exhibit a strong binding affinity to dentin due to its increased permeability, which facilitates their absorption into organic connective tissues and the odontoblast process [51]. This interaction results in the precipitation of proteins and the formation of a protective film, which may contribute to reducing the transmission of external stimuli by effectively sealing the dentinal tubules [33]. In the current investigation, EDS analysis revealed traces of Sr in specimens treated with strontium acetate, indicating that Sr can be retained or integrated on the dentin surface, thereby contributing to the occlusion of dentin tubules.

TheraSol also contains MFP as an active agent. Sodium monofluorophosphate (Na_2PO_3F) alleviates TS primarily by promoting dentin tubule occlusion and dentin remineralization, reducing the transmission of pain-inducing stimuli [52]. Once applied, MFP breaks down, releasing fluoride ions (F^-). These ions integrate into the hydroxyapatite structure of the enamel and dentin, forming fluoroapatite, which is more resistant to acid attacks and wear. Additionally, fluoride reacts with calcium and phosphate in saliva and dentin, forming CaF_2 precipitates. These deposits help block opened dentinal tubules, reducing the ability of external stimuli to reach the underlying nerve endings [53]. This property of MFP was observed in SEM images, where occlusion of dentin tubules was detected. Meanwhile, EDS analysis revealed a fluorine content on the dentin surface of the TheraSol-treated specimens.

A primary limitation of this laboratory study was its inability to accurately mimic the intricate conditions of the oral environment. Factors such as saliva composition, enzymatic activity, bacterial presence, and mechanical forces from mastication and brushing, which

https://doi.org/10.5395/rde.2026.51.e8

can influence the long-term efficacy of desensitizing agents, are not accurately simulated in laboratory conditions. Additionally, the alleviation of TS symptoms could not be assessed *in vitro*. Additionally, the use of extracted teeth, which lack the dynamic interactions with pulp tissue and blood circulation, may affect the penetration and retention of the tested agents. Another limitation was that the application methods and exposure times used *in vitro* may not precisely reflect real-life clinical use, potentially leading to overestimation or underestimation of the agents' effectiveness. Furthermore, the durability of tubule occlusion over time, particularly under conditions of acidic or abrasive challenges, may not be adequately assessed in short-term laboratory studies, necessitating further *in vivo* research to validate the findings. Finally, the use of human teeth from different patients, which are inherently non-standardized, may introduce variability into the results.

CONCLUSIONS

Considering the limitations of this laboratory study, it can be deduced that the tested desensitizing agents were capable of occluding the dentin tubules to a great extent. Among the desensitizing agents, differences in their effectiveness were observed due to discrepancies in their formula composition. Particularly, Perfect Protect presented the highest degree of occlusion of dentin tubules, followed by TheraSol and UltraEZ, which did not differ from each other. From a clinical perspective, these findings suggest that Perfect Protect may offer greater potential for reducing post-bleaching sensitivity associated with tooth bleaching. However, clinicians should interpret these results cautiously, as laboratory conditions cannot fully replicate the complexity of the oral environment. Further *in vivo* studies are necessary to affirm the effectiveness of the treatments.

CONFLICT OF INTEREST

Dimitrios Dionysopoulos is an Associate Editor of *Restorative Dentistry and Endodontics* and was not involved in the review process of this article. The authors declare no other conflicts of interest.

FUNDING/SUPPORT

This research was funded by VOCO GmbH and the Research Committee of Aristotle University of Thessaloniki, Grand Number: 10468.

ACKNOWLEDGMENTS

This research was funded by VOCO GmbH and the Research Committee of Aristotle University of Thessaloniki, Project Code 10468. The authors would like to thank Dr. Stavros Oikonomidis for his technical support in SEM observations and EDS analysis of the specimens, which were conducted in the Solid State Physics Division, Department of Physics, Aristotle University of Thessaloniki, Greece. Finally, the authors acknowledge with thanks VOCO GmbH and ABC Kinitron IKE for providing the products for the purposes of the current study.

AUTHOR CONTRIBUTIONS

Conceptualization: Dionysopoulos D, Tolidis K. Methodology: Dionysopoulos D, Mourouzis P. Validation: Papageorgiou S. Formal analysis, Visualization: Mourouzis P. Investigation, Supervision, Project administration, Funding acquisition: Dionysopoulos D. Data curation: Tolidis K. Writing – original draft: Dionysopoulos D, Mourouzis P. Writing – review & editing: Tolidis K, Papageorgiou S. All authors read and approved the final manuscript.

DATA SHARING STATEMENT

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

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