Original Article

Comparative Evaluation of the Effectiveness of Three Commercially Available Desensitizing Mouthwash on Dentinal Tubule Occlusion: An *In Vitro* Scanning Electron Microscopic Study

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ABSTRACT

Aim: To compare and evaluate the effectiveness of three commercially available desensitizing mouthwashes on dentinal tubule occlusion *in vitro* using a scanning electron microscope (SEM). **Materials and Methods:** Twenty-seven premolar maxillary teeth were collected and cleaned to remove debris and tissue using an ultrasonic scaler. Enamel was removed with the help of a high-speed plain cut tungsten carbide fissure bur under continuous water spray. Dentinal discs measuring 5mm × 5mm × 3mm were prepared from the coronal region of the extracted teeth using a double-sided carborundum disc and straight micromotor handpiece. Using carborundum paper, the discs were polished and washed with distilled water. The discs were etched using 37% phosphoric acid to remove any smear layer that was caused by the grinding process and to simulate dentinal hypersensitivity by opening the dentinal tubules. The specimens were again washed and stored in distilled water until use. Samples were then randomly allocated into Group A: calcium sodium phosphosilicate mouthwash, Group B: potassium nitrate mouthwash, and Group C: dipotassium oxalate monohydrate mouthwash. The specimens were immersed in a test tube filled with the respective mouthwash and vigorously shaken for 60s for simulating the natural mouth rinsing action. This procedure was repeated for 7 days, twice daily. To compare and assess the proportion of dentinal tubule occlusion, all the samples were processed and examined under an SEM. **Results:** The efficacy of the mouthwash on the obliteration of dentinal tubules was compared using the Kruskal–Walis analysis of variance test followed by the *post hoc* Mann–Whitney *U* test for pairwise comparison. Group A showed completely occluded tubules in 5 (55.6) and mostly occluded tubules in 4 (44.4). Group B showed mostly occluded in 4 samples (44.4) and partially occluded in 5 samples (55.6) and Group C showed completely occluded tubules in 3 samples (33.3) and mostly occluded tubules in 6 samples (66.7). A *P* value of 0.05 or less is regarded as statistically significant. **P* ≤ 0.05 is statistically significant. Between Group A and Group B as well as between Group B and Group C, there was a statistically significant difference. However, a statistically significant difference between Group A and Group C does not exist. **Conclusion:** The calcium sodium phosphosilicate group was shown to have the most occlusion of the dentinal tubule, followed by dipotassium oxalate monohydrate and potassium nitrate group.

Keywords: *Dentin hypersensitivity, mouthwash, scanning electron microscope*

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Introduction

Dentin hypersensitivity (DH) is a common condition that compromises the quality of life and has a 25%–46% rise in prevalence among people 18–70 years old. Maxillary premolars followed by maxillary molars are most commonly affected by hypersensitivity, whereas incisors are the least affected. DH is characterized by short sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic, or chemical, and which cannot be ascribed to any other form of dental defect or pathology.[1] Chief etiological factors include enamel loss owing to erosion, abrasion, abfraction, and/or denudation of the root surface by overlying cementum and periodontal tissue loss or gingival recession.[2,3] This also occurs due to aggressive oral hygiene practices and an acidic diet which accelerate the loss of tooth structure. Irrespective of the cause of the exposure of dentin, the open dentinal tubules are a common feature that provides a direct link between the tooth pulp and the oral environment.

The occlusion of dentinal tubules is caused by the use of physical or chemical substances externally on denuded surfaces, which creates an artificial layer on the outer surface of dentinal tubules by depositing an obliterating material on the dentin surface and reducing the diameter of tubules, blocking dentinal fluid diffusion, and resulting in a decrease in DH.[4] Various agents are used for treating DH, including lasers, desensitizing dentifrices, mouthwash formulations, dentin sealing agents, periodontal soft tissue grafting, etc. However, no treatment modality has been regarded as the gold standard treatment of dental hypersensitivity. Desensitizing dentifrices have been used for a long time; however, they have certain disadvantages like the need for an armamentarium, and may cause discomfort while brushing on the sensitive dentin areas.[5]

Dentifrice use has a minimal amount of exposure to non-dental areas, whereas, in mouthwash formulations, the entire oral cavity is exposed to the therapeutic agent. Mouthwash formulations serve the advantage of ease of use, cost-effectiveness, and accessibility to all areas of the mouth. They also circumvent the discomfort caused by the osmolarity of the toothpaste while brushing the sensitive dentin areas.[4] The consumer practice of rinsing after tooth brushing rather than rinsing immediately after mouthwash use, which permits mouthwash components to stay in the oral cavity after usage, is another benefit of desensitizing mouthwash. Moreover, mouthwashes are specifically useful in specific conditions such as after surgical interventions or when dentifrices or topical agents cannot be applied for any reason. Also, most of the population does not possess the motivation and skill to effectively use mechanical means (toothbrush, floss), therefore emphasizing the importance of adjunctive chemical (mouthwash) agents.

Table 1: Pairwise comparison of the dentinal tubules obliteration

P value (Mann–Whitney *U* test)	Group A	Group B	Group C
Group A	–	**0.003***	0.436
Group B	**0.003***	–	**0.011***
Group C	0.436	**0.011***	–

Bold value means significant

*$P \leq 0.05$ is statistically significant

Limited studies have been conducted to examine the effectiveness of desensitizing agents in mouthwash formulations. There is a need to determine the effect of desensitizing mouthwash formulations for treating dentinal hypersensitivity. Thus, the objective of the present study was an *in vitro* comparison of 5% calcium sodium phosphosilicate, 3% potassium nitrate, and 5% dipotassium oxalate monohydrate which are used in the treatment of dentinal hypersensitivity to evaluate and compare the dentinal tubule occlusion on dentinal discs after the application of the desensitizing agents and analyzing under a scanning electron microscope (SEM) [Table 1].

Materials and Methods

Setting and design

This study was conducted at Bharati Vidyapeeth Dental College and Hospital, Pune, India. A total of 27 human maxillary premolar teeth were extracted for orthodontic purposes and gathered from the Oral & Maxillofacial Surgery Department. Prior to beginning the study in July 2023, Bharati Vidyapeeth University's institutional ethics and research bodies granted their approval.

The inclusion criteria were human maxillary premolar teeth, extracted for orthodontic reasons, teeth with intact crown and root surfaces, and teeth unaltered by the extraction procedures. The exclusion criteria were teeth with carious lesions, teeth with restorations or fractures, teeth with pulpal or periapical diseases, and teeth with fluorosis.

Sample preparation

The extracted human premolar teeth were cleaned for debris and tissue using an ultrasonic scaler. Enamel

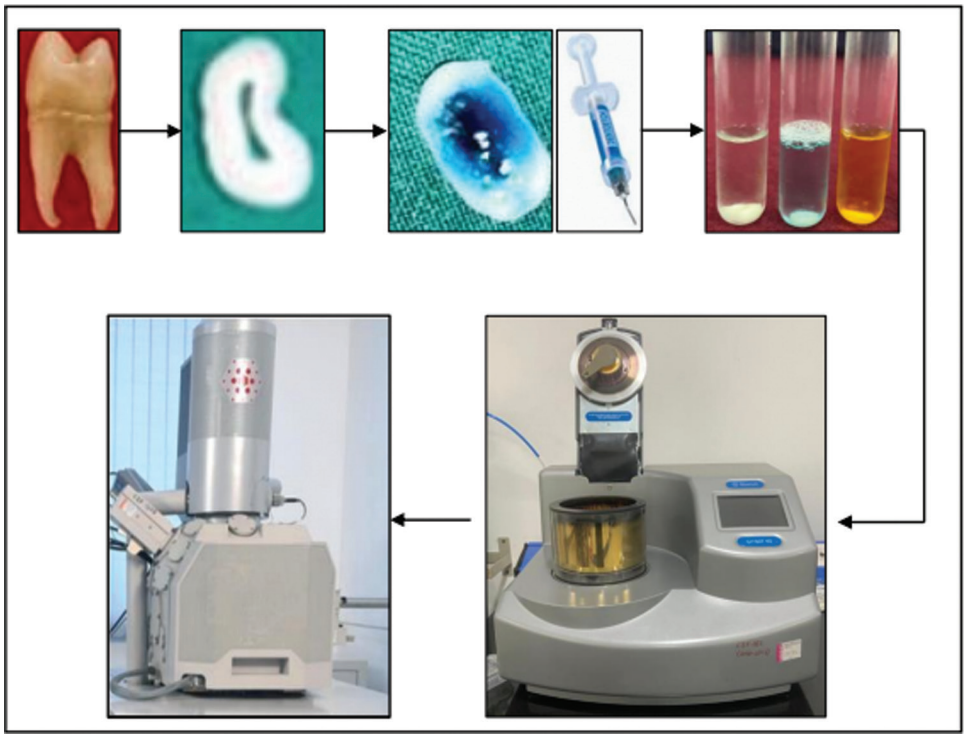

Figure 1: Preparation of samples: dentin discs were prepared from extracted maxillary premolar teeth; discs were etched with phosphorous acid; discs were immersed in respective mouthwash; discs were gold sputter coated; and examined under SEM

was removed with the help of a high-speed plain cut tungsten carbide fissure bur under continuous water spray. The specimens were placed in normal saline until dentin discs were prepared.

Dentinal discs measuring 5 mm × 5 mm × 3 mm were prepared from the coronal region of the extracted teeth using a double-sided carborundum disc and straight micromotor handpiece. Using carborundum paper, the discs were then polished and washed with distilled water. The discs were etched using 37% phosphoric acid to remove any smear layer that was caused by the grinding process and to expose the dentinal tubules to simulate dentinal hypersensitivity. Specimens were again washed and stored in distilled water until use [Figure 1].

Experimental groups and their treatments

The estimated sample size for the study is 27 (nine per group). The sample size was calculated using GPower software. Twenty-seven samples were allocated randomly into groups of 3 commercially available desensitizing mouthwash, comprising nine samples in each. Group A—dentinal discs treated with commercially available desensitizing mouthwash containing 5% calcium sodium phosphosilicate; Group B—dentinal discs treated with commercially available desensitizing mouthwash containing 3% potassium nitrate; and Group C—dentinal discs treated with commercially available desensitizing mouthwash containing 5% dipotassium oxalate monohydrate.

Procedure

The discs were immersed in a test tube filled with the respective mouthwash fitted with a rubber stopper at the top and shaken vigorously for 60 s to simulate natural mouth rinsing action. Samples were stored in distilled water when not in use. This process was repeated for seven days twice daily.

After the 7-day experiment was over, the specimens were air-dried and prepared for analysis under the SEM for the evaluation of the dentinal tubule occlusion percentage produced. To compare the efficiency of dentinal tubule occlusion, all samples were treated and examined under an SEM.

Scanning electron microscopy

Individual specimens were on a metal stub mounted. About 25 nm of gold was sputter coated onto the samples for 10 min and observed under SEM (FESEM: FEI Nova NanoSEM 450 EDS: Bruker XFlash 6I30) under 2000× magnification. To determine the total number of tubules, the number of open tubules, the number of completely occluded tubules, and the number of partially occluded tubules, photomicrographs of dentin discs at a magnification of 2000× were captured. To prevent bias, the analysis was performed by an independent, blind examiner.

Statistical analysis

SPSS version 25 was used for analyzing the data. All quantitative data were tabulated using means and standard deviations. Qualitative data were tabulated using numbers and percentages. Comparison among the three groups will be done using one-way analysis of variance (ANOVA) test followed by the *post hoc* Bonferroni test (if the data is parametric) and Kruskal–Walis ANOVA followed by Mann–Whitney *U* test (if the data is non-parametric) [Table 2]. A *P* value of 0.05 or less will be regarded as statistically significant. The number of tubules evident in each of the 2000× images was counted to provide a measure of tubule occlusion efficacy. Descriptive statistics were expressed as numbers and percentages. The efficacy of the three types of mouthwash on the obliteration of dentinal tubules was compared using the Kruskal–Walis ANOVA test followed by the *post hoc* Mann–Whitney *U* test for pairwise comparison. A $P \leq 0.05$ was considered statistically significant in the above test. All the analyses were conducted using SPSS version 25.

Results

Utilizing an SEM, the current study assessed the impact of dentinal tubule occlusion caused by three widely available desensitizing mouthwashes comprising calcium sodium phosphosilicate, potassium nitrate, and dipotassium oxalate monohydrate. Figures 2B, 2C, 2D reproduces the SEM pictures of each group at the conclusion of the 7-day period. A statistically significant difference was seen in the dentinal tubule occlusion among the three groups at the end of the seventh day. *$P \leq 0.05$ is statistically significant.

According to the assessment standards of scanning electron microscopy scoring,[6] microphotographic pictures were visually examined based on the patency of dentinal tubules.

1. Occluded (100% of tubules occluded)
2. Mostly occluded (50%–100% of tubules occluded)
3. Partially occluded (25%–50% of tubules occluded)
4. Mostly unoccluded (<25% of tubules occluded)
5. Unoccluded (0% of tubules occluded)

The assessment of the specimens using SEM revealed that before the application of desensitizing mouthwash and following the application of 37% phosphoric acid, exposure of all the dentinal tubules was revealed in the SEM microphotographs [Figure 2A]. The mean number of tubules occluded was maximum in calcium sodium phosphosilicate, followed by dipotassium oxalate monohydrate and potassium nitrate. Group A (calcium sodium phosphosilicate) [Figure 2B] showed completely occluded tubules in 5 (55.6) and mostly occluded tubules in 4 (44.4). Group B (potassium nitrate) [Figure 2C] showed mostly occluded tubules in 4 (44.4) and partially occluded tubules in 5 (55.6), and Group C (dipotassium oxalate monohydrate) [Figure 2D] showed completely occluded tubules in 3 (33.3) and mostly occluded tubules in 6 (66.7). *$P \leq 0.05$ is statistically significant. There was a statistically significant difference between Group A and Group B and Group B and Group C. However, no statistically significant difference exists between Group A and Group C.

Discussion

Chronic tooth problems with dentinal hypersensitivity are a common and unpleasant condition yet least successfully managed. Although a significant amount of research has been carried out, the clinical management of DH is largely empirical.

Dentin, in contrast to enamel, is a permeable tissue that is, traversed by fluid-filled dentinal tubules.[7] The primary structural element of the tooth is the dentin, which is mostly made up of 30,000–40,000 tubules filled with dentinal fluid. The dentinal tubule's diameter widens as it approaches the pulpal area. They have a diameter of 2.5 mm close to the pulp, 1.2 mm close to the midpoint of the dentin, and 0.9 mm at the DEJ, tapering from the inner to the outermost surface.[8] As fluid flow depends on the fourth power of the radius; the dentinal tubule's width is important. As the tubule diameter doubles, there is a 16-fold increase in the fluid flow. Compared to non-sensitive teeth, sensitive teeth have roughly eight times as many tubules and nearly

Table 2: Comparison of the dentinal tubules obliteration between the three groups

Patency of dentinal tubules	Group A, *N* (%)	Group B, *N* (%)	Group C, *N* (%)	*P* value (Kruskal–Walis analysis of variance)
Occluded	5 (55.6)	0	3 (33.3)	**0.002***
Mostly occluded	4 (44.4)	4 (44.4)	6 (66.7)	
Partially occluded	0	5 (55.6)	0	
Mostly unoccluded	0	0	0	
Unoccluded	0	0	0	

Bold value means significant

*$P \leq 0.05$ is statistically significant

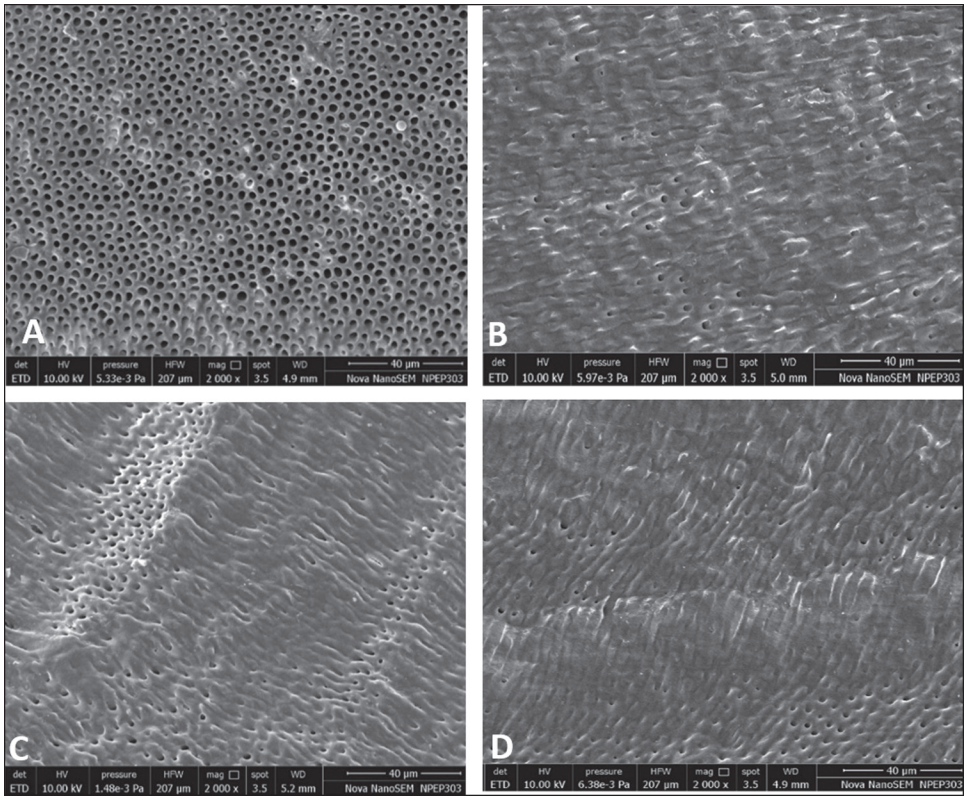

Figure 2: (A) Open dentinal tubules seen in untreated dentinal discs under SEM; (B) SEM image of Group A: calcium sodium phosphosilicate; (C) SEM image of Group B: potassium nitrate; (D) SEM image of Group C: dipotassium oxalate monohydrate

twice as many at the buccal cervical region. The DH episodic state is brought on by the changing tubule patency caused by the production and removal of smear layers.[9] There is a clear relationship between DH and tubule thickness.[10,11] In an *in vitro* study conducted by Ling *et al*.[12] on dentinal discs under the SEM, they found out that the surface area of dentin, its thickness, and its characteristics can be controlled. Dentinal hypersensitivity caused by hydraulic conductance is greater with the coronal dentin as compared with radicular dentin. The measured hydraulic conductivity and the density and diameter of tubules appear to be positively correlated.[12]

An ideal treatment for hypersensitivity should mimic the body's natural desensitizing process, inducing changes in dentin leading to faster and lasting occlusion of dentin tubules.[9,13] Moreover, the treatment should have ease of application with no side effects. According to the Grossman criteria, an ideal desensitizing agent delivery method should have long-lasting effects, not irritate pulp, cause pain, be simple to use, and not discolor teeth.[14] Treatment modalities can be available in the form of dentifrices, mouthwashes, gels, sprays, or agents to be topically applied, i.e., glass ionomer cement, varnishes, resin composite, and periodontal membranes. At home, treatments are simpler and cost-effective and simultaneously can be used to alleviate generalized dental sensitivity. Compliance, difficulty of distribution to precise areas, late onset of action, and need for repeated use are some drawbacks of at-home treatment. In-office procedures address DH that is, confined to one or a few teeth, are more complex and time-consuming, and necessitate many patient visits. Compared to pastes and gels, mouthwash formulations are a compatible delivery vehicle that serves the advantages of simplicity, cost-effectiveness, ease of use, and access to all areas of the mouth. They also have the ability to circumvent the discomfort by the osmolarity of the toothpaste while brushing sensitive dentin surfaces.[14]

The desensitizing agents in the form of mouthwash formulations chosen in this study are calcium sodium phosphosilicate, potassium nitrate, and dipotassium oxalate monohydrate. An SEM was used for the evaluation of each mouthwash in occluding tubules. Calcium sodium phosphosilicate is a bioactive glass material that reacts with saliva to form hydroxyapatite-like crystals on the surface of dentin. It was formerly introduced as a bone material with remineralization potential,[14] and has been proven effective at physically

occluding dentinal tubules.[15] This newly formed mineralized layer of dentin has the same mineral content as bone, enamel, and dentin. Also, it acts as a barrier against oral fluids preventing further DH.[15] Analysis under the SEM showed that the application of bioglass creates an apatite layer which leads to the occlusion of dentinal tubules. Hypersensitivity has long been managed with potassium nitrate. By diffusing along the dentinal tubules and depolarizing the nerve cells, it blocks neural transmission at the nerve synapse. By diffusing along the dentinal tubules, potassium salts (potassium nitrate, potassium citrate, or potassium chloride) depolarize the nerve cells and make them resistant to excitatory stimuli. An *in vivo* study demonstrated that the use of toothpaste containing 5% potassium nitrate on hypersensitive teeth was significantly more effective in immediate pain reduction than using placebo toothpaste.[16] In a separate study by Hall *et al*., the effectiveness of an experimental oral rinse containing 3% potassium nitrate (KNO_3) in the treatment of DH was compared to the use of the same fluoride toothpaste alone when used as an adjuvant to brushing.[17] By depolarizing the nerve in the dentinal tubules, potassium nitrate stops the transmission of pain. The results showed that using a 3% KNO_3 oral rinse twice daily in addition to brushing with fluoride toothpaste significantly improved dental health compared to using fluoride toothpaste alone.[18] By blocking the dentin tubule orifices with a thin layer of calcium oxalate crystals that is, resistant to acid, as well as by inhibiting the activity of sensory nerves, potassium oxalate has been shown to lessen dentin sensitivity.[5] Studies on oxalate salts are particularly extensive. When compared to artificial saliva, desensitizing treatments that contain oxalate were much more effective at occluding the dentinal tubules. The findings showed that after an acidic challenge, oxalate-containing desensitizing drugs significantly and permanently occlude the open dentinal tubules.[19]

In the present study, calcium sodium phosphosilicate was shown to have the highest dentinal tubule occlusion followed by dipotassium oxalate monohydrate and potassium nitrate, proving calcium sodium phosphosilicate to be most effective in treating dentinal hypersensitivity.

Similar results were shown in a study conducted by Vaddamanu *et al*. in their systematic review stated that 5% calcium sodium phosphosilicate containing toothpaste was more effective in reducing DH compared to many other dentinal tubule occluding molecules.[20] Also, some studies have been performed which showed potassium nitrate to be inferior to calcium sodium phosphosilicate.[21,22] Chen *et al*.[23] concluded that ACC showed more occlusion after treatment when comparing the efficacy of red propolis extract, calcium sodium phosphosilicate, and arginine–calcium carbonate in occluding dentin tubules on prepared dentin discs. Although desensitizing toothpaste only partially obliterated the dentin tubules, an animal investigation on rats showed that potassium nitrate was effective in tubular occlusion and decreased dentin permeability.[24]

Limitations

The results of the present study were similar to the above-listed previously performed studies which stated calcium sodium phosphosilicate to be the most effective in occluding dentinal tubules and providing relief from dentinal hypersensitivity. Results should be extended cautiously because factors like salivary components and an acidic pH in a clinical setting may affect tubule blockage. The results must be confirmed in light of the patient's sense of sensitivity and quality of life as dentinal hypersensitivity is subjective in nature. *In vivo* investigations should also be carried out on a larger sample size to establish healthy competition amongst these desensitizing agents in the oral environment.

Conclusion

Calcium sodium phosphosilicate is a highly effective agent for maximum occlusion of dentinal tubules and hence is able to successfully treat dentinal hypersensitivity, followed by dipotassium oxalate monohydrate and potassium nitrate.

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Conflict of interest

There are no conflicts of interest.

Author contributions

TK: principal investigator, conducted the study, wrote the manuscript; PL: conducted the study, and revised manuscript; VD: revised the manuscript; NB: revised the manuscript. All authors have approved the final draft of the manuscript.

Ethical Policy and Institutional Review Board Statement

Prior to beginning the study in July 2023, Bharati Vidyapeeth University's institutional ethics and research bodies granted their approval.

Patient declaration of consent

Not applicable.

Data availability statement

Nil.

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