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Evaluation of dentinal tubule occlusion by Fluoridated and Non-fluoridated Bioactive Glass and Propolis desensitizers by scanning electron microscopy

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A R T I C L E I N F O

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Dentinal tubule occlusion
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A B S T R A C T

Objective: Dentin hypersensitivity is a worldwide epidemiological dental health issue, bioactive toothpaste has been demonstarted in alleviating dentin hypersensitivity along with propolis. Hence their comparison as desensitizing agents in dentinal tubule occlusion is carried out in this research. This study aimed to evaluate the efficacy of Fluoridated Bioactive Glass dentifrice, Non-fluoridated Bioactive Glass dentifrice, and Propolis containing dentifrice in dentinal tubule occlusion using scanning electron microscopy (SEM) and its resistance to acid.

Materials and methods: This is an In vitro study design with permanent maxillary and mandibular premolars, which were divided into four groups. Group 1 – Fluoridated bioactive glass (FBaG) Toothpaste; Group 2 – Bioactive glass (BaG) Toothpaste; Group 3 – Propolis Toothpaste; Group 4 – Saline. Dentin discs were obtained from the samples. 37 % phosphoric acid was used to etch and expose the dentinal tubules. Rubber cup was used to apply the desensitizing agent. Samples were then subjected to 6 % citric acid. The degree of occlusion was evaluated using SEM after application of desensitizing agent and after application of citric acid.

Results: Statistical analysis was calculated and results obtained showed that specimens treated with FBaG and BaG showed highest degree of tubule occlusion with a mean value of (2.33) followed by Propolis with mean values of (3.00) and least number of occluded tubules after application of desensitizing agent. Propolis showed highest degree of occlusion with a mean value of (2.33) followed by FBaG (2.66) and BaG (3.00) after application of citric acid.

Conclusion: Study concluded that FBaG and BaG-containing dentifrice showed a significant degree of dentinal tubule occlusion. Propolis demonstrated better resistance to acid attack.

1. Introduction

Dentin hypersensitivity (DH) is characterized by short, sharp pain arising from exposed dentin in response to a stimuli. The stimulus can be thermal, tactile, osmotic or chemical form and which cannot be described to any other form of dental defect or pathology.[1] DH prevalence ranges from 4 % to 57 %, according to reports.[2,3] There's no difference between men and women and is most frequently seen in patients aged between 20 and 30 years. The most commonly affected tooth by DH is the buccal surface of maxillary and manidbular premolars.[4] According to the “hydrodynamic theory” proposed by Brannstrom, fluid can pass via exposed tubules of dentin, as a result stimulating the dental pulp nerve endings.[5,6] Therefore the treatment of DH is focussed on sealing the open dentinal tubules.

Table 1- Showing different desensitizing agents used in the study.

Treatment modality of DH is mainly by:

1. Occlusion of open tubules: sealing the patent dentinal tubules[7]
2. Desensitizing treatments: focusing on the pulpal nerves[8]

Grossman established the requirements for the perfect desensitizer in 1935. He stated that the substance or procedure should not cause pulpal irritation and should be painless and easy to apply. It should be permanently effective and should respond quickly and should not cause any discoloration. However, no material qualify each of these prerequisites.[9]

Today's regenerative medicine has been transformed by biomaterials such as bioactive glass (BaG), It results in innovative biological

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Table 1
Different Desensitizing agents used in the study.

Groups	Desensitizing Agent
Group 1	FBaG; Elsenz® Biomin-F, (Group Pharmaceuticals Limited, India)
Group 2	BaG; NovaMin Sensodyne® Repair & Protect (GlaxoSmithKline, UK)
Group 3	Propolis; Superbee® Propolis Toothpaste (Nature's Care, Australia)
Group 4	Saline; Normal Saline (Fresenius Kabi, India)

procedures such as tissue repair and drug administration.[10] Larry L. Hench created the first bioactive glass in 1969.[11] Bioactive glass-containing toothpastes have drawn a lot of scrutiny because of their ability to achieve dentinal tubule blockage which in turn minimize sensitivity.[12,13] Bioactive Glass is made up of 45 % silicon dioxide, 24.5 % calcium oxide, 24.5 % sodium oxide, and 6 % phosphorus pentoxide. It consist of hydroxyl carbonate-apatite, which is made up of crystal and has similar mineral makeup of teeth.[14] The content of fluoride present in BaG has the ability to remineralize the demineralized enamel. Bioactive glass dentifrice is considered to enhance oral health.[15] BioMin (FBaG)[16] toothpaste and NovaMin (BaG)[14] toothpaste are examples that stand out.

The active component of NovaMin is calcium sodium phosphosilicate which allows to adhere to the tooth surface and commence remineralization of enamel when saliva or any another wet medium is encountered.[17] When NovaMin comes into contact with bodily fluids, it interacts and rapidly releases phosphate, sodium, and calcium ions.[18] BioMin's unique feature is the tiny BaG particles which allow it to enter dentin channels and settle on the tooth's surface. BioMin F, contains flouride and was first made available in 1990. When it diffuses in saliva, it can produce fluorapatite, and it keep releasing fluoride for up to 12 hours after brushing.[19] Apis mellifera L bees generate propolis, which is a naturally occurring resin. Propolis is a potential medication to decrease DH because of its high flavonoid content, which possesses bactericidal, antioxidative, analgesic properties and also inhibit viral and fungal growth and aid in reducing inflammation.[20,21]

Even though dentifrices can block the tubules in dentin, everyday exposure to acidic beverages may remove the smear layer, which could reverse or decrease tubule obstruction.[22] Acid exposure on tooth surface on a regular basis has been found to increase dentin hypersensitivity's prevalence, and the intake of acidic beverages has increased in the modern lifestyle.[23] Therefore, it is worthwhile to look for active substances that can resist acid in addition to obstructing the dentinal tubules. Hence, this study aimed to evaluate the efficacy of Fluoridated Bioactive Glass dentifrice, Non-fluoridated Bioactive Glass dentifrice, and Propolis containing dentifrice in dentinal tubule occlusion using scanning electron microscopy (SEM) and its resistance to acid.

2. Materials and Methods

This in vitro study was approved by the Institutional Review Board (ECASM-AIMS-2024-077). The samples were prepared in the Department of Conservative dentistry and Endodontics, Amrita School of Dentistry, Kochi, Kerala. The SEM Analysis was done in the in the Department of Marine Geology and Geophysics, CUSAT, Kochi, Kerala.

2.1. Sample selection

The Permanent maxillary and mandibular premolars extracted for orthodontic treatment were collected as samples for the study. Teeth with abrasion, attrition, detectable cracks, caries, restorations and hypoplasia were excluded from the study.

2.2. Sample preparation

Teeth samples were cut at right angle to their longitudinal axis using an IsoMet (Buehler 5000, Illinois, USA) to create dentin discs from the middle coronal dentin that were 1.0 ± 0.1 mm thick. After removing enamel, a silicon carbide paper with a grit of 600 was used to smooth out any surface imperfections and make the surface uniformly smooth. To make certain that the polishing abrasive was entirely eliminated, specimens underwent sonication (Digital Ultrasonic Cleaner, Codyson Electrical Co., Ltd. China) for 10 min and were then extensively rinsed using saline solution. Etching of dentin was done for 5 s using 37 % of phosphoric acid (D-Tech Etching Gel) to expose the dentinal tubules. Distilled water was used to rinse the etchant from dentin disc. To eliminate any remaining etchant, the samples were sonicated for 5 min after using distilled water to rinse. Until further use, the specimens were stored in Phosphate-buffered solution (PBS). Fig. 1depicts a flowchart summarizing the experimental procedure.

2.3. Sample size calculation

Based on the Mean and SD of dentinal tubule occlusion scores after acid challenge among FBaG group (3.68 ± 0.79), BaG group (3.09 ± 0.80), Arginine Calcium Carbonate (ACC) group (4.3 ± 0.62) and saline group (5 ± 0) were observed in an earlier publication[24] and 95 % confidence and 80 % power, the minimum sample size comes to 1/Group for the pair control vs BaG, 3/Group and 6/Group for the pair control vs ACC group. So, the minimum sample size calculated was 6 in each group (6*4 = 24).

The 24 dentin discs were divided into 4 groups (Table 1).

2.4. Specimen treatment

A double-sided tape was used to secure the dentin disc (n = 6) on glass slides with the polished face oriented up. PBS was used to moisten the samples and the products were applied to the specimens using a rubber cup connected to a handpiece with slow speed (NSK, Japan). After 5 min, to clear the surfaces of any remaining debris, the dentin discs were washed with distilled water. For scanning electron microscope (SEM) (ZEISS EVO Family, Germany) analysis, from each group three dentin disc were selected at random.

2.5. Application of citric acid

The rest of the specimens (n = 3) were subjected to 6 % of citric acid with a pH of 2 for a minute on Petri plate and thoroughly rinsed off for 2 min with distilled water.

2.6. Evaluation of dentinal tubule occlusion by SEM analysis

SEM assessed the extent of occlusion of tubules of dentin. A 10 kV working voltage was used to analyse the specimens. At 2000×, the SEM pictures were recorded. During the SEM analysis of each specimen, the images were obtained from the centre of each specimen to maintain the standardization in analysis.

Dentin specimens were assessed following each of the below mentioned conditions.:

1. After application of Desensitizing agent – To assess level of occlusion observed in the dentinal tubules
2. After application of Citric acid – To assess occluded tubule resilience against acid

All the specimens were analysed, tubule blockage degree was assessed and rated by two skilled, blinded endodontists. A tubule occlusion classification system by Dessai et al., was used for scoring. On a scale of 1–5 scoring was done (1) occluded (100 % of occluded tubules); (2) mostly occluded (More than 50 percent but less than 100 percent of the tubules were occluded); (3) partially occluded (More than 25 percent but less than 50 percent of tubules were occluded); (4) mostly unoccluded Less than 25 percent of tubules were occluded); (5) unoccluded

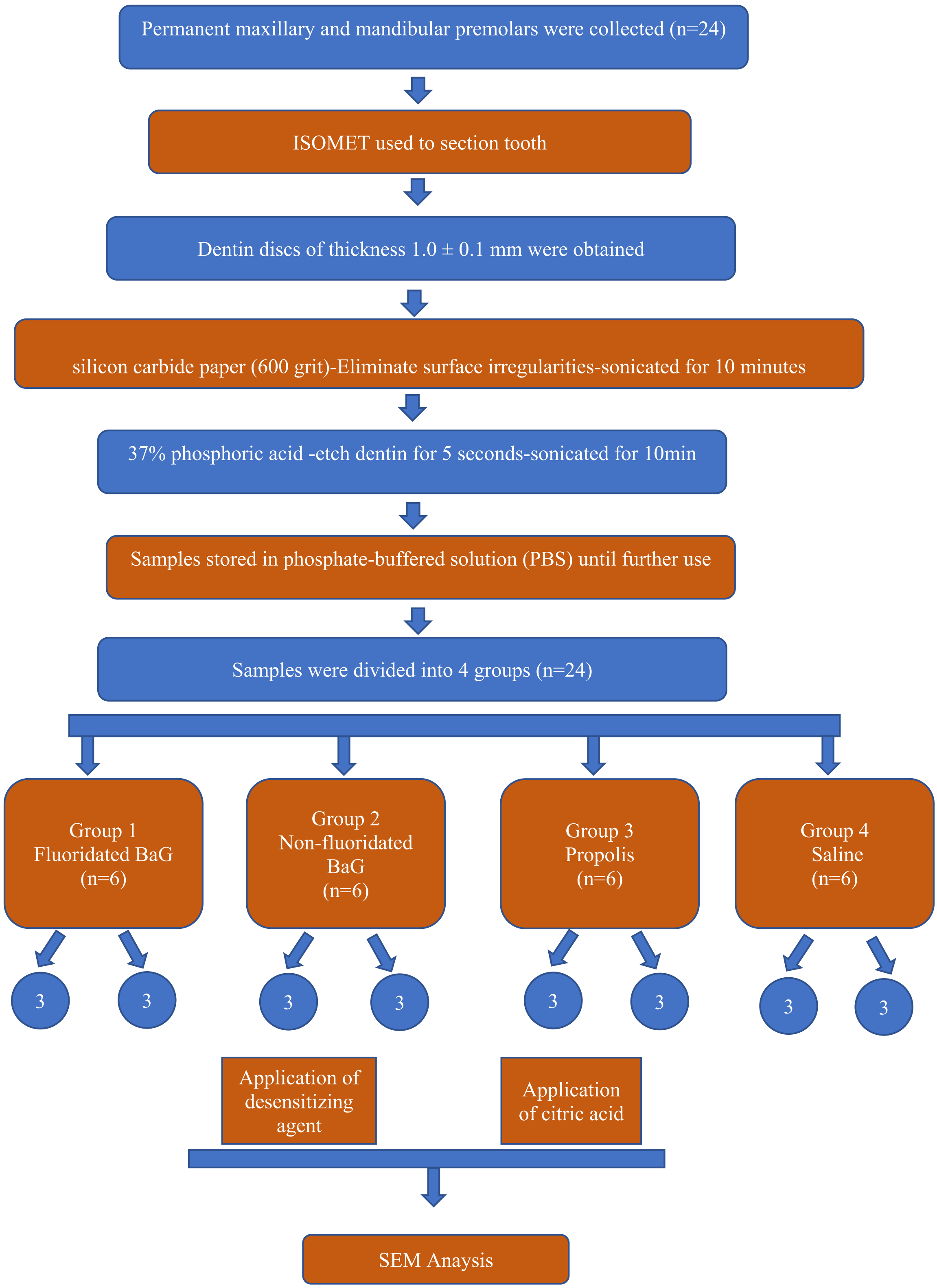

Fig. 1. Flowchart summarizing the experimental procedure.

(0 %, no occlusion of tubules seen)[24].

2.7. Statistical analysis

The IBM SPSS 20.0 version was used for the statistical analysis. Since, the sample size is less we cannot do any statistical test for checking normality. Test and assuming normality we used parametric test. Statistically significant difference was calculated using One way Anova. Multiple Comparison was calculated using Post hoc Test (Bonferroni Test). Statistical significance difference between Test and Post Acid Challenge was calculated using Independent sample *t*-test. The level of significance was considered at 5 %, i.e., $p < 0.05$ was deemed statistically significant in this research.

3. Results

(Fig. 2) depicts representative scanning microscopic image of the dentin surface with fully opened dentinal tubules after etching with phosphoric acid. Dentin discs applied with Fluoridated bioactive glass (FBaG) (Fig. 3a), Non-fluoridated BaG (Fig. 4a) showed most significant occlusion with 2.33 mean value score with no significant intergroup difference followed by Propolis with mean values of (3.00) (Fig. 5a) and least number of occluded tubules after test (Chart 1) (Table 2).

After applying a desensitizing agent, citric acid was utilized to mimic the oral environment's resistance to acid caused by acidic meals and beverages based on a study by Hongal et al.,[6]

Propolis showed most significant occlusion with mean value of (2.33) (Fig. 5b) followed by Fluoridated bioactive glass FBaG (2.66) (Fig. 3b) and Non-fluoridated BaG (3.00) (Fig. 4b) (Chart 2).

On comparing Fluoridated bioactive glass FBaG, Non-fluoridated BaG and Propolis group, all the groups showed statistically significant differences with saline group after application of desensitizing agent (Table 3). After application of citric acid, among the different groups the findings were not statistically significant. Hence, Post hoc Test was not done. Both FBaG and BaG group depicted peritubular deposition of material within the tubules. The samples treated with propolis demonstrated crystal like deposition within the dentinal tubules. After application of citric acid, in the FBaG and BaG group most tubules were in occluded state. More occluded tubules were seen in Propolis group. Saline treated specimens demonstrated unchanged tubule patency after application of desensitizing agent and after application of citric acid (Fig. 6a and b).

4. Discussion

According to reports, 15 % of people worldwide have dentine hypersensitivity.[25]

There is currently no entirely dependable therapy, one agent may work well in one situation but not in another. The hunt for the perfect desensitizing agent is still ongoing. As a first line of therapy, people with DH typically opted an at-home desensitizing toothpaste.[26] The addition of glass particles with bioactivity to toothpaste formulas appears to be a potential approach in novel dental care field. When bioactive glass particles come into contact with oral secretions, they participate in an intriguing and complex process termed as bioactivity.[27]

In 2021 the initial toothpaste with fluoride and bioglass got

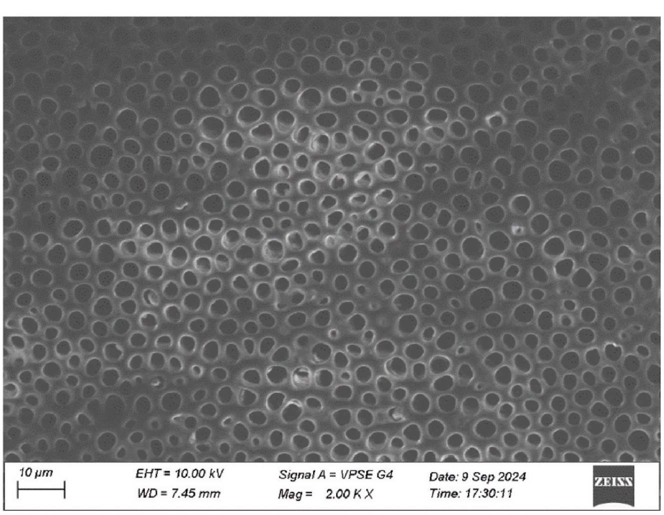

Fig. 2. Sem image of dentin showing the degree of tubule occlusion after etching with phosphoric acid.

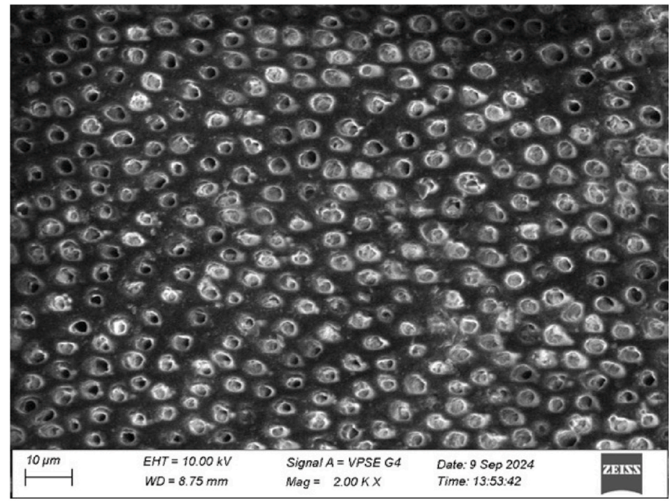

Fig. 3a. Sem image of dentin showing the degree of tubule occlusion in FBaG Group after application of desensitizing agent.

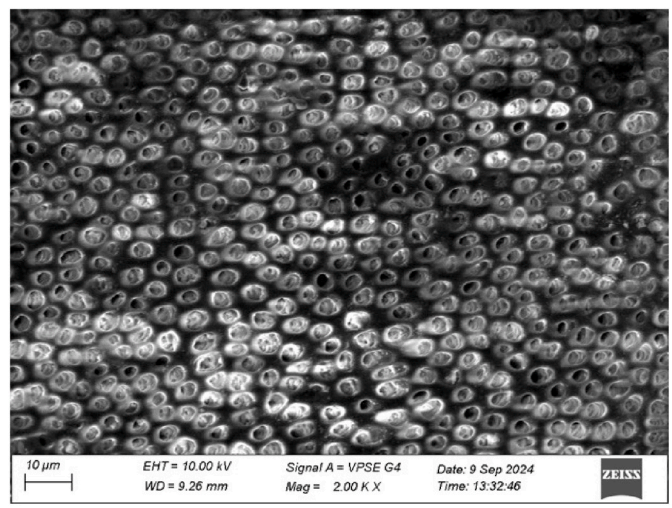

Fig. 3b. Sem image of dentin showing the degree of tubule occlusion in FBaG Group after application of citric acid.

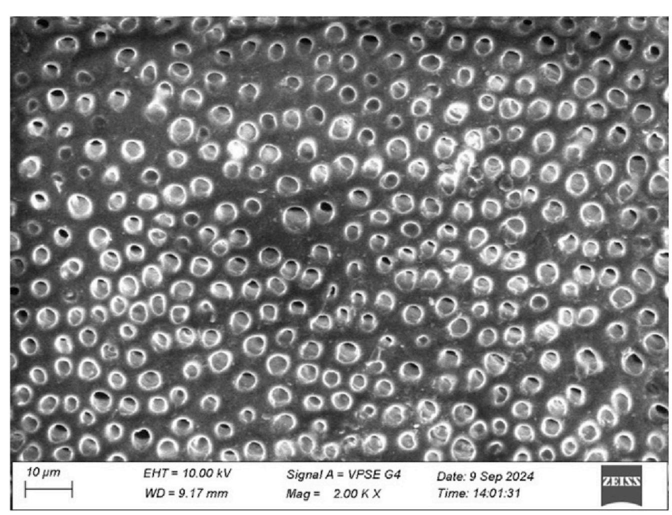

Fig. 4a. Sem image of dentin showing the degree of tubule occlusion in BaG Group after application of desensitizing agent.

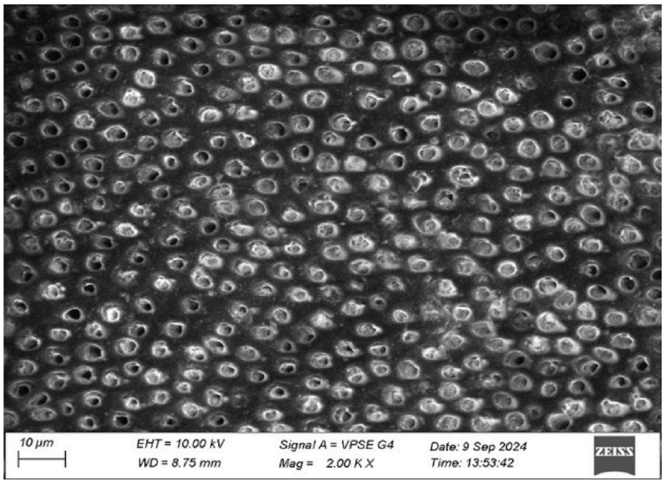

Fig. 4b. Sem image of dentin showing the degree of tubule occlusion in BaG Group after application of citric acid.

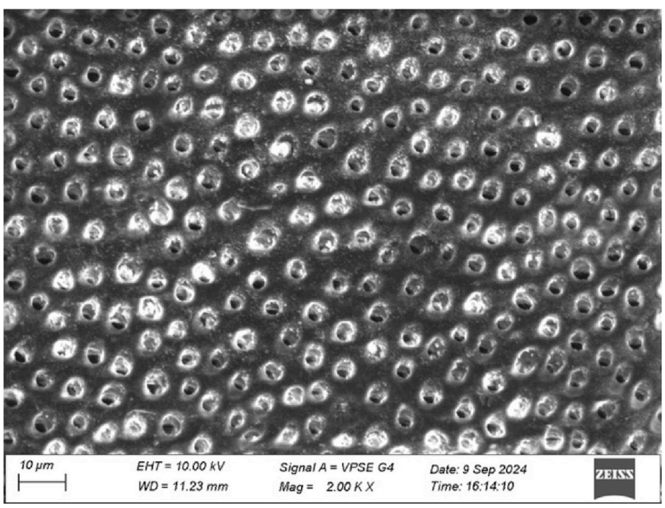

Fig. 5a. Sem image of dentin showing the degree of tubule occlusion in Propolis Group after application of desensitizing agent.

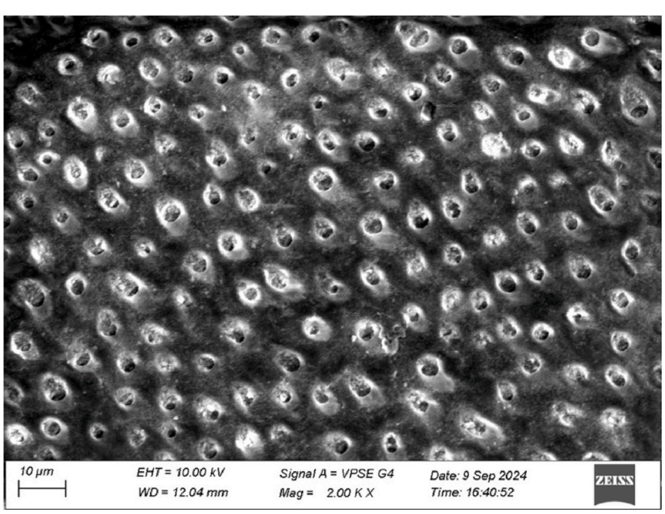

Fig. 5b. Sem image of dentin showing the degree of tubule occlusion in Propolis Group after application of citric acid.

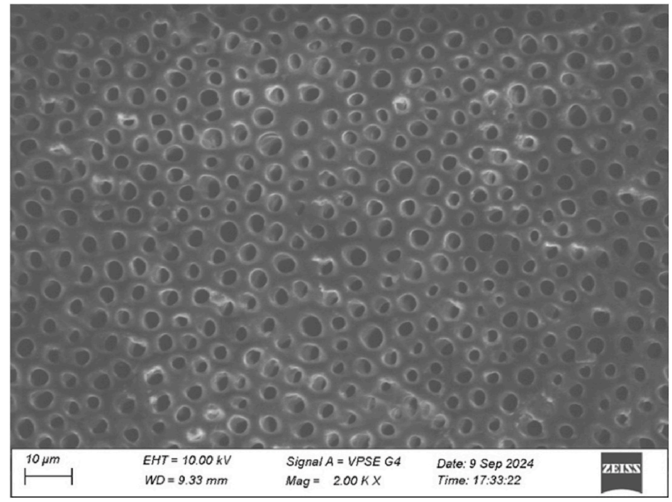

Fig. 6a. – Sem image of dentin showing the degree of tubule occlusion in Saline Group after application of desensitizing agent.

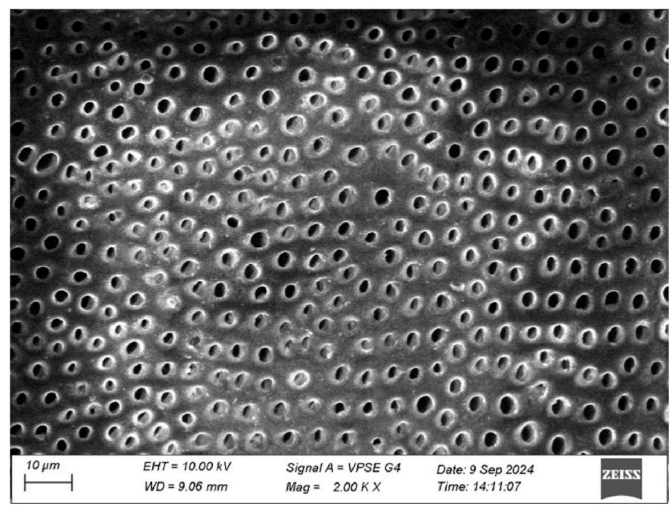

Fig. 6b. Sem image of dentin showing the degree of tubule occlusion in Saline Group after application of citric acid.

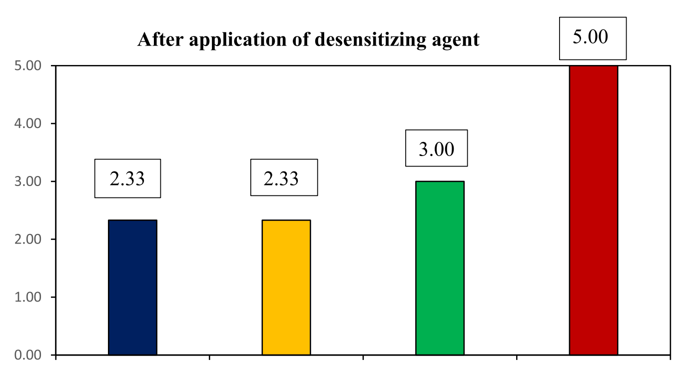

Chart 1. Degree of dentinal tubule occlusion after application of desensitizing agent.
FBaG and BaG shows highest degree of occlusion, followed by Propolis, Saline group showed no change.
FBaG: Fluoridated bioactive glass; BaG: Bioactive glass.

Table 2
One-way ANOVA was used to assess the tubule occlusion scores after application of desensitizing agent and following application of citric acid.

	Group 1 FBaG	Group 2 BaG	Group 3 Propolis	Group 4 Saline	F	P
After application of Desensitizing agent-Mean values	2.33 ±0 .57	2.33 ±0 .57	3.00 ± 0	5.00 ± 0	28.66	<0.001
After application of Citric Acid	2.66 ± 1.15	3.00 ± 1.73	2.33 ±0 .57	5.00 ± 0	3.69	0.062

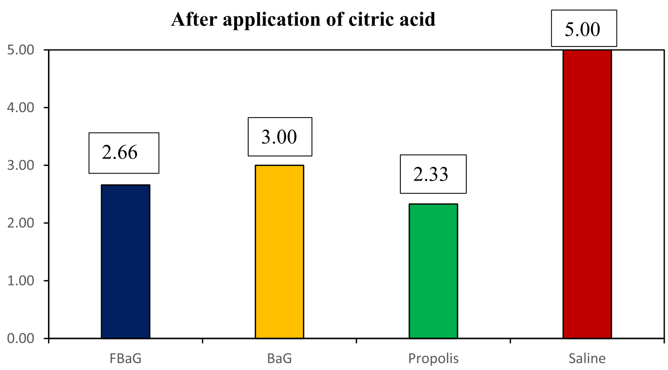

Chart 2. Degree of dentinal tubule occlusion after application of citric acid. Propolis shows highest acid resistance followed bt FBaG and BaG, Saline group showed no change.
FBaG: Fluoridated bioactive glass; BaG: Bioactive glass.

Table 3
– Post hoc Test (Bonferroni Test) after application of desensitizing agent.

Pair wise comparison	p-value
FBaG - BaG	1.000
FBaG - Propolis	0.483
FBaG - Saline	<0.001
BaG - Propolis	0.483
BaG - Saline	<0.001
Propolis - Saline	0.002

approved by U.S. Food and Drug Administration. By progressively regulating the discharge of ions of calcium, phosphate and fluoride over definite post brushing time interval, this dentifrice can increase fluorapatite that is acid resistant on surface of tooth and on exposed tubules of dentin.[28]

Based on the findings of this study, both fluoridated bioactive glass (FBaG) and Non-fluoridated BaG showed better tubule occlusion in contrast to propolis. After application of citric acid Propolis showed highest degree of occlusion followed by fluoridated bioactive glass FBaG and Non-fluoridated BaG.

In contrast to non-fluoridated BaG toothpastes, fluoridated bioactive glass (FBaG) toothpastes have a greater phosphate concentration with Calcium Fluoride (CaF_2), and have smaller average particle sizes. It creates fluorapatite on teeth instead of hydroxyapatite, which has much greater ability to resist acid by products of bacteria and encourages remineralization, especially when combined with the ions of calcium and phosphate that are liberated from the glass.[29]

Additionally, higher phosphate concentration improves the ability to produce apatite crystal, and the presence of CaF_2 accelerates glass disintegration.[29,30] After 2 min of brushing, Biomin (fluoro calcium phosphosilicate) immediately occluded the dentinal tubules. It was discovered that the dentinal tubules remained blocked even after using 6 % citric acid for 30 s. Additionally, by inhibiting the synthesis of metabolic acids, fluoro calcium phosphosilicate suppressed the metabolism of caries-causing bacteria.[31] Unlike other calcium-based products that exhibit a calcium explosion at first, NovaMin releases calcium continuously.[31] The occlusion of patent tubules of dentin by Novamin (calcium sodium phosphosilicate) is confirmed by both in vitro and clinical in situ investigations. Clinical research shows that toothpaste containing 5 %–7.5 % of calcium sodium phosphosilicate are an excellent way to treat DH.[19]

Bioactive flavonoids are among the powerful compounds found in propolis.[32] Studies confirm the ability of bioflavonoids to crystallize inside dentinal tubules and block the tubules.[33] According to studies, propolis concentrations ranging from 10 % to 30 % did not improve treatment effectiveness, suggesting that even lower amounts might be therapeutic.[34,35]

After application of citric acid, Propolis showed highest degree of occlusion followed by fluoridated bioactive glass FBaG and Non-fluoridated BaG. Propolis had both an immediate and longer-lasting impact. Its ability to seal tubules, by restricting the flow of fluid within, may be the cause of instant alleviation of sensitivity. The stability of the deposits that propolis forms may be the reason for its long-lasting effects.[32] Propolis has been demonstrated to enhance healing by promoting the production of collagen, cell metabolism, and a number of enzymes.[36]

Furthermore, the fluor-hydroxyapatite formed by BioMin F is resistant to dissolution by acid by 10 times than the hydroxyapatite formed by NovaMin, according to the BioMin manufacturer.[37] Other in vitro experiments have shown Novamin's ability to withstand acidic beverage challenges.[38–41] Only certain facets of any natural system can be examined in vitro.

4.1. *Limitations of the study*

Despite showing efficient tubule occlusion in vitro, the results might not accurately represent clinical outcomes. The durability of occlusion can be changed by oral factors like salivary flow, pH variations, plaque biofilm, and enzymatic activity. Furthermore, mechanical stresses from mastication or brushing, as well as temperature variations from food and drink, were not simulated. Therefore, although encouraging, these results require long-term clinical research and in vivo validation.

5. Conclusion

5.1. *Key findings*

Within the limits of this in vitro study, both fluoridated and non-fluoridated bioactive glass containing dentifrices showed a notable ability to occlude dentinal tubules. Propolis further enhanced resistance to acid challenge.

5.2. *Clinical implications*

BaG-based dentifrices may be recommended for reducing dentin hypersensitivity, while propolis could be beneficial in managing erosive or non-carious lesions.

5.3. *Future directions*

In vivo and long-term clinical studies are required to validate the durability and real-world effectiveness of these desensitizing agents.

Ethical Approval

This in vitro study was approved by the Institutional Review Board (ECASM-AIMS-2024-077).

Source of funding

Nil.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Nil.

List of Abbreviations

DH	Dentin hypersensitivity
FBaG	Fluoridated Bioactive Glass
BaG	Bioactive glass
ACC	Arginine Calcium Carbonate
PBS	Phosphate-buffered solution
SEM	scanning electron microscopy
Calcium Fluoride	CaF2

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