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Scanning Electron Microscopic Assessment of Dentinal Tubule Occlusion Induced by Various Desensitizing Dentifrices

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Abstract:

- ***Aim:***
  The present in vitro investigation was designed to comparatively assess the dentinal tubule occluding ability of three commercially available desensitizing dentifrices containing Novamin, nano-hydroxyapatite, and propolis.

- ***Materials and Methods:***
  Forty extracted human premolars and molars were utilized to prepare standardized dentin discs from the mid-coronal region. To simulate dentinal hypersensitivity, the specimens were treated with 37% phosphoric acid for 30 seconds to remove the smear layer and open the dentinal tubules. The samples were randomly allocated into four groups (n = 10): Group I—control (saline), Group II—Novamin-based dentifrice, Group III—nano-hydroxyapatite-based dentifrice, and Group IV—propolis-based dentifrice. The dentifrices were applied once daily for two minutes over a period of seven days. Following treatment, the extent of dentinal tubule occlusion was evaluated using scanning electron microscopy (SEM).

- ***Results:***
  Specimens treated with the nano-hydroxyapatite dentifrice demonstrated the highest degree of dentinal tubule occlusion, followed by the Novamin-containing dentifrice. The propolis-based dentifrice showed partial tubule occlusion, while the control group exhibited predominantly patent tubules.

- ***Conclusion:***
  All tested desensitizing dentifrices were capable of occluding dentinal tubules to varying extents. Among them, nano-hydroxyapatite was found to be the most effective. Propolis-containing dentifrice, owing to its natural origin and moderate efficacy, may be considered a viable alternative for the management of dentinal hypersensitivity.

Keywords: *Dentinal Hypersensitivity, Dentinal Tubule Occlusion, Novamin, Nano-Hydroxyapatite, Propolis, Scanning Electron Microscopy.*

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I. INTRODUCTION

Dentinal hypersensitivity (DH) is a commonly encountered clinical condition that presents as a short, sharp pain arising from exposed dentin in response to thermal, tactile, osmotic, or chemical stimuli, in the absence of any other identifiable dental pathology. It affects a substantial proportion of the adult population, with reported prevalence rates ranging widely due to differences in study design, diagnostic criteria, and population characteristics (1). The condition significantly impacts patient comfort and quality of life, thereby making its effective management an important aspect of routine dental care.

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The etiology of dentinal hypersensitivity is multifactorial and is primarily associated with the exposure of dentinal tubules following loss of enamel or cementum. Contributing factors include improper or aggressive tooth brushing, gingival recession, periodontal therapy, erosion, abrasion, attrition, chemical insults, and certain esthetic dental procedures such as tooth bleaching. These factors increase dentin permeability by exposing and enlarging dentinal tubules, thereby predisposing the tooth to hypersensitive responses (2).

Several theories have been proposed to explain the mechanism of pain associated with dentinal hypersensitivity, including the odontoblastic transduction theory, neural theory, and hydrodynamic theory. Among these, the hydrodynamic theory proposed by Brännström is the most widely accepted. According to this concept, external stimuli result in rapid movement of fluid within open dentinal tubules, which stimulates mechanoreceptors at the pulpal ends of the tubules and elicits pain (3).

Management strategies for dentinal hypersensitivity are directed toward either decreasing the excitability of pulpal nerves or reducing dentin permeability by occluding exposed dentinal tubules. Numerous in-office and at-home treatment modalities have been advocated, such as dentinal bonding agents, varnishes, liners, restorative materials, soft tissue grafts, lasers, and mouth rinses. However, desensitizing dentifrices continue to be the most widely recommended first-line option due to their non-invasive nature, cost-effectiveness, and ease of patient compliance (4).

Desensitizing dentifrices act primarily through two mechanisms: neural desensitization and tubule occlusion. Potassium-based compounds reduce pain transmission by depolarizing nerve fibres, whereas tubule-occluding agents function by forming insoluble precipitates within dentinal tubules, thereby limiting fluid movement and reducing hypersensitivity (5).

Among the various tubule-occluding agents incorporated into contemporary dentifrices, calcium sodium phosphosilicate (Novamin) has demonstrated significant bioactivity. In the presence of saliva, Novamin releases calcium and phosphate ions that facilitate the formation of a hydroxycarbonate apatite layer over exposed dentin and within dentinal tubules, closely resembling natural tooth mineral (6). Similarly, nano-hydroxyapatite has emerged as a biomimetic material with promising desensitizing potential due to its nanometric particle size and chemical similarity to enamel and dentin, enabling effective penetration and occlusion of dentinal tubules (7).

In recent years, interest has also grown in naturally derived agents such as propolis for the management of dentinal hypersensitivity. Propolis is a resinous substance produced by bees and is rich in bioflavonoids, waxes, essential oils, and minerals. Previous investigations have suggested that propolis can partially occlude dentinal tubules and may additionally promote reparative dentin formation, contributing to its desensitizing effect (8).

Considering the increasing availability of desensitizing dentifrices with diverse mechanisms of action, comparative evaluation of their effectiveness is essential. Therefore, the present in vitro study was conducted to evaluate and compare the dentinal tubule occluding efficacy of dentifrices containing Novamin, nano-hydroxyapatite, and propolis using scanning electron microscopy.

II. MATERIAL AND METHOD

➢ *Sample Selection* -

Forty freshly extracted human premolars and molars were selected for the study.

➢ *Inclusion Criteria*-

- Intact, non-carious extracted premolars and molars.
- Teeth obtained from individuals aged between 18 and 40 years.
- Absence of developmental defects, fractures, craze lines, or wasting diseases.

➢ *Exclusion Criteria*-

- Carious or restored teeth.
- Teeth obtained from individuals below 18 years or above 40 years of age.
- Teeth with developmental anomalies, fractures, craze lines, or signs of attrition, erosion, or abrasion.

➢ *Sample Preparation*-

Following extraction, teeth were thoroughly cleaned of debris and soft tissue remnants and stored in 10% formalin solution until use. Using a water-cooled diamond disc mounted on a slow-speed handpiece, two horizontal sections were made perpendicular to the long axis of each tooth above the cemento-enamel junction to obtain mid-coronal dentin blocks. The surrounding enamel was removed to produce standardized dentin specimens measuring approximately 5 mm × 5 mm × 2 mm.

To simulate hypersensitive dentin, the specimens were etched with 37% phosphoric acid for 30 seconds to remove the smear layer and expose the dentinal tubules, followed by thorough rinsing with distilled water.

➢ *Group Allocation and Treatment Protocol*-

The prepared specimens were randomly assigned to four groups (n = 10):

- Group I: Control (normal saline)
- Group II: Novamin-containing desensitizing dentifrice
- Group III: Nano-hydroxyapatite-containing desensitizing dentifrice
- Group IV: Propolis-containing desensitizing dentifrice

Specimens in Groups II–IV were brushed with the respective dentifrices using a rubber prophylactic cup mounted on a slow-speed handpiece for two minutes daily over seven consecutive days. After each brushing cycle, the

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samples were rinsed with distilled water and stored in artificial saliva. Group I specimens were maintained in normal saline throughout the study period.

➢ *Scanning Electron Microscopy Analysis-*

After completion of the treatment protocol, the specimens were air-dried, mounted on aluminum stubs, and sputter-coated with a thin layer of palladium. SEM evaluation was carried out using a scanning electron microscope at 500× magnification and an accelerating voltage of 20 kV. Photomicrographs obtained from the central region of each specimen were analyzed for the presence of open, partially occluded, and completely occluded dentinal tubules.

➢ *Statistical Analysis-*

The percentage of dentinal tubule occlusion was calculated for each specimen using the formula:

$$\text{Percentage of tubule occlusion} = \frac{\text{Number of occluded tubules}}{\text{Total number of tubules}} \times 100$$

Statistical analysis was performed using SPSS software version 20.0. Mean values and standard deviations were calculated. Intergroup comparisons were carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

III. RESULT

Dentinal specimens in Group I demonstrated predominantly open dentinal tubules on SEM examination (Figure 1). In Group II, the photomicrographs revealed a greater number of dentinal tubules exhibiting uniform occlusion (Figure 2). Specimens from Group III showed extensive tubule sealing, characterized by either complete occlusion or marked narrowing of the tubule lumen (Figure 3). In contrast, Group IV samples exhibited partial tubule occlusion, with several dentinal tubules remaining patent (Figure 4).

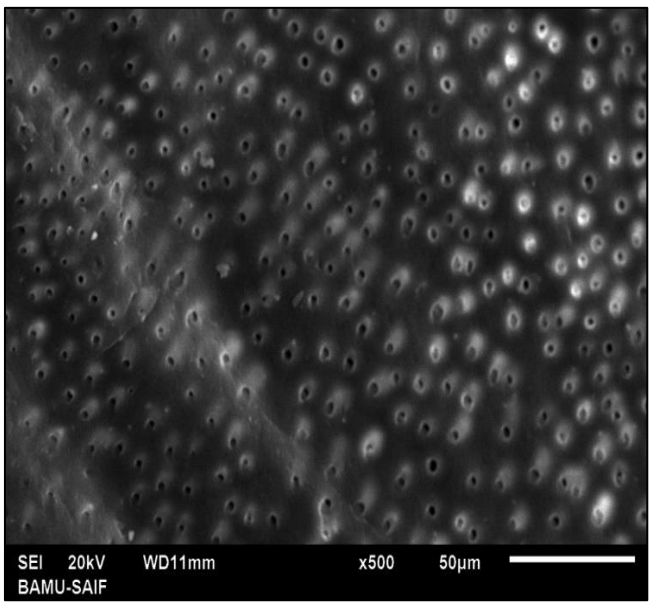

Fig 1 Group I Demonstrated Predominantly Open Dentinal Tubules on SEM Examination

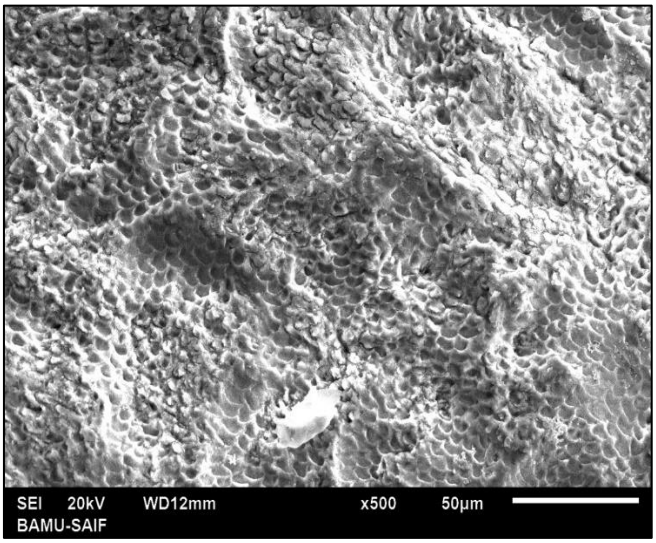

Fig 2 Group II, the Photomicrographs Revealed a Greater Number of Dentinal Tubules Exhibiting Uniform Occlusion

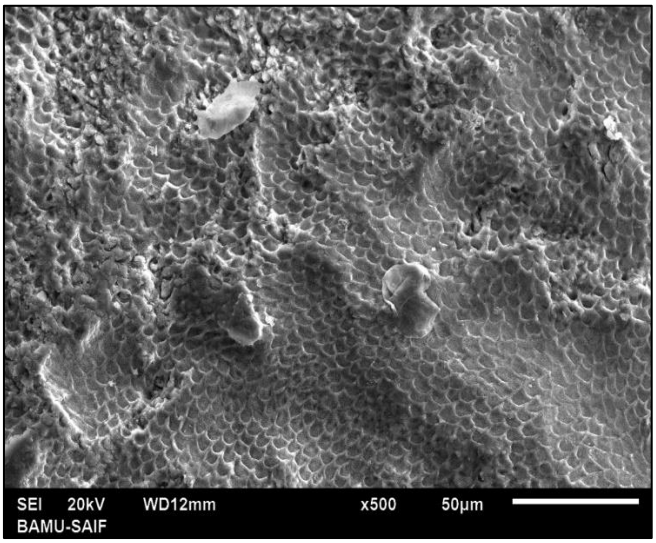

Fig 3 Group III Showed Extensive Tubule Sealing, Characterized by Either Complete Occlusion or Marked Narrowing of the Tubule Lumen

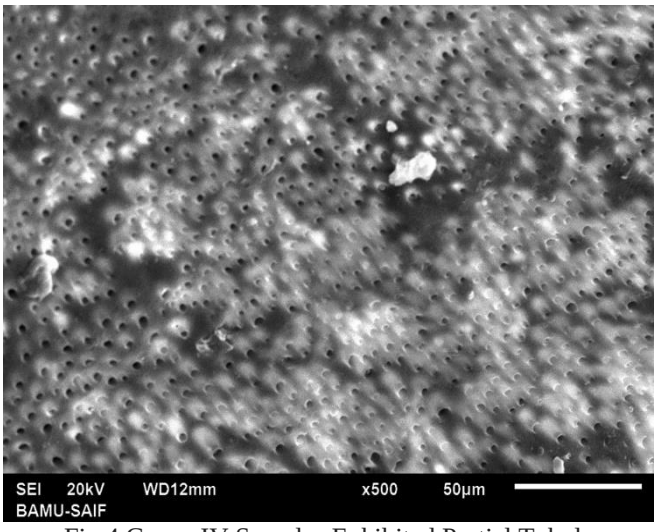

Fig 4 Group IV Samples Exhibited Partial Tubule Occlusion, with Several Dentinal Tubules Remaining Patent

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The percentage of dentinal tubule occlusion observed in the different experimental groups is presented in Table 1. Group I (control) demonstrated minimal tubule occlusion, with a mean value of 0.892 ± 0.198. In contrast, specimens treated with desensitizing dentifrices showed markedly higher levels of tubule occlusion. Group III (nano-hydroxyapatite) exhibited the highest mean percentage of occluded dentinal tubules (96.336 ± 2.675), followed by Group II (Novamin) (83.549 ± 3.986). Group IV (propolis) demonstrated a comparatively lower mean tubule occlusion (64.227 ± 5.401). One-way analysis of variance (ANOVA) revealed a statistically significant difference in dentinal tubule occlusion among the four groups ($p < 0.05$). Intergroup comparison using post-hoc Tukey's test (Table 2) showed a statistically significant difference between Group II and Group III (mean difference = −12.787; $p < 0.05$), Group II and Group IV (mean difference = 19.322; $p < 0.05$), and Group III and Group IV (mean difference = 32.109; $p < 0.05$).

Table 1 Mean Percentage of Dentinal Tubule Occlusion Observed in the Experimental Groups Following One-Way ANOVA.

Group	Mean (%)	Standard Deviation
Group I (Control)	0.892	0.198
Group II (Novamin)	83.549	3.986
Group III (Nano-hydroxyapatite)	96.336	2.675
Group IV (Propolis)	64.227	5.401

Table 2 Pairwise Comparison of Dentinal Tubule Occlusion Between Groups Using Tukey's Post-hoc Test.

Group Comparison	Mean Difference	Level of Significance
Group II vs Group III	−12.787	P < 0.05*
Group II vs Group IV	19.322	P < 0.05*
Group III vs Group IV	32.109	P < 0.05*

IV. DISCUSSION

Dentinal hypersensitivity occurs as a result of exposure of dentinal tubules due to the loss of protective enamel or cementum, commonly associated with gingival recession, periodontal therapy, erosion, abrasion, attrition, and other non-carious tooth surface loss. This exposure allows external stimuli to induce dentinal fluid movement, leading to hypersensitive responses (9). The management of dentinal hypersensitivity remains challenging due to its multifactorial etiology and the wide range of therapeutic options currently available. Nonetheless, effective occlusion of exposed dentinal tubules remains a key determinant in reducing dentin permeability and alleviating symptoms (10).

The two principal approaches for managing dentinal hypersensitivity include suppression of neural activity to reduce pain perception and mechanical or chemical occlusion of dentinal tubules to restrict fluid movement. Tubule occlusion is achieved through the deposition of materials that physically block or seal the tubules, thereby reducing the hydrodynamic mechanism responsible for pain transmission (11).

In the present study, the nano-hydroxyapatite-containing dentifrice exhibited the highest degree of dentinal tubule occlusion among all experimental groups. This superior occluding ability can be attributed to the nanoscale size of hydroxyapatite particles, which facilitates their penetration into open dentinal tubules and promotes the formation of a mineralized layer within the tubule lumen (12). These findings are in agreement with previous studies that reported enhanced tubule occlusion and improved desensitizing efficacy of nano-hydroxyapatite-based formulations (13).

The Novamin-containing dentifrice also demonstrated a significant increase in dentinal tubule occlusion when compared with the control and propolis groups. Novamin, a calcium sodium phosphosilicate, releases calcium and phosphate ions upon interaction with saliva, leading to the formation of a hydroxycarbonate apatite layer that deposits on exposed dentin surfaces and within dentinal tubules (14). The findings of the present study are consistent with earlier in vitro investigations that reported reduced dentin permeability and effective tubule sealing following Novamin application (15). Furthermore, several clinical studies have documented the efficacy of Novamin-containing dentifrices in reducing dentinal hypersensitivity (16,17). Variations in specimen preparation techniques and application protocols may explain minor discrepancies observed among different studies (18).

The propolis-containing dentifrice evaluated in this study showed moderate dentinal tubule occlusion compared to saline. This observation supports previous in vitro studies that demonstrated a reduction in dentin permeability with the application of propolis-based products (19). Clinical evidence has also indicated a significant reduction in dentinal hypersensitivity following the use of propolis-containing formulations (20,21). The desensitizing effect of propolis is believed to result from the interaction of its bioactive components, particularly bioflavonoids and waxes, with the dentin surface, leading to partial sealing of dentinal tubules (8). In addition, propolis has been reported to enhance the expression of transforming growth factor beta-1 (TGF-β1), which plays an important role in odontoblast differentiation and reparative dentin formation (22).

Several laboratory methods have been employed to evaluate the efficacy and mechanism of action of desensitizing agents, including dentin permeability tests, confocal laser scanning microscopy, and dispersive X-ray analysis. In the present study, scanning electron microscopy was selected as the analytical method due to its ability to provide high-resolution visualization of dentinal surfaces and

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tubule morphology without causing damage to the specimens, making it a reliable tool for assessing dentinal tubule occlusion (1,23).

V. LIMITATIONS

As an in vitro investigation, the present study could not fully replicate the complex conditions of the oral environment. Factors such as salivary flow, dietary habits, brushing technique, and parafunctional activities may influence the clinical performance of desensitizing dentifrices. Further long-term in vivo studies are therefore recommended to validate the findings.

VI. CONCLUSION

➢ *Within the Limitations of this in Vitro Study, the Following Conclusions may be Drawn:*

- All tested desensitizing dentifrices were capable of occluding dentinal tubules.
- Nano-hydroxyapatite-containing dentifrice demonstrated the highest level of tubule occlusion.
- Propolis-based dentifrice showed moderate efficacy and may be considered a natural alternative for managing dentinal hypersensitivity.

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