

# Comparative Evaluation of Two Novel Natural Desensitizing Agents on Dentinal Tubule Occlusion: A Scanning Electron Microscopy Study

Prem P Kar<sup>1</sup>, Prahlad A Saraf<sup>2</sup>, Laxmikant Kamatgi<sup>3</sup>, Preeti Naik<sup>4</sup>

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## ABSTRACT

**Aim:** The present *in vitro* study is aimed to compare the effectiveness of dentinal tubule occlusion of two natural-based desensitizing toothpaste (Bentodent Desensitizer and Fang Farm Mint) and Novamin-based toothpaste (Shy-NM) under scanning electron microscope (SEM).

**Materials and methods:** A total of 60 root dentin discs (3 mm-thick) were obtained from recently extracted permanent premolar teeth and were randomly divided into four groups based on the desensitizing toothpaste used, each group with 15 samples: Group I: Control, group II: Shy-NM, group III: Bentodent desensitizer, group IV: Fang Farm Mint. Samples were brushed for 2 min twice daily with a soft toothbrush with respective pea size amount of toothpaste for 14 days manually. The samples were evaluated for dentin tubule occlusion under SEM at  $\times 4000$  magnification.

**Results:** Statistical analysis was done using one-way ANOVA and Tukey *post hoc* multiple procedures. All test groups showed significantly more dentinal tubule occlusion when compared to control group. Shy-NM showed significantly more dentinal tubule occlusion when compared to Bentodent desensitizer and Fang Farm Mint.

**Conclusion:** All desensitizing toothpastes were effective in dentine tubule occlusion compared to the control group. Shy-NM provides effective dentin tubule occlusion compared to other toothpastes and thereby can be considered a promising option to relieve dentinal hypersensitivity (DH).

**Clinical significance:** Shy-NM can effectively reduce DH. Bentodent desensitizer and Fang Farm Mint can be a safer alternative to relieve DH.

**Keywords:** Dentin hypersensitivity, Desensitizing toothpaste, Natural, Novamin, Scanning electron microscopy.

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## INTRODUCTION

Dentinal hypersensitivity (DH) is one of the most common complaints from patients in dental clinics. It is defined as a short sharp pain that arises from exposed dentine in response to non-noxious stimuli, typically thermal, evaporative, tactile, osmotic, or chemical, and that cannot be ascribed to any other form of dental defects or diseases.<sup>1</sup>

The occurrence rate of DH usually ranges from 4 to 74%.<sup>2,3</sup> It is usually seen in individuals between 20 and 40 years of age. Dentinal hypersensitivity develops in two phases: lesion localization and lesion initiation. Etiological factors include poor oral hygiene, premature contact, gingival recession due to periodontal therapy, faulty tooth brushing, exogenous/endogenous nonbacterial acids, dietary habits, and low pH mouth rinses. It is usually seen more in females than males.<sup>4</sup> It is more observed in the Bucco cervical region of canine and premolar teeth in the maxilla.<sup>5,6</sup>

The hydrodynamic theory is a widely accepted theory to explain the mechanism of DH.<sup>7,8</sup> A wide variety of products are available that can be used to relieve the pain and discomfort caused by DH. These products can be categorized based on their mode of action. The first category is nerve desensitizing agents which consist of potassium salts which are believed to function by entering the dentin tubule and depolarizing the nerve thereby reducing DH. The second category of desensitizing agents acts by plugging the open dentinal tubules.

Shy-NM is a novel desensitizing toothpaste that contains Novamin/CSPS (calcium sodium phospho silicate). Novamin when encounters saliva releases sodium, calcium, and phosphate

<sup>1-4</sup>Department of Conservative Dentistry and Endodontics, PMNM Dental College and Hospital, Bagalkot, Karnataka, India

**Corresponding Author:** Prem P Kar, Department of Conservative Dentistry and Endodontics, PMNM Dental College and Hospital, Bagalkot, Karnataka, India, Phone: +91 7259194585, e-mail: kar0908@gmail.com

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ions. Sodium ions cause an elevation of pH whereas calcium and phosphate ions form a calcium phosphate layer. The calcium phosphate layer crystallizes into hydroxycarbonate apatite occluding dentinal tubules, as shown in scanning electron microscope (SEM) analysis as demonstrated by Gillam *et al.*<sup>9-11</sup>

In recent times, people are more inclined towards the use of natural-based desensitizing toothpastes as an alternative, due to lesser side effects and similar action. Bentodent Desensitizer is a novel natural-based desensitizing toothpaste containing bentonite clay, propolis, and guava leaf extract. Bentonite clay along with Propolis and guava leaf extract seals the dentinal tubules relieving DH.<sup>11</sup>

Fang Farm Mint Toothpaste is another novel natural-based desensitizing toothpaste. This paste claims to contain pure nano-hydroxy apatite which remineralizes the teeth and reduces DH.

The paste is free from chemicals such as SLS, triclosan, parabens, and fluoride. Also, they have essential oils and ayurvedic extracts which prevent oral diseases and long-term health.<sup>12</sup>

To date, no research has been attempted using Fang Farm mint toothpaste, and only a few studies done on Bentodent desensitizer toothpaste. In the present study, we aimed to compare the effectiveness of dentinal tubule occlusion of two natural-based desensitizing toothpaste (Bentodent Desensitizer and Fang Farm Mint) and Novamin-based toothpaste (Shy-NM) under SEM.

## MATERIALS AND METHODS

### Sample Size Estimation

This experimental study was conducted from July 2024 to September 2024 (two months duration) in the Department of Conservative Dentistry and Endodontics, PMNM Dental College and Hospital, Bagalkot, Karnataka for the duration of around following approval from the Institutional Ethical Committee (PMNMDCH/679/2024-25) The sample size was calculated using G\*Power software (version 3.1.9.7., Heinrich Heine-Universität, Dusseldorf, Dusseldorf, Germany).<sup>13</sup> It was used with an effect size of 0.90, an alpha value of 0.05, and a power of 95%. The results estimated a total number of 60 required samples.

### Tooth Selection

Sixty Human Permanent premolar teeth from both arches were extracted following orthodontic treatment, which was collected from the Department of Oral Surgery of the same hospital and stored in distilled water (4°C) and used within 1 month of extraction.<sup>14,15</sup>

### Inclusion and Exclusion Criteria

Caries-free crowns and roots, and teeth without any attrition, abrasion, and erosion were included in the study. Teeth with caries, developmental anomalies, root canal treatment, color change, and rough root surface were excluded from the study.<sup>16</sup>

### Dentin Disc Preparation

All samples were first scaled and cleaned manually with Hu-Friedy universal currettes to remove the soft tissue and debris attached to the teeth and were then rinsed in distilled water. Afterward, each tooth was sectioned perpendicularly to the long axis of the tooth at the cemento-enamel junction (CEJ) and 3 mm apical, then a third cutting to make buccal and lingual halves using a diamond disc. In this way, 60 root dentin discs (3mm thick) were prepared. Then all dentin discs were placed with their buccal surfaces facing upwards into self-cured acrylic resin. The cementum layer in the buccal half of the dentin discs was removed using a high-speed water-cooled diamond bur. To standardize and obtain a flat and uniform surface, all discs were smoothed using 600 grit silicon carbide (SiC) polishing papers for 1 minute; the polished samples were then placed in distilled water and sonicated for 5 minutes to remove the polishing abrasive. After sonication, the samples were rinsed with saline. Then, all specimens were exposed to 17% ethylene diamine tetra acetic acid solution for 3 minutes to remove the smear layer and then rinsed with distilled water to expose dentin tubules.<sup>17,18</sup>

### Experimental Groups

The mounted dentin samples were then randomly divided into four groups (Study design, Fig. 1):

Group I: No treatment (control,  $n = 15$ ).

Group II: Samples treated with Shy-NM, Group Pharmaceuticals Ltd, India ( $n = 15$ ).

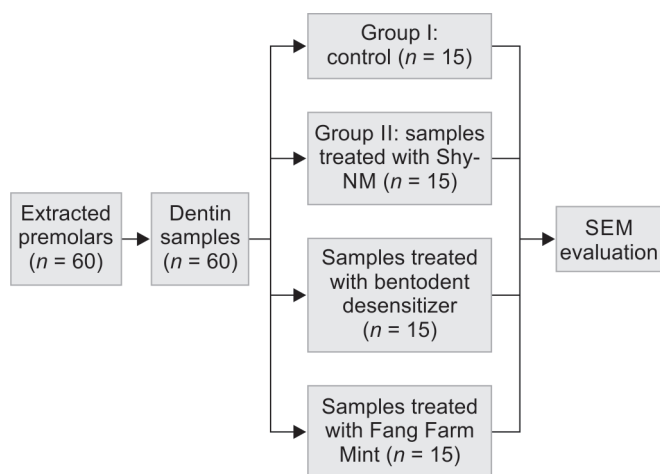


Fig. 1: Study design

Group III: Samples treated with Bentodent Desensitizer, Indian Dental Organization, Punjab, India ( $n = 15$ ).

Group IV: Samples treated with Fang Farm Mint, Couch Commerce Pvt. Ltd., India ( $n = 15$ ).

### Brushing Technique

A separate toothbrush was used for each group. Samples were brushed for 2 min twice daily with a soft toothbrush with respective pea size amounts of toothpaste for 14 days manually to completely cover the surface of the discs and at a 90° inclination to the dentin surface. The samples were rinsed under running water to remove the toothpaste and were stored in distilled water.

### Scanning Electron Microscope Analysis

After the last brushing session, the samples were thoroughly washed in distilled water followed by 70% ethanol to remove any debris on the surface. Samples were fastened to a metal support with a ribbon of carbon after which samples were conditioned in a vacuum desiccator to allow for evaporation. On complete drying, they were sputter-coated with a thin layer of gold (50 nm) in the sputter coating machine and photomicrographs were taken using SEM (Phenom Prox SEM, Thermo Fischer Scientific) at 4000× and 15 kV.

### Statistical Analysis

The percentage of occluded tubules was obtained by dividing the total number of occluded tubules by total number of tubules in the photomicrographs (Fig. 2). This result was then multiplied by 100 to obtain the percentage of occluded tubules for each photomicrograph. Results were statistically analyzed using one-way ANOVA and Tukey *post hoc* multiple procedure test in SPSS software version 24 (SPSS, Chicago, USA). The level of statistical significance was set at 0.05.

## RESULTS

Among all the groups the mean percentage of dentinal tubule occlusion was highest in Shy-NM (77.09), followed by Bentodent Desensitizer (62.52), Fang Farm Mint (60.66), control group (10.07) (Fig. 3 and Table 1).

There was a statistically significant difference in the mean percentage of dentinal tubule occlusion among the dentin samples between the groups (Table 2).

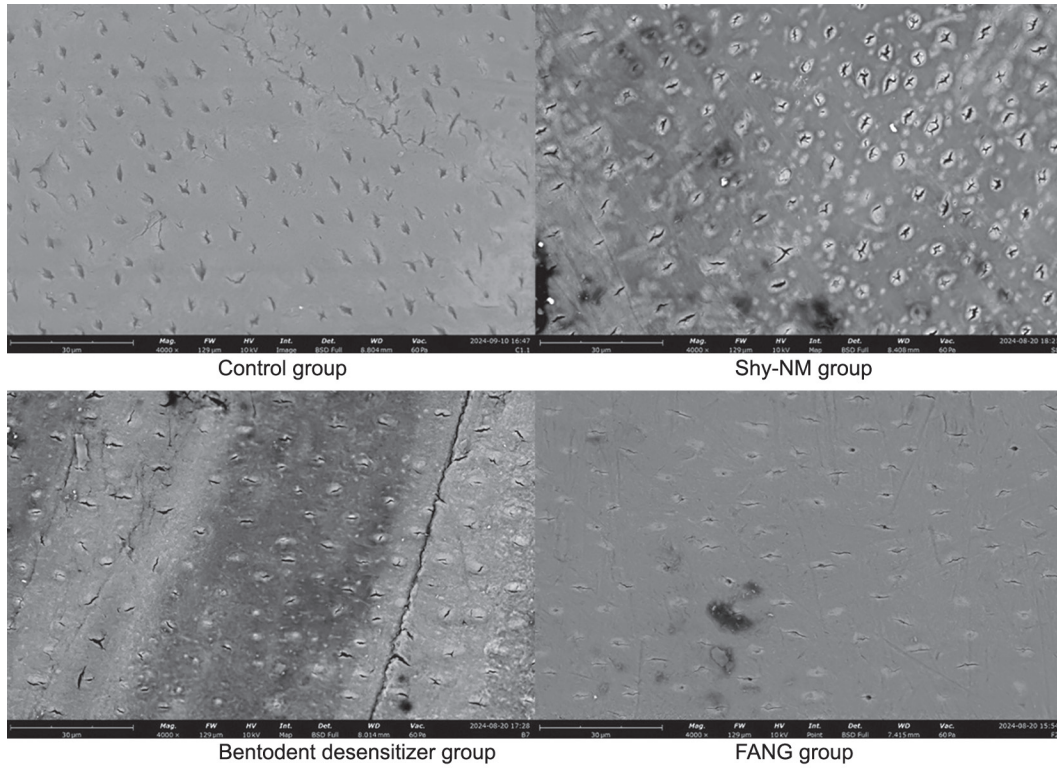


Fig. 2: Scanning electron microscope photomicrograph (x4000) for all groups

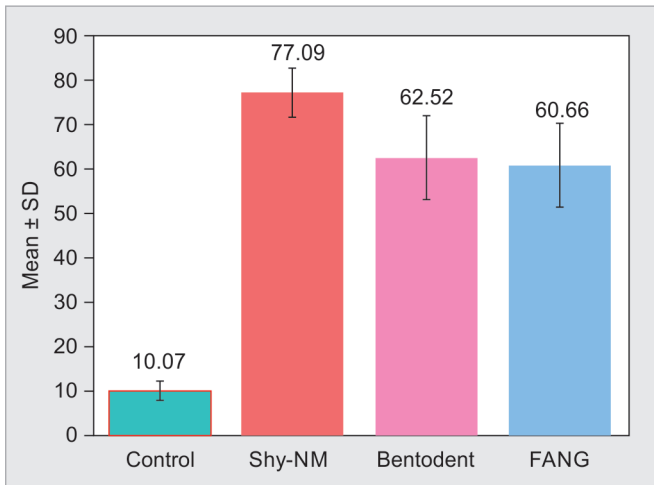


Fig. 3: Comparison of three desensitizing kinds of toothpaste with mean percentage of dentinal tubule occlusion

Table 1: Mean percentage of dentin tubule occlusion

Toothpaste	N	Mean	Std. Dev.	Std. Err.	95% CI for mean	
					Lower	Upper
Control	15.00	10.07	2.17	0.56	8.86	11.27
Shy-NM	15.00	77.09	5.59	1.44	73.99	80.18
Bentodent	15.00	62.52	9.37	2.42	57.33	67.71
FANG	15.00	60.66	9.29	2.40	55.51	65.80

All test groups showed significantly more tubule occlusion as compared to control groups. Shy-NM showed significantly more

Table 2: Comparison of three desensitizing toothpastes with mean percentage of dentin tubule occlusion by one-way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	3	38580.09	12860.03	244.7463	0.0001*
Within groups	56	2942.48	52.54		
Total	59	41522.57			

\*p < 0.05

Table 3: Pair-wise comparison of three desensitizing kinds of toothpaste with a mean percentage of dentinal tubule occlusion by Tukey's post hoc multiple procedure

Toothpaste	Control	Shy-NM	Bentodent	Fang
Mean	10.07	77.09	62.52	60.66
Std. Dev.	2.17	5.59	9.37	9.29
Control	-			
Shy-NM	p = 0.0002*	-		
Bentodent	p = 0.0002*	p = 0.0002*	-	
FANG	p = 0.0002*	p = 0.0002*	p = 0.8951	-

\*p < 0.05

tubule occlusion when compared to Bentodent Desensitizer and Fang Farm Mint. There was no statistically significant difference in tubular occlusion among Bentodent Desensitizer and Fang Farm Mint (Table 3).

Thus, the efficacy of Shy-NM toothpaste was found greater compared to other toothpastes. Shy-NM can give promising results in alleviating DH pain.



## DISCUSSION

In recent times, people have been more inclined towards the use of natural-based desensitizing toothpaste as an alternative, due to lesser side effects and similar action. Bentodent Desensitizer and Fang Farm Mint are two novel natural-based desensitizing toothpaste that claim to relieve dentinal hypersensitivity naturally by occluding the dentinal tubules. Thus, the present study was conducted to evaluate the efficacy of dentin tubule occlusion of two natural-based desensitizing toothpaste (Bentodent desensitizer and Fang Farm Mint) and Novamin-based toothpaste (Shy-NM) under SEM.

In the present study percentage of occluded tubules was obtained by dividing the total number of occluded tubules by the total number of tubules in the photomicrographs. This result was then multiplied by 100 to obtain the percentage of occluded tubules for each photomicrograph.<sup>18</sup>

In the present study separate toothbrush was used for each group. Samples were brushed for 2 minutes twice daily with a soft toothbrush with respective pea size amounts of toothpaste for 15 days manually to completely cover the surface of the discs and at a 90° inclination to the dentin surface. The samples were rinsed under running water to remove the toothpaste and were stored in distilled water.<sup>19,20</sup>

In the present study, SEM analysis was done according to Kripal et al.<sup>21</sup> and Gupta et al.<sup>22</sup> Photomicrographs were taken using SEM (Phenom Prox SEM, Thermo Fischer Scientific) at 4000x and 15 kV.

In the present study, Shy-NM was used as a gold standard desensitizing toothpaste to which 2 novel natural-based desensitizing toothpaste (Bentodent desensitizer and Fang Farm Mint) are compared. It contains Novamin/CSPS, which when exposed to saliva results in information on the calcium phosphate layer.<sup>23</sup> The calcium phosphate layer crystallizes into hydroxycarbonate apatite occluding dentinal tubules. Already many studies have been conducted evaluating its potential for dentinal tubule occlusion. It has shown promising results in the past.<sup>24</sup>

Bentodent Desensitizer is a novel natural-based desensitizing toothpaste containing bentonite clay, propolis, and guava leaf extract. Bentonite clay along with Propolis and guava leaf extract seals the dentinal tubules relieving DH. Also, few studies were done on Bentodent desensitizer toothpaste. So, this novel natural-based toothpaste was used in the present study.<sup>25</sup>

Fang Farm Mint Toothpaste is another novel natural-based desensitizing toothpaste. It contains pure nano-hydroxy apatite and is free from chemicals such as SLS, triclosan, parabens, and fluoride. Also, they have essential oils and ayurvedic extracts which prevent oral diseases and long-term health. Till now no research has been attempted for Fang Farm Mint Toothpaste evaluating its potential for occluding dentinal tubules and efficacy in relieving DH. So, this novel natural-based toothpaste was used in the present study.<sup>26</sup>

Among all the desensitizing toothpaste tested in this study, Shy-NM (Novamin-based) toothpaste showed significant maximum tubular occlusion. The results are from the study done by Shah et al.<sup>27</sup> The significant occlusion of dentinal tubules can be attributed to the active ingredient in Shy-NM i.e. CSPS (calcium sodium phosphosilicate). When CSPS encounters saliva Na<sup>+</sup> ions are released, elevating the pH into the range essential for HCA (Hydroxycarbonate apatite) formation. Minerals like Ca<sup>2+</sup> and P<sup>5+</sup> ions are released from CSPS to form a calcium-phosphate layer. The calcium-phosphate layer crystallizes into HCA and seals the opened dentinal tubules.

Shy-NM occluded significantly more dentinal tubules than Bentodent Desensitizer. This result is by Sonali and Kusum<sup>28</sup> where

Shy-NM showed slightly better results than Bentodent. The results can be attributed to the active ingredient in Shy-NM i.e. CSPS (calcium sodium phosphosilicate). This results in the formation of the HCA layer which seals the opened dentinal tubules.

In the present study, Shy-NM occluded significantly more dentinal tubules than Fang Farm Mint. On the contrary efficacy of 15% nano-hydroxyapatite-based toothpaste was greater compared to Novamin-based toothpaste in the study done by Kulal et al.<sup>29</sup> The results can be attributed to the active ingredient in Shy-NM i.e. CSPS which results in the formation of HCA layer which seals the opened dentinal tubules. Fang Farm Mint may have to have a lesser concentration of nano-hydroxyapatite (<15%) resulting in lesser dentin tubule occlusion than Shy-NM.

The Bentodent desensitizer used in the present study resulted in significantly more tubular occlusion compared to the control group. Also, it showed better dentinal tubule occlusion than Fang Farm Mint Toothpaste although there was no statistical significance among them. The results can be attributed to the presence of Bentonite clay, propolis, and guava leaf extract altogether which seals the dentinal tubules relieving DH. Propolis in the Bentodent Desensitizer is a product of honeybees, resinous supposed to obstruct the dentinal tubules alleviating DH pain.<sup>30</sup>

The limitations of the study are the limited sample size, and the estimation of the toothpaste was made in the absence of any acid challenge, which normally occurs in the mouth. Future long-term research and comparative clinical-based studies must be performed to certify the efficacy of using these products for the relief of DH.

The finding of the present study encourages the use of Shy-NM as an effective desensitizing toothpaste as it re-calcifies the tooth naturally, and provides fast and long-lasting relief of DH. Bentodent desensitizer and Fang Farm Mint can be a safer alternative option for relieving DH due to their lesser side effects.

## CONCLUSION

Within the limitations of the present study, it can be concluded that all desensitizing toothpastes were effective in dentine tubule occlusion when compared to the control group. Brushing twice daily with Shy-NM for 14 days produced effective dentinal tubule occlusion and thereby can relieve pain associated with DH effectively when compared to other desensitizing toothpaste. Among the desensitizing toothpastes, Shy-NM proved to be the most effective followed by Bentodent desensitizer and Fang Farm Mint. Further long-term research and comparative clinical-based studies must be performed to certify the efficacy of using these products for the relief of DH.

## ORCID

Prem P Kar  <https://orcid.org/0009-0001-5806-2760>

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