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Original Article

Dentistry Section

# Dentinal Tubule Occluding Efficacy of Three Nano Biomaterials Containing Toothpastes on Simulated Hypersensitive Dentin: An SEM and EDX In-vitro Analysis

NEHA MUNDHADA[1], CHETANA MAKADE[2], PRATIMA SHENOI[3], DEEPASHRI TEKAM[4], SIMRAN GUPTA[5]




## ABSTRACT

**Introduction:** Dentin Hypersensitivity (DH) is a common dental issue characterised by sharp pain in response to stimuli. Desensitising toothpastes provide lasting relief by sealing dentinal tubules. In recent years, new home-use desensitising products incorporating nano biomaterials have been developed.

**Aim:** To evaluate the efficacy of nano biomaterials such as Nano-hydroxyapatite (n-HAp), Novamin, and Pro-Argin in occluding dentinal tubules for the treatment of DH.

**Materials and Methods:** An in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at Ranjeet Deshmukh Dental College and Research Centre, Nagpur, Maharashtra, India, from October 2017 to April 2019. A total of 105 extracted maxillary premolars were divided into five groups: a no-treatment group (n=5), a control group (Sodium Fluoride) (n=25), and three experimental groups (n=25) using n-HAp, Novamin, and Pro-Argin, respectively. An Oral B cross-action toothbrush with a customised jig machine was used along with the experimental toothpaste for two minutes daily for two months. All samples were sectioned and subjected to Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) for chemical characterisation. The data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) for comparison of the mean number of dentinal tubules and Calcium to Phosphorous (Ca/P) ratio. Paired comparisons were carried out using Tukey's post-hoc test.

**Results:** It was observed that the n-HAp-containing toothpaste had the highest number of completely occluded tubules (546.00±49.96), and the Ca/P ratio of enamel (1.87±0.41) was higher than that of the other experimental groups ($p<0.0001$).

**Conclusion:** The n-HAp-containing toothpaste reported the highest efficacy in occluding dentinal tubules followed by Novamin, Pro-Argin, and regular toothpaste at the end of two months. All three tested toothpastes containing nano biomaterials had remineralisation potential.


**Keywords:** Desensitising toothpaste, Nanoparticles, Scanning electron microscope

## INTRODUCTION

Dentin Hypersensitivity (DH) is an agonising clinical condition with a prevalence rate ranging between 4% to 74% [1]. The age group most commonly affected by DH falls within the range of 20 to 40 years, with its peak incidence at the end of the third decade, and a slightly higher predilection observed in females. The teeth most frequently affected by DH are canines, followed by first premolars, incisors, second premolars, and molars [2].

The severity of DH depends on the width of the dentinal tubules. The rate of fluid flow is directly proportional to the fourth power of the radius, i.e., 5 μ, 1.2 μ, and 1 μ at the pulpodentinal junction, middle dentin, and dentinoenamel junction, respectively [3]. If the tubular diameter doubles, there is a 16-fold increase in fluid flow. Sensitive teeth have tubules that are twice as wide at the buccal cervical area compared to non sensitive teeth [4].

There is a myriad of desensitising agents and proprietary products available, such as fluorides, potassium nitrate, oxalate-containing compounds, dentin bonding agents, Portland cement, propolis, and lasers [5]. Treatment with these agents occludes the dentinal tubules by depositing precipitates, resulting in decreased dentinal permeability [6].

Recently, novel biomaterials like nHAp, Novamin, and Pro-Argin have been introduced for the treatment of DH, working on the principle of dentinal tubule occlusion [7].

The n-HAp, expressed chemically as $Ca_{10}(PO_4)_6(OH)_2$, is the most biocompatible material that has gained wide acceptance in biomedicine and dentistry in recent years. These particles easily penetrate dentinal tubules and block fluid movement within the tubules when combined with various agents [8]. n-HAp facilitates surface remineralisation by creating a biomimetic apatite layer on both enamel and dentin surfaces. This process is driven by the unique chemical and physical properties of n-HAp particles, which closely mimic the constituents of enamel mineral [9].

Novamin/Calcium Sodium Phosphosilicate (CSPS) is a bioactive glass and highly biocompatible material. Novamin forms Hydroxycarbonate Apatite (HCA) upon contact with body fluids, which is chemically similar to enamel and dentin [10]. In saliva, sodium ions from Novamin particles quickly start exchanging with hydrogen cations. This swift ion exchange enables the release of calcium and phosphate ions from the particle structure. A temporary rise in pH occurs, promoting the precipitation of calcium and phosphate from both the particles and saliva, resulting in the formation of a calcium phosphate layer on the exposed dentin surface [11].

Pro-Argin, a combination of the amino acid arginine and calcium carbonate naturally found in saliva, has been recognised for its potential oral health benefits. Pro-Argin technology depends on the interaction between arginine and calcium carbonate, which occlude the dentinal tubules, thereby preventing the flow of dentinal fluid and reducing DH [12].

The study by Bologa E et al., compared the efficacy of three desensitising toothpastes containing dontodent sensitive, Dr. Wolff's Biorepair, and Sensodyne repair and Protect on dentinal tubule occlusion [13]. However, there is limited information available in the literature regarding the effectiveness of n-HAp, Novamin, and

Pro-Argin in occluding dentinal tubules for the treatment of DH. To address this gap, the present study aimed to comparatively evaluate the effectiveness of desensitising toothpaste containing n-HAp, Novamin, and Pro-Argin in occluding exposed dentinal tubules.

## MATERIALS AND METHODS

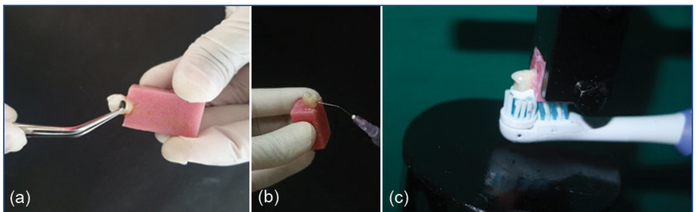
The in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at Ranjeet Deshmukh Dental College and Research Centre, Nagpur, Maharashtra, India, from October 2017 to April 2019. Approval was obtained from the Institutional Ethics Committee (IEC/VSPMDCRC/04/2017).

**Inclusion and Exclusion criteria:** Freshly extracted mature human permanent maxillary premolars from patients aged 18-25 years, extracted for orthodontic or periodontal purposes, were selected for the study. The exclusion criteria included teeth with caries, trauma, fractures, or other defects such as root calcification, root resorption, incompletely formed apices, developmental anomalies, and severe curvatures. The samples were cleaned, disinfected, and stored according to the guidelines set by the Occupational Safety and Health Administration (OSHA) and Centres for Disease Control and Prevention (CDC). They were stored in 10% formalin at room temperature.

**Sample size calculation:** The sample size calculation was based on a previous study [7] where only Scanning Electron Microscopy was used to evaluate dentinal tubule occlusion, and the difference between the groups was statistically significant with a sample size of 10 teeth per group. Thus, considering a study power of 80% and a confidence interval of 95%, 25 samples were allocated per group (four groups), and five teeth were included in the no-treatment group (negative control). Therefore, a total of 105 teeth were included in the study.

### Study Procedure

A standardised Class V cavity was prepared on the buccal surface of the tooth 1 mm coronal to the Cementoenamel Junction (CEJ). The dentin surface was then polished with emery paper and washed with distilled water. A 30% phosphoric acid was used for 15 minutes to simulate hypersensitive dentin by removing the smear layer, followed by thorough washing with distilled water. All samples were stored in distilled water after treatment with 30% phosphoric acid [Table/Fig-1a-c].




**[Table/Fig-1]:** Sample standardisation: (a) Class V cavity preparation; (b) Sample etched with 30% Phosphoric acid followed by distilled water; (c) Samples mounted on customised jig stimulating tooth brushing.

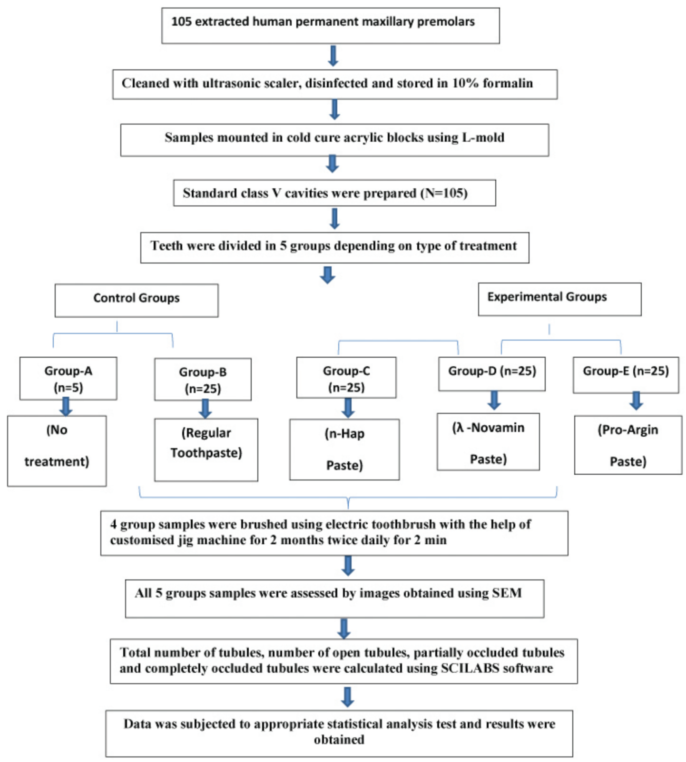
Based on the desensitising toothpastes used, the samples were allocated using a computer-assisted randomisation technique into five groups as follows:

**Control groups:**

- **Group A (Negative control):** No treatment (n=5).
- **Group B (Positive control):** α-Regular toothpaste containing Sodium Fluoride (n=25) (Colgate toothpaste) (Midtown Manhattan, New York City).

**Experimental groups:**

- **Group C:** Toothpaste containing n-HAp (n=25) (Apagard Premio toothpaste) (Apatite Co., Ltd., and Dental Kagaku Co., Ltd., Japan).
- **Group D:** Toothpaste containing Novamin (n=25) (Sensodyne Repair and Protect) (Global Healthcare Products, India).
- **Group E:** Toothpaste containing Pro-Argin (n=25) (Vantej Toothpaste) (Group Pharmaceutical, India).




**[Table/Fig-2]:** Methodology algorithm.

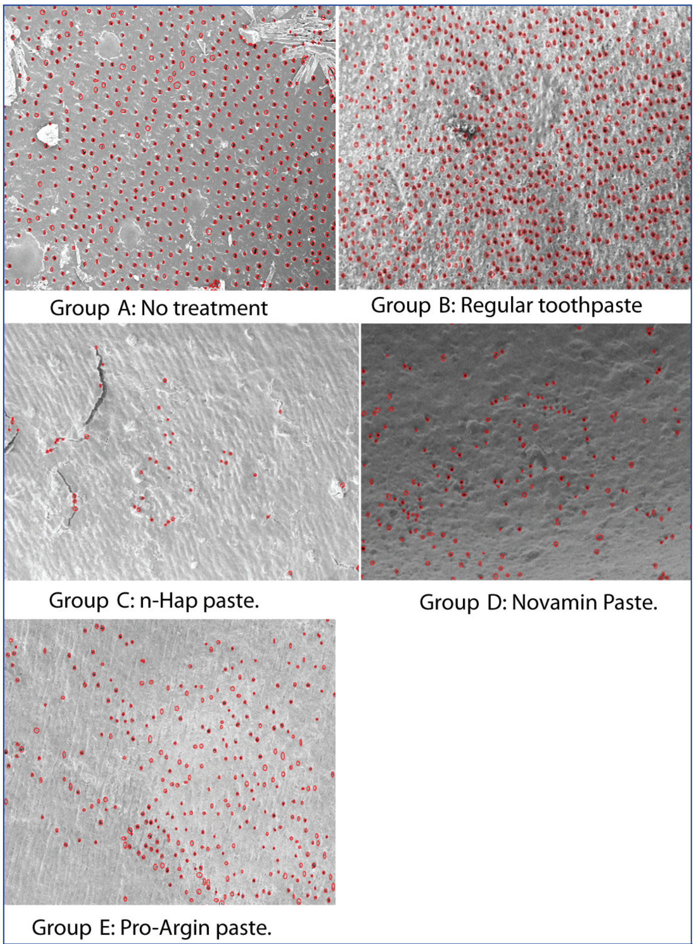
Each treatment group (group B, group C, group D, group E) samples were brushed using a pea-sized amount of each paste along with 1 mL of artificial saliva (ICPA Wet Mouth, India), two minutes twice daily for two months at approximately 0.9 N load with an Oral B CrossAction toothbrush using a customised jig machine [Table/Fig-1c]. The samples were stored in artificial saliva (ICPA Wet Mouth, India). After the treatment, all samples along with group A were dried and prepared for analysis by SEM. The methodology algorithm has been summarised in [Table/Fig-2].

**Scanning Electron Microscope (SEM) observation:** All the specimens were dehydrated using increasing concentrations of ethanol, dried, and fixed by immersing them in Hexamethyldisilazane (HMDS) for 10 minutes. They were then left for 24 hours on filter paper inside a covered glass vial at room temperature. Following gold sputter coating (SC701AT, Elionix, Tokyo, Japan), the cross-sections of the dentinal tubules were examined using an SEM (JSM-5310LV, JEOL, Tokyo, Japan). The counting of the total number of dentinal tubules, open dentinal tubules, partially occluded dentinal tubules, and completely occluded dentinal tubules from the obtained images was done using Scilabs software (version 5.5.2) (Rungis, France), and the reported data were evaluated for statistical analysis [Table/Fig-3].

**Energy Dispersive X-Ray Spectroscopy (EDS) analysis:** EDS analysis was performed to characterise the newly formed mineralised layer on the dentin surface. The randomly selected specimens were dehydrated in the same manner as described above. The surfaces were polished, carbon-coated, and then examined using field emission SEM/EDS (S-4500, Hitachi, Hitachinaka, Japan). A quantitative elemental analysis of calcium and phosphorus was performed on the formed layer of toothpaste and the crystallites, with nine randomly selected areas analysed for each sample.

## STATISTICAL ANALYSIS

The data on the total number of open, partially occluded, and completely occluded dentinal tubules, as well as the mineral content in the form of Ca/P ratio, were obtained and summarised in terms of mean, standard deviation, median, and range for each study group. The comparison of the mean number of dentinal tubules




**[Table/Fig-3]:** Scanning electron microscopy images analysis using SCILAB software for showing occluded dentinal tubules after application of toothpastes.

and the Ca/P ratio was statistically performed using a one-way ANOVA. The paired comparisons between groups were carried out using Tukey's post-hoc test. The statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corporation, USA) for each type of dentinal tubule, and the inferences were obtained. The level of statistical significance was tested at a 5% level.

| Groups | Open dentinal tubules (Mean±SD) | Partially occluded dentinal tubules (Mean±SD) | Completely occluded dentinal tubules (Mean±SD) |
|---|---|---|---|
| Group A (n=5) | 583±20.48 | NA | NA |
| Group B (n=25) | 292±63.55 | 72.68±20.49 | 218.76±62.46 |
| Group C (n=25) | 1±2.47 | 36.04±49.63 | 546.00±49.96 |
| Group D (n=25) | 2±3.18 | 79.68±74.21 | 501.80±75.59 |
| Group E (n=25) | 9±19.93 | 143.80±67.36 | 430.44±72.66 |

**[Table/Fig-4]:** Descriptive statistics for number of open partially occluded and completely occluded dentinal tubules across different study groups.

## RESULTS

Group A showed the highest number of open dentinal tubules. The comparison of the mean number of open dentinal tubules using ANOVA across different study groups differed significantly (p<0.0001) [Table/Fig-4]. The pair-wise comparison [Table/Fig-5] of the mean difference in open dentinal tubules between group A and all other groups showed statistically significant differences with p<0.0001. A similar observation was made for group B. However, the mean differences between group C, group D, and group E were statistically insignificant (p>0.05).

The comparison of the mean number of partially occluded dentinal tubules using ANOVA across different study groups differed significantly (p<0.0001 [Table/Fig-4]). In [Table/Fig-6], it was observed from the pairwise comparison of the mean number of partially occluded dentinal tubules that the mean difference between group B and group E was statistically significant with p<0.0001. Furthermore, the mean difference between group C and group D was statistically significant with a p-value of 0.039. However, the mean difference of group B was statistically insignificant compared to group C and group D (p>0.05).

| Paired groups | | Mean difference | p-value | 95% confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| Group A | Group B | 291.44 | <0.0001 | 246.60 | 336.28 |
| | Group C | 582.04 | <0.0001 | 537.20 | 626.88 |
| | Group D | 581.48 | <0.0001 | 536.64 | 626.32 |
| | Group E | 574.24 | <0.0001 | 529.40 | 619.08 |
| Group B | Group C | 290.6 | <0.0001 | 264.71 | 316.49 |
| | Group D | 290.04 | <0.0001 | 264.15 | 315.93 |
| | Group E | 282.8 | <0.0001 | 256.91 | 308.69 |
| Group C | Group D | -0.560 | 1.000 | -26.45 | 25.33 |
| | Group E | -7.800 | 0.918 | -33.69 | 18.09 |
| Group D | Group E | -7.240 | 0.937 | -33.13 | 18.65 |

**[Table/Fig-5]:** Pairwise comparison of mean number of open dentinal tubules across different study groups.
Tukey's post-hoc test; p<0.05: significant

| Paired groups | | Mean difference | p-value | 95% confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| Group B | Group C | 36.640 | 0.110 | -5.40 | 78.68 |
| | Group D | -7.000 | 0.972 | -49.04 | 35.04 |
| | Group E | -71.12 | <0.0001 | -113.16 | -29.08 |
| Group C | Group D | -43.64 | 0.039 | -85.68 | -1.60 |
| | Group E | -107.76 | <0.0001 | -149.80 | -65.72 |
| Group D | Group E | -64.12 | 0.001 | -106.16 | -22.08 |

**[Table/Fig-6]:** Pairwise comparison of the mean number of partially occluded dentinal tubules all experimental groups.
Tukey's post-hoc test; p<0.05: significant

| Paired groups | | Mean difference | p-value | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| Group B | Group C | -327.24 | <0.0001 | -376.00 | -278.48 |
| | Group D | -283.04 | <0.0001 | -331.80 | -234.28 |
| | Group E | -211.68 | <0.0001 | -260.44 | -162.92 |
| Group C | Group D | 44.200 | <0.0001 | -4.56 | 92.96 |
| | Group E | 115.56 | <0.0001 | 66.80 | 164.32 |
| Group D | Group E | 71.36 | 0.001 | 22.60 | 120.12 |

**[Table/Fig-7]:** Pairwise comparison of mean number of completely occluded dentinal tubules across all experimental groups using Tukey's post-hoc test.
Tukey's post-hoc test; p<0.05: significant

group C showed the highest number of completely occluded dentinal tubules. The comparison of the mean number of completely occluded dentinal tubules was statistically significant with p<0.0001. It was observed from the pairwise comparison [Table/Fig-7] of the mean number of completely occluded dentinal tubules that the mean difference in completely occluded dentinal tubules between group B (positive control) and all other experimental groups was statistically significant with p<0.0001. Additionally, the mean difference of group C differed significantly from group D and group E with p<0.0001.

**EDX analysis:** EDX analysis was used to determine the calcium and phosphorus content (in weight %) of the groups. Group A has been excluded from the analysis because toothpaste was not applied to them. The mean Ca/P ratio of enamel after the application of toothpaste in group B was 0.28±0.005, group C was 1.87±0.41, group D was 1.82±0.06, and group E was 1.76±0.35. A pairwise comparison of the mean Ca/P ratio showed a statistically significant difference between group B and the other groups (p<0.001). However, no statistically significant difference was observed between group C, D and E [Table/Fig-8].

| Pairwise comparisons | | Tukey's $HSD_{0.05}$=1.1216 $HSD_{0.01}$=1.3492 | $Q_{.05}$=3.9224 $Q_{.01}$=4.7183 |
|---|---|---|---|
| Group B v/s Group C | M1=0.28 M2=1.87 | 2.39 | Q=8.36 (p<0.001)* |
| Group B v/s Group D | M=0.28 M3=4.17 | 3.52 | Q=12.32 (p<0.001)* |
| Group B v/s Group E | M1=0.28 M4=1.76 | 4.70 | Q=16.42 (p<0.001)* |
| Group C v/s Group D | M1=1.87 M5=1.82 | 5.78 | Q=20.22 (p=0.146) |
| Group C v/s Group E | M2=1.87 M3=1.76 | 1.13 | Q=3.95 (p=0.624) |
| Group D v/s Group E | M2=1.82 M4=1.76 | 2.30 | Q=8.06 (p=0.189) |

**[Table/Fig-8]:** Pairwise comparison of Ca/P ratio using EDX analysis across the groups.
*p<0.05: significant

## DISCUSSION

The present study showed that n-HAp-containing toothpaste reported the highest efficacy in occluding dentinal tubules, followed by Novamin, Pro-Argin, and regular toothpaste at the end of two months, which is essential for the treatment of DH, as the treatment of dentinal hypersensitivity is quite challenging. It emphasises the use of a material that chemically reacts, physically occludes, and adheres densely to dentinal tubules to significantly reduce the possibility of reopening the occluded tubules and prevent their recurrence.

In the present study, the regular toothpaste group (group B) exhibited an average of 292 open dentinal tubules, 72.68 partially occluded tubules, and 218.76 completely occluded tubules. The regular toothpaste contained calcium carbonate, silica, sodium silicate, Sodium Monofluorophosphate (NaMFP) (1000 ppm), and sodium lauryl sulfate. These abrasive agents may contribute to the partial or complete occlusion of dentinal tubules through continuous deposition on the exposed dentin surface [14].

Group C contains n-HAp particles, which are biomimetic minerals composed of calcium and phosphate ions. These particles deposit in demineralised dentin and resemble the inorganic components of teeth. The n-HAp particles possess strong surface bioactivity and biocompatibility, actively inducing remineralisation. Their smaller size and high reactivity enable the deposition of these particles in dentinal tubules. Hence, in the present study, the mean number of open tubules was 1, partially occluded tubules were 36.01, and completely occluded tubules were 546, representing the highest number of occluded dentinal tubules [12].

In Group D (Novamin), the mean numbers of open, partially occluded, and completely occluded tubules were 2, 79.68, and 501.80, respectively. These results can be explained by the composition of Novamin particles, which consist of CSPS with 25% sodium, 25% calcium, 6-8% phosphate, and silica. Novamin has a strong affinity for binding to collagen; thus, when it contacts dentin with high collagen content, more Novamin binds to the exposed dentinal surfaces, physically occluding the dentinal tubules. However, the main drawback of using Novamin is that it requires a longer duration for apatite formation and dentinal tubule occlusion [13].

In group E (Pro-Argin), the mean numbers of open (9), partially occluded (143.80), and completely occluded tubules (430.44) were reported. This aligns with the mechanism in which arginine physically adsorbs onto the surface of calcium carbonate, creating a positively charged agglomerate that easily adheres to the negatively charged exposed dentin surfaces. The enzymatic and microbiological effects of human saliva could not be evaluated in the present study because artificial saliva was used [15]. The result is in accordance with the study conducted by Shah S et al., (2017), which evaluated the efficacy of Novamin and Pro-Argin-containing desensitising dentifrices on the occlusion of dentinal tubules. They concluded that Novamin-containing toothpaste showed uniform and maximum tubular occlusion (95.58%), whereas Pro-Argin showed a mean tubular occlusion rate of 89.90% under SEM [16].

The comparative findings of the present study correspond with the previous studies [Table/Fig-9] [7,13,14,16-20].

The present study revealed that nHAP-containing toothpaste showed the highest increase in calcium and phosphorus wt.%

| S no. | Authors name and year | Place of study | Number of subjects | Material's compared | Parameter assessed | Conclusion (percentage of dentinal tubule occlusion) |
|---|---|---|---|---|---|---|
| 1. | Kulal R et al., (2016) [7] | Odisha, India. | 40 disc shaped dentin samples | 15% Nano-hydroxyapatite (n-HAp) crystal, 5% Novamin, 8% Proargim | Pre- and Post-treatment SEM analysis | n-HAp showed 98.1%, Novamin 83.1%, Pro-Argin showed 69.1% tubule occlusion |
| 2. | Shah S et al., (2018) [16] | Maharashtra, India | 70 dentinal blocks | Novamin, 8% Arginine and Calcium, Carbonate, 10% Strontium hloride | Scanning Electron Microscope (SEM). | Novamin showed 95.58% of dentinal tubule occlusion |
| 3. | Hongal S et al., (2019) [18] | Karnataka, India | 40 dentinal disc | Strontium Chloride, Potassium Nitrate, Pro-Argin, Bioactive Glass | SEM study | Bioactive glass was 85.49% |
| 4. | Bologa E et al., (2020) [17] | Oradea, Romania | 40 human teeth | Three different n-HAp toothpastes: Karex, Biorepair Plus Sensitive, Dr. Wolff's Biorepair | A SEM and EDX check the obliteration of tubules. | 100% (Score 1) of tubules occluded was seen in Karex, Biorepair Plus Sensitive. |
| 5. | Midha V et al., (2021) [14] | Haryana, India | 100 dentin slices | 5% Novamin, Propolis, 5% Potassium Nitrate, 8% arginine | SEM analysis check the obliteration of tubules. | 5% Novamin has 95.8±2.20, Propolis has 33.84±11.76, 5% potassium nitrate has 51.12±11.76 and 8% arginine 73.70±13.35 percentage of occluded tubules. |
| 6. | Ashraf R and Aidaros N, (2021) [20] | Egypt | 40 dentinal disc | Nano seashell, Sodium Fluoride and Signal Complete 8 (Hydrogenated Starch Hydrolysate) | SEM. | The combination of Nano seashell and Sodium Fluoride resulted in a notable increase in occluded dentinal tubules. Mineral deposits formed by Sodium Fluoride exhibited greater resilience in acidic conditions compared to those formed by Nano seashell. |
| 7. | Krishna EN and Prakash S, (2022) [19] | Karnataka, India | 24 dentinal disc | 5% Potassium Nitrate, Pro-Argin | SEM. | Mean value of number of occluded dentinal tubules is 1,647 with 5% Potassium Nitrate and 6,376 with Pro-Argin |
| 8. | Bologa E et al., (2023) [13] | Oradea, Romania | 40 human dentin disks | Hydroxyapatite and Sodium fluoride, 20% Microcrystalline Zinc HAP Novamin | SEM, EDX analysis and Vickers hardness test. | 70% tubule occlusion in Hydroxyapatite and Sodium fluoride group |
| 9. | Present study | Maharashtra, India | 105 dentin disc | n-HAp, Novamin, Pro-Argin | SEM, EDX analysis | n-HAp has 546 completely occluded tubules, |

**[Table/Fig-9]:** Similar studies from the literature [7,13,14,16-20].

after remineralisation, followed by Novamin. This finding was in accordance with Kumar A et al., (2015). A commercially available nHAP paste was evaluated for its effectiveness in preventing demineralisation under polarised light microscopy. Novamin® (CSPS) is a type of bioactive glass that becomes reactive upon contact with bodily fluids, leading to the deposition of HCA. This mineral closely resembles the enamel and dentin minerals, thereby promoting remineralisation of enamel [21].

The possibility of arginine's remineralisation effect in this study can be attributed to its remaining protein form [19]. Alkali-stable fluorapatite is created when albumin adsorbs onto fluorides and draws calcium and phosphate. The topographical polar surface area of L-arginine monohydrochloride (128 $A^2$) is significantly smaller-more than 10 times-than that of albumin (1530 $A^2$). This size difference facilitates the diffusion of arginine-fluoride for the remineralisation of sub-surface lesions, as well as the creation of a reservoir of arginine-fluoride that can be released during acid attacks [22].

### Limitation(s)

The present study was an in-vitro study; it cannot fully simulate the dynamics of oral conditions in their entirety. As a result, the findings may need to be extrapolated to in-vivo conditions, and desensitising toothpaste may require more time to show results. Therefore, long-term studies need to be conducted. However, the present findings could open up new possibilities for reducing DH using such novel biomaterials.

## CONCLUSION(S)

In the present study, the number of dentinal tubules occluded in all experimental groups was significantly higher when compared to regular toothpaste. All three tested toothpastes containing nano biomaterials showed remineralisation potential, with the highest being found in nHAp, followed by Novamin, Pro Argin, and Regular toothpaste at the end of two months. Therefore, considering the findings of the present study, it can be concluded that n-HAp, a novel biomaterial, is a potential treatment modality for DH.

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PARTICULARS OF CONTRIBUTORS:
1. Consultant Endodontist, Department of Conservative Dentistry and Endodontics, VSPM Dental College and Research Centre, Nagpur, Maharashtra, India.
2. Associate Professor, Department of Conservative Dentistry and Endodontics, VSPM Dental College and Research Centre, Nagpur, Maharashtra, India.
3. Professor and Head, Department of Conservative Dentistry and Endodontics, VSPM Dental College and Research Centre, Nagpur, Maharashtra, India.
4. Senior Resident, Department of Dentistry, AIIMS, Bhopal, Madhya Pradesh, India.
5. Postgraduate, Department of Conservative Dentistry and Endodontics, VSPM Dental College and Research Centre, Nagpur, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Chetana Makade,
Associate Professor, Department of Conservative Dentistry and Endodontics,
VSPM Dental College and Research Centre, Nagpur-440019, Maharashtra, India.
E-mail: makade.chetana@gmail.com



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