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# Occlusion of Dentinal Tubules by Nanohydroxyapatite and Zinc Carbonate Hydroxyapatite-Based Toothpastes as Desensitizing Agents: An in Vitro Study

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**ABSTRACT**

**Objectives:** Dentin hypersensitivity (DH), characterized by a short, sharp, and intense pain response to a stimulus, is the most commonly encountered patient complaint. Various in-office and at-home methods including the recently introduced nano-hydroxyapatite (n-HA) and zinc carbonate-hydroxyapatite (Zn-CHA)-based products reduce the DH discomfort. This study compared the effectiveness of commercially available n-HA and Zn-CHA toothpastes for occlusion of dentinal tubules for management of DH.

**Materials and Methods:** In this in vitro study, 40 tooth samples were randomly assigned to 4 groups (n=10): negative control): brushing without toothpaste,Aclaim n-HA toothpaste,Biorepair Zn-CHA toothpaste, andShyNM calcium sodium phosphosilicate (CSPS) toothpaste. After demineralization with 17% ethylenediaminetetraacetic acid, the mounted specimens were brushed with an electric pressure-sensitive toothbrush and the respective toothpaste. Occlusion of dentinal tubules was subsequently evaluated under a scanning electron microscope (SEM). Elemental composition of the occluding plug was analyzed by energy-dispersive X-ray spectroscopy (EDX).

**Results:** The test n-HA and Zn-CHAand the positive control (CSPS) groups revealed significantly higher tubular occlusion compared to the negative control group (p= 0.001). The highest tubular occlusion was achieved in n-HA and Zn-CHA groups (80%); while negative control showed no occlusion (0%). Elemental analysis of dentinal plug showed significant differences in carbon, oxygen, calcium, and phosphorus levels among the groups ($p<0.05$).

**Conclusion:** The tubular occlusion capacity of Zn-CHA and n-HA were similar to CSPS-based toothpaste, and CHA in Zn-CHA enabled the formation of stable dentinal plugs.

**Keywords:** Dentin Sensitivity; Dentifrices; Dentin Desensitizing Agents; Hydroxyapatites; Metal Nanoparticles; Toothpastes

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## INTRODUCTION

Short, sharp, severe, and exaggerated dental pain and discomfort are the hallmarks of dentin hypersensitivity (DH), that arise from exposed dentin in response to stimuli that are typically thermal, evaporative, tactile, osmotic, or chemical, and cannot be attributed to any other type of dental disease or defect [1]. Lesion initiation and localization are the two successive steps of DH etiopathogenesis [2]. The defect, i.e., exposure of dentinal tubules extending from

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dentin to pulp, is caused by the loss of enamel or gingiva covering the dentin [3]. The dentinal tubules are more frequent and wider in sensitive dentin compared with non-sensitive cervical dentin, according to histological analysis. Clinically, a tubular diameter of 0.83µm has been reported in cases with DH [4].

Both "at home" and "in-office" strategies are used for the management of DH [5]. Potassium salts such as oxalates, citrates, and acetates are present in some therapeutic desensitizing agents; they decrease the intra-tubular nerve fiber excitability by obstructing axonic conduction [6]. But the majority of at-home pastes work by the application of coagulating substances, obstructing the dentinal fluid flow and plugging the tubules [2]. However, none of these procedures is highly effective to obstruct the dentinal tubules [7]. Bioactive glass toothpastes, such as ShyNM, which are based on calcium sodium phosphosilicate (CSPS), may be effective for tubular occlusion. In vitro studies showed that occlusion of dentinal tubules by bioactive glass was resistant to acid challenges, and outperformed comparable commercially available pastes [8,9].

Aclaim's nano-hydroxyapatite toothpaste (n-HA) has demonstrated encouraging tubular occlusion in clinical testing [10]. Since n-HA possesses a special remineralizing ability, it is used as a biomimetic substitute for tubular occlusion [11]. Similarly, metal nanoparticles (NPs) were added to toothpastes because of their superior surface area to volume ratio and increased bioavailability [12]. They enter demineralized dentin, where they adhere to collagen and produce precursors of unstructured calcium/phosphate that are necessary for tubular plugging [13,14]. Metal NPs like those of zinc (Zn-NPs) showed improved mechanical qualities, increased maturity, and faster functional dentin remodeling when applied on citric acid-etched dentin [15]. In vitro, a Zn-carbonate n-HA toothpaste (Zn-CHA; BioRepair Sensitive (Dr. K. Wolff GmbH, Bielefeld, Germany) more successfully blocked the tubules than comparable products [16]. After using it for 4 and 8 weeks, it considerably reduced the clinical symptoms of DH [17]. This finding implies that Zn-NPs function as efficient desensitizers.

Despite the fact that there are sufficient data to support the HA's efficacy as a desensitizer, comparison of various HA-based treatments has not been extensively performed. Thus, the purpose of this study was to compare the occlusion of dentinal tubules caused by desensitizing toothpastes based on commercially available Zn-CHA and n-HA.

## MATERIALS AND METHODS

The present in vitro study was conducted according to the guidelines of the Checklist for Reporting In Vitro Studies (Supplementary Table 1) after receiving approval from the institutional ethics committee, Kasturba Medical College, Manipal (#694/2020).

***Eligibility criteria:***

Forty anonymized human premolars and molars extracted for periodontal reasons were selected, and grossly carious teeth, teeth with short root trunks, and those with developmental anomalies/defects were excluded.

***Sample Size estimation, randomization, and allocation:***

The sample size was calculated with an estimated power of 80% with a confidence interval of 95% based on mean and standard deviation values from a previous study [18]. Thus, a sample size of 10 dentin blocks per study group was derived, leading to a final sample size of 40.

A simple randomization method was followed; wherein, the specimens were assigned an integer value from 1 to 40. An online software tool (www.randomizer.org) was used for randomization, based on which, four sets of 10 specimens were assigned to each study group: - negative control (only brushing), n-HA group Aclaim toothpaste (Group Pharmaceuticals, Malur, India), Zn-CHA group (Biorepair Advanced Toothpaste; Coswell Group, Bologna, Italy) and CSPS group (ShyNM; Group Pharmaceuticals, Malur, India; positive control) (Table 1).

**Table 1.** Study groups and material composition

| Study Group | Name of Toothpaste | Composition |
|---|---|---|
| **Negative Control** | No toothpaste | - |
| **n-HA group** | Aclaim toothpaste (Group Pharmaceuticals, India) | Sorbitol, glycerine, silica, purified water, hydroxyapatite, cocamidopropyl betaine, hydroxyethyl cellulose, titanium dioxide, flavour, sodium saccharin |
| **Zn-CHA group** | Biorepair Advanced Toothpaste (Coswell Group, Bologna, Italy) | Aqua, zinc carbonate hydroxyapatite, hydrated silica, sorbitol, glycerine, xylitol, silica, aroma, cellulose gum, zinc PCA, sodium myristoyl sarcosinate, sodium methyl cocoyl taurate, tetra potassium pyrophosphate, sodium saccharin, zinc citrate, citric acid, ammonium acryloyldimethyltaurate/VP copolymer, benzyl alcohol, phenoxyethanol, sodium benzoate, limonene. |
| **CSPS group** | ShyNM Toothpaste (Group Pharmaceuticals, India) (positive control) - | Glycerine, polyethylene glycol 400, silica, calcium sodium phosphosilicate, sodium lauryl sulphate, titanium dioxide, flavour, carbomer, potassium acesulfame. |

***Preparation of dentin discs:***

After complete removal of debris, each specimen was stored in 10% formalin. The specimens were then cut into dentin discs measuring 5×5×3mm using a carbide bur, which were subsequently mounted in acrylic blocks (20×20×10mm) and stored in artificial saliva (Wet Mouth; ICPA, India) (Fig. 1). Next, 600-grit silicon carbide abrasive paper (600P silicon Carbide Paper; Polar Star, India) was used to polish the discs. Before applying the desensitizing agents, the specimens were etched for 2 minutes with 17% ethylene diamine tetra acetic acid (Prevest DenPro Limited, India).

**Fig 1.** Specimen preparation: Selected premolar and molar teeth were sectioned into three sections of crown, root trunk and root using a carbide wheel bur. Root trunk was further sectioned into dentin blocks measuring 5×5×3mm.

***Brushing protocol:***

A customized wooden jig with a 15"×10" base and two vertical pillars was fabricated to standardize the brushing parameters (Fig. 2).

**Fig 2.** A customized wooden jig to standardize the brushing protocol: A wooden jig with two vertical pillars with a customized toothbrush-holding depression (for the selected toothbrush – Oral B Pro 2000N) and a specimen-holding platform was designed to standardize the brushing protocol.

The first pillar ended in a platform holding the specimen; whereas, the second pillar included a customized concavity to hold the electric toothbrush (Oral B Pro 2000N; Proctor and Gamble, USA). A cuboidal (2×2×1cm) undermined surface in the platform floor provided an accurate fit for the acrylic blocks and prevented any movement of the specimens during brushing. The height of the vertical pillars was adjusted to place the toothbrush at a 90-degree angle relative to the specimens [19]. The etched specimens were positioned on the cuboidal groove of the jig to achieve complete stability during brushing movements. After the toothbrush placement, a pea-sized amount of the test toothpaste was applied over the dentin surface, followed by brushing for 2 minutes for 14 days according to the previously established protocols [19]. The pressure-sensitive sensor of the electric toothbrush standardized the brushing force. The specimens were washed with water and immersed in artificial saliva between the application sessions.

***Scanning electron microscope (SEM) assessment and energy dispersive X-ray spectroscopy (EDS/EDX):***

The treated acrylic blocks were rinsed with water, dried, and trimmed after the last application. After thorough cleaning in a high-vacuum system, the specimens were sputtered with a thin layer of gold (Quorum SC7620, Laughton, United Kingdom). The coated specimens were then mounted on carbon-coated aluminum stubs, and positioned in the inner chamber of the SEM (Zeiss EVO MA18 SEM with Oxford EDS-X-act, Jena, Germany) for analysis. Micrographs were captured at 5kV and 2000x magnification. Five photomicrographs were obtained from each specimen, and each specimen's mode value was taken into consideration while scoring the degree of occlusion of dentinal tubules. The scoring criteria were as follows:

Score 1 indicated complete occlusion (all tubules were occluded), score 2 indicated substantial occlusion (50%-100% of tubules were occluded), score 3 indicated partial occlusion (25%-50% of tubules were occluded), score 4 indicated generally open (<25% of tubules were occluded), and score 5 indicated no tubular occlusion (0% tubular occlusion) [20]. Elemental analysis of both the dentin plugs and the treated specimens was conducted using an EDX spectrometer (Zeiss EVO MA18 SEM with Oxford EDS-X-act, Jena, Germany). Presence of carbon (C), oxygen (O), calcium (Ca), phosphorus (P), nitrogen (N), silicon (Si), and sodium (Na) was qualitatively evaluated.

***Statistical analysis:***

Descriptive statistics for explanatory and outcome variables were calculated using median and interquartile ranges for quantitative variables (based on the normality test - Shapiro-Wilk test), frequencies, and proportions for qualitative variables. Inferential statistics such as the chi-square test were used to associate tubular occlusion with the groups. An analysis of variance (ANOVA) test was applied to compare the mean tubular occlusion among the groups, and then pairwise comparisons were performed using the Bonferroni test. The Kruskal-Wallis and Mann-Whitney U tests compared the levels of the elements among the groups. The significance level was set at 5%.

## RESULTS

In this study, 10 tooth specimens were treated with the allocated desensitizing toothpastes in each group. Photomicrographs revealed regular and wide open dentinal tubules in negative control while substantial occlusion of dentinal tubules was observed in the test n-HA and Zn-CHA groups and the CSPS positive control group (Fig. 3).

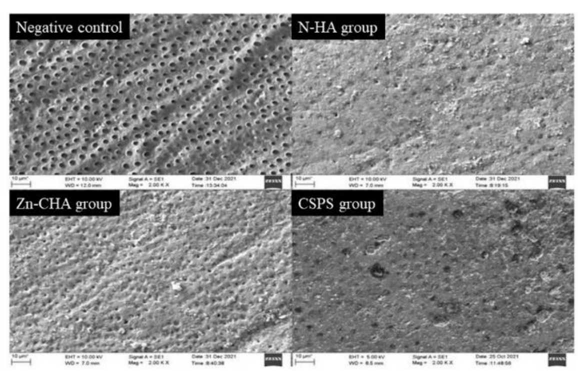


**Fig 3.** Representative photomicrographs from four groups: Negative control group shows "open" status of dentinal tubules (score 5). N-HA and Zn-CHA Groups show "occluded" status of tubules (score 1). CSPS Group shows mostly occluded status of tubules (score 2).

The Chi-square test revealed that in n-HA and Zn-CHA groups, 80% of the tubules were completely occluded while 20% were mostly occluded (p =0.001, Table 2).

In CSPS group, 70% of the tubules were completely occluded while 30% were mostly occluded (p =0.001). However, in negative control group, 0% of the tubules showed complete occlusion; 20% were mostly open, and 80% were completely open. Overall, 57.5% of the tubules were occluded, 17.5% were mostly occluded, 5% were mostly open, and 20% were completely open.

ANOVA showed significant differences in the mean tubular occlusion scores among the groups (p =0.001, Table 3). Comparing the test groups i.e. n-HA, Zn-CHA and positive control CSPS groups with the negative control group using the Bonferroni post-hoc test showed significant tubular occlusion in the test groups (p =0.00, Table 3). However, there was no significant difference in tubular occlusion when the test groups (n-HA, Zn-CHA and positive control CSPS groups) were compared to each other (p =1.0).

**Table 2.** Percentage of tubular occlusion achieved in different treatment groups

| Tubular occlusion | | Groups: Negative control | n-HA group | Zn-CHA group | CSPS group | Total | Chi-square value | P-value |
|---|---|---|---|---|---|---|---|---|
| **Occluded** | Count | 0 | 8 | 8 | 7 | 23 | 40.49 | 0.001* |
| | % | 0.0% | 80.0% | 80.0% | 70.0% | 57.5% | | |
| **Mostly occluded** | Count | 0 | 2 | 2 | 3 | 7 | | |
| | % | 0.0% | 20.0% | 20.0% | 30.0% | 17.5% | | |
| **Partially occluded** | Count | 0 | 0 | 0 | 0 | 0 | | |
| | % | 0.0% | 0.0% | 0.0% | 0.0% | 0% | | |
| **Mostly open** | Count | 2 | 0 | 0 | 0 | 2 | | |
| | % | 20.0% | 0.0% | 0.0% | 0.0% | 5.0% | | |
| **Open** | Count | 8 | 0 | 0 | 0 | 8 | | |
| | % | 80.0% | 0.0% | 0.0% | 0.0% | 20.0% | | |
| **Total** | Count | 10 | 10 | 10 | 10 | 40 | | |
| | % | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | | |

*Significant

**Table 3.** Comparison of the mean tubular occlusion scores among and between the groups

| Group | Tubule occlusion score (mean±SD) | ANOVA Pvalue | Pairwise comparisons | Mean difference | Post hoc Bonferroni test P value |
|---|---|---|---|---|---|
| **Negative control (n=10)** | 4.80±0.42 | 0.001* | Negative control v/s n-HA | 3.60 | 0.000* |
| | | | Negative control v/s Zn-CHA | 3.60 | |
| | | | Negative control v/s CSPS | 3.50 | |
| **n-HA group (n=10)** | 1.20±0.42 | | n-HA v/s Zn-CHA | 0.00 | 1.000 |
| | | | n-HA v/s CSPS | -0.10 | |
| **Zn-CHA group (n=10)** | 1.20±0.42 | | Zn-CHA v/s CSPS | -0.10 | |
| **CSPS group (n=10)** | 1.30±0.48 | | | | |

*Significant

Comparing the elemental levels with the Kruskal-Wallis test showed significant differences in the levels of C (p=0.001), O (p=0.001), Ca (p=0.002), and P (p=0.005) among the groups (Table 4). Further comparisons with the Mann-Whitney U test showed significantly higher levels of C in negative control group when compared with other three groups i.e. n-HA, Zn-CHA and CSPS (p=0.000). However, O was significantly higher in the n-HA, Zn-CHA and CSPS groups when

**Table 4.** Comparison of elemental levels in dentinal plugs among and between the groups

| Element | Group | Median (IQR) | Kruskal-Wallis test P value | Pairwise comparison | Mann Whitney U post hoc test U value | p value |
|---|---|---|---|---|---|---|
| Carbon | Negative control | 35.12 (5.43) | 0.001* | Negative Control v/s n-HA | 0.00 | 0.000* |
| | n-HA group | 25.46 (8.49) | | Negative Control v/s Zn-CHA | 0.00 | 0.000* |
| | Zn-CHA group | 25.895 (9.84) | | Negative Control v/s CSPS | 0.00 | 0.000* |
| | | | | n-HA v/s Zn-CHA | 42.00 | 0.545 |
| | CSPS group | 20.705 (6.12) | | n-HA v/s CSPS | 31.00 | 0.151 |
| | | | | Zn-HA v/s CSPS | 24.00 | 0.049 |
| Oxygen | Negative control | 39.665 (2.9) | 0.001* | Negative Control v/s n-HA | 4.00 | 0.001* |
| | n-HA group | 48.925 (6.31) | | Negative Control v/s Zn-CHA | 4.00 | 0.001* |
| | Zn-CHA group | 45.205 (3.03) | | Negative Control v/s CSPS | 13.00 | 0.005* |
| | | | | n-HA v/s Zn-CHA | 31.00 | 0.151 |
| | CSPS group | 44.345 (4.07) | | n-HA v/s CSPS | 22.00 | 0.034 |
| | | | | Zn-CHA v/s CSPS | 39.00 | 0.406 |
| Calcium | Negative control | 16.8 (4.86) | 0.002* | Negative Control v/s n-HA | 49.00 | 0.940 |
| | n-HA group | 15.67 (8.41) | | Negative Control v/s Zn-CHA | 19.00 | 0.019 |
| | Zn-CHA group | 19.63 (4.95) | | Negative Control v/s CSPS | 1.00 | 0.000* |
| | | | | n-HA v/s Zn-CHA | 29.00 | 0.112 |
| | CSPS group | 21.025 (2.36) | | n-HA v/s CSPS | 17.00 | 0.013 |
| | | | | Zn-CHA v/s CSPS | 29.00 | 0112 |
| Phosphorus | Negative control | 8.595 (2.4) | 0.005* | Negative Control v/s n-HA | 24.00 | 0.049 |
| | n-HA group | 10.28 (3.88) | | Negative Control v/s Zn-CHA | 16.5 | 0.011* |
| | Zn-CHA group | 10.405 (2.77) | | Negative Control v/s CSPS | 8.0 | 0.001* |
| | | | | n-HA v/s Zn-CHA | 46.50 | 0.791 |
| | CSPS group | 11.8 (1.86) | | n-HA v/s CSPS | 29.00 | 0.112 |
| | | | | Zn-CHA v/s CSPS | 30.00 | 0.131 |

*Significant

compared to the negative control (p=0.001). The P was significantly higher in Zn-CHA (p=0.011) and less in CSPS group (p=0.001) when they were compared to the negative control while Ca was significantly higher in CSPS group compared to the negative control (p= 0.000). However, the difference in levels of the C, O, Ca and P was insignificant when the n-HA, Zn-CHA and CSPS groups were compared (Table 4).

## DISCUSSION

The present study evaluated and compared the occlusion of dentinal tubules and composition of dentinal plugs formed after applying n-HA, Zn-CHA, and CSPS-based desensitizing toothpastes. Even though all the agents caused significant tubular occlusion, complete closure of all the tubules was not achieved with any of the tested toothpastes.

N-HA decreases DH by obstructing the dentinal tubules with a coating of mineral HA and HA plug [7,21]. A topical state of supersaturation of calcium and phosphate ions with regard to tooth minerals is maintained by n-HA, a reservoir for these ions, that results in mineral deposition on the tooth surface [22]. Toothpastes with n-HA increase salivary calcium concentrations and seal tooth surface micropores [23-26]. Accordingly, significant amounts of calcium and phosphate ions are absorbed by the tooth surface from the saliva, dentifrices, or mouth rinses through the pores, acting as a template in the apatite deposition process, and fostering crystal integrity and growth [25].

Evidence shows that doping HA with ions including $Zn^{2+}$, $F^{-}$, $Mg^{2+}$, $Sr^{2+}$, and $CO_3^{2-}$ modifies the crystals' nucleation, growth, orientation, and solubility [27]. The elongated microaggregates with low crystallinity and high surface area produced by Zn-carbonate and HA in Zn-CHA toothpaste improve the solubility and affinity for the naturally occurring minerals in enamel and dentin [28]. Carbonate ions can partially replace the phosphate or the hydroxyl ions in the native HA crystals since they are found in small amounts in natural enamel and dentin [28, 29]. In CHA crystals, the carbonate replaces the phosphate ion, increasing the solubility of the apatite phase and lowering crystallinity [28, 30]. The Zn-NPs also have a greater viscoelastic modulus, promote total tubular blockage, activate dentin remodeling, and result in formation of a more resilient tooth structure with higher mechanical properties and abrasion resistance [15,31,32]. Moreover, Zn ions, which replace a tiny percentage of the bivalent calcium ions in Zn-CHA, are released gradually and exert an antibacterial effect [33,34].

On the other hand, research indicates that CSPS toothpastes are clinically more successful in lowering DH and generating superior tubular occlusion [35,36]. In the oral environment, CSPS releases phosphate, calcium, and sodium. They may be more beneficial than n-HA because they interact with the oral fluids to generate a crystalline hydroxycarbonate apatite layer that is physically and chemically analogous to natural tooth minerals [37]. Nevertheless, the glass particles that enclose the calcium and phosphate ions in CSPS shield them from action. The glass particles must be trapped for the calcium and phosphate to localize. Therefore, there is a delay in the desensitizing effect of CSPS, which may be equal to the n-HA toothpaste.

Tubular occlusion was expected to improve by the smaller particle size of n-HA and Zn-CHA (20 nm), and bioactive glass (<20μm), as occlusion of the exposed dentinal tubules minimizes DH by reducing permeability and avoiding fluid disturbance [38]. However, the tubular occlusion ratings attained by the three agents did not differ significantly. Additionally, as they all encourage the deposition of ion-substituted biomimetic HA on exposed dentinal tubules, they may produce similar remineralization and desensitization effects [27]. However, the acid challenge may demineralize the tubular precipitates and reopen them; thus, partial tubular obstruction achieved with these agents may offer prompt but temporary relief from DH [39]. Since all these agents produced similar tubular occlusion, they could be suggested for DH and could also be a substitute for one another [7].

Since Zn-CHA has a larger carbonate content than n-HA and CSPS toothpastes, the plug that formed after applying Zn-CHA had a higher concentration of carbon. Similarly, the composition of CSPS group showed minor quantities of sodium together with elevated calcium and phosphorus content [8].

The results of tubular occlusion in the present study might have been influenced by specific procedural adjustments and other circumstances. We utilized an electric pressure-sensitive toothbrush because prior research has shown that it is more effective than manual toothbrushes for lowering the Schiff sensitivity scores [43]. Standardization and repeatability of the application regimen were made possible by the stability of the toothbrush-specimen combination using a specially designed jig [19]. Moreover, the quantity of tubular blockage seen may vary depending on whether a pea-sized amount of toothpaste is applied over contemporary slurry-based preparations [19]. Finally, the tubular occlusion parameters may also be influenced by the anatomical site from where dentin blocks are obtained. Dentin blocks were sectioned from the cervical area of human premolar and molar root trunks in the current study because clinically noticeable sensitivity is often seen in this area. The cervical third of tooth crown may have a much different tubular density and diameter than the middle third, which was commonly utilized in earlier research [20].

The current results clearly showed that the desensitizing toothpastes containing n-HA, Zn-CHA, and CSPS obstructed the dentinal tubules. Long-term in vivo investigations are necessary for additional validation of these results, as they are primarily based on an in vitro study. Additionally, only a bioactive toothpaste made of glass was used to compare the n-HA and Zn-CHA. Zn-CHA toothpastes' desensitizing effect should be better understood by comparison with other tubular occluding agents. Evaluation of the resilience of produced dentinal precipitates to mechanical and chemical stressors is also crucial.

## CONCLUSION

The present findings demonstrated that the toothpaste containing Zn-CHA facilitated efficient tubular occlusion, on par with n-HA and CSPS. The occluding plug is probably more resistant to mechanical and chemical stimulation because of the presence of CHA in its composition. Long-term studies are required to assess the dentinal plugs' resistance to salivary and acid stimulations.

## CONFLICT OF INTEREST STATEMENT

None declared.

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