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Evaluation of two desensitizing toothpastes containing Nano-hydroxyapatite and Bioactive glass on dentinal tubule occlusion - A SEM study

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Abstract

Background: Dentin hypersensitivity is a common dental problem which is a frequent complaint among patients The recent and mostly used management of Dentinal hypersensitivity is use of Desensitizing Toothpaste. The aim of this study is evaluation of two desensitizing toothpastes containing Nano-hydroxyapatite and Bioactive Glass on dentinal tubule occlusion under Scanning Electron Microscope (SEM).

Materials and Method: Forty-five samples were prepared from the extracted sound maxillary and mandibular molar teeth. The specimens were randomly divided into 3 groups Group A- Fifteen specimens of this group were brushed with Acclaim toothpaste for 2 minutes for 14 days. Group B- Fifteen specimens of this group were brushed with Elsenz toothpaste for 2 minutes for 14 days. Group C- Fifteen specimens of control group were immersed in artificial saliva and were

brushed with distilled water for 2 minutes for 14 days. Samples were dehydrated in alcohol then sputtered coated with gold and were visualized under SEM. Tubule occlusion score among various study groups was done using Chi-square test.

Result: Group A (Aclaim) showed maximum samples having mostly occluded dentinal tubules (Score 2) whereas Group B (Elsenz) showed maximum samples having partially occluded tubules and mostly unoccluded dentinal tubules (Score 3 and Score 4). The samples of Group C (Control) showed mostly unoccluded and unoccluded dentinal tubules (Score 4 and Score 5).

Conclusion: The overall statistics revealed that Aclaim shows better occlusion of dentinal tubules compared to Elsenz and Control group.

Keywords: Dentinal hypersensitivity, Hydroxy-apatite, Bioactive glass, SEM study.

Introduction

Dentin hypersensitivity is a common dental problem which is a frequent complaint among patients. Dentin hypersensitivity is recognized as short sharp pain arising from exposed dentin in response to stimuli which are typically thermal, evaporative, tactile, osmotic or chemical that cannot be ascribed to any other dental defect or disease.[1] The occurrence rate of Dentinal hypersensitivity usually ranges from 4-57%.[2] The prevalence rate of Dentinal hypersensitivity is between 60-98% in patients suffering from periodontitis.[3]

When tooth is exposed to chronic trauma due to tooth brushing, parafunctional habits, periodontal diseases and diet containing acidic components, cervical lesions are formed in the exposed part of the root leading to dentinal hypersensitivity.[4]Dentin hypersensitivity is seen to affect patients mostly between 30 to 40 years and has been noted that it affects women more than men.[5,6] Though it can occur in any tooth, mostly it is seen to affect canines and premolars.[7] Several methods have been proposed in the management of dentinal hypersensitivity which can be divided into: Self performed therapy at home and In-office Treatment.[8] Self performed therapy at home refers to the treatment that can be self assessed by the patient at home, which are simpler and inexpensive methods like the use of Desensitizing Toothpaste/Dentifrices, Desensitizing Mouthwashes and Chewing Gums. In-office treatments refers to the treatments performed by the Dental professionals which are more complex and potent desensitizing treatment, these include application of desensitizing agents like varnishes, liners, restorative materials, adhesives and resins and recently lasers.[9]

The mechanism of action of most desensitizing agents to reduce dentinal hypersensitivity is either by blocking the neural transmission at the pulpal tissues by chemically depolarizing the nerve synapse or by physically occluding the dentinal tubules. The recent and mostly used management of Dentinal hypersensitivity is use of Desensitizing Toothpaste. Among such toothpastes, Bioactive glass containing toothpaste and Nano-hydroxyapatite containing toothpaste are gaining popularity. Bioactive glass is a ceramic material consisting of amorphous sodium calcium phosphosilicate which is highly reactive in water and the fine particle size can physically occlude the Dentinal tubules.[10] Bioactive glass (BG) has been extensively studied in the fields of tissue engineering, bone regeneration, and dentin remineralization, because of its remarkable bioactive capability in forming hydroxyl carbonate apatite (HCA) when stored in simulated body fluids.[11]

Bioactive glass is composed of silicon, sodium, calcium, and phosphorus oxides with specific percentages. In contact with a biological fluid such as saliva, Bioglass particles precipitate to form a layer which is composed of calcium and phosphate, induced by the release of these ions from the glass which can mechanically occlude dentinal tubules and lower fluid flow within the dentin.[12]

Nano-hydroxyapatite toothpaste was first introduced in Japan in 1980's [13] and are being used in oral care formulations for effective occlusion of dentinal tubules.[14] This biofunctional material has both desensitizing and remineralizing potential for the treatment of Dentinal hypersensitivity.[15] It occludes the dentinal tubules and limits the movement of the dentinal fluid.[16] Li et al. stated that having a particle size of 20 nm, nano-HA resembles the natural building blocks of enamel.[17] The nanohydroxyapatite clusters act as a calcium and phosphate reservoir, which helps in maintaining a supersaturation of ions causing their deposition on the tooth surface, and elevates the calcium concentration in the saliva, thus promoting crystal integrity and growth.[18]

The aim of the present study is evaluation of two desensitizing toothpastes containing Nano-hydroxyapatite and Bioactive Glass on dentinal tubule occlusion under Scanning Electron Microscope (SEM).

Materials and Methods

The present study was carried out in the Department of Periodontology, Himachal Institute of Dental Sciences, Paonta Sahib (H.P). Extracted human molars were used for the study.

Teeth Selection

The teeth selected for the study were obtained from the patients who reported to the Outpatient Department of Oral and Maxillofacial Surgery of Himachal Institute of Dental Sciences, Paonta Sahib (H.P) for extraction of periodontally compromised maxillary and mandibular teeth and necessarily met the following criteria:

Inclusion criteria for the study were, vital teeth at the time of extraction, sound maxillary and mandibular molar extracted due to periodontal disease, teeth without any prior restoration. **Exclusion criteria** were, presence of caries on the surface of the teeth or wasting disease, teeth which were fractured and malformations, teeth having periapical infection or non-vital teeth, teeth with congenital cementum and dentin abnormalities.

Preparation and storage of teeth

After extraction, teeth were subjected to ultrasonic scaling and root planing and then washed with distilled water. After this, they were stored in 10% formalin at room temperature till further use.

ACLAIM Toothpaste

Aclaim is a product of Group pharmaceuticals. Aclaim toothpaste contains nano-particles of hydroxyapatite. Hydroxyapatite is a naturally occurring mineral that is found in bones and the teeth. Hydroxyapatite was included in the form of nanocrystals because they dissolve easier in this form. Crystals of nanohydroxyapatite included in dental products have a dimension of 50 -1000 nm, which enables them to act like fillers. These products can penetrate and block the exposed dentinal tubules which are responsible for Dentinal Hypersensitivity.

Elsenz Toothpaste

Elsenz toothpaste comprises of Fluoro calcium phosphosilicate which is an improved form of Bioactive glass. Elsenz forms acid resistant fluorapatite and releases fluoride over a period of 8 – 12 hours. The formation of fluoroapatite layer and layer composed of calcium and phosphate induced by the release of these ions from the glass can mechanically occlude dentinal tubules and lower fluid flow within the dentin.

Method

Forty-five samples were prepared from the extracted sound maxillary and mandibular molar teeth and stored in 10% Formalin after scaling and root planing. The teeth were sectioned with diamond disc to create dentin specimens. The prepared dentin specimens were then smoothened with 1200 grit silicone carbide polishing paper. The smear layer was removed by immersing the specimens in the 17% Ethylenediaminetetraacetic acid (EDTA) for 2 minutes. After that, they were thoroughly rinsed and stored in the artificial saliva.

The specimens were randomly divided into 3 groups

Group A- Fifteen specimens of this group were brushed with Acclaim toothpaste for 2 minutes for 14 days.

Group B- Fifteen specimens of this group were brushed with Elsenz toothpaste for 2 minutes for 14 days.

Group C- Fifteen specimens of control group were immersed in artificial saliva and were brushed with distilled water for 2 minutes for 14 days.

The prepared discs were mounted on an acrylic block. A pea-size amount of assigned dentifrice was placed onto the dentin surface using powered toothbrush. The brush bristles were directed at 90º angulation to the specimen and were in constant contact with the dentin surface. The brushing duration was for 2 minutes each day for 14 days. After brushing, the specimen were washed and stored in the artificial saliva. After the last brushing session, the samples were thoroughly washed in distilled water, dehydrated with alcohol in different concentration of alcohol and sputtered coated with gold. The surface of the specimen were visualized under SEM under magnification of X2000 and the photomicrographs of the area were obtained. Percentage of the occluded tubules was obtained by dividing the total number of occluded tubules by the total number of tubules in the photomicrographs.

Results and Discussion

After observing the SEM images at a magnification of 2000X, the images were assessed independently by a trained blinded reviewer to score the level of tubule occlusion (on a categorical scale of 1-5):

Tubule occlusion scoring index given by west et al.[19]

Scores	Description
1	Occluded (100% of tubules occluded)
2	Mostly occluded (50–<100% of tubules occluded)
3	Partially occluded (25–<50% of tubules occluded)
4	Mostly unoccluded (<25% of tubules occluded)
5	Unoccluded (0%, no tubule occlusion)

Statistical Analysis

The statistical analysis was done using SPSS (Statistical Package for the Social Sciences, SPSS for Windows, version 16, IBM, USA). Descriptive statistics were calculated as frequency and percentage. The comparison of tubule occlusion

scores among various study groups was done using Chi-square test. The level of significance for the present study was fixed at a P-value of less than 0.05.

Results

The descriptive statistics for tubule occlusion scores in Group A. There were 10 (66.7%) samples with score 2, followed by 4 (26.7%) samples with score 3 and 1 (6.6%) sample with score 4. None of the samples showed a score of 1 or 5.

The descriptive statistics for tubule occlusion scores in Group B . There were 3 (20%) samples with score 2, 8 (53.3%) samples with score 3 and 4 (26.7%) sample with score 4. None of the samples showed a score of 1 or 5.

The descriptive statistics for tubule occlusion scores in Group B . There were 3 (20%) samples with score 2, 8 (53.3%) samples with score 3 and 4 (26.7%) sample with score 4. None of the samples showed a score of 1 or 5.

Comparison of tubule occlusion scores

Table 4. shows the comparison of tubule occlusion scores among the three study groups. The analysis showed that there was a statistically significant difference in tubule occlusion scores ($P<0.001$).

Following observations were found:

Group A showed statistically significant higher proportions of samples (76.9%) with score 2 compared to Group B (23.1%) and Group C (0%) as shown in table no. 2&3. No statistically significant difference was found in relation to score 2 between Group B and Group C shown in table no.4.

Group B showed statistically significant higher proportions of samples (66.7%) with score 3 compared to Group C (0%) . No statistically significant differences were found in relation to score 3 between Group A and Group B, and between Group A and Group C.

Group C showed statistically significant higher proportions (61.5%) of samples with score 4 compared to Group A (7.7%). No statistically significant differences were found in relation to score 4 between Group A and Group B, and between Group B and Group C.

Group C showed statistically significant higher proportions (100%) of samples with score 5 compared to Group A (0%) and Group B (0%).

In inter group comparison of tubule occlusion scores between study **Group A** and study **Group B**, both groups had zero samples with score 1, for score 1 no statistically significant difference was seen between the groups . Group A had 10 samples (76.9%) and Group B had 3 samples (23.1%) with score 2, for score 2 the statistical difference was significant. 4 samples (33.3%) from Group A and 8 samples (66.75) from Group B had score 3, the statistical difference between the group was non-significant for this score. For score 4, 1 sample (7.7%) from Group A and 4 samples (30.8%) from Group B got this score, the statistical difference was non-significant. None of the samples from both the groups got score 5, the statistical difference between the group for score 5 was non-significant. **Amin Meghna et al (2015)** [23] carried out an in vivo study which showed efficacy of commercially available nanohydroxyapatite paste as an effective desensitizing agent. Similar results were seen in a study carried out by **Jalaluddin M. et al (2022)** [24], where they concluded that 1% nano-hydroxyapatite containing desensitizing agent exhibited higher dentinal tubular occluding capacity

compared to the bioactive glass and tri-calcium phosphate-containing desensitizing agents.

In intergroup comparison between **Group A** and **Group C,** for score 1 both the groups had zero samples, there was no significant statistical difference between the group for this score. Group A had 10 samples (76.9%) and Group C had 0 samples (0%) with score 2, for score 2 there was statistically significant difference between the groups. 4 samples(33.3%) from Group A and 0 samples (0%) form Group C had score 3, the statistical difference between the group was non significant for this score. For score 4, Group A had 1 sample (7.7%) and Group C had 8 samples (61.5) with this score, the statistical difference between the group for this score was significant. 0 samples (0%) from Group A and 7 samples (100%) from group C had the score 5, the statistical difference between the group for this score was significant. Similar results were observed in a study conducted by **R. Kulal et al (2016)** [15], where they concluded that the ability of nanohydroxyapatite in relieving Dentinal hypersensitivity is due to the occlusion of the dentinal tubules by the nano-hydroxyapatite crystals and the formation of a protective biomimetic layer.

In intergroup comparison between the study **Group B** and study **Group C**, none of the group had samples with score 1, the statistical difference between the group for this score was non-significant. For score 2, 3 samples (66.7%) from Group B and 0 samples (0%) from group C had this score, the statistical difference for this score between the study groups was non-significant. Group B had 8 samples (66.7%) and Group C had 0 samples (0%) with score 3, for this score the statistical difference between the group was significant. 4 samples (30.8%) of Group A and 8 samples (61.5%) of Group C had score 4, the statistical difference between the study groups for this score was non-significant. For score 5, 0 samples (0%) from Group B and 7 samples (100%) from Group C had this score, the statistical difference between the groups for this score was significant. In the study conducted by **Midha Vasu et al (2021)** [25] it was reported that 5% NovaMin (bioactive glass) group showed more completely occluded dentinal tubules when compared to Propolis, 5% potassium nitrate, 8% arginine containing toothpastes. **Shah Shivani et al (2017)**[26] also concluded in their study that Novamin containing toothpaste showed the highest percentage of tubular occlusion as compared to 8% arginine and 10% strontium chloride containing desensitizing dentifrices.

The possible reason that the results of the dentin specimens treated with Aclaim surpassed those of Elsenz could be due to small particles of nanohydroxyapatite that could be easily penetrated and accumulated into the dentinal tubules than the large bioactive glass broken particles that are only physically bounded till the beginning of the bioactive reaction of these particles.

Heba H Ashlerbiney et al (2020) [22] in their study concluded that biomimetic nanohydroxyapatite–containing toothpaste proved immediate dentinal tubule occlusion compared to calcium-sodium phosphosilicate containing toothpaste, although, both types of toothpastes could have the potential to treat dentin hypersensitivity.

The overall statistics revealed that Group A (Aclaim) shows better occlusion of dentinal tubules

compared to Group B (Elsenz) and Group C (Control). However, further clinical trials should be undertaken to see efficacy of these toothpastes in actual clinical settings. Longer evaluation time and ability of toothpaste to resists acid dissolution should also be taken into account.

Table 1: Comparison of Tubule Occlusion Scores among the study groups

			Group				P value
			Group A	Group B	Group C	Total	
		%	76.9%	23.1%	0%	100.0%	
	Score 3	Count	4	8	0	12	<0.001*
		%	33.3%	66.7%	0%	100.0%	
	Score 4	Count	1	4	8	13	
		%	7.7%	30.8%	61.5%	100.0%	
	Score 5	Count	0	0	7	7	
		%	0%	0%	100.0%	100.0%	

*Statistically significant (Chi-square value = 39.846)

Each subscript letter denotes a subset of Group categories whose column proportions do not differ significantly from each other at the .05 level.

Table 2: Comparison of Tubule Occlusion Scores between the study Group A and Group B

Tubule Occlusion score	Group A	Group B	Significance Level
Score 1	0%	0%	Non-significant
Score 2	76.9%	23.1%	Significant
Score 3	33.3%	66.7%	Non-significant
Score 4	7.7%	30.8%	Non-significant
Score 5	0%	0%	Non-significant

Table 3: Comparison of Tubule occlusion Scores between the study Group A and Group C

Tubule Occlusion score	Group A	Group C	Significance level
Score 1	0%	0%	Non-significant
Score 2	76.9%	0%	Significant
Score 3	33.3%	0%	Non-significant
Score 4	7.7%	61.5%	Significant
Score 5	.0%	100.0%	Significant

Table 4: Comparison of Tubule Occlusion Scores between the study Group B and Group C

Tubule Occlusion score	Group B	Group C	Significance level
Score 1	0%	0%	Non-significant
Score 2	23.1%	0%	Non-significant
Score 3	66.7%	0%	Significant
Score 4	30.8%	61.5%	Non-significant
Score 5	.0%	100.0%	Significant

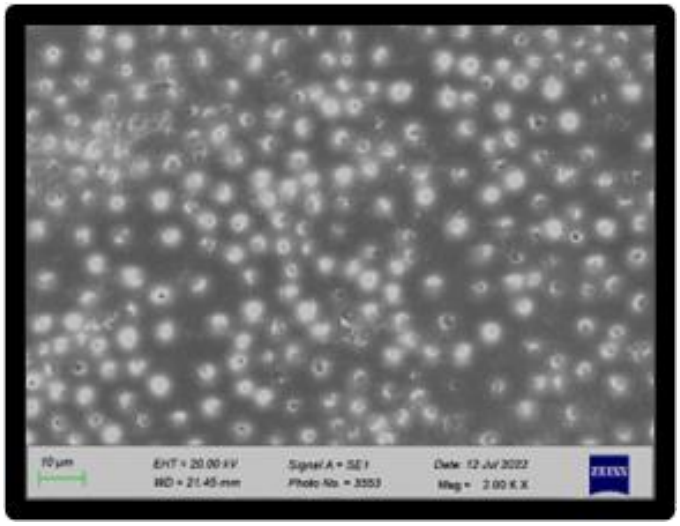

Fig 1: Photomicrographs of root dentin samples with mostly occluded dentinal tubules- Score 2

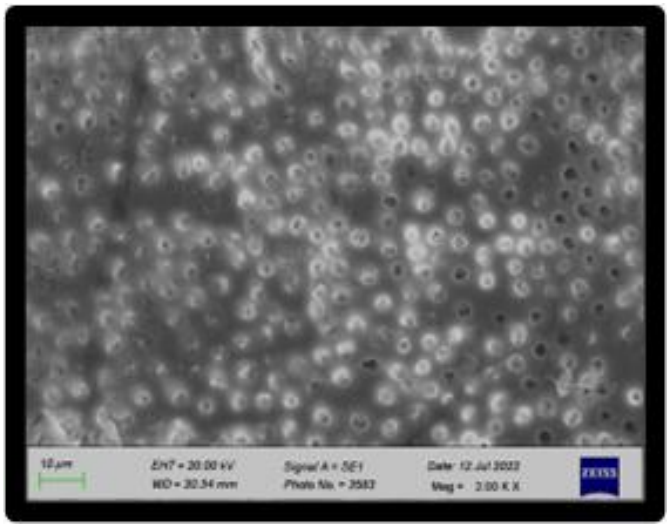

Fig 2 : Photomicrograph of root dentin samples With partially occluded dentinal tubules- Score 3.

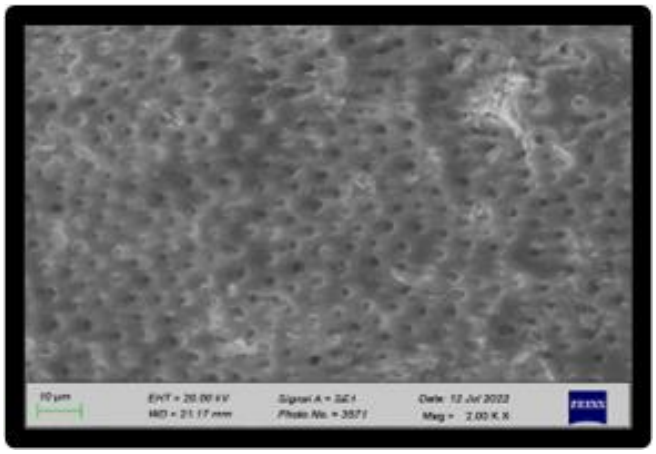

Fig 3: Photomicrograph of root dentin sample with all unoccluded dentinal tubules – Score 5.

Conclusion

In the current study, 45 multirooted teeth were taken, then scaling and root planing was performed. Sectioning of the samples was done followed by the smear layer removal by immersing the samples in 17%EDTA for 2 minutes, which were then thoroughly rinsed and stored in artificial saliva. The samples were divided randomly into 3, Group A was treated with Aclaim toothpaste, Group B treated with Elsenz toothpaste and Group C was treated with Distilled water. The duration of brushing period was 2 minutes each day for 14 days. The samples were dehydrated with alcohol in different concentrations after which they sputter coated with gold, and visualized under SEM at a magnification of X2000.

Within the limit of the present study, Aclaim showed maximum samples having mostly occluded dentinal tubules (Score 2) whereas Elsenz showed maximum samples having partially occluded tubules and mostly unoccluded dentinal tubules (Score 3 and Score 4). Control showed mostly unoccluded and unoccluded dentinal tubules (Score 4 and Score 5). However, further clinical trials should be undertaken with greater sample size to see the efficacy of these toothpastes and their ability to resist acid dissolution should be assessed.

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