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EFFECT OF COMMERCIALLY AVAILABLE NANO-HYDROXYAPATITE CONTAINING DESENSITIZING TOOTHPASTE/MOUTHWASH AND COMBINATION OF BOTH ON DENTINAL TUBULAR OCCLUSION: A SEM ANALYSIS

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Abstract

Aim

The aim of the present in-vitro study is to evaluate and compare the dentinal tubule occlusion using a n-HAP containing toothpaste / n-HAP containing mouthwash and the combination of two under scanning electron microscope (SEM).

Materials and Methods

Extracted premolars and canine were collected and stored in 10% formalin. Dentin blocks measuring 8 mm × 5 mm× 2 mm were prepared from the crown part adjacent to CEJ. The specimens were randomly divided into 3 groups with 5 specimens each. Group1 -Toothpaste group, Group 2 - Mouthwash group and Group 3 - Combination group. The samples were treated twice daily as per the assigned group for 28 days. The percentage of the occluded

dentinal tubules and the open area of tubules was assessed at baseline, 7th, 14th, 21st and 28th day by subjecting the samples for electro micrograph through scanning electron microscope.

Results

Combination group showed higher percentage of occluded dentinal tubules and least area of open tubules as compared to toothpaste group followed by mouthwash group at 7th, 14th, 21st, and 28th day respectively.

Conclusion

It can be concluded that brushing twice daily with n-HAP containing toothpaste along with usage of n-HAP containing mouthwash for duration of 28 days produced good dentinal tubule occlusion and may be useful in the treatment of dentinal hypersensitivity.

Key words: Dentinal tubules, Dentine hypersensitivity, Mouthwash, Toothpaste.

INTRODUCTION

Dentine hypersensitivity is one of the most painful long term problems of the teeth and has the lowest success rate.[1] Dentine hypersensitivity (DH) is defined as a "short sharp pain arising from exposed dentine most commonly at the tooth cervical area in response to thermal, tactile evaporative, osmotic or chemical stimuli but which cannot be ascribed to any other dental defects, diseases or restorative treatments".[2] About 30,000 tubuli per mm² of 1-2 μm in diameter present in dentine that extends at angle of 90° from the dentine surface to the tooth pulp.[3]

Usually, canines and premolars are the teeth affected by dentine hypersensitivity. Wasting disease, recession of gingiva and inappropriate brushing techniques are the main etiologic factor for dentin hypersensitivity.[4]

There are three major theories of dentine hypersensitivity. The direct stimulation theory[5] states that nerve terminals passes via pulp and dentin and merge with the dentinoenamel junction. Odontoblasts acts as a receptors and transfer signal to a nerve endings explained by the odontoblast receptor theory[6]. A more recent idea has came in existence due to the drawbacks of these two theories, which states that sensitivity develops because of the movement of fluid within the dentinal tubules that stimulates mechanoreceptors on the pulpal nerve ending known as the fluid flow theory[7].

There are two methods to reduce hypersensitivity that includes occlusion of the dentinal tubules physically and chemically blockage of neural transmission due to altered nerve synapses.[8] The recently developed interest in nanotechnology introduced the application of nano-hydroxyapatite in the field of dentistry.[9]

Wide variety of toothpaste have been developed to decrease tooth hypersensitivity. Toothpastes contains potassium ions that depolarise sensory pulpal nerves and interrupt the transmission of pain stimuli.[10] In comparison of the traditional toothpaste nanohydroxyapatite is suggested to inhibit tooth sensitivity more successfully because it biochemically binds both collagen and hydroxyapatite from dentine and easily occlude the dentinal tubules because of its nano-sized diameters.[11]

To the best of our knowledge, the literature on comparison of efficacy of dentinal tubular occlusion by n-HAP containing toothpaste/mouthwash and combination of both is missing. The aim of the present in-vitro study is to evaluate and compare the efficacy of commercially available nano-hydroxyapatite (n-HAP) containing toothpaste/mouthwash and the combination of two for dentinal tubule

occlusion under scanning electron microscope (SEM).

MATERIALS AND METHODS

This In-vitro study was conducted in the Department Of Periodontology, Rama Dental College, Hospital and Research Center, Kanpur, Uttar Pradesh, India in collaboration with IIT, Kanpur (Department Of Materials Science And Engineering), Uttar Pradesh, India.

Required extracted premolars and canines were collected from Department of Oral surgery and from some private clinics.

INCLUSION CRITERIA

- Caries free crown and root.
- Teeth free from periodontal disease.
- Teeth without any attrition, abrasion and erosion.

EXCLUSION CRITERIA

- Teeth with restorations.
- Teeth with external resorption and developmental anomalies.
- Teeth with fractures.
- Hypoplastic teeth
- Endodontically treated teeth

METHODOLOGY

SPECIMEN PREPARATION:-

Premolars and canine were collected and stored in 10% formalin after extraction. Gross debris have been removed from teeth and stored in distilled water. Then the teeth were sectioned mesiodistally in horizontal manner by using a diamond disc bur and straight micromotor hand piece.

EXPERIMENTAL DESIGN:-

Dentin blocks were prepared from the crown part adjacent to CEJ measuring 8mm × 5 mm×2mm. To get rid of any cutting debris the blocks were rinsed in distilled water for half minute to remove particulate matter resulting from cutting. 17% EDTA solution were used for 5 minutes to etch the dentinal disk. For 30 seconds, the treated samples were thoroughly rinsed with distilled in order to eliminate remaining debris.

Then, the specimens were randomly divided into 3 groups with 5 specimens in each group.

Group 1 - n-HAP containing toothpaste

Group 2 - n-HAP containing mouthwash

Group 3 - Combination of n-HAP containing toothpaste and mouthwash.

In group 1 specimens were brushed with toothpaste for 2 minutes twice daily for 28 days. After brushing, the samples were washed with distilled water.

In group 2 specimens were vigorously shaked in the mouthwash for 1 minutes twice daily for 28 days.

In group 3 specimens were brushed with toothpaste for 2 minutes and after 30 minutes rinsed with mouthwash for 1 minute twice daily for 28 days.

Measurements of specimens of each group was taken at baseline, 7^{th}, 14^{th}, 21^{st}, and 28^{th} day. The baseline recordings for each group acted as the control for that particular group. The blocks were stored in distilled water during the experimental period.

PREPARATION OF THE SPECIMENS FOR SEM ANALYSIS

The sample disks were dried in hot air oven for 8 hours. Sputter coating of samples was done with the layer of gold measuring 1.5 -3 nm. The extent of tubular occlusion was assessed using a Carl Zeiss EVO 50 WSEM. All specimens were observed under 4000X magnification and a beam voltage of 15Kv. The photographs were saved and analyzed for dentinal tubule occlusion and changes in the open area of tubules. (Figure 1-3.)

Steps involved in SEM analysis are as follows:

1. Gold sputtering

The sample disks were dried in hot air oven for 8 hours and later mounted on aluminium stubs using a conductive carbon tape. Sputter coating of samples were done with the layer of gold measuring 1.5 -3 nm to aid conductivity for further WSEM analysis.

2. Teeth mounted in SEM machine

Samples were mounted inside the SEM machine to focus the electron beam. The signals produced due the interaction of beam's electrons with the atoms of the specimen, gives information about its composition, surface topography and other electrical properties. These interactions and effects were analyzed and results into an image.

3. Vaccumization

Vaccumization was produced inside the electron microscope to facilitate the electrons signals from the sample to the detector for better imaging.

4. Image processing.

The generated signals were captured by electrical detectors, then transformed into digital images and finally displayed on a screen as digital image.

ASSESSMENT OF DENTINAL TUBULAR OCCLUSION

By observing in the SEM images, dentinal tubule occlusion was evaluated and expressed in percentages. The dentinal tubules were segregated into three categories depending on the percentage of occlusion:-[12]

1. Unoccluded (when the occlusion was 0%)
2. Partially occluded (when the occlusion was < 75%)
3. Completely occluded (when the occlusion was ≥75%)

ASSESSMENT OF OPEN AREA OF DENTINAL TUBULES

The open area of the dentinal tubules was manually outlined and the value of area within the outline was automatically calculated by software (Image J Version 1.47, National Institute of Health, USA).

STATISTICAL ANALYSIS

The data was recorded in MS Excel sheet and was further subjected to statistical analysis using SPSS software 24. Statistical significance was recorded at $P \leq 0.05$.

Repeated measure ANOVA test was performed for intragroup comparison of open area of tubules at different time intervals in all three group. One way ANOVA test was performed to compare the percentage of unoccluded, partially occluded and completely occluded tubules and for comparison of open area of tubules between three groups at different time intervals.

Results

Table 1. shows that percentage of occluded dentinal tubules was higher in combined group followed by toothpaste and mouthwash group at 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively.

Table 2. shows intragroup comparison of mean open area of tubules which was reduced from baseline to 28days and was statistically significant in all three groups.

Table 3. Overall intergroup comparison of open area of tubules showed statistically significant difference among three groups with combined group showing least area followed by toothpaste group and mouthwash group at 7^{th},14^{th}, 21^{st} and 28^{th} day respectively.

DISCUSSION

Gingival recession, wasting diseases such as attrition, erosion, abrasion and abfraction causes loss of enamel as well as cementum exposes the dentinal tubules which results in teeth sensitivity.[13] In 1935, Grossman said that a perfect desensitizing agent should be act rapidly, long lasting effective, safe to pulp, simple on application and do not causes staining of the teeth.[14]

Toothpaste and mouthwash are most frequently form of desensitizing agents used for reducing hypersensitivity because of its low cost, easy to use and at home application.[15]

In 1980,[16] toothpastes based on n-HAP was first discovered in Japan and are used as oral care products for occlusion of dentinal tubules effectively. It can be easily binds with the dentinal tubules, results in their occlusion and reducing DH. It also binds with the dentine apatite and tooth enamel because it is biologically active, chemically reactive and have higher surface area.[17]

In the current study, extracted canine and premolars were used because these teeth are most affected by dentinal hypersensitivity .[18]

Using the extracted teeth, cervical dentine blocks were prepared from the crown part adjacent to CEJ measuring 5mm × 8 mm× 2mm. Since, the dentin hypersensitivity mainly occurs in cervical areas so, the use of cervical dentin blocks approximately simulated the clinical scenario.[19]

17% EDTA solution were used to etch the dentinal disks for 5 minutes, to remove smear layer from dentinal disk and to stimulates the open dentinal tubules of the sensitive teeth.[20]

In mouthwash and combined groups the dentinal disks were vigorously shaken to copy the clinical circumstances which occurs during oral rinsing.[20]

SEM investigation was selected for this in-vitro study because it is a non-destructive procedure and also provides high resolution to observe the treated dentinal tubules.[21]

Since, the tooth is nonconductive so, prior to SEM analysis, sputter coating of dentinal disks with a thin layer (1.4-2.5 nm) of gold was done to made them conductive, eliminates the electrons from the disks, enhances the quality and resolution of images during imaging.[22]

Nowadays, nano-hydroxyapatite is available in the form of toothpaste and mouthwash. The present study evaluated the percentage of occluded dentinal tubules and changes in the open area of dentinal tubules after applying commercially available n-HAP containing toothpaste or mouthwash or combination of both by SEM analysis that gave detailed image of micromorphological changes in dentinal tubules.

Here, the percentage of occluded dentinal tubules increased in all the three groups at 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively.

Also, the combined group showed better dentinal tubules occlusion (85.12%) than

The percentage of occluded dentinal tubules predict efficacy of n-HAP as desensitizing agent and changes in open area of tubules helps us to understand that the occlusion of dentinal tubules is a gradual process which might takes multiple days to multiple weeks in order to achieve complete occlusion of dentinal tubules.

The result for toothpaste group is supported by the studies done by Jena et al[23] and Kulal et al[10]. A 14 days study done by Jena et al[24] who investigated and compared the efficacy of occluded dentinal tubules by four different dentifrices i.e. potassium salts, bioactive glass, fluoride and 15% n-HAP, reported that 15% n-HAP containing toothpaste showed 68.015 mean percentage of occluded tubule at 14^{th} day from the baseline. Kulal et al[10] also conducted a study for 7days to evaluate and compared the effects of nano hydroxyapatite, novamin and proargin containing desensitizing agents on dentinal tubule occlusion and said that nano hydroxyapatite containing desensitizing agents showed 97.62% occlusion of dentinal tubules on the 7^{th} day as compared to baseline.

The results for the mouthwash group is supported by the study done by Hill R.G et al[24] and Saini N et al[18] . Hill R.G et al[24] performed a study to evaluate the occlusion of dentinal tubules and reduced fluid flow by using five desensitizing oral rinse containing either nanohydroxyapatite, zinc substituted HA, potassium oxalate, arginine and potassium nitrate and found that after 30 seconds treatment, nanohydroxyapatite containing desensitizing oral rinse showed that most of the dentinal tubules were occluded. Also, Saini N et al[18] in their 14 days study with n-HAP containing mouthwash reported 75% completely occluded dentinal tubules at 14th day from baseline. toothpaste group (80.84%) followed by mouthwash group (71.03%) at 7^{th}, 14^{th},

21st and 28th day respectively in the present study.

The comparison of n-HAP containing toothpaste or mouthwash or combination of both has not yet been reported in the literature. Though a 60 days study with different desensitizing agents performed by George et al[8] who compared the use of novamin based desensitizing toothpaste, mouthwash, and their combination, found that by the 28th day the percentage of completely occluded dentinal tubules was similar that is 100% in toothpaste group and combination group followed by mouthwash which had only 60% of the dentinal tubules as completely occluded whereas 40% were partially occluded. In the present study, both intra and inter group comparison showed that though the open area of dentinal tubules was decreased in all the three group from 7th to 14th, 21st and 28th day respectively but combined group showed least area of open tubules than toothpaste group followed by mouthwash group.

There is no study in literature which compare n-HAP containing toothpaste or mouthwash or combination of both related to changes in open area of dentinal tubules.

Better performance of toothpaste group as compared to mouthwash group can be explained firstly, because of the presence of hydrated silica in abrasive form and secondly, because of the formation of smear layer during brushing which is further pushed into dentinal tubules.

The best result shown by combined group can be attributed due to dual action shown by toothpaste and mouthwash. The action of toothpaste along with the vigorous shaking of the dentinal disc samples in mouthwash daily would have provide additional deposition of n-HAP crystals in the dentinal tubules.[18]

Here, the present study have some limitations such as limited sample size and the placement of dentinal discs in the distilled water after application of toothpaste and mouthwash was unable to simulate the oral conditions. The percentage of occluded dentinal tubules were counted manually which is subjected to a human error.

CONCLUSION

It can be concluded that brushing twice daily with n-HAP containing toothpaste along with usage of n-HAP containing mouthwash for duration of 28 days produced good dentinal tubule occlusion and may be useful in the treatment of dentinal hypersensitivity. Further research including clinical studies is required to provide evidence for the durability of occluded dentinal tubules after using combination of n-HAP containing toothpaste and mouthwash.

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LEGENDS

Figure 1. SEM images of toothpaste group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

Figure 2. SEM images of mouthwash group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

Figure 3. SEM images of combined group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

Table 1. Intergroup comparison of percentage of unoccluded, partially occluded and completely occluded tubules at different time intervals.

Table 2. Intragroup comparison of open area (Pixel) of tubules at different time intervals.

Table 3. Intergroup Comparison of open area (Pixel) of tubules at different time intervals.

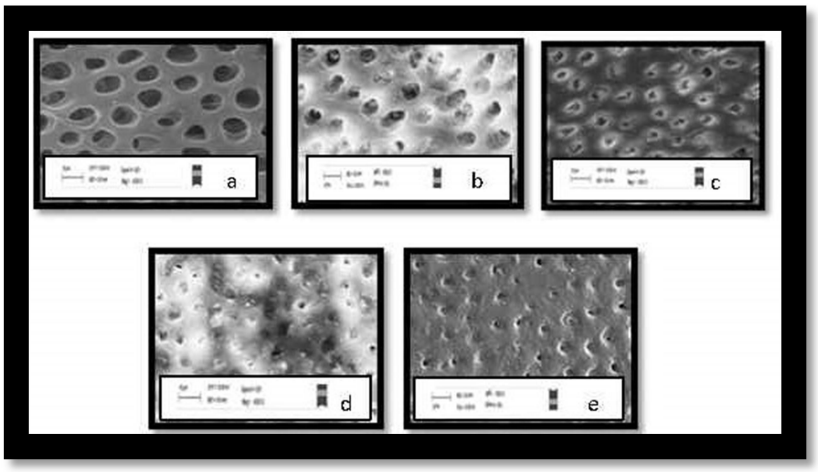

Figure 1. SEM images of toothpaste group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

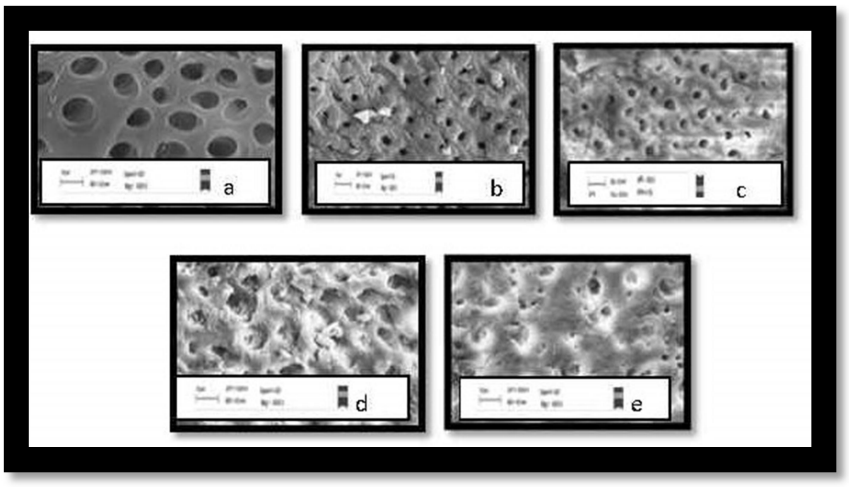

Figure 2. SEM images of mouthwash group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

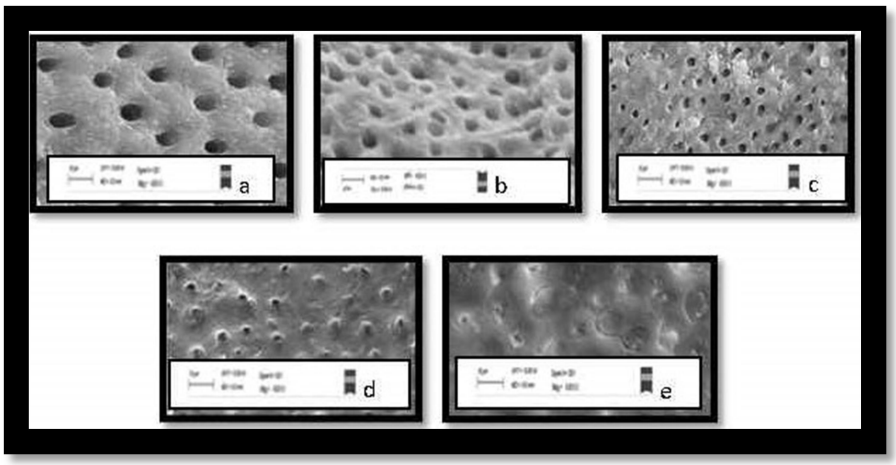

Figure 3. SEM images of combined group at a. Baseline b. 7th day c.14th day d.21st day e.28th day.

Table 1. Intergroup comparison of percentage of unoccluded, partially occluded and completely occluded tubules at different time intervals.

Interval		Toothpaste		Mouthwash		Combined		p value
		Mean (%)	**SD**	**Mean (%)**	**SD**	**Mean (%)**	**SD**	
Baseline	**Unoccluded**	100	0.00	100	0.00	100	0.00	----
	Partially occluded	0	0.00	0	0.00	0	0.00	----
	Completely occluded	0	0.00	0	0.00	0	0.00	----
7th day	**Unoccluded**	36.52	5.68	53.11	8.41	26.07	6.30	<0.001*
	Partially occluded	39.43	7.46	32.70	9.62	35.25	4.74	0.392
	Completely occluded	25.87	5.73	14.16	6.20	38.92	4.21	<0.001*
14th day	**Unoccluded**	23.00	4.66	33.16	5.70	14.46	4.68	<0.001*
	Partially occluded	31.58	4.64	33.20	4.23	29.98	4.38	0.534
	Completely occluded	44.84	6.45	34.47	8.28	55.53	5.98	0.002*

21st day	**Unoccluded**	13.27	3.62	22.56	3.61	5.30	3.15	<0.001*
	Partially occluded	28.22	2.63	27.00	6.38	18.34	4.38	0.579
	Completely occluded	58.45	5.46	52.24	7.63	76.36	6.14	<0.001*
28th day	**Unoccluded**	3.34	2.50	9.70	2.86	2.22	0.45	<0.001*
	Partially occluded	15.82	9.08	19.86	3.80	14.88	2.91	<0.001*
	Completely occluded	80.84	8.06	71.03	4.68	85.12	3.78	<0.001*

- One-way ANOVA test; * indicates statistically significant difference at p≤0.05 Ok

Table 2. Intragroup comparison of open area (Pixel) of tubules at different time intervals.

Groups		Baseline	7th day	14th day	21stday	28th day	p value
Toothpaste	**Mean**	1142.7	409.96	271.28	124.22	50.12	0.002*
	SD	318.7	38.07	13.15	18.73	12.34	
Mouthwash	**Mean**	1143.28	506.24	332.38	162.76	136.55	0.002*
	SD	319.00	109.27	63.48	35.82	43.9	
Combination	**Mean**	1141.7	316.82	161.30	108.45	43.22	0.001*
	SD	316.7	94.10	39.88	21.11	13.45	

Repeated measure ANOVA test; * indicates statistically significant difference at p≤0.05

Table 3. Intergroup Comparison of open area (Pixel) of tubules at different time intervals.

Interval	Toothpaste		Mouthwash		Combination		p value
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Baseline	1142.7	318.7	1143.28	319.0	1141.7	319	0.7
7th day	409.96	38.07	506.24	109.27	316.82	94.1	0.001*
14th day	271.28	13.15	332.38	63.48	161.3	39.88	0.001*
21st day	124.22	18.73	162.76	35.82	108.45	21.11	0.001*
28 days	50.12	12.34	136.55	43.9	43.22	13.45	0.001*

One way ANOVA test; * indicates statistically significant difference at p≤0.05