

# A Comparison of Various Dentifrices Containing Such as Bioactive Glass, Hydroxyapatite & Potassium Nitrate on Dentinal Tubules Occlusion: A SEM Study

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## ABSTRACT

**Background:** Dentine hypersensitivity is a common problem found mostly in adult population ranging from 4 to 74%. Recently bioactive glass and synthetic hydroxyapatite have been used for treating dentine hypersensitivity. The present study was done to evaluate qualitatively the effect of three desensitizing dentifrices containing Bioactive Glass, Hydroxyapatite and Stannous Fluoride on the patency of dentinal tubules by Scanning Electron Microscopy at 7 days, 1month, and 3 months interval.

**Materials & Methods:** 40 freshly extracted sound premolars extracted for orthodontic purpose from the patients of age group 15 to 25 years were used as specimens. The extracted teeth were cleansed by soaking in 3% sodium hypochloride for 10 minutes and then washed with running water and stored in distilled water for further use. All the specimens were ultrasonicated (at 42000 Hz, 5 cycles) for 12 min in distilled water to remove residual smear layer and to open the dentine tubule to simulate hypersensitive cervical dentin.

**Results:** The percentage of dentinal tubular occlusion in Group1, Group 2, Group 3 and Group 4 were compared at various time intervals i.e. at 1week, 1month and 3 months. There was a highly significant ( $P < 0.001$ ) relationship at 7day interval. The relationship between various groups at 1 month and 3 month was also highly significant ( $P < 0.001$ ).

**Conclusion:** We concluded that Bioactive glass dentifrice showed the highest percentage of tubule occlusion, as it precipitated the hydroxycarbonate apatite over the entire dentin surface followed by Hydroxyapatite, Stannous fluoride and Distilled water where the tubule occlusion was mainly by the silica abrasive.

**Keywords:** Dentine Hypersensitivity, SEM, Dentinal Occlusion, Dentifrices.

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## INTRODUCTION

Dentine hypersensitivity is defined as a sharp pain arising from exposed dentin as a result of various stimuli such as heat, cold, chemical or osmotic, and that cannot be explained as arising from any other form of dentinal defect or pathology. Dentine hypersensitivity is a common problem found mostly in adult population ranging from 4 to 74%. This wide variation in prevalence may be due to various factors like chronic trauma from tooth brushing, gingival recession, erosion of enamel, anatomical factors, etc.

The incidence of tooth hypersensitivity increases with age, and is attributed to the general increase in exposed root surfaces of the teeth from periodontal disease, or cyclic loading fatigue of the thin enamel near the cemento-enamel junction (CEJ).<sup>1-3</sup> The currently accepted theory for tooth hypersensitivity is the hydrodynamic theory proposed by Brannstrom in 1966.<sup>4,5</sup> This theory is based on

the concept that open dentinal tubules allow fluid flow through the tubules, which excites the nerve ending in the dentinal pulp. Clinical replicas of sensitive teeth viewed under a Scanning Electron Microscope (SEM) reveal varying numbers of open or partially occluded dentinal tubules.<sup>6</sup> In general, tubules are not seen at the tooth root surface because of the cementum covering the tooth root, or because of a smear layer of dentinal debris 2-5 microns thick that covers the tooth surface and masks the tubules. There have been two basic approaches to the treatment and prevention of dentinal hypersensitivity. The first approach is to treat the tooth with a chemical agent that penetrates into the dentinal tubules and depolarizes the nerve synapse, which reduces sensitivity by preventing the conduction of pain impulses (e.g., potassium nitrate). The second approach is to treat the tooth with a chemical or physical agent that creates a deposition layer

and mechanically occludes dentinal tubules, which reduces sensitivity by prevention of pulpal fluid flow (e.g., potassium oxalate, ferric oxalate, strontium chloride).<sup>7-9</sup> Recently bioactive glass and synthetic hydroxyapatite have been used for treating dentine hypersensitivity. Bioactive glass (Calcium sodium phosphosilicate) is a highly biocompatible material that was originally developed as bone regenerative materials. It is known to deposit hydroxycarbonate apatite into the exposed dentinal tubules and mechanically occlude them.<sup>10</sup> Dentifrice containing synthetic hydroxyapatite powder is known to be highly effective in fortifying and remineralizing the tooth surface. The present study was done to evaluate qualitatively the effect of three desensitizing dentifrices containing Bioactive Glass, Hydroxyapatite and Stannous Fluoride on the patency of dentinal tubules by Scanning Electron Microscopy at 7 days, 1month, and 3 months interval.

**MATERIALS AND METHODS**

40 freshly extracted sound premolars extracted for orthodontic purpose from the patients of age group 15 to 25 years were used as specimens. The extracted teeth were cleansed by soaking in 3% sodium hypochloride for 10 minutes and then washed with running water and stored in distilled water for further use.

**Dentine Specimen Preparation**

Dentine specimens of size 3x3x2mm were prepared from the cervical 1/3<sup>rd</sup> of each premolar using double-sided diamond disk attached to water-cooled air-rotor<sup>†</sup> and straight hand piece<sup>‡</sup> at slow speed. The specimens were randomly divided into 4 groups of 10 dentin blocks. These dentin blocks were mounted on 2mm thick polyvinyl plastic plate of using Cyanoacrylate adhesive (super glue). Each dentine block was polished with sof-lex polishing discs of coarse, medium, fine, extra fine disks and final polishing was done with prismagloss fine and extra fine polishing pastes containing 1 and 0.2 μ alumina abrasives. All the specimens were ultrasonicated (at 42000 Hz, 5 cycles) for 12 min in distilled water to remove residual smear layer and to open the dentine tubule to simulate hypersensitive cervical dentin.

Specimens of groups 1, 2 and 3 were brushed with dentifrices slurries prepared by diluting 2gm of dentifrice with 6 ml of distilled water. Brushing was performed with Colgate actibrush which is a battery powered tooth brush with soft round bristle and oscillation speed of 8000 rpm/min. Brushing was done for 2 minutes for each dentine block from equidistance by attaching bristle protector to the tooth brush shaft. After each brushing session specimens were washed under running tap water and then kept in normal saline at room temperature.

**Statistical Analyses**

Dentinal tubule occlusion in different groups are compared by one way Analysis of variance (ANOVA) and multiple dependent variables within the group were compared using Post Hoc Tukey HSD significance level for rejection of null hypothesis was set at α=0.05.

**RESULTS**

The percentage of dentinal tubular occlusion in Group1, Group 2, Group 3 and Group 4 were compared at various time intervals i.e. at 1week, 1month and 3 months. There was a highly significant (P<0.001) relationship at 7day interval. The relationship between various groups at 1 month and 3 month was also highly significant (P<0.001) [Table 1].

Intercomparison of percentage of dentinal tubule occlusion between Group 1, Group 2, Group 3 and Group 4 at 7 days was assessed. Percentage of dentinal tubules occlusion in Group 1 when compared with Group 2 showed a highly significant relationship (P<0.001). Also comparison was made between Group 3 and group 4 which was highly significant (P<0.001). Percentage of dentinal tubules occlusion in Group 2 when compared to group 3 was Non-significant (P=0.655) and when Group 2 was compared to group 4 it was highly significant (P<0.001). Group 3 percentage of dentinal tubules occlusion as compared with Group 4. This relationship was highly significant (P<0.001) (Table 2).

**Table 1: Intercomparison of dentinal tubule occlusion between Group 1, Group 2, Group 3 and Group 4 at various**

Interval	F	Significance
7 day	148.73	<0.001 **
1 month	493.74	<0.001 **
3 month	890.65	<0.001 **

**Table 2: Intercomparison of dentinal tubule occlusion between Group 1, Group 2, Group 3 and Group 4 at various intervals by ANOVA test.**

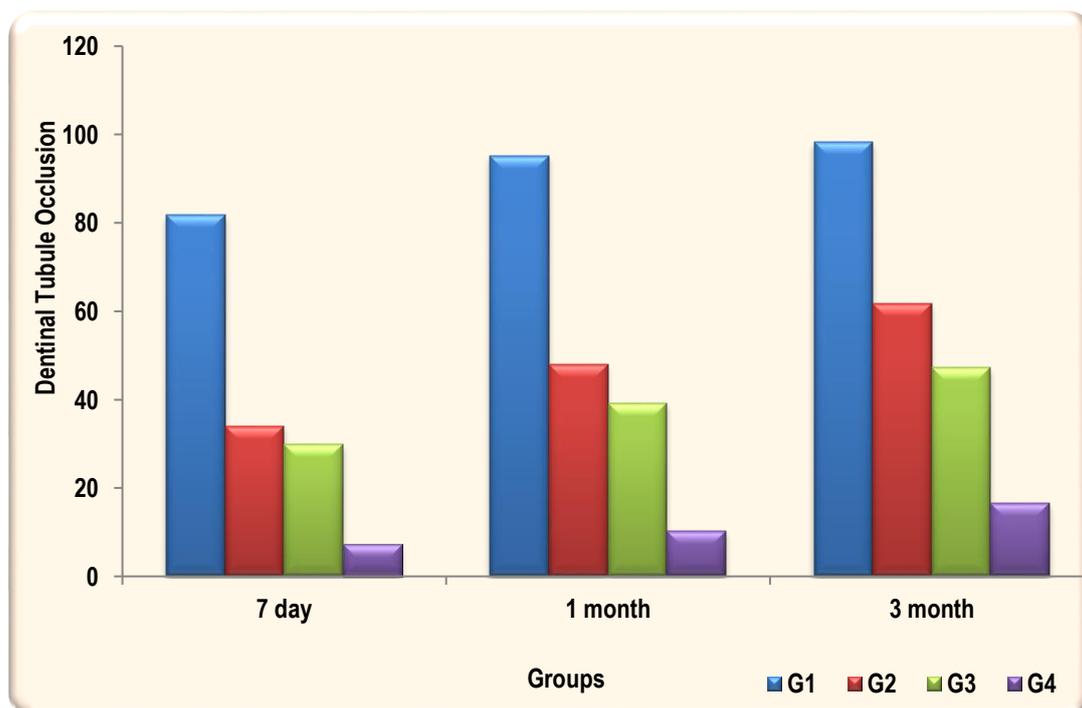
(I) Dentinal tubule occlusion	(J) Dentinal tubule occlusion	Mean Difference (I-J)	Std. Error	Significant	95% Confidence Interval	
					Lower Bound	Upper Bound
G1	G2	47.70*	3.63	<0.001**	37.91	57.49
	G3	51.92*	3.63	<0.001**	42.13	61.71
	G4	74.55*	3.63	<0.001**	64.76	84.33
G2	G1	-47.70*	3.63	<0.001**	-57.49	-37.91
	G3	4.22	3.63	.655	-5.56	14.00
	G4	26.84*	3.63	<0.001**	17.06	36.63
G3	G1	-51.92*	3.63	<0.001**	-61.71	-42.13
	G2	-4.22	3.63	.655	-14.00	5.56
	G4	22.62*	3.63	<0.001**	12.84	32.41
G4	G1	-74.55*	3.63	<0.001**	-84.33	-64.76
	G2	-26.84*	3.63	<0.001**	-36.63	-17.06
	G3	-22.62*	3.63	<0.001**	-32.41	-12.84

**Table 3: Intercomparison of dentinal tubule occlusion between Group 1, Group 2, Group 3 and Group 4 by Tukey HSD at 1 month**

(I) Dentinal tubule occlusion	(J) Dentinal tubule occlusion	Mean Difference (I-J)	Std. Error	Significant	95% Confidence Interval	
					Lower Bound	Upper Bound
G1	G2	47.20*	2.24	<0.001**	41.16	53.24
	G3	56.02*	2.24	<0.001**	49.98	62.06
	G4	84.87*	2.24	<0.001**	78.83	90.91
G2	G1	-47.20*	2.24	<0.001**	-53.24	-41.16
	G3	8.81*	2.24	<0.001**	2.77	14.85
	G4	37.66*	2.24	<0.001**	31.62	43.70
G3	G1	-56.02*	2.24	<0.001**	-62.06	-49.98
	G2	-8.81*	2.24	<0.001**	-14.85	-2.77
	G4	28.84*	2.24	<0.001**	22.80	34.88
G4	G1	-84.87*	2.24	<0.001**	-90.91	-78.83
	G2	-37.66*	2.24	<0.001**	-43.70	-31.62
	G3	-28.84*	2.24	<0.001	-34.88	-22.80

**Table 4: Intercomparison of dentinal tubule occlusion between Group 1, Group 2, Group 3 and Group 4 by Tukey HSD at 3 month**

(I) Dentinal tubule occlusion	(J) Dentinal tubule occlusion	Mean Difference (I-J)	Std. Error	Significant	95% Confidence Interval	
					Lower Bound	Upper Bound
G1	G2	36.54*	1.60	<0.001**	32.21	40.88
	G3	51.04*	1.60	<0.001**	46.70	55.37
	G4	81.81*	1.60	<0.001**	77.48	86.15
G2	G1	-36.54*	1.60	<0.001**	-40.88	-32.21
	G3	14.49*	1.60	<0.001**	10.15	18.82
	G4	45.27*	1.60	<0.001**	40.93	49.60
G3	G1	-51.04*	1.60	<0.001**	-55.37	-46.70
	G2	-14.49*	1.60	<0.001**	-18.82	-10.15
	G4	30.77*	1.60	<0.001**	26.44	35.11
G4	G1	-81.81*	1.60	<0.001**	-86.15	-77.48
	G2	-45.27*	1.60	<0.001**	-49.60	-40.93
	G3	-30.77*	1.60	<0.001**	-35.11	-26.44



**Graph 1: Intergroup comparison of mean value of dentinal tubule occlusion in Group 1 (Bioactive Glass), Group 2 (Hydroxyapatite), Group 3 (Stannous Fluoride) and Group 4 (Distilled Water) at various interval**

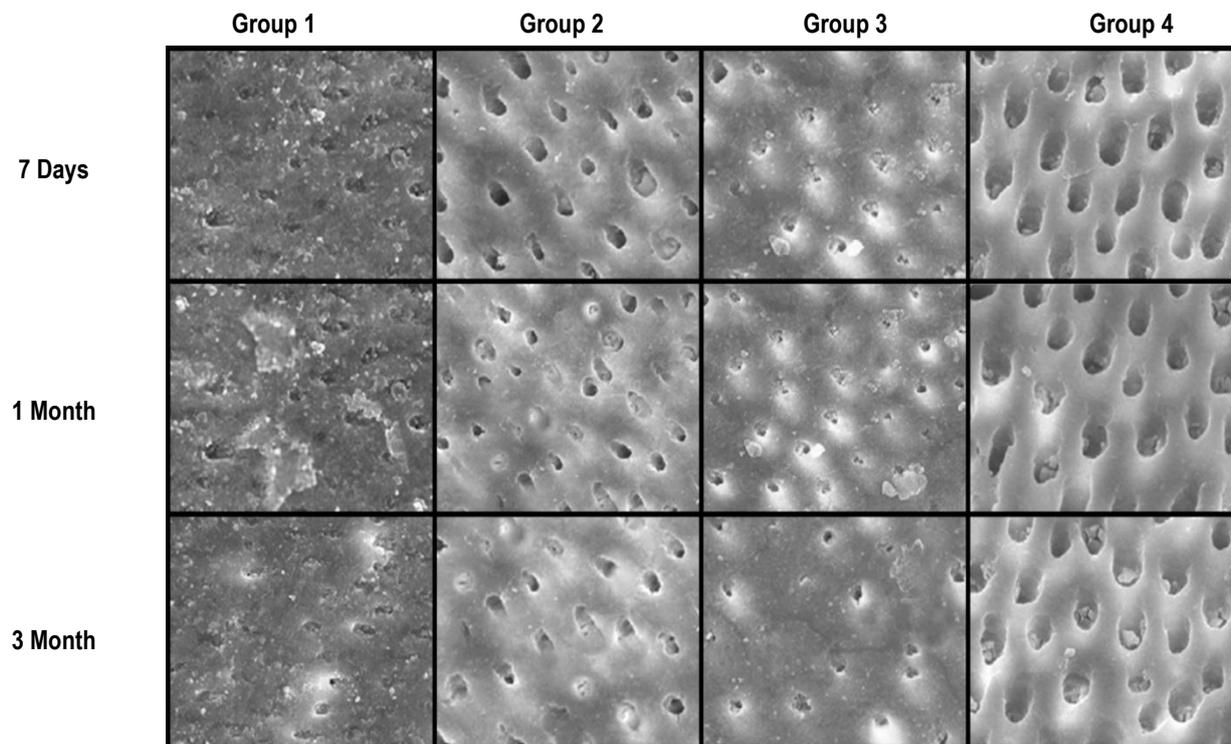


Figure 1: Dentinal tubules occlusion in various interval in different groups by SEM study.

The comparison of percentage of dentinal tubules occlusion of all the groups namely Group 1, Group 2, Group 3 and Group 4 at 1 month as well as 3 months was highly significant ( $P < 0.001$ ) [Table 3, Table 4, Graph 1].

## DISCUSSION

The present study was done to evaluate qualitatively the effect of three desensitizing dentifrices containing Bioactive Glass, Hydroxyapatite and Stannous Fluoride on the patency of dentinal tubules by Scanning Electron Microscopy.

The percentage of dentinal tubule occlusion in Group 1 was 81.7%, Group 2 was 34%, Group 3 and Group 4 was 29.8% and 7.1% respectively at 7 days. Maximum dentinal tubule occlusion was seen in Group 1. This can be supported by the fact that microscopic particles of Bioactive Glass when exposed to water release mineral ions. These ions form hydroxyapatite crystals, a form of hard and strong mineral found in mineralized teeth.<sup>11</sup>

Also the study done by D.G. Gillam et al<sup>10</sup> concluded that possible deposition on the exposed dentine surface may be either in the form of bioactive glass or more likely as precipitation of calcium phosphate following ion exchange on the surface of the bioactive glass when in contact with the aqueous environment. Marini et al<sup>12</sup> also explained that bioactive glass is chemically calcium sodium phosphosilicate which is highly biocompatible. These materials are reactive when exposed to body fluids and deposit hydroxycarbonate apatite, a mineral that is chemically similar to the mineral in enamel and dentine.

In Group 2 percentage of dentinal tubule occlusion was  $34.04 \pm 10.75$  at 7 days. This can be supported by the study done by Mukai Y, Tomiyama K et al<sup>13</sup> whose results suggested that powdered apatite glass ceramics are able to effectively occlude the dentinal tubules. They found that the mean tubular occlusion was 32.70%. This occlusion may be attributed to the deposition of both hydroxyapatite and silica crystals, typical granular deposition

in the open tubule without changing the orifice diameter of any tubule. Kodaka T et al<sup>14</sup> analyzed the powdered hydroxyapatite (brushite and monoteite) in the dentifrice by X-ray diffraction method and the peaks of hydroxyapatite confirmed the presence of brushite and monoteite crystals. They concluded that Hydroxyapatite crystals act as abrasives and may be effective in treating dentin hypersensitivity.

In Group 3 partial dentinal tubular occlusion was seen at 7 days. This can be supported by the fact that the small fraction of the fluoride initially applied to dentin is retained in the insoluble apatite form, thus making the lattice more stable and less soluble in acid.<sup>15</sup> The invitro study done by Miller et al<sup>16</sup> on root dentin treated with stannous fluoride concluded that partial or complete occlusion of dentin tubules is by a tin-rich surface deposit formed within one week use of an anhydrous 0.4% stannous fluoride gel.

The least percentage of dentinal tubule occlusion was observed in Group 4 at 7 days. This goes in hand with study done by Pashley et al<sup>17</sup> who suggested that despite the reduction in radius of the dentinal tubule lumen and complete occlusion of some tubules, control Group (distilled water) still had most of the tubule orifice open even after seven days. On the contrary the study done by Carlo prati et al<sup>18</sup> showed that the dentin permeability increased when the brushing was done in the presence of smear layer but the reduction in the permeability was smaller.

In this study when the percentage of dentinal tubules occlusion at 7 days of Group 1 (Bioactive Glass) was compared with that of the other three groups (Group 2, Group 3 and Group 4). Group 1 showed a highly significant relationship ( $P < 0.001$ ). The comparison of the percentage of dentinal tubules occlusion in Group 2 (Hydroxyapatite) with Group 3 (Stannous Fluoride) was statistically not significant ( $P = 0.655$ ). However there was a highly significant relationship when Group 2 when compared with Group 4 ( $P < 0.001$ ) and also when Group 3 was compared with Group 4 ( $P < 0.001$ ). This suggests that the maximum occlusion of dental

tubules at 7 days was observed in the specimens treated with bioactive glass followed by hydroxyapatite, stannous fluoride and distilled water. This can be explained by the study done by Sharma et al<sup>19</sup> who compared the efficiency of bioactive glass and stannous fluoride for treating hypersensitivity. They found that after one week bioactive glass group had a better response than stannous fluoride. Shreya Shetty, Ramesh kohad and Ramreddy Yeltiwar<sup>20</sup> compared the use of hydroxyapatite desensitizing toothpaste with distilled water. Hydroxyapatite treated teeth showed statistically significant reduction in hypersensitive symptoms ( $P<0.001$ ) when compared to treated with distilled water.

The mean  $\pm$  SD of percentage of dentinal tubule occlusion was assessed in Group 1, Group 2, Group 3 and Group 4 at 1 month interval. The mean  $\pm$  SD of percentage of dentinal tubule occlusion in Group 1 was  $95.181\pm 2.724$ , Group 2 was  $47.974\pm 6.748$ , Group 3 was  $39.155\pm 6.640$  and Group 4 was  $10.308\pm 1.879$ . In vitro study done by J.S Wefel<sup>21</sup> has demonstrated that a hydroxyl-carbonate apatite layer is formed on dentine blocks after brushing for one month with bioactive glass. This layer would be persistent for some period of time after applications of the bioactive glass containing dentifrice would be discontinued. The clinical study conducted at the using 7.5% bioactive glass dentifrice formulation showed a 55% reduction in sensitivity after 4-weeks.

The percentage of dentinal tubular occlusion in Hydroxyapatite Group was better than stannous fluoride Group. This can be suggested by the results of the SEM studies that showed hydroxyapatite occluding almost 50% of the dentinal tubules predominantly with apatite mineral not only on the dentin surface but also deep inside the dentinal tubules to a depth of 10 to 15  $\mu\text{m}$  from dentin surface with the formation of the smear layer.<sup>22,23</sup> Ellingsen and Rolla<sup>24</sup> examined stannous fluoride treated dentine surfaces by scanning electron microscopy and electron microprobe analysis after a period of one month. They observed a dense layer of tin and fluoride containing globular particles blocking about 40% of the dentinal tubules. Blong and associates<sup>25</sup> in their clinical study found that a 0.4% stannous fluoride gel was an effective agent in the control of pain associated with hypersensitive dentine. The use of the gel up to a minimum of 4 weeks was necessary to achieve satisfactory results. In this study the group where distilled water was used showed a marginal increase in the occlusion of dentinal tubules probably because of the formation of the smear layer.

The intercomparison of the occlusion of dentinal tubules at 1 month between all the groups showed highly significant relationship ( $P<0.001$ ). Marini I, Checchi I, Greenspan D<sup>26</sup> compared the use of sodium calcium phosphosilicate (Bioactive glass), stannous fluoride and potassium nitrate over a period of 4 weeks. The results of this study suggested that sodium calcium phosphosilicate was statistically better than stannous fluoride and potassium nitrate. Comparative study of hydroxyapatite and distilled water was done by Slosarczyk A et al<sup>27</sup> They observed in the SEM study that the mechanical incorporation of the hydroxyapatite grains in the abraded dentine obturated some dentinal canaliculi were as it was negligible in specimens obturated with distilled water.

The maximum occlusion of dentinal tubules was observed in Group 1 after 3 month. Almost all the dentinal tubules (98.3%)

were occluded when observed in SEM. Litkowski et al<sup>28</sup> in their clinical trial for eight weeks and showed efficacy of bioactive glass containing toothpaste in significantly reducing patients perceived pain to stimuli with daily use. The subjects were followed up with interviews up to twelve weeks after cessation. These data indicated a significant reduction in hypersensitivity. Du MQ et al<sup>29</sup> compared 5% Novamin containing toothpaste (bioactive glass) with strontium chloride containing toothpaste. Novamin containing toothpaste rapidly relieves tooth sensitivity. The dentinal tubule occlusion in Group 2 was 61.7%. Su Hwan K et al<sup>30</sup> evaluated the clinical efficiency of hydroxyapatite as a desensitizing agent as well as examined the SEM photographs of tooth specimens treated with hydroxyapatite over a period of twelve weeks. In the clinical trial most of the subjects obtained sustained relief from hypersensitivity. The SEM photographs of the teeth specimens showed effective tubule occlusion sufficiently augmenting the results of the clinical study. This it was assumed that hydroxyapatite penetrate the tubules to a sufficient depth to bring about desensitization. In Group 3 the percentage of dentinal tubule occlusion was 47.2%. Thrash et al<sup>31</sup> compared 0.4% stannous fluoride gel to aqueous 0.717% fluoride solution and a placebo at 2, 4, 8 and 16 weeks interval following a twice daily application. The results indicated subjects who applied 0.4% stannous fluoride reported significantly less sensitivity probably suggesting the stannous fluoride help in reducing thermal sensitivity. The Group 4 specimens where distilled water was used showed a marginal increase ( $16.51\pm 3.12$ ) in the occlusion of the dentinal tubules probably because of the formation of smear layer. As such till date no clinical study or SEM study has been done to compare the efficacy of bioactive glass and hydroxyapatite. A clinical study comparing bioactive glass with stannous fluoride for a period of twelve weeks was done by Sharma et al<sup>19</sup> At the twelve weeks' time point, maximum reduction of sensitivity was seen in subjects who were instructed to use bioactive glass containing dentifrice. However the study failed to described the duration of use of the desensitized toothpaste.

Most of the data in this study has been compared with clinical studies. There are a very few SEM studies done with a limited number of desensitising tooth pastes and short duration to study the effectiveness of these agents to occlude the dentinal tubules. However there is no such data ever reported to explain the invitro blockade of the dentinal tubules over a period of three months. This study is unique as it has attempted to study the effect of selected desensitizing agents namely Bioactive glass, Hydroxyapatite and Stannous fluoride using distilled water as control on dentinal tubular occlusion using scanning electron microscopy for a three month period which is the first of its kind.

It can be inferred from the interpretation of the results of this study that the use of dentifrice containing bioactive glass (Novamin) occluded almost all the dentinal tubules (98%). The physical occlusion of bioactive glass particles begins when material comes in contact with aqueous environment such as saliva. Sodium ions in the particles immediately begin to exchange with hydrogen cations. This rapid release of ions allows calcium ions in the particle structure, as well as phosphate ions to be released from bioactive glass. This initial series of reactions occurs within seconds of exposure, and the release of the calcium and phosphate ions continues as long as the particles are exposed to the aqueous environment. A localized transient increase in pH

occurs during the initial exposure of the material due to the release of sodium ions. This increase in pH helps to precipitate the calcium and phosphate ions from the Novamin particles, along with calcium and phosphorus found in saliva to form a layer of calcium phosphate. As the particle reactions continue and the deposition of calcium and phosphorus complexes continues to crystallize into hydroxycarbonate apatite which is chemically and structurally equivalent to biological apatite. This thus forms continuity with the existing tooth structure there by leading to an almost permanent occlusion of the dentinal tubules. This can be the probable reason for the almost complete tubular occlusion observed in this study.

When the hydroxyapatite containing dentifrice comes in contact with the aqueous environment like saliva forms an amorphous gelatinous material with submicron sized loosely joined particles. After a few minutes (20-30 minutes) the amorphous material crystallizes. The approximate size of the crystallites is about 5-10 µm which are ideal for their penetration into the dentinal tubules. However the amount of the crystals formed is less. Thus the numbers of tubules occluded are relatively less than that of novamin. In addition the depth of penetration is limited.<sup>22</sup>

Stannous fluoride has shown under SEM analysis that it causes partial or complete occlusion of the dentinal tubules which is more a precipitation reaction having weak physical forces. It forms tin rich surface globules on the orifice of the dentinal tubules. This suggests that stannous fluoride has to be continuous applied to be effective as a desensitising agent. Thus it may not be very effective in severe cases of hypersensitivity.<sup>16</sup>

The use of distilled water as a desensitizing agent is Non-significant. Only partial tubular occlusion was observed in this study as a result of fraction of obliteration of the dentinal tubules due to the formation of smear layer by mechanical brushing.<sup>17</sup>

## CONCLUSION

We concluded that Bioactive glass dentifrice showed the highest percentage of tubule occlusion, as it precipitated the hydroxycarbonate apatite over the entire dentin surface followed by Hydroxyapatite, Stannous fluoride and Distilled water where the tubule occlusion was mainly by the silica abrasive. Further studies with larger sample size and longitudinal studies with various age groups should be considered as the pain threshold varies amongst the different age groups to confirm the findings of this study.

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