

Comparative Evaluation of Desensitizing Dentifrices containing BioMin[®], Novamin[®] and Fluoride on Dentinal Tubule Occlusion before and after a Citric Acid Challenge – A scanning Electron Microscope *in-vitro* Study

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Abstract

Objective: The purpose of this study was to evaluate the *in vitro* effectiveness of two different bioglass-containing commercial desensitizing toothpastes together with a fluoride containing dentifrice as a control on dentinal tubule occlusion before and after a citric acid challenge and immersion in artificial saliva.

Methodology: Forty-five dentine specimens with patent tubules were randomly divided into 3 groups (n=15). Group A: brushing with Biomin (Elsenz[®]); Group B: brushing with Novamin (Sensodyne Repair[®]); and a control Group C: brushing with fluoride (Colgate Total[®]). In each group, treated specimens were further subdivided into Subgroup A: directly undergoing SEM, Subgroup B and C soaked in 0.3% citric acid and artificial saliva (Wet mouth[®]) for 5 minutes respectively. The percentage of tubule occlusion (%OCT) of the representative images from each group was analyzed using an environmental scanning electron microscopy and were scored by a blind review.

Conclusion: The %OCT with BioMin[®] containing dentifrice was significantly higher than NovaMin[®] and a control i.e., fluoride containing dentifrice. Biomin[®] and Novamin[®] containing dentifrices showed significant citric acid resistant compared to the fluoride containing dentifrice although the BioMin[®] containing dentifrice significantly showed better resistant to a citric acid challenge than the NovaMin[®] containing dentifrice. Immersion in artificial saliva resulted in an increase in tubular occlusion for all groups which was insignificant.

Keywords: Bioactive glasses (Biomin and Novamin); Citric acid; Artificial saliva; Environmental scanning electron microscope; Dentine hypersensitivity; Dentinal tubule

Introduction

Dentine hypersensitivity (DH) is characterized by short, sharp pain arising from exposed dentine in response to stimuli that are typically thermal, tactile, osmotic or chemical in nature [1]. Removal of the enamel covering the crown of the tooth or denudation of the root surface occurring due to loss of the cementum secondary to a disease process are the main etiologies causing exposure of dentine that culminate in dentine hypersensitivity [2].

The two main approaches of treating dentine hypersensitivity include the interference of nerve transmission and occlusion of the open dentinal tubules [3]. Numerous in-office and over-the-counter products, such as varnishes, liners, restorative materials, dentinal adhesives, dentifrices, mouthwashes etc. have been used to reduce dentine hypersensitivity. Even with the vast amount of published data it has not been possible to reach a consensus about the product that represents the gold standard for the treatment of dentine hypersensitivity. Dentifrices are the most widely used over-the-counter desensitizing agents [4] that involve the application of active agents such as potassium nitrate, oxalates, strontium-based compounds, citrate-based compounds, stannous fluoride etc. Most of these cause occlusions of the dentinal tubules which decrease both dentine permeability and fluid movement thereby reducing hypersensitivity [4].

The recent additions to the plethora of active ingredients in dentifrices for the treatment of dentine hypersensitivity include a bioactive glass formulation (NovaMin[®]) [5]. NovaMin[®], is a bioactive glass which when exposed to an aqueous media provides calcium and phosphate ions forming a hydroxy-carbonate apatite like layer that is chemically similar to that present in enamel and dentine. More recently a patented bioactive glass toothpaste (BioMin[®]) which is also marketed

in India (Elsenz, Group Pharmaceuticals Ltd., India) containing fluoride with high phosphate content and small particle size has been developed.

Although saliva plays a pivotal role in naturally reducing dentine hypersensitivity by transporting and plugging calcium and phosphate into the dentinal tubules, this natural process may be insufficient to induce rapid occlusion and reduce dentine hypersensitivity in most individuals. Although it has been extensively demonstrated that some dentifrices are able to reduce dentine permeability *in vitro* there is little information regarding their effects under a simulated oral environment, subsequent to an acid challenge and artificial saliva immersion. There are limited published data on comparing the effectiveness of these two Bioglass products or on their ability to withstand an acid challenge which could in turn reverse the tubular ability of the Bioglass particles as well any reduction in permeability (e.g. fluid flow within the dentinal tubules). As exposure to acid and saliva could reverse the reduction in permeability caused by the desensitizers and dentine bonding agents, an ideal dentifrice needs not only to reduce the dentine permeability, but also maintain the occlusion effects in the face of an acid challenges and artificial saliva immersion [6].

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Aim

The aim of this *in vitro* study was to comparatively evaluate the effects of two desensitizing dentifrices containing BioMin®, NovaMin® and a Fluoride containing dentifrice as a control on dentinal tubule occlusion before and after a citric acid challenge and immersion in artificial saliva using an environmental scanning electron microscopy (ESEM).

Materials and Methods

This *in vitro* study was performed on forty-five dentine discs specimens which were obtained from permanent third molar human teeth at MGM Dental College and Hospital, Navi Mumbai, India. The study purpose was explained, and informed consent was obtained from subjects whose extracted teeth were used for the study. The effects of two commercially available dentifrices containing BioMin (Elsenz® Group Pharmaceuticals Ltd., India) and NovaMin (SENSODYNE Repair® Group Pharmaceuticals Ltd., India) together with a control dentifrice containing fluoride (COLGATE Total® Colgate-Palmolive Pvt. Ltd., India) on dentinal tubule occlusion were assessed by an environmental scanning electron microscopy (ESEM) analysis with and without a citric acid challenge and artificial saliva immersion.

Preparation of dentine specimens

Extracted permanent third molar human teeth which underwent surgical extractions were stored in normal saline. Teeth free from periodontal disease, caries lesion, teeth with restorations and endodontic treatment, crown fracture, attrition, abrasion, erosion, external resorption, and developmental anomalies were selected. The collected teeth were debrided thoroughly to remove any remaining debris, and periodontal remnants using an ultrasonic scaler (Satalec®, Acteon Group, Gustave, France) and the root surfaces were planed using curettes (Gracey curettes 1–2, Hu-Friedy, Chicago, IL, USA) to remove the tissue remnants. After debridement, the teeth were stored in 0.5% thymol for a period not more than 1 month before using them for the preparation of dentine discs.

Twelve dentine discs with a thickness of approximately 0.6 mm-1

mm were obtained by placing cuts perpendicular to the long axis of the tooth from the region between the apical limit of the dentino-enamel junction and the coronal limit of the pulp chamber based on Pashley’s dentin- disc model using a diamond disc (22 mm diameter) (SS White, Lakewood, NJ, USA). On average two to three usable specimens were obtained from each tooth. The dentine discs that were not cut properly or were not of uniform size were discarded. The surface of each dentine disc was polished with a 600-grit silicon carbide paper for 30 s using a back and forth motion. The smear layer was removed by immersion all the dentine discs in 6% citric acid for two minutes. The discs were removed from the citric acid solution and were immersed in de-ionized water for 30 seconds. Dentine discs were then fractured longitudinally using dental pliers in four quadrants to obtain 48 discs specimens. 45 discs specimens were then mounted on the paraffin wax blocks to receive one of the three desensitizing agents.

The forty-five specimen discs were randomly divided using computer generated randomization using SPSS software into three groups: Groups I, II and III each group comprising of fifteen specimens as follows (Figure 1):

Group I (n=15): Specimens were treated with a BioMin® containing dentifrice

Group II (n=15): Specimens were treated with a NovaMin® containing dentifrice

Group III (n=15): Specimens were treated with a fluoride containing dentifrice

Treatment regimen

All discs specimens of Groups I, Group II and Group III were gently rinsed using a drop of sterile saline following which a dab-on application of BioMin®, NovaMin® or fluoride containing dentifrice dispensed on brushed with a dab-on application of the undiluted respective dentifrice with circular motion using a powered toothbrush (Oral-B Pro-Health Precision Clean Electric Toothbrush) of circular head for two minutes.

Following application, the specimens in each group were further

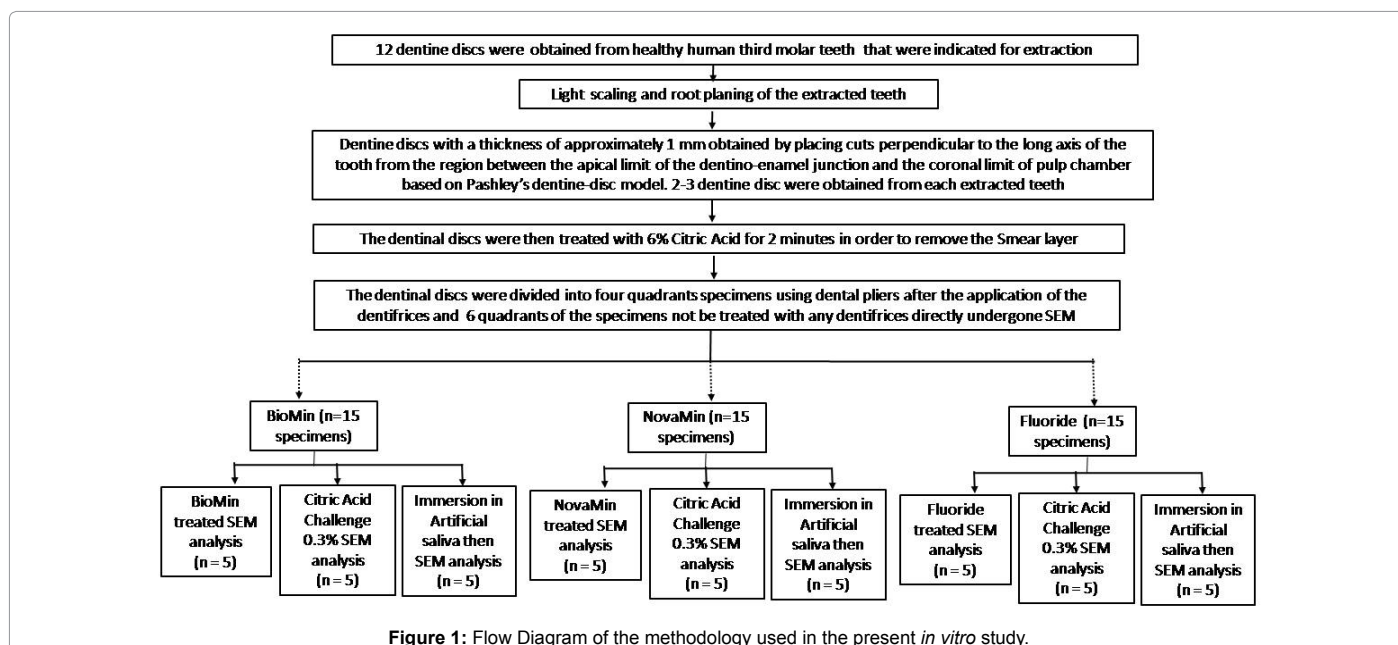


Figure 1: Flow Diagram of the methodology used in the present *in vitro* study.

divided into three subgroups A, B and C consisting of five specimens each. Specimens from all subgroups were immersed in a plastic jar filled with distilled water and stirred for one min with a plastic stirrer to ensure removal of any excess desensitizing agent. The discs in each group were treated in the following manner (Figure 1):

Subgroup IA: Specimens treated with a BioMin® containing dentifrice

Subgroup II A: Specimens treated with a NovaMin® containing dentifrice

Subgroup III A: Specimens treated with a fluoride containing dentifrice

Subgroup I B: Specimens treated with a BioMin® containing dentifrice followed by citric acid challenge

Subgroup II B: Specimens treated with a NovaMin® containing dentifrice followed by citric acid challenge

Subgroup III B: Specimens treated with a fluoride containing dentifrice followed by citric acid challenge

Subgroup I C: Specimens treated with a BioMin® containing dentifrice followed by artificial saliva immersion

Subgroup II C: Specimens treated with a NovaMin® containing dentifrice followed by artificial saliva immersion

Subgroup III C: Specimens treated with a fluoride containing dentifrice followed by artificial saliva immersion

Citric acid challenge

Specimens from subgroup IB, IIB and IIIB were then immersed in a Petri dish containing 0.3% citric acid with sodium hydroxide buffer (NaOH) with pH of 3.2 for five minutes. Following a citric acid challenge, they were immersed in a jar of de-ionized water and stirred for one minute to ensure removal of any excess citric acid and were lightly dried using an air blast.

Artificial saliva treatment

Specimens from subgroup IC, IIC and IIIC were then immersed in a petri dish containing artificial saliva (Wet Mouth®) (simulating the oral environment) for five minutes, they were immersed in a jar of de-ionized water and stirred for one min to ensure removal of any excess of saliva and were lightly dried using an air blast.

Preparation of the specimens for ESEM analysis

Dentine discs specimens were dried in the desiccator (under an infrared lamp for additional 15 minutes) and then later mounted on to aluminum stubs using a conductive carbon tape. These were then sputter coated with platinum for further ESEM analysis. Micrographs were taken at various magnifications (2500x and 5000x).

Assessment of tubular occlusion

The extent of tubule occlusion was assessed using an environmental scanning electron microscope (Regional Sophisticated Instrumentation Centre (RSIC), IIT Bombay). Specimens were sputter coated with platinum (Pt) to aid conductivity and examined at an operating voltage of 15 kV in the secondary electron mode. The images were taken at various magnifications (2500x and 5000x) and then assessed for the level of tubule occlusion (on a scale of 1-5) for 2500x magnification by three independent blind reviewers, in accordance with the ranking

system established below that visualizing the extent of occlusion (visual score) [7,8].

- 1: Occluded (100% of tubules occluded).
- 2: Mostly occluded (75% of tubules occluded)
- 3: Equally occluded/unoccluded (50% of tubules occluded)
- 4: Mostly unoccluded (25% of tubules occluded)
- 5: Unoccluded (0%: no tubule occlusion).

The percentage of tubule occlusion (% OCT) was evaluated using the formula [9] for 5000x magnification:

$$\% \text{ OCT} = \text{Number of occluded tubules} \times 100$$

$$\% \text{ OCT} = (\text{Number of occluded tubules} \times 100) \div \text{Total number of tubules}$$

Statistical Analysis

The statistical analysis was performed using 'Medcalc 64 bit' Version 17.6 (Medcalc software bvba, Belgium). Data for the percentage of occluded tubules was expressed as descriptive (mean, \pm standard deviation). The distributions of %OCT were checked for normality using Shapiro-Wilk test and the scores of the three reviewers blinded to the dentifrices were averaged. Since the data was non-parametric, the three groups were compared for differences in the %OCT using a Kruskal-Wallis test. The three groups were compared for differences in the % OCT using One-Way ANOVA. Post-hoc pair-wise comparisons were made using Wilcoxon test. The analysis was completed using two-sided tests at alpha 0.05 (95% C.L.).

Results

Descriptive statistics showing the mean values, standard deviation

Subgroup	Mean \pm SD	p value
I A	88.09 \pm 4.38	p<0.05 $\alpha\mu$
II A	78.66 \pm 4.70	p<0.05 $\#\mu$
III A	35.36 \pm 6.58	p<0.05 $\#\alpha$
I B	56.76 \pm 6.16	p<0.05 $\alpha\mu$
II B	45.80 \pm 8.16	p<0.05 $\#\mu$
III B	6.64 \pm 2.70	p<0.05 $\#\alpha$
I C	88.74 \pm 4.31	p<0.05 μ
II C	81.02 \pm 5.07	p<0.05 μ
III C	32.70 \pm 4.74	p<0.05 $\#\alpha$

Table 1: Descriptive statistics showing the mean values, standard deviation of percentage of occluded tubules (%) for subgroups IA, IIA, IIIA, IB, IIB, IIIB, IC, IIC and IIIC results of one-way ANOVA for subgroup IA, IIA and IIIA; IB, IIB and IIIB; IC, IIC and IIIC (#: Group I ; α : Group II; μ : Group III (p<0.05)).

Subgroup	Mean \pm SD	p value
I A	88.09 \pm 4.38	p<0.05 α
I B	56.76 \pm 6.16	p<0.05 $\#\mu$
I C	88.74 \pm 4.31	p<0.05 α
II A	78.66 \pm 4.70	p<0.05 α
II B	45.80 \pm 8.16	p<0.05 $\#\mu$
II C	81.02 \pm 5.07	p<0.05 α
III A	35.36 \pm 6.58	p<0.05 α^*
III B	6.64 \pm 2.70	p<0.05 $\#\mu$
III C	32.70 \pm 4.74	p<0.05 α

Table 2: Descriptive statistics showing the mean values, standard deviation of percentage of occluded tubules (%) for subgroups IA, IIA, IIIA, IB, IIB, IIIB, IC, IIC and IIIC and results of one-way ANOVA for subgroup IA , IB and IC; IIA , IIB and IIC; IIIA, IIIB and IIIC (#: subgroup A ; α : subgroup B; μ : subgroup III (p<0.05)).

of percentage of occluded tubules (%) for subgroups IA, IIA and IIIA; IB, IIB and IIIB; and IC, IIC and IIIC results of one-way ANOVA for subgroups (Tables 1 and 2).

Table 1 showing that the mean value of the percentage of occluded tubules for IA ($88.09\% \pm 4.38\%$) was higher as compared to IIA ($78.66\% \pm 4.70\%$) and IIIA ($35.36\% \pm 6.58\%$) which was statistically significant ($p < 0.05$, (Table 1) and (Figure 2), also the mean value of the percentage of occluded tubules for IB ($56.76\% \pm 6.16\%$) was higher as compared to IIB ($45.80\% \pm 8.16\%$) and IIIB ($6.64\% \pm 2.70\%$) with statistical significance ($p < 0.05$) (Table 1) and (Figure 2).

Table 2 shows the mean value between the subgroup A, B and C of each group. The mean value of IC ($88.74\% \pm 4.31\%$) was higher than the mean values of IA ($88.09\% \pm 4.38\%$) but did not show any significance ($p > 0.05$) (Table 2 and Figure 2), however the mean values of IA ($88.09\% \pm 4.38\%$) and IC ($88.74\% \pm 4.31\%$) was statistical significantly higher to

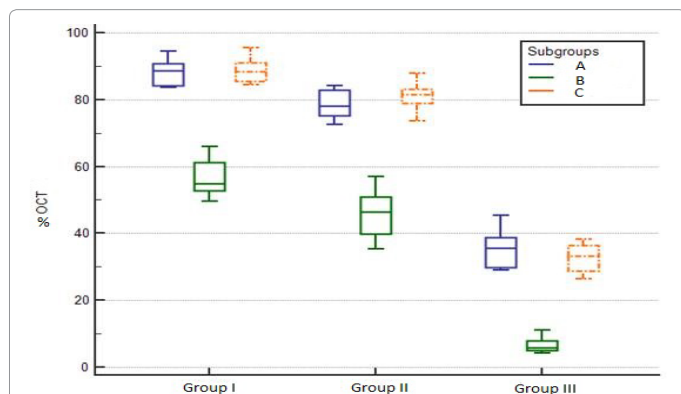


Figure 2: Multiple comparison of percentage of occluded tubules between group I, II and III.

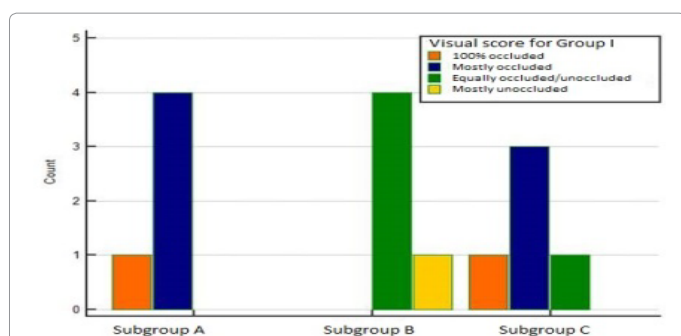


Figure 3: Comparison of grades between subgroups IA, IB and IC.

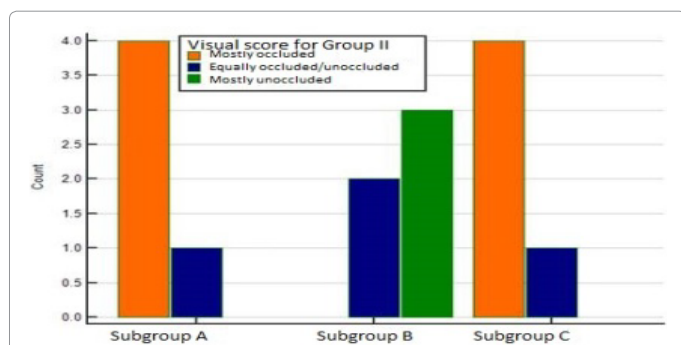


Figure 4: Comparison of grades between subgroups IIA, IIB and IIC.

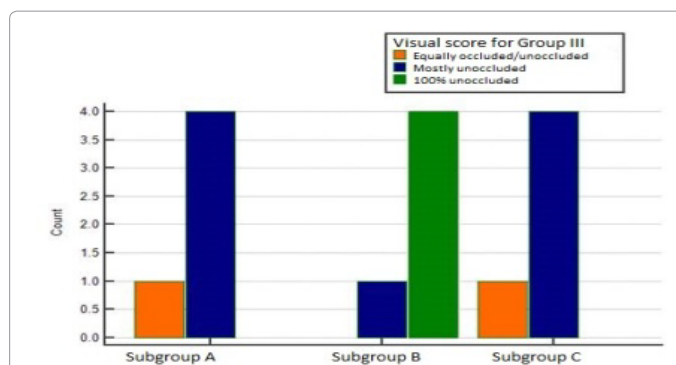
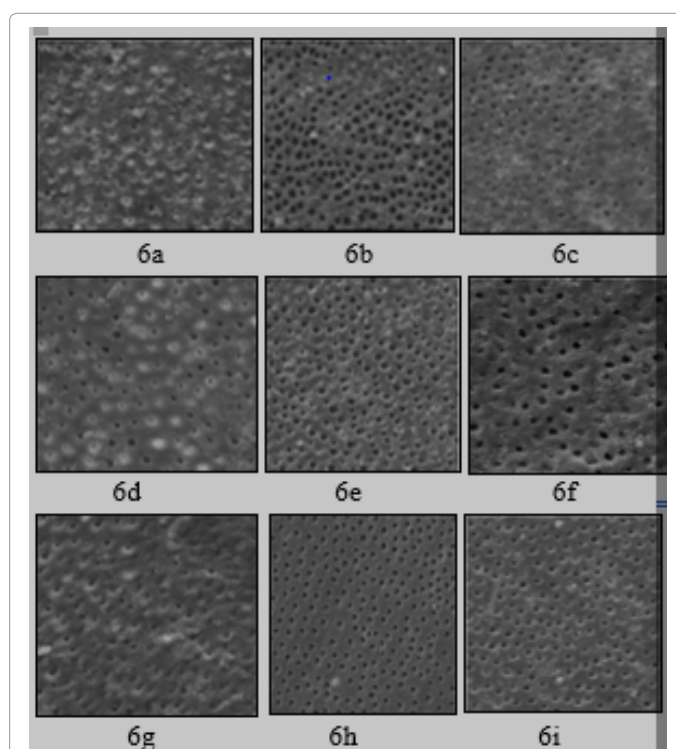


Figure 5: Comparison of grades between subgroups IIIA, IIIB and IIIC.



6a: subgroup IA, 6b: Subgroup IB, 6c: Subgroup IC, 6a: subgroup IIA, 6b: Subgroup IIB, 6c: Subgroup IIC 6a: subgroup IIIA, 6b: Subgroup IIIB, 6c: Subgroup IIIC.

Figure 6: (a-i) ESEM images at 2500x (visual score according to visual examination).

that of IB ($56.76\% \pm 6.16\%$) ($p < 0.05$) (Table 2 and Figure 2). Similarly, the mean value of IIC ($81.02\% \pm 5.07\%$) was higher than mean values of IIA ($78.66 \pm 4.70\%$) but did not show any significance ($p > 0.05$) (Table 2 and Figure 2), however the mean values of IIA ($78.66\% \pm 4.70\%$) and IIC ($81.02\% \pm 5.07\%$) was statistical significantly higher to that of IIB ($45.80\% \pm 8.16\%$) ($p < 0.05$) (Table 2 and Figure 2). Whereas the mean value of IIIC ($35.70\% \pm 4.74\%$) and IIIA ($35.36\% \pm 6.58\%$) were higher to that of IIIB ($6.64\% \pm 2.70\%$) which was statistically significant ($p < 0.05$) (Table 2 and Figure 2).

For subgroup IA 80% belonged to rank 2; followed by 20% showing rank 1 (Figure 3) and for subgroup IIA 80% belonged to rank 2 and remaining 20% showed occlusion as rank 3 (Figure 4) whereas subgroup IIIA 80% belonged to rank 4 and 20% belonged to rank 3 (Figure 5). For the subgroup IB, 80% samples belonged to rank 3

whereas 20% belonged to rank 4 (Figure 3) whereas for subgroup IIB 60% belonged to rank 3 and remaining 40% showed occlusion as rank 4 (Figure 4) whereas subgroup IIIB 80% belonged to rank 5 and 20% belonged to rank 4 (Figure 5). For subgroup IC, 60% samples belonged to rank 2 where as 10% of each belonged to rank 1 and 3 (Figure 3) whereas for subgroup IIC 80% belonged to rank 2 and remaining 20% showed occlusion as rank 3 (Figure 4) whereas subgroup IIIC 80% belonged to rank 4 and 20% belonged to rank 3 (Figure 5) (Figures 6a-6i) (Figures 7a-7i).

Discussion

The aim of the present study was to compare the desensitizing effects of Biomin® and Novamin® containing dentifrices, following a citric acid challenge as there currently does not appear to be any published studies comparing these two desensitizing dentifrices. This present *in-vitro* study demonstrated that the Biomin® and Novamin® containing dentifrices together with the fluoride control can provide considerable tubular occlusion of the patent dentinal tubules after two minutes of application. Upon comparison, the Biomin® containing dentifrice, showed better percentage of dentinal tubule occlusion as compared to a Novamin® containing dentifrice and the fluoride control. Several *in vitro* and *in vivo* investigations have shown the efficacy of different toothpastes in occluding dentinal tubules [10-17]. The amorphous sodium calcium phospho-silicate present in the Novamin® containing toothpaste showed a strong attraction for collagen [17]. Due to the high collagen content of dentine, Novamin® binds to the exposed dentine surfaces and physically occlude the dentine tubules [10].

Burwell et al. [18] conducted a series of studies showing that a single application of NovaMin® with a concentration of above 3%, either in a daily-use dentifrice or a professionally applied prophylaxis paste, was

effective at blocking at least 75%-95% of the open tubules. Furthermore, these studies also demonstrated that a single application of NovaMin® in these models resisted a repeated acid challenge. Biomin® containing dentifrice ($56.76 \pm 6.16\%$) (Figure 7b) showed a greater percentage of occluded tubules compared to the Novamin® containing dentifrice ($45.80 \pm 8.16\%$) (Figure 7e), which was statistically significant.

Saliva naturally occludes the patent dentinal tubules by transporting calcium and phosphate ions into the tubules to induce tubule plugging and by forming a surface protective layer of a salivary glycoprotein with calcium and phosphate [19]. However, this process of natural tubule occlusion is very slow and the tubule plugging may be easily removed by both dietary acid and physical insult (e.g., tooth brushing), thus rendering it neither effective nor reliable in providing lasting relief of dentine hypersensitivity. In the present *in vitro* study, there was a small increase in the percentage of dentinal tubule occlusion in all three dentifrice groups which were not statistically significant.

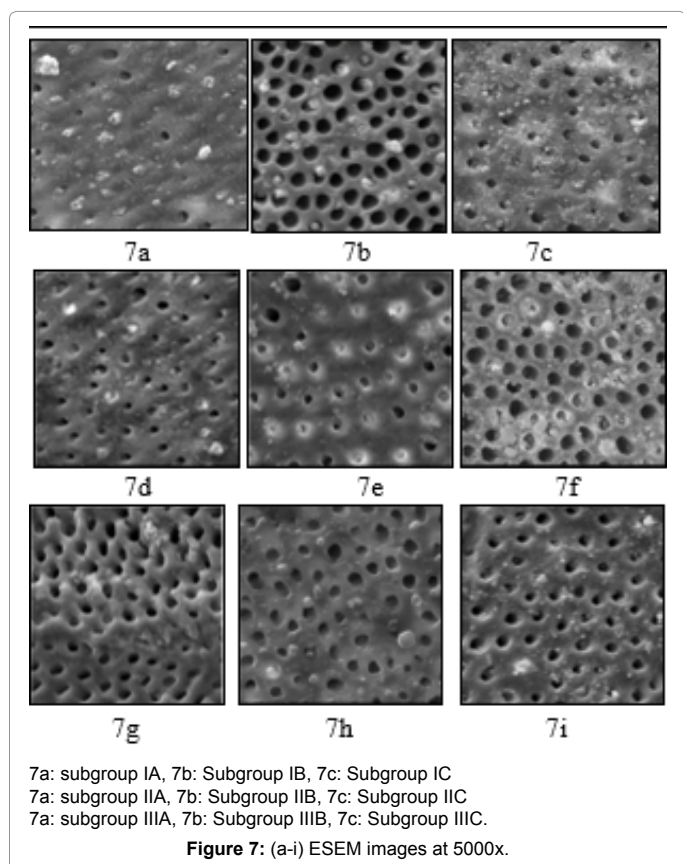
However, this study should be interpreted with caution before extrapolating these results into dental practice, considering the nature and limitations of the *in vitro* experimentation. The desensitizing effect of these agents on vital teeth can be determined only in a clinical situation. The manual counting of the dentinal tubules for calculating the percentage of tubule occlusion is subjective to human error. *In vitro* dentine permeability tests in human dentine sections are also required to supplement the results from the tubular occlusion aspect of this type of study. Furthermore, in order to demonstrate a desensitizing effect of both Biomin® and Novamin® it is essential to conduct a well-controlled randomized Clinical Trial to directly compare their effectiveness in reducing Dentine Hypersensitivity over a minimum six weeks

Conclusion

The results from the present study would suggested that the percentage of dentinal tubule occlusion following an application of a Biomin® containing dentifrice onto a dentine surface was statistical significant from the Novamin® and fluoride containing dentifrices. Biomin® and Novamin® containing dentifrices demonstrated resistance to a citric acid challenge and both dentifrices were statistically different to a fluoride containing dentifrice, the BioMin® containing dentifrice significantly showed better resistant to a citric acid challenge compared to the NovaMin® containing dentifrice. Both Biomin® and Novamin® dentifrices demonstrated an increase in the percentage of dentinal tubule occlusion although there were no statistical differences between the two groups. Both these dentifrices were statistical different to a fluoride dentifrice. Immersion in artificial saliva however, resulted in an increase in tubular occlusion for all groups although there were no statistical differences between the groups.

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