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International Journal of Current Research
Vol. 9, Issue, 06, pp.53115-53122, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF DESENSITIZING TOOTH PASTES CONTAINING 5% CALCIUM SODIUM PHOSPHOSILICATE AND 8% ARGININE CALCIUM CARBONATE ON DENTINAL TUBULE OCCLUSION AND DENTINE PERMEABILITY – AN IN VITRO STUDY

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ARTICLE INFO

Article History:
Received 21st March, 2017
Received in revised form
16th April, 2017
Accepted 27th May, 2017
Published online 30th June, 2017

Key words:

Dentin Hypersensitivity,
Calcium Sodium Phosphosilicate,
Arginine Calcium Carbonate,
Scanning Electron Microscope,
Stereomicroscope.

ABSTRACT

Introduction: First line therapy of dentin hypersensitivity typically involves the use of desensitizing toothpastes. Two such agents in the market are 5% Calcium Sodium Phosphosilicate (CSPS) and 8% Arginine Calcium Carbonate (ACC), which have unique mechanisms of blocking the dentinal tubules in a natural bio-mimetic way. So the purpose of this in vitro study was to evaluate and compare the efficacy of dentinal tubule occlusion using Scanning Electron Microscope (SEM) and dentinal permeability by using Stereomicroscope (SM) after the application of these agents.

Methods: Total 60 samples were included in this study. 30 samples were used for SEM analysis and 30 others for SM analysis. Each of these 30 samples were again divided into three groups as: distilled water-Group-I, 5% CSPS-Group-II and 8% ACC-Group-III. The dentine surface morphology was evaluated using SEM after brushing and after acid treatment. Dentine permeability analysis using Evans blue dye was evaluated by using SM. Statistical analysis was done using one-way ANOVA and Chi-square test. Tukey post hoc test was used for multiple comparisons.

Results: CSPS and ACC test groups showed statistically significant difference in dentinal tubule occlusion and in permeability reduction than the respective DW control ($p<0.001$). Comparing the mean permeability differences between different groups, after the treatment, CSPS group showed a higher permeability reduction and number of occluded tubules.

Conclusion: 5% CSPS shows promise as a desensitizing agent, which not only occludes the dentinal tubules, but also reduces the tubule permeability and shows very good resistance to acid treatment than 8% ACC.

Citation: Abdul Hakeem and Sunil Sathyanarayana, 2017. "Comparative evaluation of desensitizing tooth pastes containing 5% calcium sodium phosphosilicate and 8% arginine calcium carbonate on dentinal tubule occlusion and dentine permeability – an in vitro study", *International Journal of Current Research*, 9, (06), 53115-53122.

INTRODUCTION

Dentine hypersensitivity is one of the commonest dental problems affecting approximately 8-57% of adult population and is associated with pain due to exposure of dentin to oral environment. (Pinto *et al*., 2010) It has a multi-factorial etiology and involves the interaction of erosion, abrasion, saliva, bio-film/pellicle and all factors exposing dentinal tubules (Markowitz and Pashley, 2007). Based on hydrodynamic theory, the occlusion of dentinal tubules is the main method to treat dentin hypersensitivity by decreasing fluid shift inside the dentinal tubules and thereby relieving symptoms of sensitivity (Wang *et al*., 2011). Even though numerous approaches have been attempted to relieve the pain caused by dentinal hypersensitivity, the longevity and the efficacy of the desensitizing effect (Schuurs *et al*., 1995) were questionable (Morris *et al*., 1999). In order to fulfill these deficiencies, new products with novel technologies are being developed constantly. Two such agents in the market are Calcium Sodium Phosphosilicate (5%) and Arginine Calcium Carbonate (8%), which have unique mechanisms of blocking the dentinal tubules in a natural bio-mimetic way. Calcium Sodium Phosphosilicate (CSPS) acts as a bio-mimetic mineralizer, matching the body's own mineralizing traits. When CSPS materials are exposed to an aqueous environment such as water or saliva, there is an immediate release of sodium ions from the CSPS particles resulting in a localized, transient increase in pH. This helps to precipitate the calcium and phosphate ions from the CSPS particle, along with the calcium and phosphorus found in saliva to form a calcium phosphate (Ca-P) layer. As the particle reactions continue and the deposition of Calcium and Phosphorus complexes continue, this layer crystallizes into hydroxycarbonate apatite, which is chemically and structurally equivalent to biological apatite.

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This unique attribute of the composition of CSPS sets it apart from all other materials that have been shown to act as physical occluding materials (Greenspan, 2010). Arginine Calcium Carbonate (ACC) contains Arginine and Calcium Carbonate. Arginine is a positively charged amino acid which can be found naturally in saliva. It is believed that the reaction between arginine and calcium carbonate imitates the natural process of desensitization by saliva. Arginine reacts with Calcium Carbonate to form a paste-like plug at physiological pH (pH 6.5–7.5). This physically plugs the open dentinal tubules (sealing the exposed dentin), adheres to the walls of the tubules and reacts with the calcium and phosphate ions of the tubular fluid, making its adherence on the tubular wall more secure (Kleinberg I. SensiStat, 2002). So the purpose of this study was to evaluate and compare the efficacy of dentinal tubule occlusion by using Scanning Electron Microscope (SEM) and dentinal permeability by using Stereomicroscope (SM) after the application of 5% CSPS and 8% ACC.

MATERIALS AND METHODS

Sample preparation for SEM and SM analysis

30 human premolar teeth extracted for orthodontic reasons were collected from the Department of Oral surgery, Dayananda Sagar College of Dental Sciences, Bangalore. Teeth were cleaned thoroughly in running water and stored in 10% formalin until required. Teeth were then sectioned (transverse sections) with a diamond disc to create 1mm dentin discs. Each disc was conditioned with 6% citric acid for 2 minutes and rinsed with distilled water in order to expose the dentinal tubules and simulate hypersensitive dentin according to the experimental approach proposed by Pashley *et al* (1981). The specimens were stored in artificial saliva during the experimental period. Specimens were randomly divided using coin toss method into 3 groups of 10 specimens each. Group-I consisted of specimens brushed for 2 minutes twice daily for seven days with distilled water (DW). Group-II consisted of specimens brushed for 2 minutes twice daily for seven days with 5% CSPS dentifrice. Group-III consisted of specimens brushed for 2 minutes daily for seven days with 8% ACC.

Dentine tubule occlusion using SEM

After brushing all specimens were dehydrated, dried and sputter-coated with gold palladium alloy. Then, the specimens were examined under a SEM (QUANTA 2000) at 20 kV acceleration voltages. Standardized images of the dentine discs were acquired at a magnification of 8000X. Three images were acquired per disc.

Evaluation of SEM images

Analysis of the specimens was done and percentage of occluded tubules were obtained by dividing the total number of occluded tubules from the total number of tubules in each photomicrograph. This result was multiplied by 100, to obtain the percentage of occluded tubules for each photomicrograph. Each SEM photomicrograph was assesed for the percentage of completely occluded tubules, partially occluded tubules and un-occluded tubules.

Dentin permeability analysis by dye penetration test using SM

Thirty human premolars (Ten teeth per group) were used for this analysis. Cavities of 0.3mm depth and 0.8mm width were prepared on the buccal surfaces (2 mm coronal to the CEJ) of the premolar using a standard #245 carbide bur (S.S. White) with active tip 0.3 mm. Prior to the treatment, the dentin of all the cavities were treated with 6% citric acid to remove the smear layer and to open the dentinal tubules. The teeth were brushed with an electric toothbrush (JSW H 27 tooth brush) using the experimental agents (tests: 1g of the toothpaste with 1ml of distilled water; control: 2ml of distilled water). This procedure was performed once daily for 4 days. After each daily treatment, 5µL of 5% Evans blue dye solution (LOBA CHEMIE) was applied with a dropping pipette onto each dentin cavity and was left to penetrate for 5 mins. Samples were sectioned longitudinally (bucco-lingual sections). Stereomicroscopic analysis was done under obliquely reflected light using Stereomicroscope (Carl Zeiss, Discovery 20). Stereomicroscopic photomicrographs were captured and images were transferred to analyze using Lext image software. Each image was calibrated individually using a standard scale (µm). The depth of penetration and area of penetration were calculated. Two measurements (upper and lower region in the analyzed area) were taken for each image, indicating the depth of the dye infiltration (length of dye penetration), and a mean was calculated for each specimen. The area of dye penetration was calculated by using the software. The same examiner performed all of the measurements.

Statistical analysis

Data was entered in Microsoft excel and analyzed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package. Statistical analysis was done using one-way Analysis of variance (ANOVA) and Chi-square ($\chi 2$) test. Tukey post hoc test was used for multiple comparisons.

RESULTS

Permeability analysis

Dye penetration observed in all groups after the application of the desensitizing agents is shown in table-1 (depth of dye penetration) and table-2 (area of dye penetration). While comparing the dentin permeability between the groups, the mean depth of penetration of CSPS group (Group-II) was found to be 1.319±0.156mm, while that of DW group (Group-I) was 3.205±0.228mm. The mean value observed for ACC group (Group-III) was 2.135±0.157 mm. The difference in depth of dye penetration between the group was statistically significant ($P<0.001$) (Table-1, Graph-1). Based on the result, it was found that CSPS group showed the least depth of penetration whereas the highest depth of penetration was observed for DW group. While comparing the dentin permeability between the groups, the mean area of penetration of CSPS group (Group-II) was found to be 1.621± 0.181mm^2, while that of DW group (Group-I) was 3.75±0.236mm^2. The mean value observed for ACC (Group-III) was 2.361± 0.251mm^2. The difference in depth of dye penetration between groups was statistically significant ($P<0.001$) (Table-2, Graph-2). From the result it was found that CSPS group showed the least area of penetration whereas the highest area of penetration was observed for DW group. CSPS and ACC test groups showed statistically significant permeability reduction than the respective DW control ($p<0.001$). Comparing the mean permeability differences between different groups, after the treatment, CSPS group showed a better permeability reduction.

The Stereomicroscopic images of samples treated with different test agents are shown in Figures 1a-3b.

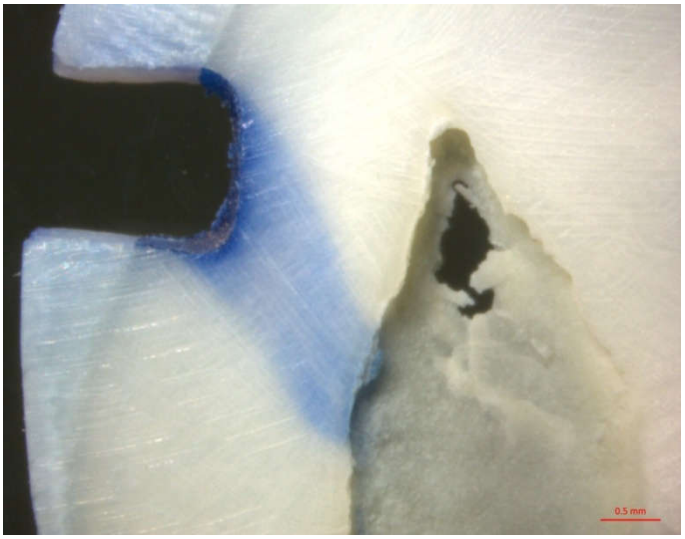


Fig. 1a. SM photograph of DW treated sample showing dye penetration up to the pulp chamber

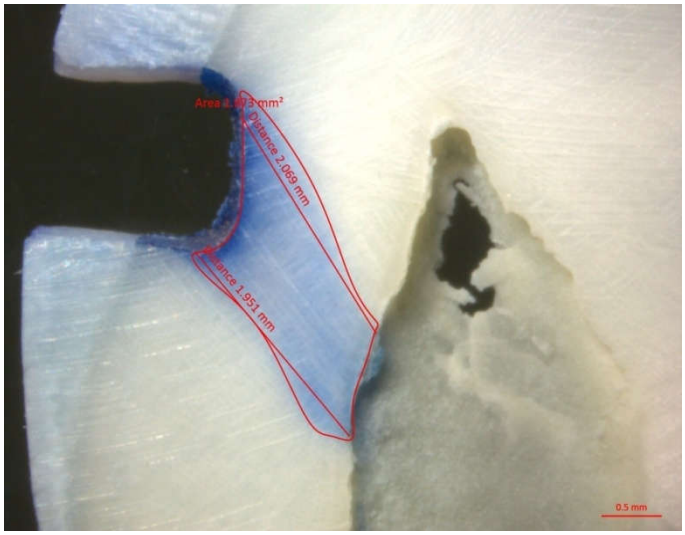


Fig. 1b. Measurement of depth and area of dye penetration by using LEXT software

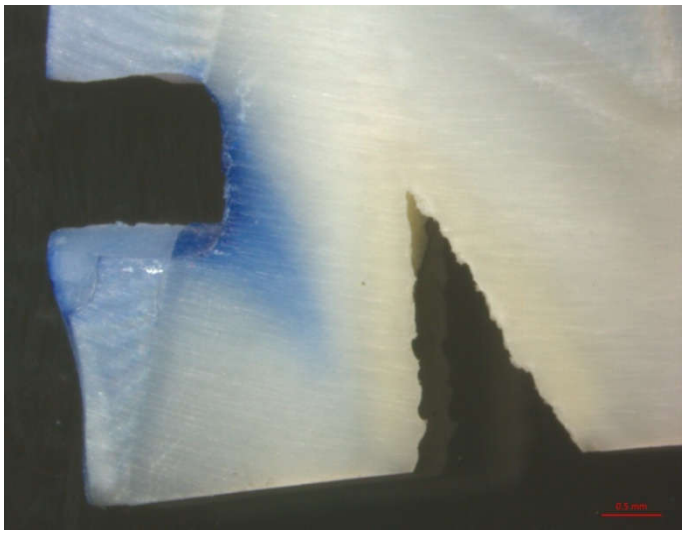


Fig. 2a. SM photograph of CSPS treated sample showing dye penetration into the dentin

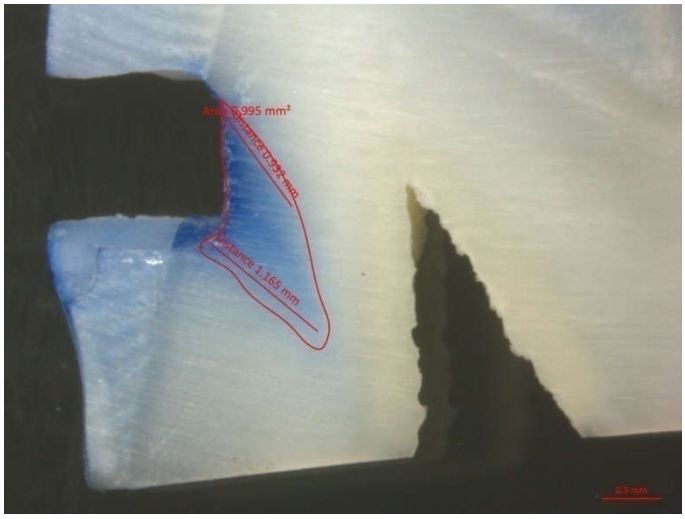


Fig. 2b. Measurement of depth and area of dye penetration by using LEXT software

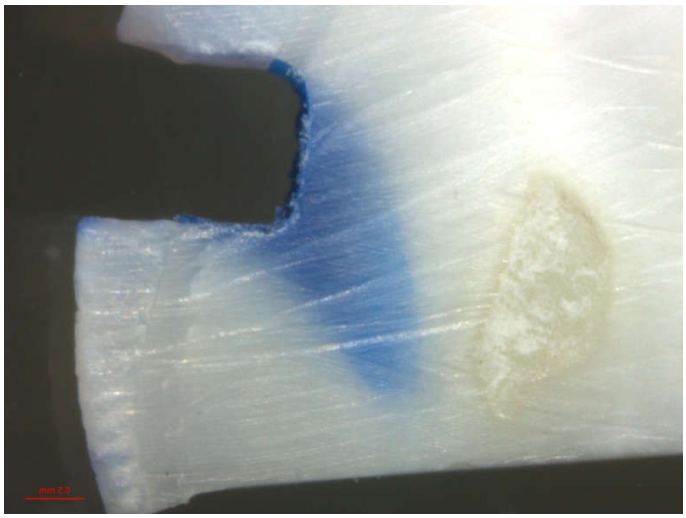


Fig. 3a. SM photograph of ACC treated sample showing dye penetration near to the pulp chamber

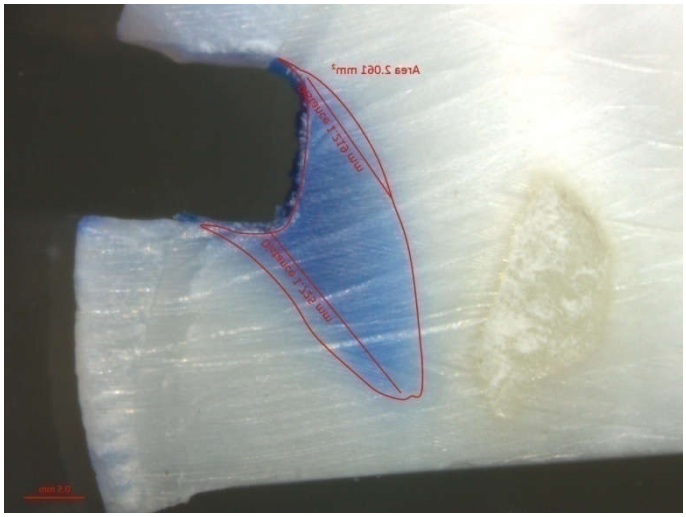


Fig. 3b. Measurement of depth and area of dye penetration by using LEXT software

SEM analysis

The SEM examination showed variable degrees of dentinal tubule coverage by the agents used.

Table 1. Comparison of mean distance of dye penetration between the groups

Groups	N	Mean	SD	'F' value	'p' value	Between the Group Comparison					
						1 vs 2		1 vs 3		2 vs 3	
						Diff.	P value	Diff.	p value	Diff.	P value
Group 1	10	3.205	0.228	266.478	*<0.001*	1.886	*<0.001*	1.070	*<0.001*	-0.816	*<0.001*
Group 2	10	1.319	0.156								
Group 3	10	2.135	0.157								

Table 2. Comparison of mean values of area of dye penetration between the study groups

Groups	N	Mean	SD	'F' value	'p' value	Between the Group Comparison					
						1 vs 2		1 vs 3		2 vs 3	
						Diff.	P value	Diff.	p value	Diff.	P value
Group 1	10	3.750	0.236	232.224	*<0.001*	2.130	*<0.001*	1.389	*<0.001*	-0.740	*<0.001*
Group 2	10	1.621	0.181								
Group 3	10	2.361	0.251								

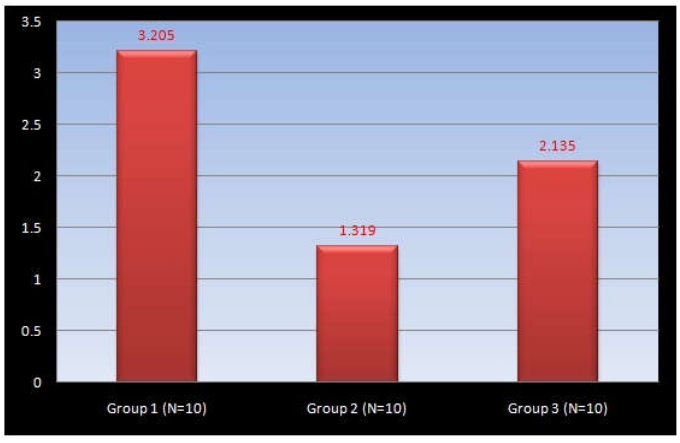
Table 3. Comparison of percentage of tubule occlusion between the groups after the application of test agents and after the acid challenge

	Groups	Type			Total	χ^2 value	'p' value
		Completely Occluded Tubule	Partially Occluded Tubule	Open Tubule			
Before	Group I	11	77	255	343	636.099	**<0.001**
		3.2%	22.4%	74.3%	100.0%		
	Group II	276	42	10	328		
		84.1%	12.8%	3.0%	100.0%		
	Group III	218	97	26	341		
		63.9%	28.4%	7.6%	100.0%		
After	Group I	6	60	265	331	552.993	**<0.001**
		1.8%	18.1%	80.1%	100.0%		
	Group II	252	54	21	327		
		77.1%	16.5%	6.4%	100.0%		
	Group III	170	119	63	352		
		48.3%	33.8%	17.9%	100.0%		

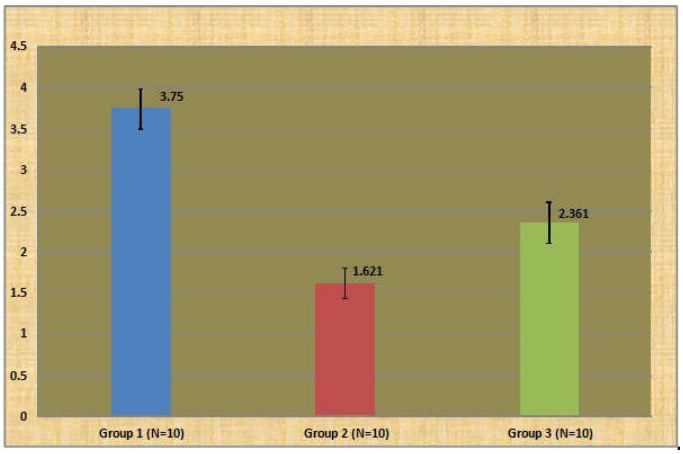
Table 4. Intra group comparison of percentage of occlusion of tubules before and after the acid treatment

Groups		Type			Total	χ^2 value	'p' value
		Completely Occluded Tubule	Partially Occluded Tubule	Open Tubule			
Group 1	Before	11	77	255	343	3.560	0.169
		3.2%	22.4%	74.3%	100.0%		
	After	6	60	265	331		
		1.8%	18.1%	80.1%	100.0%		
Group 2	Before	276	42	10	328	6.493	*0.039*
		84.1%	12.8%	3.0%	100.0%		
	After	252	54	21	327		
		77.1%	16.5%	6.4%	100.0%		
Group 3	Before	218	97	26	341	23.392	*<0.001*
		63.9%	28.4%	7.6%	100.0%		
	After	170	119	63	352		
		48.3%	33.8%	17.9%	100.0%		

Statistically significant differences ($p<0.001$) (Table-3, Graph-3) were found between the test and control agents after seven days of brushing. DW Group SEM images for the DW group are shown in figures (Figure-4a). The result of the SEM revealed complete occlusion in only 3.2% dentinal tubules and 22.4% remained partially occluded. Dentin tubules that remained open were 74.3%. Thus DW group showed a highly significant difference ($p<0.001$) (Table-3, Graph-3) for the open tubule category. DW group showed more number of open tubules and less partially occluded tubules, than before the acid challenge. After the 6% citric acid challenge, (Figure-4b) the dentine surfaces brushed with water exhibited smooth surfaces without any remnants of debris, and wider tubular openings were observed in most dentinal tubules. No intra-group statistically significant difference ($p>0.05$) (Table-4, Graph-4) was observed.



Graph 1. Comparison of mean distance of dye penetration between the groups

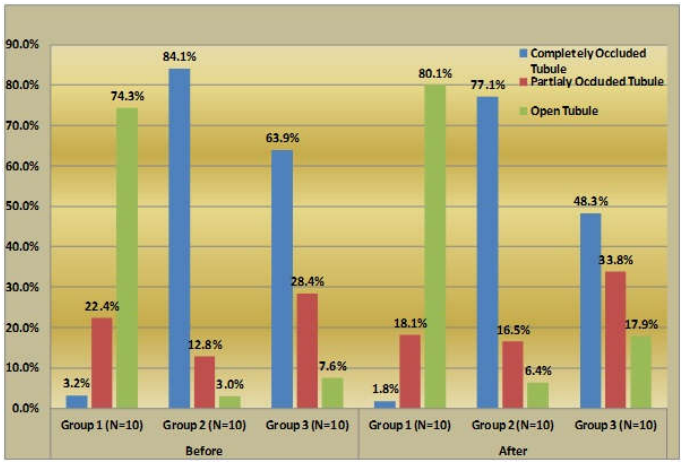


Graph 2. Comparison of mean values of area of dye penetration between the study groups

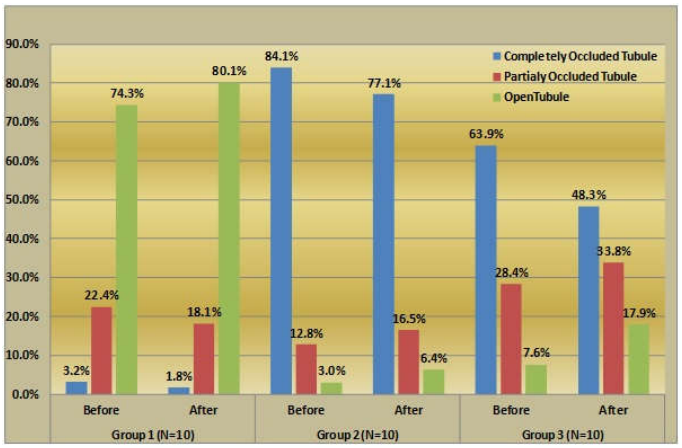
CSPS group

The SEM images of CSPS group showed 84.1% tubules were completely occluded and 12.8% showed partially occluded tubules. The percentage of the non-occluded dentinal tubules was 3.02%. Visual assessment of the SEM images (Figure-5a) revealed varying degrees of surface deposits and levels of tubule occlusion. The CSPS dentifrice formed a heterogeneous covering over the dentine and obliterated majority of tubule openings. In this group a highly statistical significance ($p<0.001$) (Table-3, Graph-3) was found in the category of complete tubule occlusion. After the acid challenge with 6% citric acid, the percentage of completely occluded tubules was reduced to 77.1% and that of partially occluded tubules was increased to 16.5%. Tubules which remained open were 6.4%.

SEM images (Figure-5b) showed that the surface become more homogenous with most of the dentinal tubules occluded, after the acid challenge. Intra-group statistically significant difference ($p<0.001$) was seen (Table-4 and Graph-4).



Graph 3. Comparison of percentage of tubule occlusion between the groups after the application of test agents and after the acid challenge



Graph 4. Intra group comparison of percentage of occlusion of tubules before and after the acid treatment

ACC group

The SEM images of ACC group showed, 69.3% tubules were completely occluded and 28.4% were partially occluded. Dentinal tubules that remained open were 7.6%. The SEM images (Figure-6a) showed a less crystalline natured surface compared to the CSPS group. After acid challenge the ACC showed an increase in the partially occluded tubules and a reduction in completely occluded tubules. After acid challenge the SEM images (Figure-6b) showed more homogenous surface and more number of partially occluded tubules. Intra-group statistically significant difference ($p<0.001$) was seen (Table-4 and Graph-4).

DISCUSSION

The aim of this study was to compare the efficacy of 5% CSPS and 8% ACC containing desensitizing agents by using stereomicroscope for dentine permeability analysis and SEM for checking tubule occlusion. In this study, to simulate the oral condition, samples were stored in artificial saliva after each brushing (Arrais *et al*., 2003). It has been proposed that saliva can solubilize material adhering to teeth. Therefore, it is essential to evaluate whether desensitizing agent could occlude dentinal tubules effectively under the circumstance similar to the oral environment. In this study, a magnification of 8000X was used for evaluating the dentinal tubule occlusion. The highest percentage of tubule occlusion was seen in CSPS group (84.1%).

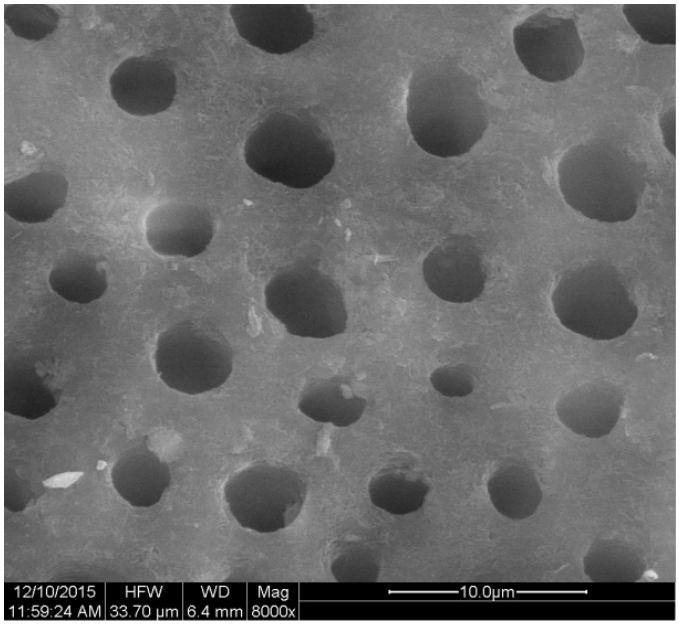


Fig. 4a. Morphology of dentinal tubules treated with distilled water seen under scanning electron microscope (8,000X)

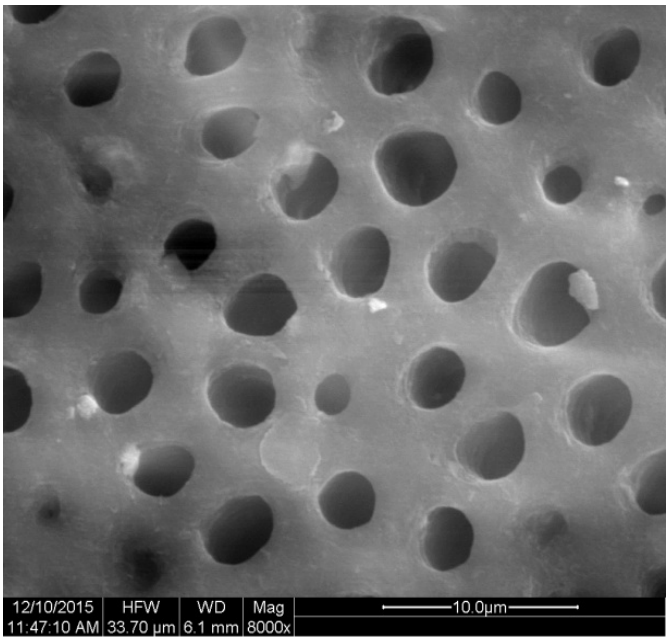


Fig. 4b. After acid challenge

Similar results were observed by Litkowski (1998) and Du Min *et al*. (2008), who demonstrated that CSPS has the ability to rapidly occlude the dentinal tubules and releases calcium continuously to maintain the occlusion of the dentinal tubules. SEM micrograph of specimen treated with CSPS showed more crystalline layer on the dentin surface. These findings are in accordance with the study conducted by Earl *et al*. (2011). They found that CSPS when applied on the dentin surface transforms from an amorphous material to a crystalline hydroxyapatite like material, which helps in occluding the dentinal tubules.

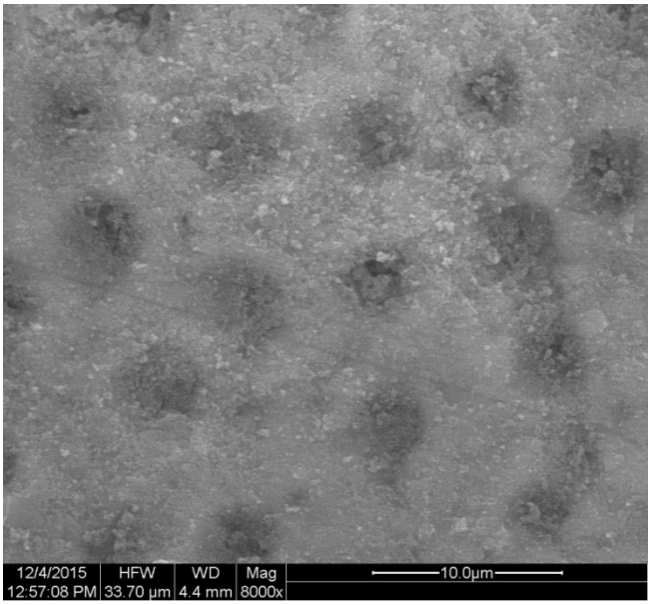


Fig. 5a. Morphology of dentinal tubules treated with CSPS, under scanning electron microscope (8,000X)

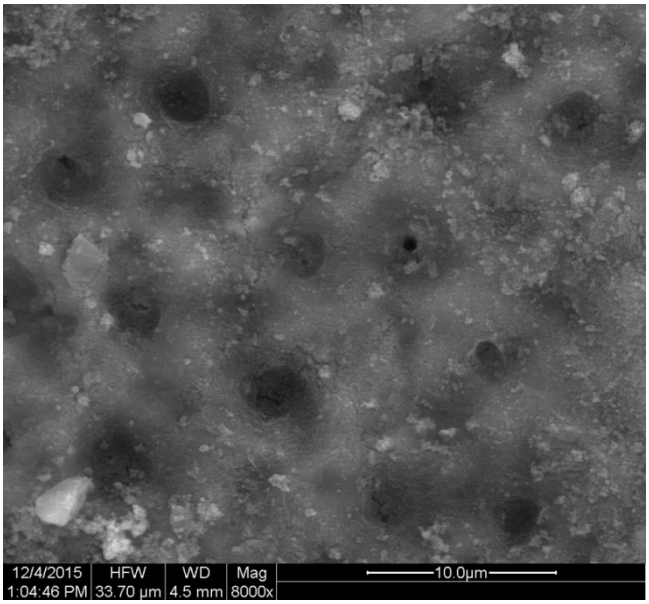


Fig. 5b. After acid challenge

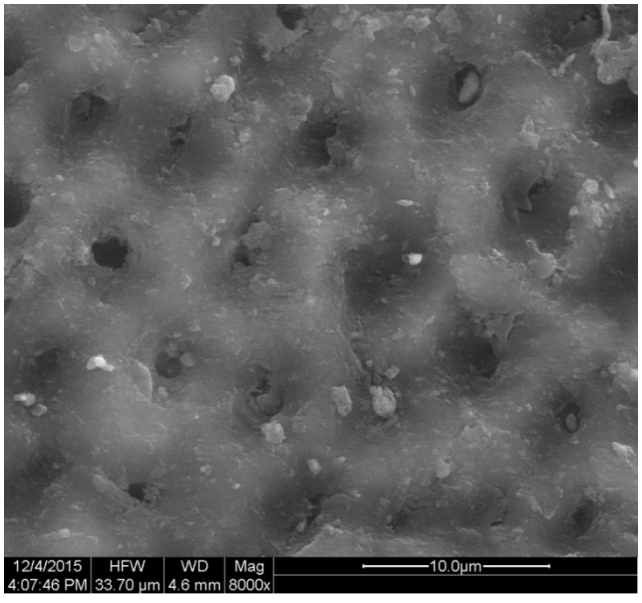


Fig. 6a. Morphology of dentinal tubules treated with ACC, under scanning electron microscope (8,000X)

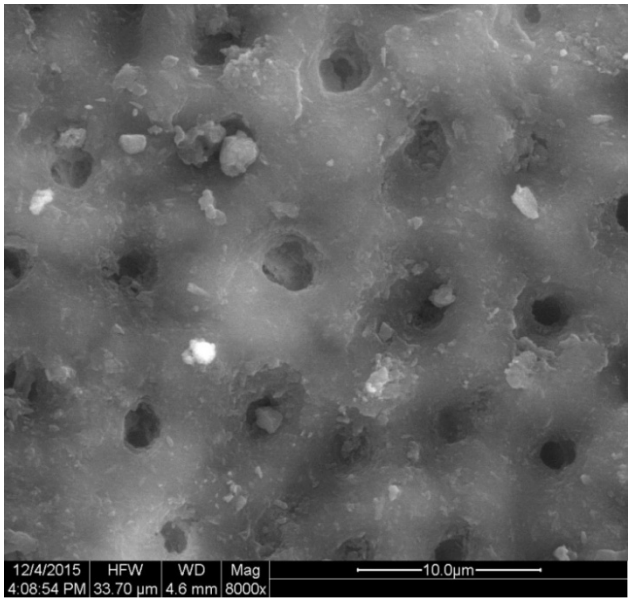


Fig. 6b. After acid challenge

Another in vitro study by Wang *et al*. (2011) also revealed that CSPS dentifrice was highly effective in occluding the dentinal tubules. The ACC showed moderate percentage (69.3%) of complete tubular occlusion compared to CSPS by forming a plug. This agent showed highest percentage (28.4%) of partially occluded tubules amongst all groups. These findings are in accordance with other studies (Sauro *et al*., 2011). However, some contradictory result is also seen in the literature. Petrou *et al*. (2009) noticed that the paste containing ACC was highly effective in occluding the dentinal tubules. This variation in the result may be due to the differences in the parameters and methodology used in the respective studies. Among all the groups, CSPS showed highest number of tubule occlusion compared to all groups and the results were statistically significant. The modern day diet has become increasingly acidic making it one of the commonest causes of dentinal hypersensitivity. In order to evaluate the efficacy of CSPS & ACC to overcome the acidic challenges in the oral cavity, a similar situation was created by exposing the sample to the 6% citric acid challenge (Wiegand *et al*., 2007). Also this step was done to ensure the prepared dentin surface was free of any smear layer or plugs that might be confused with treatment materials. Citric acid was chosen for this purpose as it is one of the commonest content of fruit juices and acidic food. Results showed that the CSPS treated specimens produced high acid resistance compared to other agents. A possible explanation for CSPS conferring a superior degree of protection against demineralization is that acid exposure could also be acting to accelerate the chemical degradation of CSPS, increasing the local concentration of calcium and phosphate, which in turn could provide a degree of local buffering and inhibit further demineralization of the dentine surface (Parkinson and Willson, 2011). Another explanation could be that, as a collagen rich surface is exposed following repeated acid exposure, the dentine surface affords a greater surface area for nucleation sites for the dissolution of CSPS (Ore´fice *et al*., 2009). CSPS is known to adsorb onto collagen and this attraction to collagen increases as the CSPS precipitates. ACC showed less acid resistance compared with CSPS. In this study, dentin permeability analysis was done by using 5% Evans blue similar to the study conducted by Pinto *et al*. (2010). The distilled water group showed highest permeability compared to other agents. This confirms that the distilled water group contains

highest number of open tubules. CSPS treated specimens showed least permeability compared to other agents in this study. This may be due to the presence of higher number of occluded dentinal tubules. This complete occlusion prevents infiltration of dye into the tubules. These results are in accordance with the study conducted by Wang *et al*. (2011). The specimens treated with ACC exhibit less permeability compared to distilled water group and more permeability when compared with CSPS group. This may be due to the presence of higher number of partially occluded dentinal tubules. These findings are in accordance with the study conducted by Sauro *et al*. (2011). Markowitz and Pashley, described that the permeability depends on number and diameter of dentinal tubules, and the reduction of patent dentinal tubules could decrease dentin permeability (Markowitz and Pashley, 2008). This finding implies that, the tubule occlusion is directly related to the reduction in dentin permeability. Though efficacy of CSPS was tested and proven in this in vitro study, a long term evaluation in a well designed randomized clinical trial needs to be done to confirm the superiority of CSPS over other agents.

Conclusion

Within scope of this in vitro study, it can be concluded that, higher percentage of tubular occlusion and least permeability was seen in specimens treated with 5% CSPS followed by 8% ACC and DW. Thus, CSPS shows promise as a desensitizing agent, which not only occludes the dentinal tubules, but also reduces the tubule permeability and shows very good resistance to acid treatment. The results of this study needs to be evaluated in a well designed randomized clinical trial.

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