ORIGINAL RESEARCH ARTICLE

EVALUATION OF THE DENTINAL TUBULE OCCLUSION ABILITY OF SODIUM FLUORIDE IN THREE DIFFERENT COMMERCIALLY AVAILABLE FORMS: A SEM STUDY

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ABSTRACT

Background: The aim of the present study was to evaluate the dentinal tubule occluding ability of commercially available sodium fluoride in three different forms such as varnish, dentifrice and gel by SEM.

Material and Methods: An in-vitro study conducted on eighty periodontally affected extracted teeth. Longitudinal and cross-sections of dentine were distributed into 4 groups each containing 20 specimens such as group A (control), group B (varnish), group C (Dentifrice) and group D (Gel) and were treated with varnish, dentifrice and gel for one week. Specimens were mounted on metal stubs and analysed by Scanning Electron Microscope under 3000x and 5000x magnification.

Results: Group A, control group revealed exposure of dentinal tubules which were completely open. In the Group B (varnish), statistically significant occlusion of dentinal tubules under longitudinal and cross-section of specimen was observed (p value <0.05). In the Group C containing dentifrice, partial obliteration of the tubules was observed but it was statistically significant as compare to control group (p value <0.05). In the Group D containing gel, minimal obliteration of the tubules was observed but it was statistically significant as compare to control group (p value <0.05). On comparison of Group C and Group D the tubule occlusion is statistically non-significant (p value $=0.186$).

Conclusion: NaF varnish showed the highest percentage of tubule occlusion, and proved to be best desensitizing agent and most effective in occluding Dentinal Tubules when compared with that of NaF dentifrice and NaF gel.

Keywords: Dentinal hypersensitivity, Varnish, Gel, Denitrifies

INTRODUCTION

Dentine hypersensitivity is defined as sharp pain arising from the exposed dentin typically in response to chemical, thermal, tactile, or osmotic stimuli that cannot be explained as arising from any other form of dentinal defect or pathology.[1]

Dentine hypersensitivity is a common problem found mostly in adult population ranging from 4 to 74% and is a significant clinical problem which can arise due to the removal of protective enamel layer by attrition from occlusal wear, abfractions, parafunctional habits, abrasive tooth brushing or erosion from acidic diet. On the other hand, gingival recession, periodontal disease and also improper tooth brushing can expose root surfaces.[2, 3] however it still remains a poorly understood area and consequently there appears to be no permanent treatment for

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this clinical condition. Various hypotheses have been proposed to explain the mechanism of dentine hypersensitivity, but the hydrodynamic theory given by Gysi in 1900 and later scientifically explained by Branstrom in 1966 is the most commonly accepted theory.[4-7]

The hydrodynamic theory is based on the concept that fluid within the dentinal tubule can flow inward or out ward direction, depending on pressure differences in the surrounding tissue. A stimulus that makes contact with a tooth surface where there is exposed dentin in addition to patent tubule causes fluid flow and alter direction. This fluid flow within the tubules serves as a medium to excite intradental nerves, which is perceived as pain by the patient.[5]

Patency and permeability of dentin tubule in hypersensitive dentin was confirmed by several studies. These finding explain why some patients with exposed cervical dentine exhibits dentine hypersensitivity and others not .[7-11]

Based on principles of hydrodynamics any decrease in dentin fluid movement should result in reduction of sensitivity. In accordance with this theory Pashley in 1986 reported that dentine hypersensitivity might be reduced physiologically by formation of intratubular crystals from the dentinal fluids and saliva minerals or by the application of therapeutic chemical agent to occlude the exposed dentinal tubules.[12]

It is thought that most of these chemical compounds reduce dentinal hypersensitivity either by crystallizing inside the dentinal tubules or by forming a precipitate at the entrance of the tubule, thereby decreasing the dentinal tubular flow. [13, 14]

Dentinal hypersensitivity possesses a challenging dental problem which can be successfully managed by a very wide variety of procedures, agents and formulations applied locally, either in office or at home. Various chemical compounds have been used such as, Silver nitrate, formalin, Glycerin, Strontium chloride, Dicalcium phosphate, Potassium nitrate, Sodium fluoride, Sodium citrate, Calcium hydroxide, Resins, Potassium oxalate, Stannous fluoride, Cyanoacrylate, ferric oxalate bioactive glass and synthetic hydroxy apatite have been used for treating dentine hypersensitivity.[7-16] Bioactive glass (Calcium sodium phosphosilicate) which is highly biocompatible material that was originally developed as bone regenerative materials. These were known to deposit hydroxycarbonate apatite into the exposed dentinal tubules and mechanically occlude them.[17]

The goal of treatment of dentinal hypersensitivity ideally should be the restoration of the original impermeability of the dentinal tubules and the relief of dentinal hypersensitivity experienced by patient. A number of treatment regimen have been recommended over the years for the management of dentin hypersensitivity such as toothpaste and gels, adhesive resins and mouth rinse. The oral environment being dynamic, the desensitizing agent has to withstand the challenges of salivary dissolution, acid attack from microbes and food components as well as chemical, mechanical and thermal trauma to provide long-lasting pain relief for the patient. Lasers, on the other hand, are a promising and upcoming treatment modality and has raised another possible treatment option and become a research interest in the last decades in the management of DH. The effect of laser as desensitizing agent cause photo biomodulation in the dentin and bring about analgesia in the neural complex cause thermal changes which encourages recrystallization of dentin to cause occlusion of the tubules and formation of a smear layer which is much more resistant in the oral environment.

Sodium fluoride have been incorporated into oral hygiene products to reduce dentinal hypersensitivity since decades for treating dentine hypersensitivity and which is available in a variety of forms. Currently, fluoride is one of the most effective preventive option and the use of fluoridated varnishes with sodium fluoride (in high concentrations) as the active ingredient has been advocated to increase time of action of sodium fluoride in contact with exposed dentin, thus aiming to enhance its effectiveness in decreasing dentine sensitivity The mechanism of action for sodium fluoride is chemical precipitation of sodium ions which occludes dentinal tubules, thus, preventing the stimulation of free nerve endings.

There are limited studies in the literature showing the effective management of dentinal hypersensitivity using sodium fluoride in various forms, therefore present study was conducted to evaluate and compare the efficacy sodium fluoride in three different commercial available forms under scanning electron microscope to visualize the extent of the occlusion of dentinal tubules.

MATERIAL AND METHODS

Present in-vitro study was conducted in the department of Periodontology, Teerthanker Mahaveer Dental College, Moradabad. Periodontally weak human extracted teeth were collected from Oral Surgery Department of Teerthanker Mahaveer Dental College. The study protocol was reviewed and approved by the Ethical committee of Teerthanker Mahaveer University.

STUDY DESIGN: An in-vitro study to compare and evaluate the efficacy of commercially available forms of sodium fluoride by SEM.

SAMPLE SIZE:

An in-vitro SEM study was conducted to evaluate the dentinal tubule occlusion ability of sodium fluoride in three different commercially available forms such as varnish, dentifrice and gel. 80 extracted teeth were randomly assigned to following 4 treatment groups of 20 each.

GROUP A: 20 extracted teeth without any desensitizing agent.

GROUP B: 20 extracted teeth with varnish containing sodium fluoride.

GROUP C: 20 extracted teeth with dentifrice containing sodium fluoride.

GROUP D: 20 extracted teeth with gel containing sodium fluoride.

Analysis of dentin surface was done with scanning electron microscopy to evaluate the occlusion of dentinal tubules under longitudinal and cross-section.

INCLUSION CRITERIA:

- Freshly extracted human periodontally weak teeth with history of cervical hypersensitivity.
- Patient with cervical abrasion.

EXCLUSION CRITERIA:

- History of use of professional desensitizing treatment
- Carious and or restored teeth
- Tooth related with any kind of prosthesis

Dentin Sample Preparation:

- Extracted human teeth were collected .The teeth were cleaned thoroughly and stored in 10% formalin (pH 7) at room temperature.
- Eighty dentin discs each with thickness of 3mm were carefully cut perpendicular to the long axis of the tooth apical to the cementoenamel junction i.e. cross-section and parallel direction i.e. longitudinal section by means of a low speed water cooled diamond saw.(figure 3 & 4)
- The smear layer was subsequently removed by dipping the dentin discs into 1% citric acid solution (pH 7.4) for 2 minutes. The etched dentin disc was rinsed with deionized water. The specimens were equally distributed into 4 groups each containing 20 specimens.

Specimens were randomly divided into four groups each with twenty dentine specimens

Group-A: Specimens were immersed in normal saline for one week

Group-B: Specimens were treated with 1 to 1.5 ml varnish (as recommended) once for every 3 to 6 months using applicator.

Group-C: Specimens were brushed with dentifrice for 2 min twice per day for 7 days.

Group-D: Specimens were brushed with gel for 2 min twice per day for 7 days.

After each brushing session specimens were washed under running tap water and then kept in normal saline filled plastic container.

SAMPLES PREPARATION FOR SCANNING ELECTRON MICROSCOPY

Specimens obtained after the treatment were mounted on metal stubs and dried in a silica gel vacuum desiccators for 15 minutes. To perform SEM analysis, the samples were sputter-coated with 25nm of gold. Microanalysis of the dentin surface of longitudinal and cross-section was obtained using a scanning electron microscope at 20 kV at 3000x and 5000x. (Figure 5 & 6)

EVALUATION OF DENTINAL TUBULES UNDER SEM

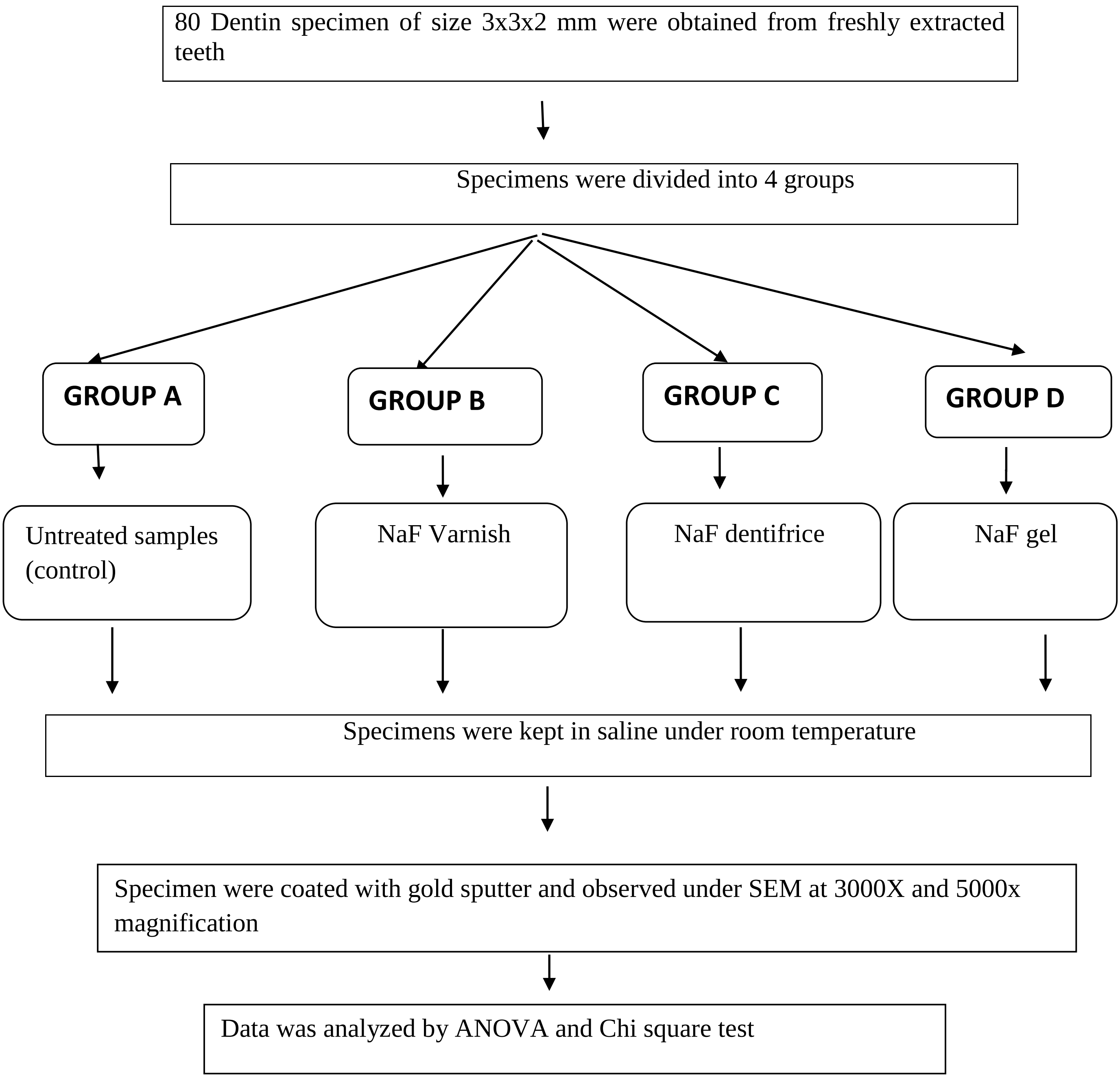
SEM analysis of the specimen was done and percentage of occluded tubules were calculated. Each SEM photograph was assessed for:

- Percentage of un-occluded tubules.
- Percentage of minimal occluded tubules
- Percentage of partially occluded tubules.
- Percentage of completely occluded tubules

GRADING: Dentin specimens were treated using desensitizing agents and these specimen were given score according to Tubule Occlusion Brushing Assay (TUBA) scale.[18] Specimens were graded blindly to treatment on a 6-point scale from 0: open tubuli to 5: no open tubuli, intact smear layer.

Score 0: open tubule
Score 1: 0 - 20%
Score 2: 20 – 40%
Score 3: 40 – 60%
Score 4: 60 – 80%
Score 5: 80 – 100% with no open tubuli and intact smear layer

INVESTIGATION DESIGN:



SEM analysis:

After the last brushing session, dentine specimens were stored in beaker shaker machine for 2 hrs and then washed with distilled water and thin layer of gold sputter coating was done(MED 010- Geol, Japan). Photomicrographs were taken using Scanning Electron Microscope (DSM 840-A-Geol.Japan) from the center of 3x3x2mm of longitudinal and cross-section dentine block at 3000X and 5000x magnification.

RESULT

Group A (n=20): The group A comprised of 20 controlled specimen which presented a score of 0, All of the specimens (100%) scored 0 indicating all opened dentinal tubules within the study group. (Table II).

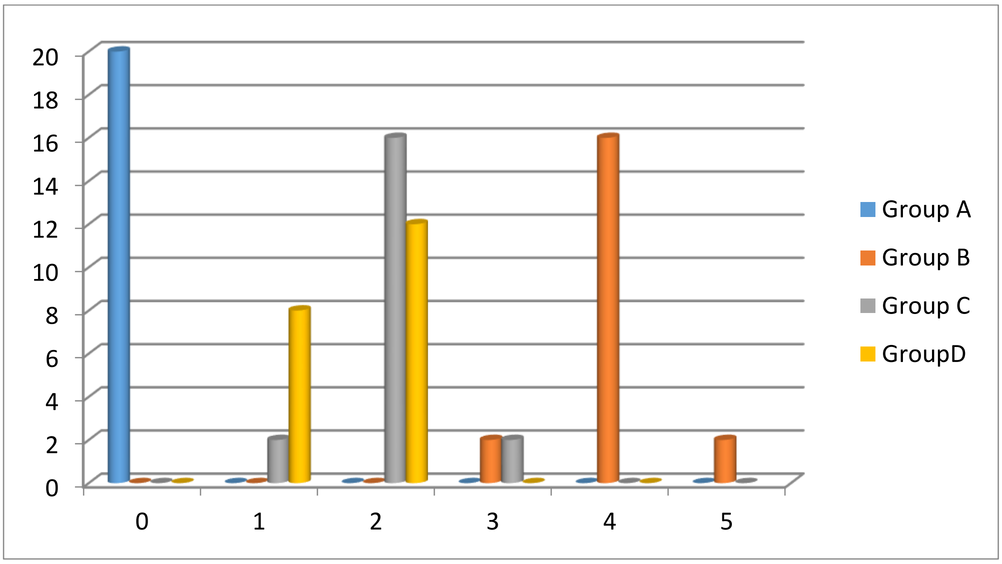
Group B (n=20): The group B comprised of 20 specimen treated with varnish in which zero specimens (0%) presented a score of 0, Zero specimens (0%) presented a score of 1 and zero (0%) specimens scored 2. Two specimens (10%) scored 3, sixteen specimens (80%) presented a score 4 and two (10%) specimens scored 5 indicating complete occlusion of dentinal tubules with intact smear layer within the study group. (Table II)

Group C (n=20): The group C consisted of 20 specimens treated with dentifrice. Zero specimen (0%) presented a score of 0, two specimens (10%) presented a score of 1, and sixteen (80%) specimens scored 2. Two specimens (10%) scored 3, Zero specimen (0%) scored 4 and 5 indicating partial occlusion of tubules. (Table II)

Group D (n=20): The group consisted of 20 specimens treated with Gel. Zero specimen presented a score of 0, eight specimens (40%) presented a score of 1 and twelve (60%) specimens scored 2. Zero specimens (0%) scored 4 and 5 indicating partial occlusion of tubules. (Table II)

TUBA Scores	Group A n=20	Group B n=20	Group C n=20	Group D n=20
(0)	20 (100%)	0	0	0
(1)	0	0	2 (10%)	8(40%)
(2)	0	0	16 (80%)	12(60%)
(3)	0	2(10%)	2 (10%)	0
(4)	0	16(80%)	0	0
(5)	0	2(10%)	0	0

Table I: Illustrates the comparison among the different groups using TUBA scale.



Graph I: Comparison of TUBA scores among different groups

On the basis of ANOVA and chi-square test there is a significant difference of scores between different groups. Table II and III showing Group A specimens i.e. control group scored 0, group B specimens (varnish), scored 3, 4 & 5. Group C specimens (dentifrice) scored 2 & 3 and group D specimens (gel) scored 1 & 2. Comparison of scores among different groups which depicts that all specimen of group A falls under score 0, group B had a significant range between score 4 and 5, group C falls under score 1, 2 and 3.

Group D specimens scored 1 and 2. (Graph I & II)

Comparison of percentage of tubule occlusion in group A and B. There was a significant difference among both the groups. Group B specimen showed the most occluded dentinal tubules.

On the basis of ANOVA and chi-square test there is a significant difference of scores between different groups (p value <0.05).Table IV and V showing the group B comprised of 20 specimen treated with varnish in which zero specimens (0%) presented a score of 0, Zero specimens (0%) presented a score of 1 and zero (0%) specimens scored 2. Two specimens (10%) scored 3, sixteen specimens (80%) presented a score 4 and two (10%) specimens scored 5 indicating complete occlusion of dentinal tubules with intact smear layer within the study group.

On the basis of ANOVA and chi-square test there is a significant difference of scores between different groups (p value <0.05).The group C consisted of 20 specimens treated with dentifrice. Zero specimen (0%) presented a score of 0, two specimens (10%) presented a score of 1, and sixteen (80%) specimens scored 2. Two specimens (10%) scored 3, Zero specimen (0%) scored 4 and 5 indicating partial occlusion of tubules. (Table VI,VII and Graph IV).

On the basis of ANOVA and chi-square test there is a significant difference of scores between different groups (p value <0.05).The group consisted of 20 specimens treated with Gel. Zero specimen presented a score of 0, eight specimens (40%) presented a score of 1 and twelve (60%) specimens scored 2. Zero specimens (0%) scored 4 and 5 indicating partial occlusion of tubules. (Table VIII,IX and graph V)

On the basis of ANOVA and chi-square test there is a significant difference of scores between different groups (p value <0.05).NaF varnish group showed the most significant value (p value< 0.05) than dentifrice and gel.(Table XII,XIII and Graph VII)

Group C scored 1, 2 & 3 and group D specimen scored 1 & 2. On the basis of ANOVA and chi-square test on comparison of dentinal tubule occlusion ability within dentifrice and gel data was not statistically significant (p value >0.05).(Table XIV,XV and graph VIII)

DISCUSSION

Dentin hypersensitivity is one of the most painful and least predictably treated chronic conditions in dentistry and it still remains a challenging problem to a clinician. It is a significant clinical problem which can arise due to various etiological factors such as, due to the removal of protective enamel layer by attrition from occlusal wear, abfractions, parafunctional habits, abrasive tooth brushing or erosion from acidic diet etc. The approaches used in the treatment and prevention of cervical dentinal hypersensitivity are tubular occlusion and/or the blockage of nerve activity.[15]

The difficulty found in treating dentinal hypersensitivity is expressed by the enormous number of techniques and therapeutic alternatives to relieve it.[16]

Several methods and materials, such as liners, restorative materials, dentinal adhesives, dentifrices, mouthwashes, varnishes and lasers have been used for the management of dentinal hypersensitivity. Dentinal tubule occlusive therapies for the treatment of dentinal hypersensitivity are frequently proposed because it is believed that sealing the dentinal surface diminishes the movement of fluids inside the tubule and is capable of reducing DH.[25] The use of sodium fluoride as a desensitizing agent appeared to have best therapeutic potential to alleviate dentinal hypersensitivity.[16]

The present study was conducted to evaluate and compare the dentinal tubule occluding ability of three commercially available desensitizing agents using sodium fluoride in different forms such as varnish, dentifrice and gel by SEM under 3000x and 5000x magnification.

Eighty human extracted teeth were randomly divided into four groups each with twenty dentine specimens such as group A (control), group B (varnish), group C (Dentifrice) and group D (Gel). Results of this study revealed that group B i.e. varnish completely occluded the dentinal tubules, scoring highest percentage of tuba score when compared with groups A , C and D and proved to be most effective in the management of cervical hypersensitivity.

In accordance with the studies done by Corona et al and Ritter et al, the results of present study demonstrated that varnish containing sodium fluoride, showed statistically significant reduction ($p< 0.05$) in hypersensitivity of the teeth to thermal stimuli when compared with a control ($p>0.05$), lacking the active ingredients .[29,32]

Dentine hypersensitivity highlights the different stimuli inducing the pain, of these cold or evaporative stimuli are usually identified as the most intensive. If the hydrodynamic theory has to be accepted for the dentine hypersensitivity mechanism, then the lesion must have the dentinal tubules open at the dentin surface and should be patent to pulp, which has been confirmed by several SEM studies by Yoshiyama, Pashley, Absi.[10, 15, 19,23] These studies provide evidence demonstrating the presence of as much as 8 times greater number of open dentinal tubules and 2 times wider the diameter on hypersensitive dentine compared to non-sensitive dentine. The SEM study in accordance with sensitive and non-sensitive dentin showed significantly greater number of open tubules in sensitive dentin.[11]

Pashley found that the hydraulic conductance of tissue expresses the ease with which fluid can move across the unit surface area under a unit pressure per unit of time. This information has important implication for treatment strategies, reducing the number of open tubules or decreasing the diameter is mode of reducing the hypersensitivity by many chemical compounds.[7, 24] In present study, sodium fluoride in its various forms demonstrated the occlusion of dentinal tubules, which further causes the precipitation of sodium fluoride crystals and reduce the movement of dentinal fluids and helps in the management of dentinal hypersensitivity

Greater than 85% of the resistance to the fluid flow across the dentin towards the pulp has been provided by the smear layer produced during the brushing, grinding, polishing, cutting etc. and natural tubule occlusion can occur through the formation of the calculus or intracanal salivary minerals. Several chemical compounds have been used for the treatment of the dentine hypersensitivity with varying degree of success in the past. [15, 25, 26]

Grossman in 1935 proposed 6 characteristics that a material for treating dentinal hypersensitivity should possess. The material should be (1) Non irritating to the pulp; (2) Relatively painless on application; (3) Easy to apply; (4) Rapid action; (5) Effective for long time; (6) Non staining .[30]

The therapeutic agent's forms insoluble precipitate inside the open tubule and either completely block the tubule or reduce the diameter of the exposed tubule.[15, 19] Various therapies available for the treatment of hypersensitivity, the home-care methods generally promoted very often to about 77%. These products are generally desensitizing dentifrices, and patients are advised to brush using these dentifrices to bring about the desired occlusion of open tubules[3]. In present study, management of dentinal hypersensitivity using sodium fluoride in various commercially available forms causes the precipitation of sodium fluoride crystals which further occludes and subsequently reduce the movement of dentinal fluids within the tubules.

Addy in his study concluded that the occluded dentinal tubules resemble the clinical treatments with desensitizing chemical agents. Thus commercially available desensitizing dentifrices analyzed were Thermoseal. Nitra.Oral-B sensitive and Oravive, all of which contained silica as the abrasive agent and strontium chloride, potassium nitrate, Hydroxyapatite, and bioactive glass as active ingredients respectively.[16,29] To determine the dentinal tubule occluding ability of each of dentifrice dentine specimens were obtained from premolars to simulate the clinical hypersensitive dentine, Dentine samples were obtained at the level of dentoenamel junction of cervical third of premolars and further polishing and ultrasonication of the samples removed the smear layer occluding the dentinal tubules. All the samples were brushed for 2 min with motorized tooth brush from the equidistance with the help bristle protector to standardize the brushing pressure and strokes for each samples, since the motorized brush (Colgate actibrush) oscillates at constant speed. After each brushing session the samples were kept in artificial saliva and shaker machine to simulate the oral condition. [29] In present study among the treated groups the specimens brushed with varnish showed the highest percentage of tubule occlusion, and demonstrated highest scores among different groups as it precipitated the hydroxyapatite over the entire dentin surface followed by dentifrice and gel.

The specimens of group B in the present study were treated with varnish, exhibited higher percentage of tubule occlusion score i.e. 3 ,4 and 5 than Group C scoring 1,2 and 3 and Group D which falls under score 1 and 2 which were treated with dentifrice, gel and control groups. The Group C and the Group D were not significantly different. These specimens showed the p value (0.186) i.e. non-significant are similar to the results obtained by T.Suge et al. in their study on duration of tubule occlusion by calcium phosphate precipitation method using artificial saliva.[30]

On contrary a study by Carlo P.et al, showed that the dentin permeability increased when the brushing was done in the presence of smear layer[27] but the reduction in the permeability was smaller compare to those reported by Pashley et al. Despite the reduction in radius of the dentinal tubules lumen and complete occlusion of the some tubules, control group still had most of the tubule orifice open even after seven days.[18]

D.G.Gillam et al in their study 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 phosphorous (calcium phosphate) following ion exchange on the surface of the bioactive glass.[38]

The other possible explanation for the surface deposition was given by Marini et al. according to them 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 dentin.[42]

In the present study the tubule orifices were obliterated by the deposition of sodium fluoride ions which may be attributed in the occlusion of tubules, which were evaluated through cross-section and penetrates deep into the tubules under longitudinal section. Even though the sodium fluoride present in all the treatment groups leading to tubule occlusion was significantly different from each other. In contrast the abrasive system of dentifrice formed granular and crystal deposits or a thin deposition over dentin surface that partly or completely occluded the tubules. SEM provided the evidence that the tested agents produced their desensitizing response by tubule occlusion and thereby interfering with hydrodynamic mechanism to stop the fluid movement across the dentin.

Present study concluded that the specimen treated with desensitizing NaF varnish, NaF dentifrices and NaF gel, gave significantly higher percentage of tubule occlusion compare to the control group (p value< 0.05). NaF varnish group showed the most significant value (p value< 0.05) than dentifrice and gel. On comparison of dentinal tubule occlusion ability within dentifrice and gel data was not statistically significant (p value >0.05). Therefore, the present study demonstrated that the varnish as a desensitizing agent proved to be best over dentifrice and gel in the management of dentinal hypersensitivity.

CONCLUSION

An in-vitro SEM study was conducted to evaluate and compare the dentinal tubule occluding ability of commercially available sodium fluoride in three different forms i.e. varnish, dentifrice and gel. NaF dentifrice proved to be significantly effective when compared with NaF gel but not superior to NaF varnish.NaF varnish showed the highest percentage of tubule occlusion ,in longitudinal and cross-section and proved to be the best desensitizing agent in occlusion of dentinal tubules followed by NaF dentifrice and NaF gel.

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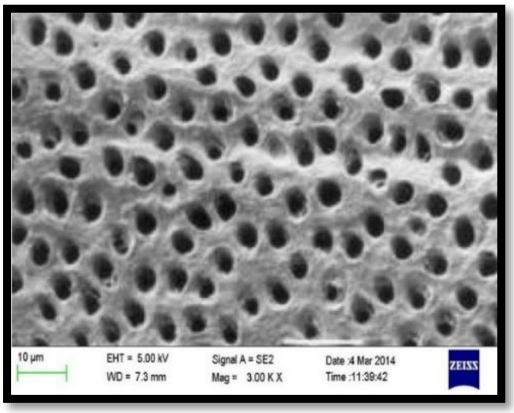
FIGURES:

GROUP A (CONTROL)



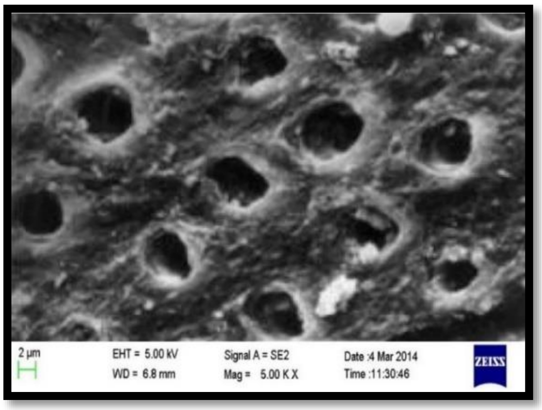
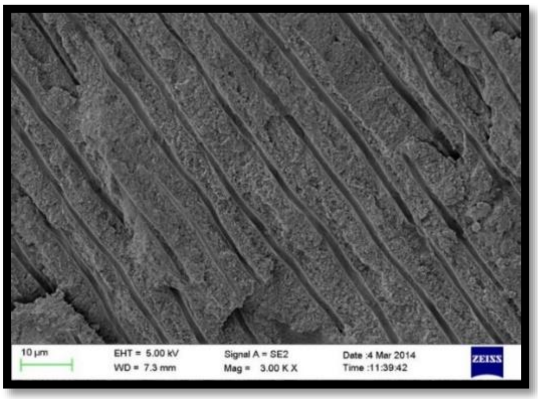


Figure 1 Figure 2

Figure: 1 & 2 Illustrating the cross-section of dentin specimen under 3000x and 5000x respectively which shows un-occluded dentinal tubules



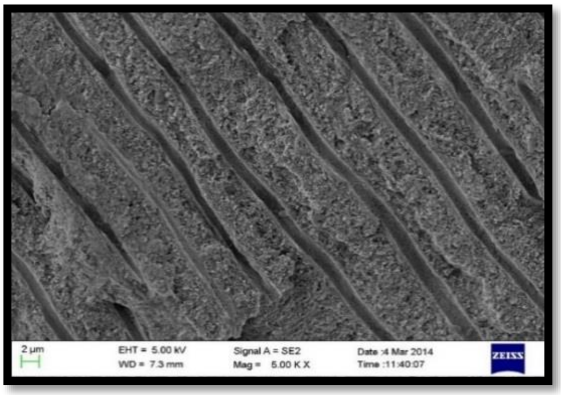
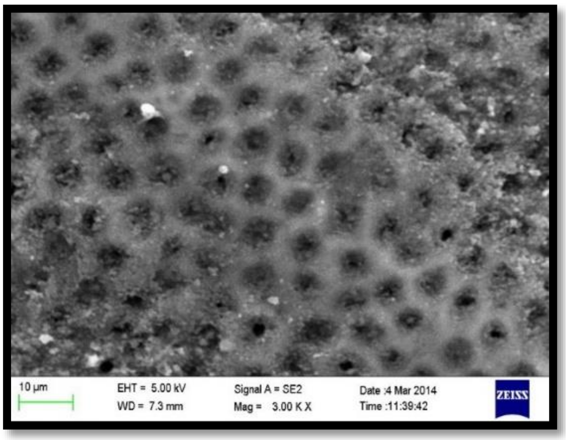


Figure 3 Figure 4

Figure: 3 & 4 illustrating the longitudinal section view of dentin specimen under 3000x and 5000x respectively.

GROUP B (NaF VARNISH)



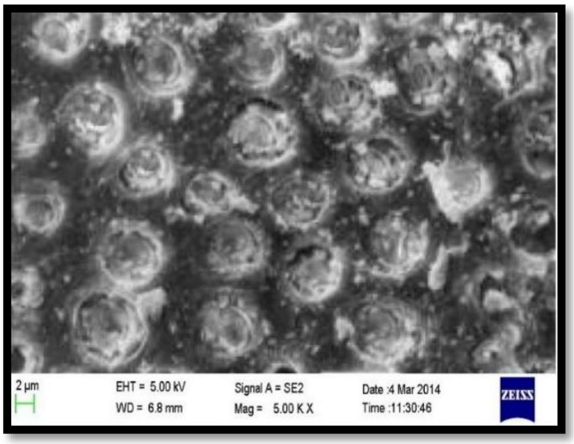
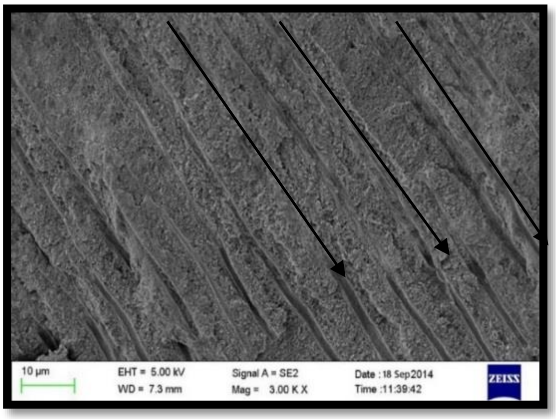


Figure 5 Figure 6

Figure 11 & 12 Illustrating the cross-section of dentin specimen under 3000x and 5000x respectively, showing complete occlusion of dentinal tubules.



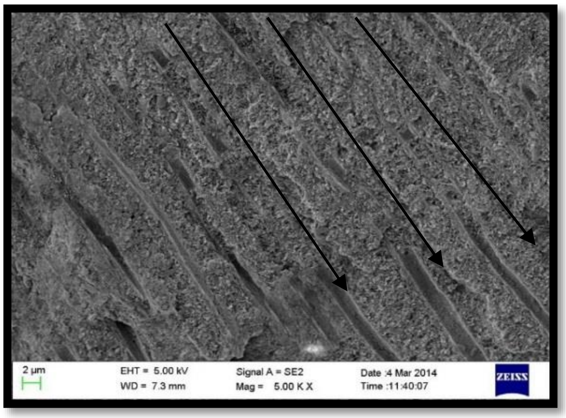
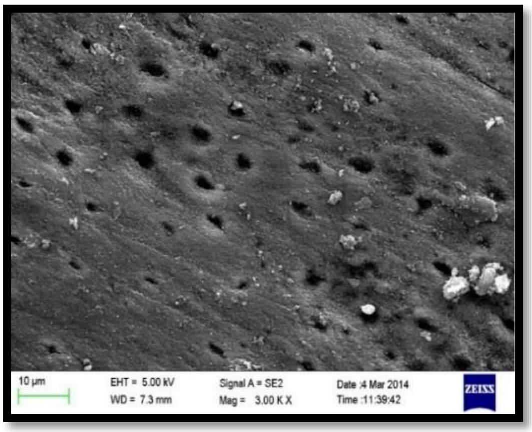


Figure 7 Figure 8

Figure: 7 & 8 Illustrating the longitudinal section of dentin specimen under 3000x and 5000x, respectively showing the occlusion of tubules to a significant length.

GROUP C (NaF DENTIFRICE)



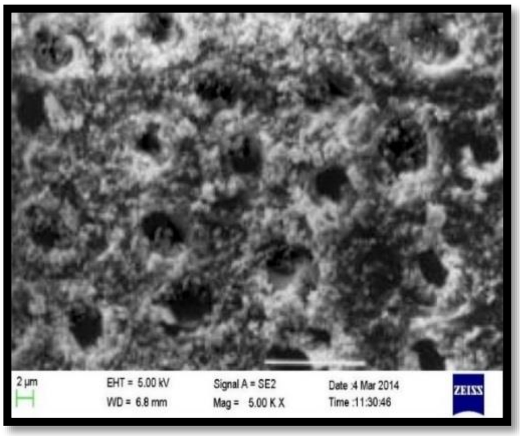
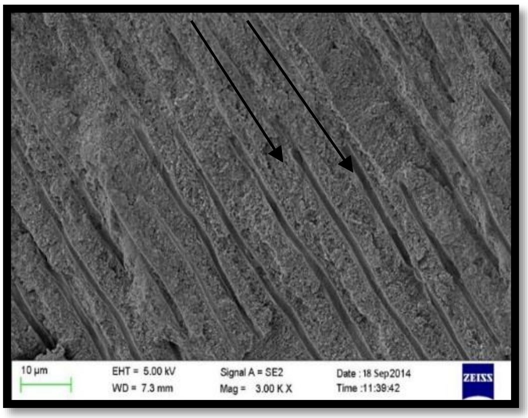


Figure 9 Figure 10

Figure: 9 & 10 Illustrating the cross-section of dentin specimen under 3000x and 5000x respectively showing the partial occlusion of dentinal tubules.



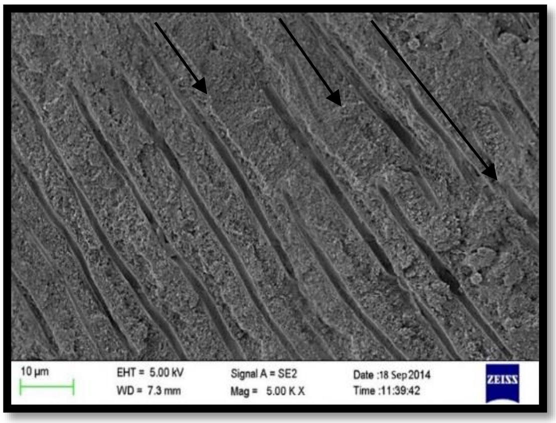
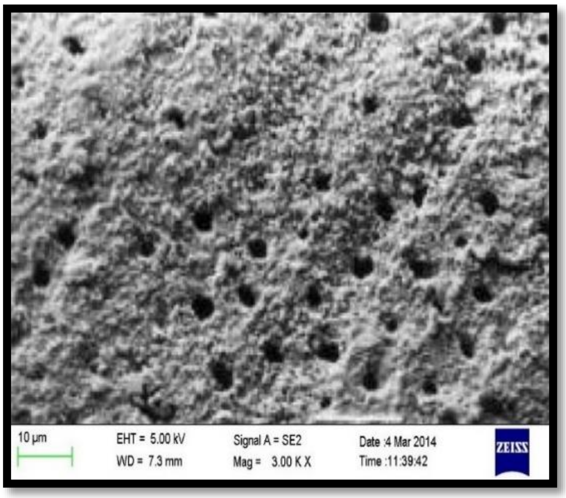


Figure 11　　　　　　　　Figure 12

Figure: 11 & 12 illustrating the longitudinal section of dentin specimen under 3000x and 5000x respectively, showing the partial occlusion of tubules length.

GROUP D (NaF GEL)



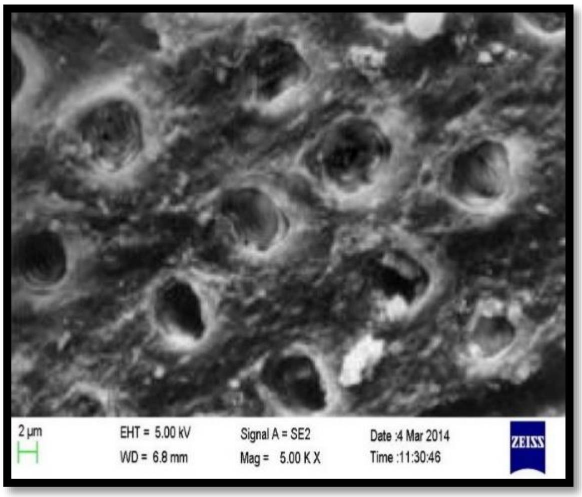
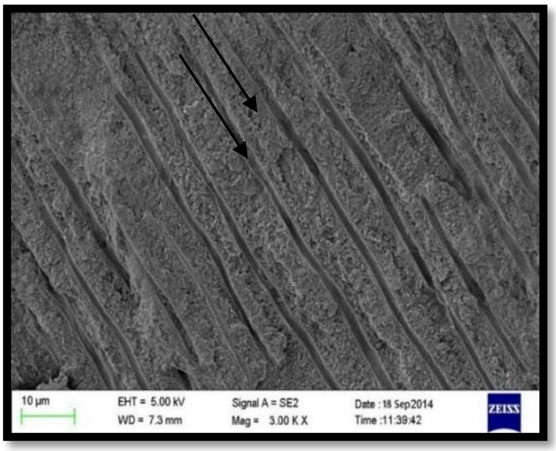


Figure 13　　　　　　　　Figure 14

Figure: 13 & 14 Illustrating the cross-section of dentin specimen under 3000x and 5000x respectively showing minimal occlusion of dentinal tubules.



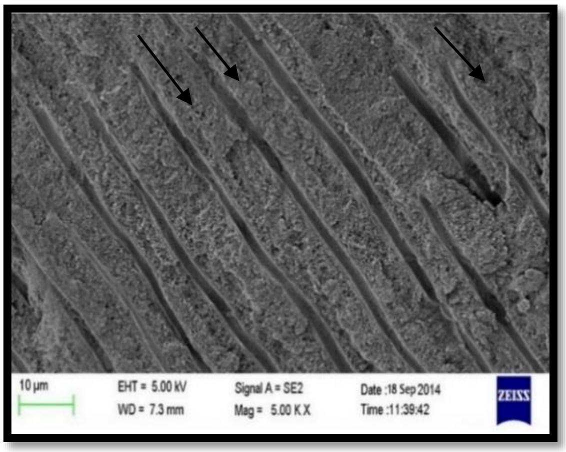


Figure 15　　　　　　　　Figure 16

Figure: 15 & 16 illustrating the longitudinal section of dentin specimen under 3000x and 5000x respectively, showing the occlusion of tubules to minimal number and length.