10.5005/jp-journals-10051-0141

ORIGINAL ARTICLE

Comparative Evaluation of Dentinal Tubule Occlusion Ability of Three Commercially Available Desensitizing Dentifrices: An *in vitro* Scanning Electron Microscope Study

[1]Kamal J Manhas, [2]Gaurav Malhotra, [3]Pradeep Shukla, [4]Preeti Shukla, [5]Varun Dahiya, [6]Prerna Kataria

ABSTRACT

Introduction: Tooth sensitivity is a very common clinical presentation with a prevalence of 4 to 57 % and mostly occurs in the age group of 30 to 40 years. The dentinal tubule exposure is principally responsible for the clinical symptoms of dentin hypersensitivity (DH). Hence, the aim of this study was to evaluate and compare the dentinal tubule occluding ability of three commercially available dentifrices (strontium chloride, potassium nitrate, and sodium monofluorophosphate) using scanning electron microscopy (SEM).

Materials and methods: Freshly extracted 50 molars were collected and stored in 10% formalin. All experiments were conducted within 2 months of extraction. The teeth were sectioned mesiodistally and ultrasonicated and were randomly divided (10 each) into five groups:

- Group I: Specimens immersed in artificial saliva for 1 week.
- Group II: Specimens brushed with distilled water only.
- Group III: Specimens brushed with strontium chloride-containing toothpaste.
- Group IV: Specimens brushed with potassium nitrate-containing toothpaste.
- Group V: Specimens brushed with sodium monofluorophosphate-containing toothpaste.

Each SEM photomicrograph was critically analyzed for exposed and occluded dentinal tubules after application of the various agents.

Results: Statistically significant difference was found out among the five groups ($p < 0.001$). It was seen that the mean percentage of occluded dentinal tubules of groups I, II, III, IV, and V was 35.47, 23.32, 75.90, 85.27, and 90.21% respectively.

Conclusion: The present *in vitro* SEM study results revealed that all the experimental agents were effective in occluding dentinal tubules as compared with the control group. The percentage of occluded tubules was found to be highest for sodium monofluorophosphate as compared with the other groups over a period of 7 days.

Keywords: Dentin sensitivity/therapy, Dentinal hypersensitivity, Desensitizing dentifrices, Scanning electron microscope study.

How to cite this article: Manhas KJ, Malhotra G, Shukla P, Shukla P, Dahiya V, Kataria P. Comparative Evaluation of Dentinal Tubule Occlusion Ability of Three Commercially Available Desensitizing Dentifrices: An *in vitro* Scanning Electron Microscope Study. Int J Oral Care Res 2018;6(1):5-11.

Source of support: Nil

Conflict of interest: None

[1]Postgraduate Student, [2,5,6]Professor, [3]Professor and Head [4]Assistant Professor

[1-3,5,6]Department of Periodontology and Implantology, Divya Jyoti College of Dental Sciences & Research, Ghaziabad, Uttar Pradesh, India

[4]Department of Biotechnology and Microbiology, IIMT College of Life Sciences, IIMT University, Meerut, Uttar Pradesh, India

Corresponding Author: Kamal J Manhas, Postgraduate Student, Department of Periodontology and Implantology, Divya Jyoti College of Dental Sciences & Research, Ghaziabad, Uttar Pradesh, India, Phone: +919906222607, e-mail: drkamalmanhas@gmail.com

INTRODUCTION

Tooth sensitivity is a very common clinical presentation which can cause considerable concern for patients. This condition is frequently encountered by dentists, endodontists, periodontists, hygienists, and dental therapists. This condition generally involves the facial surfaces of teeth near the cervical aspect and is very common in premolars and canines.[1] It is a condition often termed as "enigma being frequently encountered but poorly understood" with a prevalence of 4 to 57% and mostly occurs in the age group of 30 to 40 years.[2]

The human teeth comprise enamel, dentin, cementum, and pulp. Dentin is the main supporting structure of the tooth and consists of an organic component containing collagen fibers in a matrix of collagenous proteins and an inorganic component containing hydroxyapatite crystals. Within dentin, dentinal tubules are present, which extend from the external surface to the pulp.[3] There are approximately 30,000 to 40,000 tubules per square millimeter, which can transmit pain to the pulp if the dentin is exposed. The diameter and density of the tubules are greatest near the pulp. Tapering from the inner to the outermost surface, they have a diameter of 2.5 μm near the pulp, 1.2 μm in the midportion of the dentin, and 0.9 μm at the dentinoenamel junction.[4] Each tubule contains a Tomes fiber (cytoplastic cell process) and an odontoblast that communicates with the pulp. Within the dentinal tubules, there are two types of nerve fibers, myelinated (A fibers) and unmyelinated (C fibers). The A fibers are responsible for the sensation of DH.[5]

Dentin hypersensitivity has been defined as "A short, sharp pain arising from exposed dentinal in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defect or pathology."[6]

A modification of this definition was suggested by the Canadian Advisory Board on DH in 2003, which suggested that "disease" should be substituted for "pathology."[7]

The etiology of DH can stem from multiple sources, such as abfraction, abrasion, erosion, traumatic oral hygiene, and periodontal disease. Other factors include patient's deleterious habits, poor oral hygiene, chewing tobacco, excessive occlusal force, premature occlusal contact, and gastroesophageal reflux.[8] Cold and air stimulation are known to be the commonest stimuli while dietary acid is also shown to have a significant potential in evoking DH.[9] The resulting dentinal tubule exposure is principally responsible for the clinical symptoms of DH.

Various theories have been put forth to explain the mechanism of DH which includes odontoblastic transduction theory, neural theory, and hydrodynamic theory. The most widely accepted theory for DH is hydrodynamic theory given by Gysi[10] and later explained by Brannstrom[11] according which the exposure of dentinal tubules allows movement of intradentinal fluid leading to stimulation of A-δ fibers causing hypersensitivity. Hence, occlusion of the tubules is supposed to block the hydrodynamic mechanism and reduce dentin sensitivity.

Dentin hypersensitivity can be treated by different ways; first, by reducing the dentinal tubules hypoconduction by occluding them; second, by reducing the nerve fibers excitability and/or by a combination of these two approaches.[12]

Potassium nitrate, potassium bicarbonate, and potassium chloride are active agents that can reduce nerve excitability, while the combination treatment, such as the use of bipotassium oxalate, which has an obstructive mechanism, also has a direct action on pain receptors. Also protein-denaturing substances, such as formaldehyde, glutaraldehyde, zinc chloride, zinc iodide, phenols, concentrated alcoholic solutions, and strong or weak acids, act directly on the nerves and cause precipitation of dentinal fluid proteins that can occlude the dentinal tubules.[13]

Various treatment modalities are available to treat hypersensitivity which includes at-home and in-office treatment. At home, various toothpastes consist of a variety of dentifrices containing different constituents in which stannous fluoride, strontium chloride, and potassium oxalate[14] are the most widely used dentifrices for delivering over-the-counter desensitizing agents. Desensitizing toothpastes should utilize both or at least one of these two mechanisms of action to relieve DH. This causes occlusion of dentinal tubules which decreases both dentin permeability and fluid movement, thereby reducing hypersensitivity.[15]

The desired goal for treatment of DH is attainment of immediate and lasting relief from discomfort. Grossman stated that the therapy for DH should be nonirritant to the pulp, relatively painless on application, easily carried out, rapid in action, effective for a long period, without staining effects, and should be consistently effective.[16]

A SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. The SEM can achieve resolution better than 1 nm.

Specimens can be observed in high vacuum, in low vacuum, in wet conditions (in environmental SEM), and at a wide range of cryogenic or elevated temperatures.[17]

The aim of this SEM study was to evaluate and compare the dentinal tubule occluding ability of three commercially available dentifrices (strontium chloride, potassium nitrate, and sodium monofluorophosphate) by using SEM.

MATERIALS AND METHODS

The present *in vitro* study was conducted in the Department of Periodontology and Implantology, D.J. College of Dental College and Research, Modinagar, Uttar Pradesh, in collaboration with the Birbal Sahani Institute of Paleobotany, Lucknow, Uttar Pradesh. Freshly extracted 50 molars were collected from the Department of Oral and Maxillofacial Surgery, D.J. College of Dental Sciences and Research, Modinagar and stored in 10% formalin. All experiments were conducted within 2 months of extraction. Impacted 3rd molar and periodontally compromised molar teeth were included in the study. All carious, restored, fractured, root canal-treated teeth, teeth with developmental anomalies, and teeth that demonstrated the presence of any wasting diseases were excluded from the study.

Preparation of the Dentin Specimen

Extracted molar teeth were scaled with ultrasonic scaler and thoroughly cleaned with normal saline and stored in 10% formalin at room temperature for no longer than 2 months prior to their use. The teeth were sectioned mesiodistally using a double-sided diamond disk. One block was obtained from each flat cervical dentin surface

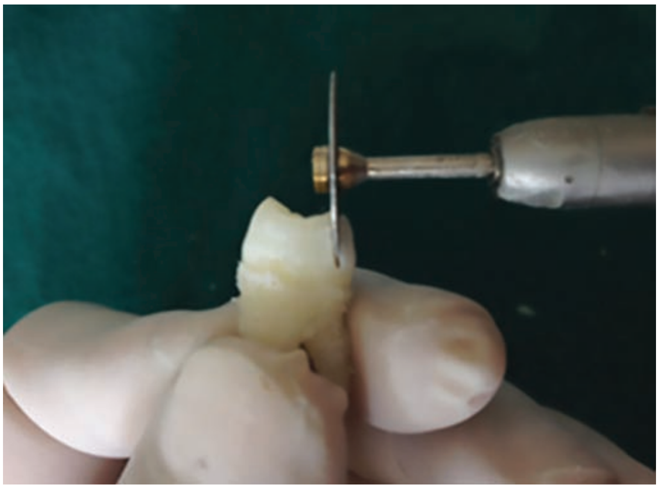


Fig. 1: Mesiodistal section (palatal)

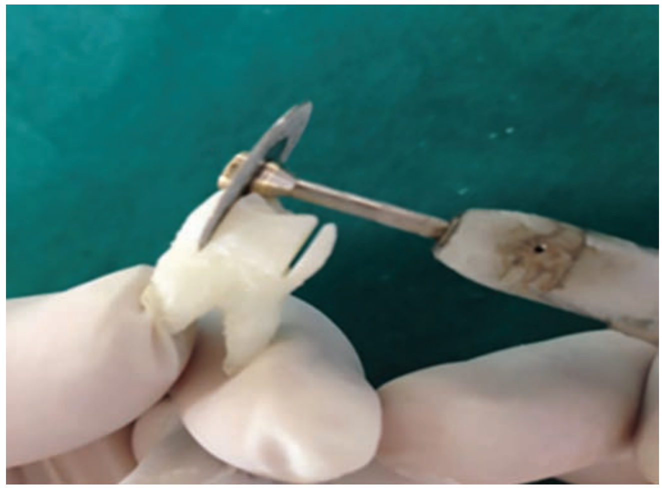


Fig. 2: Mesiodistal section (facial)

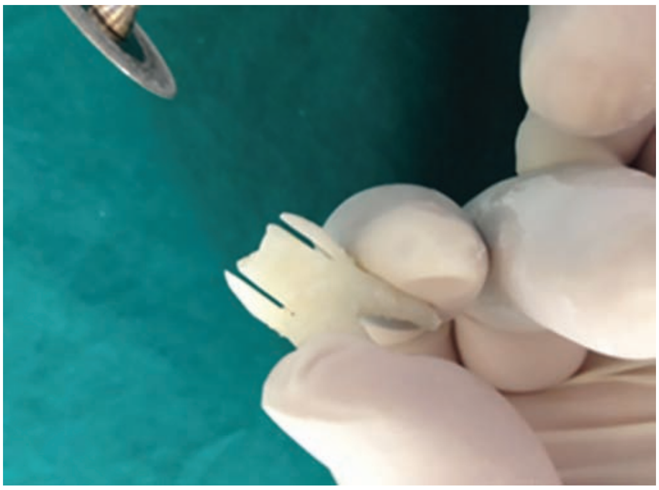


Fig. 3: Mesiodistal longitudinal section

Fig. 4: Transverse section (coronal)

Fig. 5: Transverse section (cervical)

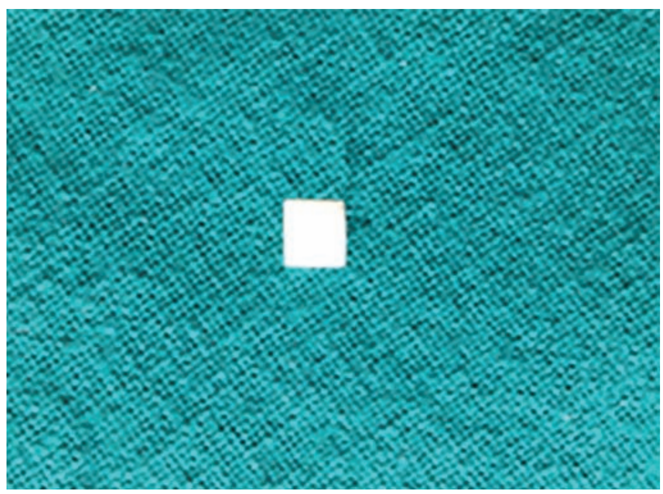


Fig. 6: Flat cervical dentin block

measuring 5 × 5 × 3 mm (L × B × H) by transverse sectioning of the tooth as shown in Figures 1 to 9. The specimens were ultrasonicated in distilled water for 12 minutes to remove residual smear layer and to open dentinal tubules.

Removal of Smear Layer

Samples were ultrasonicated in an ultrasonic cleaner with distilled water for 12 minutes for the removal of smear layer and expose the dentinal tubules to simulate

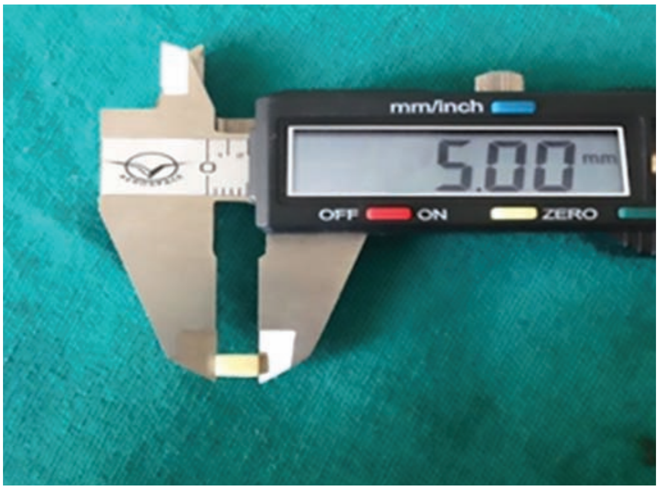


Fig. 7: Dentin block length

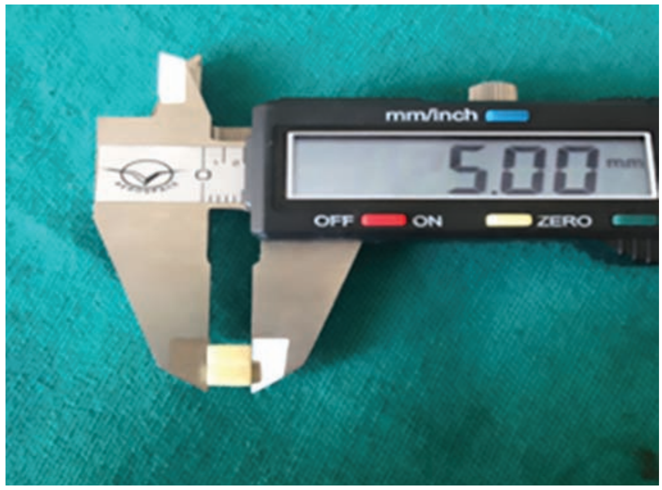


Fig. 8: Dentin block breadth

Fig. 9: Dentin block height

Fig. 10: Specimen after gold palladium coating

hypersensitive dentin. Subsequently, the sections were copiously rinsed with distilled water for a period of 30 seconds.

Collection and Storage of Teeth

After the selection of teeth, they were cleaned by irrigating with saline to get rid of blood and saliva, then the teeth were washed with distilled water and stored in 10% formalin at room temperature.

The specimens were randomly divided (10 each) into five groups:

- Group I: Specimens were immersed in artificial saliva for 1 week.
- Group II: Specimens were brushed with distilled water only.
- Group III: Specimens were brushed with strontium chloride-containing toothpaste.
- Group IV: Specimens were brushed with potassium nitrate-containing toothpaste.
- Group V: Specimens were brushed with sodium monofluorophosphate-containing toothpaste.

Each specimen from group I was immersed in artificial saliva for 1 week. Group II specimen were brushed with distilled water for 2 minutes twice daily for 7 days, and groups III, IV, and V were brushed with the dentifrice slurries, which were prepared by diluting 2 gm of the dentifrice in 6 mL of distilled water.

Preparation of Specimens for SEM Study

The SEM analysis was done at Birbal Sahani Institute of Palaeobotany, Lucknow, Uttar Pradesh. Treated specimens were mounted on aluminum stubs with a double-sided adhesive tape. Mounted samples were then placed in the sputter coater for gold-palladium coating, which was done on each sample as shown in Figure 10.

Thereafter, the specimens were examined under SEM at magnification of 1000× and a representative photomicrograph of the each specimen was randomly taken.

Scanning Electron Microscopy Analysis

Each SEM photomicrograph was critically analyzed for exposed and occluded dentinal tubules after application of strontium chloride-containing toothpaste, potassium nitrate-containing toothpaste, and sodium monofluorophosphate-containing toothpaste, immersed in artificial saliva and with distilled water only.

The total number of tubules and the number of occluded tubules were counted in each photomicrograph of all of the specimens and statistical calculations were made to obtain the result.

The surface was scanned at a magnification of 1000×. Each photomicrograph was counted and analyzed to assess the percentage of tubular occlusion. The percentage of occluded tubules was calculated using the following equation[46]:

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

This percentage represents the occlusion exhibited by the different treatments used.

Statistical Analysis

The values were represented in number (n), percentage (%), mean (μ), and standard deviation (σ). The statistical tests used were analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) test. A p-value ≤ 0.05 was considered statistically significant. Statistical Package for Social Sciences version 20 was used for analysis.

RESULTS

In the present study, we compared the dentinal tubule occluding ability of three commercially available dentifrices using SEM.

It was seen that the mean percentage of occluded dentinal tubules of groups I, II, III, IV, and V was 35.47, 23.32, 75.90, 85.27, and 90.21% respectively.

Statistically significant difference was found among the five groups ($p < 0.001$) (Table 1).

Intergroup comparison of the occluded dentinal tubules on the dentin surface after brushing with and without dentifrices was done using Tukey HSD *post hoc* multiple comparison shown in Table 2.

Graph 1 shows the mean of occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices, and there was significant result in groups III, IV, and V, and most significant in group V.

Graph 2 shows the mean of total and occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices, and there was significant difference of result in groups III, IV, and V, and most significant in group V.

Table 1: Mean of occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices

Groups	*Number*	*Mean (SD)*	*f-value*	*p-value*
I	10	23.20 (4.638)	134.269	0*
II	10	16.00 (5.164)		
III	10	58.60 (9.383)		
IV	10	66.00 (5.735)		
V	10	70.00 (8.273)		
Total	50	46.76 (23.757)		

*Highly significant, f-value-ANOVA

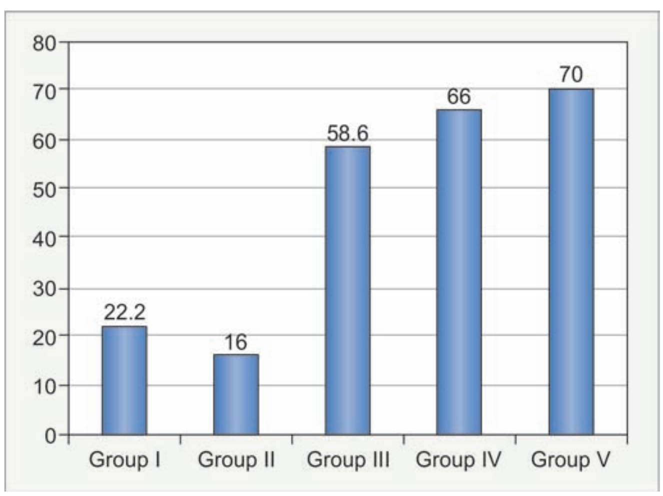
Table 2: Intergroup comparison of the occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices using Tukey HSD *post hoc* multiple comparisons

Dependent variable: occluded tubules	*(I) group*	*(J) group*	*Mean difference (I–J)*	*Standard error*	*Sig.*
Tukey HSD	1	2	7.200	3.083	0.153
		3	−35.400*	3.083	0
		4	−42.800*	3.083	0
		5	−46.800*	3.083	0
	2	1	−7.200	3.083	0.153
		3	−42.600*	3.083	0
		4	−50.000*	3.083	0
		5	−54.000*	3.083	0
	3	1	35.400*	3.083	0
		2	42.600*	3.083	0
		4	−7.400	3.083	0.134
		5	−11.400*	3.083	0.005
	4	1	42.800*	3.083	0
		2	50.000*	3.083	0
		3	7.400	3.083	0.134
		5	−4.000	3.083	0.694
	5	1	46.800*	3.083	0
		2	54.000*	3.083	0
		3	11.400*	3.083	0.005
		4	4.000	3.083	0.694

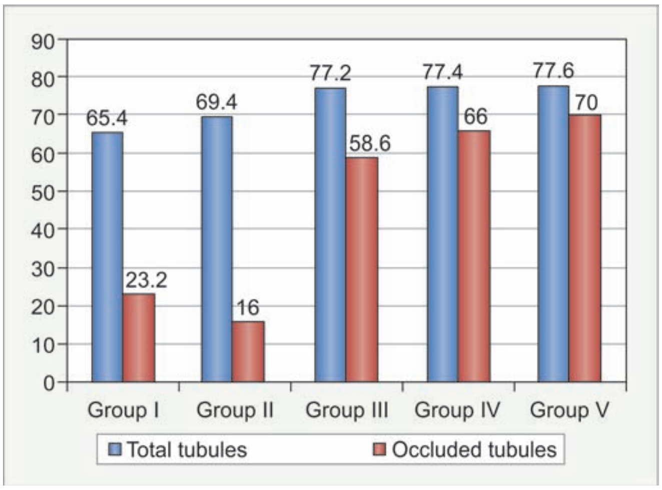
*The mean difference is significant at the 0.05 level

DISCUSSION

Dentin hypersensitivity is a painful response of the tooth to different stimuli, such as brushing, low pH beverages, occlusal overload, dental caries, and thermal changes.[18]



Graph 1: Mean of occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices



Graph 2: Mean of total and occluded dentinal tubules on the dentin surface after brushing with dentifrices and without dentifrices

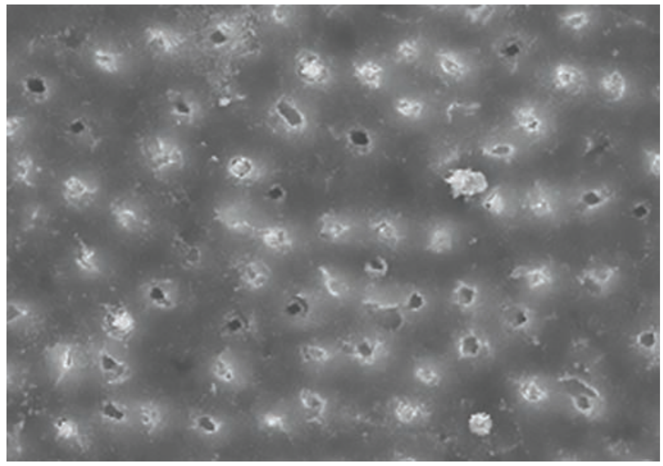


Fig. 11: Immersed in artificial saliva

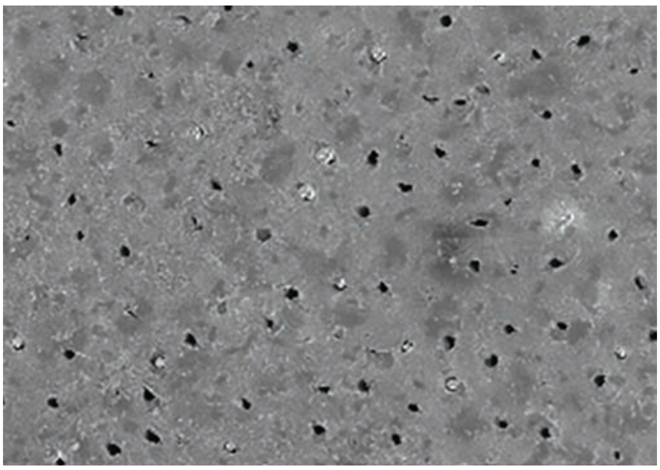


Fig. 12: Brushed with distilled water only

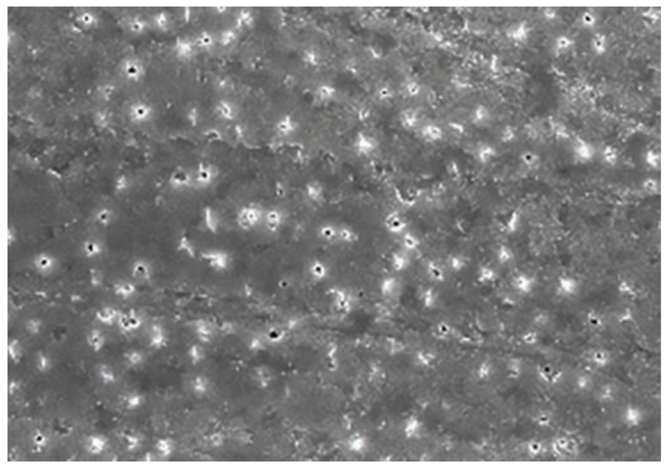


Fig. 13: Brushed with strontium chloride-containing toothpaste

The DH is characterized by a rapid onset of sharp burst of pain, lasting for seconds or minutes. The desired goal for treatment of DH is attainment of immediate as well as lasting relief from discomfort. This is achieved by application of a desensitizing agent used alone or as an adjunct to another dental procedure. Till date, no such treatment has been discovered and there is no "gold standard" by which one can assess the efficacy of the agent used.[19]

In the present *in vitro* study, the dentin blocks were divided into five groups: group I (artificial saliva); group II (distilled water only); group III (strontium chloride); group IV (potassium nitrate); and group V (sodium monofluorophosphate) and were evaluated using SEM to determine the amount of dentinal tubular occlusion (Figs 11 to 15).

Each specimen from group I was immersed in artificial saliva for 1 week. Group II specimens were brushed with distilled water for 2 minutes twice daily for 7 days, and groups III, IV, and V were brushed with the dentifrice slurries.

The SEM analysis was done and the results were: all the experimental agents, strontium chloride (Sensodyne original), potassium nitrate (Emoform), and sodium monofluorophosphate (Colgate maximum cavity protection) were effective in occluding dentinal tubules as compared with the control group. The percentage of occluded tubules was found to be highest for sodium monofluorophosphate as compared with the other groups over a period of 7 days. The occlusion of the tubules by potassium nitrate and strontium chloride showed comparable results over a period of 7 days.

Results were similar to those of Pusso-Carrasco, Hernandez in which they were effective in occluding dentinal tubules as compared with the control group and on contrary with the results of Blitzer.

CONCLUSION

The present *in vitro* SEM study results revealed that all the experimental agents, strontium chloride (Sensodyne original), potassium nitrate (Emoform), and sodium monofluorophosphate (Colgate maximum cavity protection) were effective in occluding dentinal tubules as compared with the control group. The percentage of

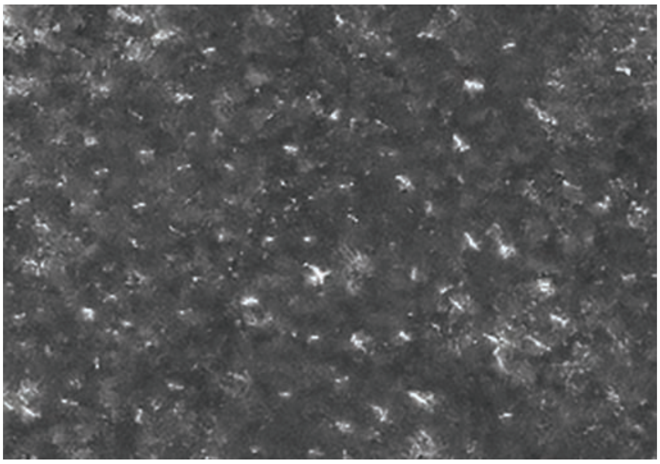


Fig. 14: Brushed with potassium nitrate-containing toothpaste

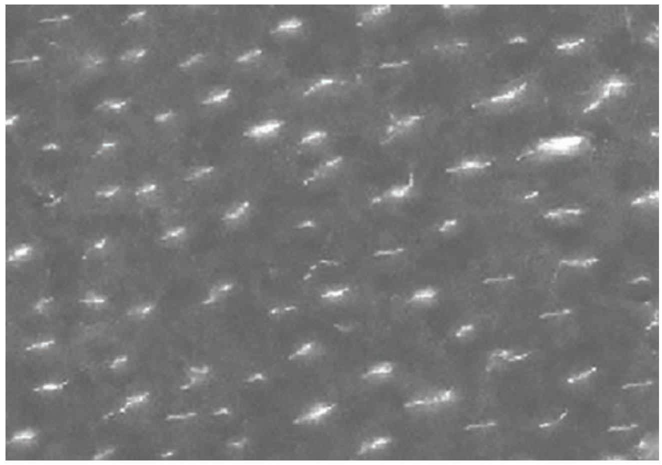


Fig. 15: Brushed with sodium monofluorophosphate-containing toothpaste

occluded tubules was found to be highest for sodium monofluorophosphate as compared with the other groups over a period of 7 days. The occlusion of the tubules by potassium nitrate and strontium chloride showed comparable results over a period of 7 days. However, further long-term *clinical* and *in vitro* studies are required to establish the role of these agents in reducing DH.

ACKNOWLEDGMENTS

The authors would especially like to acknowledge and thank Dr Chander Shekhar Joshi, Dr Ankita Gupta, and Dr Puja Jha for always being there to provide support, without which it would have been impossible to complete this work.

REFERENCES

1. Anand S, Anu Priya S, Raju A, Arun Priya S. Dentine hypersensitivity—an enigma? Indian J Dent Adv 2011;3(4):659-667.
2. Lavender SA, Petrou I, Heu R, Stranick MA, Cummins D, Sullivan RJ, Santarpia RP 3rd. Mode of action studies on a new desensitizing dentifrice containing 8.0% arginine, a high cleaning calcium carbonate system and 1450 ppm fluoride. Am J Dent 2010 May;23(Spec No A):14A-19A.
3. Lima RR, Araujo LM, Affonso PR, Maranhao KM, Antoniazzi JH, Lamarao SS. Scanning electron microscopic investigation of dentinal tubules in Cebus apella dentin. Ci Anim Bras 2009 Dec;10(4):1328-1331.
4. Nanci, A. Ten Cate's oral histology. 8th ed. St. Louis (MO): Elsevier; 2013. p. 194.
5. Bhandary S, Hegde MN. A clinical comparison of in-office management of dentin hypersensitivity in a short term treatment period. Int J Biomed Adv Res 2012;3(3):169-174.
6. Dowell P, Addy M. Dentine hypersensitivity—a review. Aetiology, symptoms and theories of pain production. J Clin Periodontol 1983 Jul;10(4):341-350.
7. Canadian Advisory Board on Dentin Hypersensitivity. Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. J Can Dent Assoc 2003 Apr;69(4):221-226.
8. Orchardson R, Gillam DG. Managing dentin hypersensitivity. J Am Dent Assoc 2006 Jul;137(7):990-998.
9. Chabanski MB, Gillam DG, Bulman JS, Newman HN. Prevalence of cervical dentine sensitivity in a population of patients referred to a specialist periodontology department. J Clin Periodontol 1996 Nov;23(11):989-992.
10. Gysi A. An attempt to explain the sensitiveness of dentine. Br Dent J 1900;43:865-868.
11. Brannstrom, M. A hydrodynamic mechanism in the transmission of pain-produced stimuli through the dentine. In: Anderson DJ, editor. Sensory mechanisms in dentine. Oxford: Pergamon; 1963. pp. 73-79.
12. Gillam DG, Orchardson R. Advances in the treatment of root dentine sensitivity: mechanisms and treatment principles. Endod Topics 2006 Mar;13(1):13-33.
13. Al-Tayeb D. Management of root-dentine hypersensitivity following non-surgical periodontal therapy: clinical and scanning electron microscopic study. Egypt Dent J 2008 Jul;54(3):1-12.
14. Miglani S, Aggarwal V, Ahuja B. Dentin hypersensitivity: recent trends in management. J Conserv Dent 2010 Oct;13(4):218-224.
15. Arrais CA, Micheloni CD, Giannini M, Chan DC. Occluding effect of dentifrices on dentinal tubules. J Dent 2003 Nov;31(8):577-584.
16. Ayad F, Ayad N, Zhang YP, DeVizio W, Cummins D, Mateo LR. Comparing the efficacy in reducing dentin hypersensitivity of a new toothpaste containing 8% arginine, Calcium carbonate and 1450 ppm Fluoride to a commercial sensitive toothpaste containing 2% potassium ion: an eight week clinical study on Canadian adults. J Clin Dent 2009;20(1):10-16.
17. Hsu KW, Marshall SJ, Pinzon LM, Watanabe L, Saiz E, Marshall GW. SEM evaluation of resin-carious dentin interfaces formed by two dentin adhesive systems. Dent Mater 2008 Jul;24(7):880-887.
18. Addy M, Urguhart E. Dentine hypersensitivity: its prevalence, aetiology and clinical management. Dent Update 1992 Dec;19(10):410-412.
19. Dababneh RH, Khouri AT, Addy M. Dentine hypersensitivity-an enigma? A review of terminology, mechanism, aetiology and management. Br Dent J 1999 Dec;187(11):606-611.