AN IN VITRO COMPARATIVE EVALUATION OF DENTINAL TUBULE OCCLUSION BY CURRENT DESENSITIZING AGENTS- A SCANNING ELECTRON MICROSCOPIC STUDY

Dilip Katakam[1], Sh. Priyadarshini[2], Divakar K. P[3], Sai Gowthami Mitta[4], T. Premlata Devi[5], Babina Chirom[6]

[1]Practitioner, Sri Sai Dental Hospital, Gudivala, Andhra Pradesh, India.
[2]Assistant Professor, Department of Conservative Dentistry and Endodontics, RIMS, Imphal, Manipur, India.
[3]Associate Professor, Department of Conservative Dentistry and Endodontics, ESIC Dental College, Gulbarga, Karnataka, India.
[4]Practitioner, Smile Dental Clinic, Vijayawada, Andhra Pradesh, India.
[5]Associat Professor, Department of Conservative Dentistry and Endodontics, RIMS, Imphal, Manipur, India.
[6]Assistant Professor, Department of Prosthodontics, Crown and Bridge and Implantology, RIMS, Imphal, Manipur, India.

ABSTRACT

BACKGROUND

Increasing life expectancy coupled with effective treatment options for dental caries and periodontal disease has enabled patients to retain their natural teeth for prolonged periods. Dentinal hypersensitivity is a very common clinical problem affecting the adult population.

Aims and Objectives- The aim of the present study was to compare the effects of topical application of three commercially available desensitizing products: nano-hydroxyapatite (Aclaim), Pro-Argin (Colgate Sensitive Pro-relief) and sodium fluoride with functionalized tricalcium phosphate (Clinpro) on dentinal tubule occlusion.

MATERIALS AND METHODS

Extracted human premolars were cut longitudinal to the tooth axis with a diamond disk and polished to obtain a flat surface with cleanly exposed dentinal tubules. Then the dentin samples were kept in a demineralising solution (Citric acid) for 40 mins. The teeth were randomly divided into three groups (n=10), according to the dentin surface treatments: Group A- Aclaim (Nano-hydroxyapatite); Group B- Colgate Sensitive Pro-relief (Pro-Argin); Group C – Clinpro (NaF with functionalized tricalcium phosphate). Brushings were done for 15 secs. twice daily for 1 week. In between all the samples were stored in artificial saliva. Then the samples were dried and observed under SEM.

RESULTS

The dentin desensitizing agents tested showed a wide range of dentinal tubule occlusion under SEM examination. Occlusion of dentinal tubules by Pro-Argin was comparatively superior to the other two agents.

CONCLUSION

The present study provides an understanding regarding the potential occluding effects of three desensitizing agents. It also explains the mechanisms of dentinal tubule occlusion by these three agents which may probably explain the reasons for varying results while treating dentinal hypersensitivity with these agents.

KEY WORDS

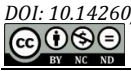
Dentinal Hypersensitivity (DHS), Dentinal Tubules, Dentinal Tubule Occlusion, Scanning Electron Microscope (SEM).

HOW TO CITE THIS ARTICLE: Katakam D, Priyadarshini S, Divakar KP, et al. An in vitro comparative evaluation of dentinal tubule occlusion by current desensitizing agents- a scanning electron microscopic study. J. Evolution Med. Dent. Sci. 2019;8(04):243-247, DOI: 10.14260/jemds/2019/53

BACKGROUND

Increasing life expectancy coupled with effective treatment options for dental caries and periodontal disease has enabled patients to retain their natural teeth for prolonged periods.[1-3] Dentinal hypersensitivity is a very common clinical problem affecting the adult population. By definition dentine hypersensitivity is "pain arising from exposed dentin, in response to external stimuli such as thermal, tactile, osmotic or chemical that cannot be explained by any other form of dental defect or pathology."[4]

'Financial or Other Competing Interest': None.
Submission 04-01-2019, Peer Review 22-01-2019,
Acceptance 24-01-2019, Published 28-01-2019.
Corresponding Author:
Dilip Katakam,
4-1-9, Parnapalli Road,
Pulivendula-516390, Kadapa District,
Andhra Pradesh, India.
E-mail: dilipkatakam@gmail.com
DOI: 10.14260/jemds/2019/53



Dentin exposure may be the result of attrition, abrasion, erosion or abfraction. Denudation of root surface due to gingival recession or non-surgical and surgical periodontal treatment can also cause Dentin Hypersensitivity.[5-10] The reported prevalence of dentine hypersensitivity varies from 8 to 57 percent.[11] Dentin hypersensitivity often occurs in patients in the age group of 30 to 40 years, but it may affect patients of any age. It affects women more often than men, though the gender difference is not statistically significant.[12] DHS most commonly occurs on the buccal aspect of permanent teeth near the CEJ, which is also the site that most frequently exhibits dentin exposure. With regard to tooth type, it has been reported that canines and premolars in either arch are the most frequently involved.[13-14] Dentine hypersensitivity is mostly seen in lower premolars followed by lower molars, upper premolars, lower incisors, upper canine, upper incisors, lower canines and upper molars.[15]

A number of theories have been proposed to explain dentin hypersensitivity. These include odontoblastic transduction theory, neural theory and hydrodynamic theory.

Of these, the hydrodynamic theory best explains the mechanism of dentin hypersensitivity (Brannstrom). According to this theory, external stimuli cause dentin fluid movement. This fluid movement results in short and sharp responses in the A delta fibers. The pain is generally transient in nature observed instantly after the stimulus but diminishing quickly thereafter.[16] In dentin hypersensitivity, the openings of dentinal tubules are patent thus promoting hydrodynamic changes to elicit a painful response. If functional radius of opened dentinal tubules decreases, permeability decreases thus reducing dentin hypersensitivity. Therefore, the treatment of dentin hypersensitivity should be directed towards occluding dentinal tubules as well as interference of nerve transmission.[17]

There a number of approaches that have been tried to manage DHS. For localized DHS, confined to a few teeth, in office approaches are popular. According to Pashley, products for in-office application are generally classified as those that do not polymerize, such as varnishes and precipitating agents, and those that undergo a setting reaction or polymerizing action, such as the conventional or resin-modified glass ionomer cements, and resinous adhesives. Among the substances indicated for these types of treatment are the oxalate, chloride and fluoride-based agents, either associated with iontophoresis, or not.

In clinical practice, generalized hypersensitivity is a frequent complaint of many patients. This is best managed by at home methods. Fluorides, stannous fluoride, potassium nitrate, mouth rinses, tooth paste are used. Many products have been used to occlude dentinal tubules with varying results.[17]

Presently several new technologies had been used to treat DHS are Mineral or ionic technologies: Fluoride; Amorphous calcium phosphate: CPP-ACP; Protein technology: Recaldent; NaF with Tricalcium phosphate technology: Clinpro; Pro-Argin technology: Colgate Sensitive Pro-relief; Novamin technology: Calcium sodium phosphosilicate and Nanotechnology: Nanohydroxyapatite.

The purpose of this study was to evaluate the ability of three current desensitizing agents Pro-Argin (Colgate Sensitive Pro-relief), NaF with tricalcium phosphate (Clinpro), Nanohydroxyapatite (Aclaim), on dentinal tubule occlusion using Scanning Electron Microscope.

MATERIALS AND METHODS

The present in vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Bangalore Institute of Dental Sciences and Research Institute in collaboration with Indian Institute of Medical Sciences, Bangalore, Karnataka. 30 human premolars extracted for orthodontic reasons were collected and sectioned longitudinally along the tooth axis with a diamond disk and polished to obtain a flat surface with cleanly exposed dentinal tubules (dentin samples). All the samples were ultrasonicated in distilled water to remove any residual smear layer. The samples were kept in a demineralizing solution (Citric Acid) for 40 min. Then all the samples were cleaned and randomly divided into 3 groups (A, B, C)-

Group A- Brushed with Nanohydroxyapatite (Aclaim) for 15 secs. twice daily for 1 week.

Group B- Brushed with Pro-Argin (Colgate sensitive Pro-relief) for 15 secs. twice daily for 1 week.

Group C- Brushed with NaF with tricalcium phosphate (Clinpro) for 15 secs. twice daily for 1 week.

In between all the samples were stored in artificial saliva. Finally, all the samples were dried and observed by Scanning Electron Microscopy. The total number of tubules and the number of occluded tubules were counted in each photomicrograph of all specimens. The statistical calculations were made to obtain the result. The surface was scanned at a magnification of 1000X. The percentage of occluded was calculated using the following equation. % of occluded dentinal tubules=number of occluded tubules x 100/ Total number of tubules

Statistics

The data was entered in a Microsoft Excel worksheet and analysed using IBM SPSS version 21.0. As the data was continuous, parametric tests were used for analysis. The statistical tests used were analysis of variance (ANOVA) with Tukey's post hoc test. The statistical significance was considered with α at 0.05.

RESULTS

In the present in vitro study, the dentin desensitizing agents tested showed a wide range of dentinal tubule occlusion, under SEM examination. The untreated areas presented a smooth appearance and open tubule orifices with smear layer (Fig. 1). Following demineralization, the smear layer was removed, and the tubules were widely opened (Fig. 2). A thin layer of covering was observed on the treated surfaces of samples with Group B (Pro-Argin) (Fig. 3a, 3b, 3c). Majority of dentinal tubules obliterated with a coat that covered the surface and infiltrated into the tubules. When compared to Group B (Pro-Argin) a less number of dentinal tubules were occluded with Group C (Clinpro) (4a, 4b). Group A (Aclaim) occluded less number of dentinal tubules when compared with other two desensitizing agents (5a, 5b). It was seen that mean percentage of occluded dentinal tubules of group A, B, C were 55.90, 69.21 and 61.84 respectively. The statistical significance found among the three groups were showed in Table 1 and 2. Graphical representation of the results was showed in Graph 1.

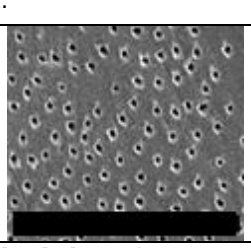


Figure 1. Dentinal Tubules Before Demineralisation Under SEM

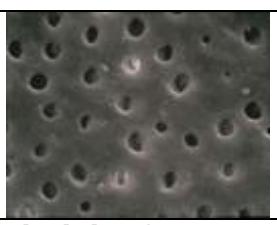


Figure 2. Dentinal Tubules After Demineralisation Under SEM

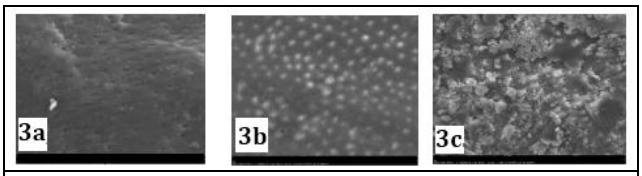


Figure 3a, Fig. 3b and Fig. 3c. Dentinal Tubules Occlusion Treated with Pro-Argin

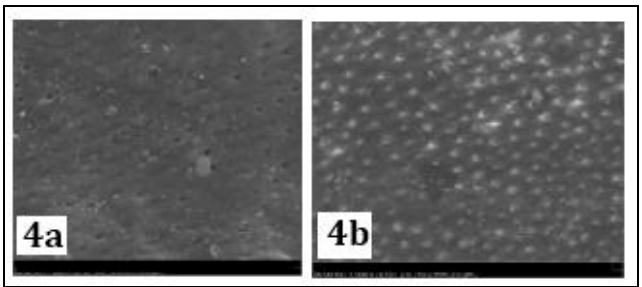


Figure 4a and Fig. 4b. Dentinal Tubules Occlusion Treated with Clinpro

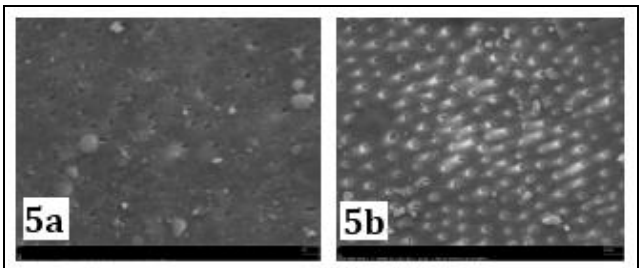


Figure 5a and Fig. 5b. Dentinal Tubules Occlusion Treated with Aclaim

Intergroup Comparison Using One-Way ANOVA					
Study Group	**N**	**Mean**	**Std. Dev.**	**ANOVA F Value**	**P value**
Group A	10	55.50	9.465	7.334	0.003*
Group B	10	69.21	8.437		
Group C	10	61.84	5.64		

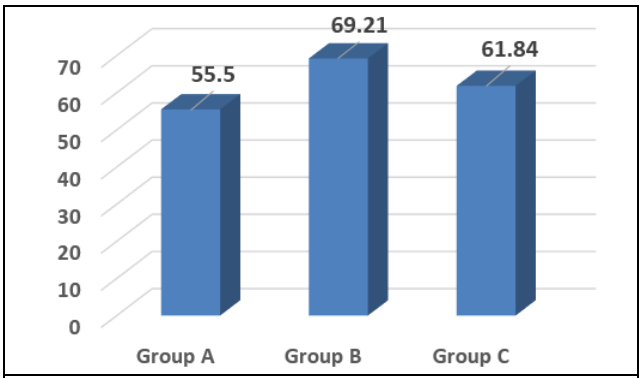
Table 1

*Statistically significant

Pair Wise Comparison Using Tukey's Post HOC Test			
Group I	**Group J**	**Mean Difference (I-J)**	**p-Value**
Group A	Group B	-13.71	0.003*
	Group C	-6.34	0.085
Group B	Group C	7.37	0.033*

Table 2

*Statistically significant



Graph 1. Mean of Occluded Dentinal Tubules on Dentin Surfaces After Brushing with Desensitizing Agents

DISCUSSION

Treating dentinal hypersensitivity can be challenging for the dental professional because of the difficulty related to measuring the pain response. There are two primary approaches in treating the dentinal hypersensitivity. They are interference of nerve transmission and occlusion of dentinal tubules. For interference of nerve transmission potassium salts are commonly used. Potassium depolarizes nerve fibers and interferes in the transmission of pain response. The main disadvantage is that the relief is neither immediate nor long lasting. The reason for this is twofold. First, the potassium ions must diffuse through the dentin tubules against a positive flow of dentin fluid. Second, these ions must build up and remain at elevated concentrations in order for the nerve fibres to remain depolarized, and this build up takes time. Further, if the treatment is discontinued, concentrations of potassium around the nerve fibres diminish and sensitivity relief is lost. Hence this method is not popular while treating dentinal hypersensitivity[18]. The second approach in treating dentinal hypersensitivity is occlusion of open dentinal tubules. For occlusion of dentinal tubules several desensitizing agents are available. Treatment can be applied by the dentist in the dental office or self – administered by the patient at home. In-office treatments are more complex and generally target dentinal hypersensitivity localized to one or a few teeth. At home methods are simple, inexpensive and can treat simultaneously generalized dentinal hypersensitivity affecting many teeth. They include agents in the form of gels, dentifrices, mouthwashes.

The aim of the present study was to evaluate the efficacy of three current desensitizing agents Pro-Argin (Colgate Sensitive Pro-relief), NaF with functionalized tricalcium phosphate (Clinpro), Nano Hydroxyapatite (Aclaim), on dentinal tubule occlusion using scanning electron microscope.

In the present SEM study Group B samples (Pro-Argin) showed more number of dentinal tubule occlusions when compared with Group C (Clinpro) and Group A samples.

The combination of arginine, an amino acid found in saliva, and calcium carbonate comprises the new Pro-Argin technology. State-of-the-art surface techniques and in vitro studies demonstrate that a new Pro-Argin desensitizing dentifrice with a whitening benefit containing 8.0% arginine, a high cleaning calcium carbonate system and sodium monofluorophosphate effectively occlude dentin tubules. These results are in complete agreement with similar studies conducted with currently marketed sensitivity products with the breakthrough Pro-Argin technology.[19] Arginine binds to the tooth surface and allows the calcium carbonate to slowly dissolve and release calcium that is readily available to re-mineralize the tooth surface. This method uses the natural attributes of saliva through the re-mineralization of tooth structure by consistently delivering calcium and phosphate into dentin tubules. Pro-Argin is a desensitizing dentifrice containing 8% arginine, a high cleaning calcium carbonate system and sodium monofluorophosphate which effectively occlude dentinal tubules. The combination of arginine and calcium carbonate acts by forming a plug of calcium and phosphate that occludes dentinal tubules. The positively charged arginine is attracted to the negatively charged surface where it helps attract and adhere calcium carbonate to dentin surface and deep into dentinal tubules

(Klienberg).[19] The association of arginine and calcium carbonate in situ provides an alkaline environment which encourages endogenous calcium and phosphate ions to deposit and further plug and occlude dentin tubules.[20] Studies by Lavender, Cummins and Wang et.al employing CLSM, SEM and AFM have provided insight into the mechanism of action of Pro-Argin technology.[21] In our study the dentin samples treated with Pro-Argin showed substantial crystal like deposits occluding and sealing the opened dentinal tubules. Similar reports by Cummins in his study in which he revealed that arginine provides an impervious seal deep within the dentinal tubule providing a robust barrier against fluid movement within the tubules.[19]

The main component of dentin is calcium phosphate, it is natural that the dentinal tubules be occluded with this mineral. Hydroxyapatite [HAP: $Ca_{10}(PO_4)_6$ (OH)] and tricalcium phosphate [TCP: $Ca_3(PO_4)_2$] are well-known bioactive materials, and their excellent biocompatibility with both hard and soft tissues. Treatment for dentin hypersensitivity based on calcium phosphate precipitation, has been reported (Imai and Akimoto, 1990). Laboratory and clinical studies have demonstrated that the combination of fluoride and functionalized β-tricalcium phosphate (fTCP) produces stronger, more acid-resistant mineral relative to fluoride, native β-TCP, or fTCP alone. In contrast to other calcium-based approaches that seem to rely on high levels of calcium and phosphate to drive re-mineralization, fTCP is a low dose system designed to fit within existing topical fluoride preparations. The functionalization of β-TCP with organic and/ or inorganic molecules provides a barrier that prevents premature fluoride-calcium interactions and aids in mineralization when applied via common preparations and procedures. Clinpro contains 950 ppm fluoride with an innovative functionalized tricalcium phosphate ingredient. The combination is designed to make fluoride, phosphate, and calcium ions available to the teeth, which may aid in sensitivity reduction. During manufacturing process, a protective barrier is created around calcium allowing it to co-exist with fluoride ions. During brushing, the toothpaste comes in contact with saliva, the barrier breaks down and makes the calcium, phosphate and fluoride readily available to teeth [22]. In the present study, dentin samples treated with Clinpro showed more number of dentinal tubule occlusion while leaving some dentinal tubules opened. Calcium in Clinpro increases the likelihood of forming calcium fluoride globules on the tooth surface. The presence of calcium fluoride can occlude dentinal tubules to reduce DHS.

The recent trend in biomaterials research is focused on nanotechnology. The search for an agent that would predictably and permanently occlude the tubules and blend with them has prompted the use of HAP which is the principal constituent of tooth. Hydroxyapatite is used in toothpaste for re-mineralization of enamel. With their high area to volume ratio, nano-sized HA particles are expected to be excellent materials for these applications. The nanoparticles of hydroxyapatite present a higher bioactivity, due to its small diameter and morphology which increases its superficial area. Its capacity of hydration and wettability allow it to release calcium and phosphate to tooth in adequate concentration. In the present study the dentin samples treated with NanoHAP also showed occlusion of dentinal tubules but in less number. In vitro tests had been shown the application of NanoHAP results in the deposition of a thick layer of occlusive hydroxyapatite inside the dentinal tubules. This deposit produces a uniform and impermeable film on the dentin, hindering the access of external stimuli to the pulp and thereby eliminating the symptoms.

The present study has been limited to two-week application (Twice daily) of the desensitizing agents on the prepared dentinal samples. Most of the dentinal tubules are occluded and if the application period is increased there are chances of occluding more no. of dentinal tubules.

CONCLUSION

This present SEM examination provided understanding of potential occluding effect of three desensitizing agents. The different mechanisms of dentinal tubule occlusion probably result in different effectiveness of hypersensitivity reduction. The results of the present study confirmed by SEM analysis of dentin samples, demonstrated that majority of dentinal tubules were obliterated by the dentin samples of Group B (Pro-Argin) followed by Group C (Clinpro) and Group A (Aclaim). However further research is required to provide evidence of durability of occlusion of these desensitizing agents under simulated clinical conditions.

REFERENCES

[1] Rees JS, Addy M. A cross-sectional study of dentin hypersensitivity. J Clin Periodontology 2002;29(11):997-1003.
[2] Ritter AV, De Dias LW, Miguez P, et al. Treating cervical dentin hypersensitivity with fluoride varnish: a randomized clinical study. J Am Dent Assoc 2006;137(7):1013-20, quiz 1029.
[3] Pinto SC, Pochapski MT, Wambier DS, et al. In vitro and in vivo analyses of the effects of desensitizing agents on dentin permeability and dentinal tubule occlusion. J Oral Sci 2010;52(1):23-32.
[4] Fu Y, Li X, Que K, et al. Instant dentin hypersensitivity relief of a new desensitizing dentifrice containing 8.0% arginine, a high cleaning calcium carbonate system and 1450 ppm fluoride: a 3-day clinical study in Chengdu, China. Am J Dent 2010;23(Spec No A):20A-7A.
[5] Ahmed TR, Mordan NJ, Gilthorpe MS, et al. In vitro quantification of changes in human dentin tubule parameters using SEM and digital analysis. J Oral Rehabil 2005;32(8):589-97.
[6] Wara-Aswapati N, Krongnawakul D, Jiraviboon D. The effect of a new toothpaste containing potassium nitrate and triclosan on gingival health, plaque formation and dentine hypersensitivity. J Clin Periodontol 2005;32(1):53-8.
[7] Brannstrom M, Linden LA, Astrom A. The hydrodynamics of the dental tubule and of pulp fluid. A discussion of its significance in relation to dentinal sensitivity. Caries Res 1967;1(4):310-7.
[8] Pashley DH, O'Meara JA, Kepler EE, et al. Dentin permeability. Effects of desensitizing dentifrices in vitro. J Periodontol 1984;55(9):522-5.
[9] Arrais CA, Micheloni CD, Giannini M, et al. Occluding effect of dentifrices on dentinal tubules. J Dent 2003;31(8):577-84.

[10] Leme PAF, Dos Santos JC, Giannini M, et al. Occlusion of dentin tubules by desensitizing agents. Am J Dent 2004;17(5):368-72.
[11] Irwin CR, McCusker P. Prevalence of dentin hypersensitivity in a general dental population. J Ir Dent Assoc 1997;43(1):7-9.
[12] Orchardson R, Gillam DG. Managing dentin hypersensitivity. J Am Dent Assoc 2006;137(7):990-8, quiz 1928-9.
[13] Addy M, Mostafa P, Newcombe RG. Dentin hypersensitivity: the distribution of recession, sensitivity and plaque. J Dent 1987;15(6):242-8.
[14] Flynn J, Galloway R, Orchardson R. The incidence of 'hypersensitivity' teeth in the west of Scotland. J Dent 1985;13(3):230-6.
[15] Brugnera A, Garini AE, Pinheiro A. Laser therapy in the treatment of dental hypersensitivity - a histologic study and clinical application. Laser Therapy 2000;12(Spe Mil Edi):6-21.
[16] Bartold PM. Dentinal hypersensitivity: a review. Aus Dent Jour 2006;51(3):212-8, quiz 276.
[17] Arrais CA, Chan DC, Giannini M. Effects of desensitizing agents on dentinal tubule occlusion. J Appl Oral Sci 2004;12(2):144-8.
[18] Cummins D. Recent advances in dentin hypersensitivity: clinically proven treatments for instant and lasting sensitivity relief. Am J Dent 2010;23(Spec No. A):3A-13A.
[19] Lavender SA, Petrou I, Heu R, et al. 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;23(Spec No. A):14A-9A.
[20] Kleinberg I. SensiStat. A new saliva-based composition for simple and effective treatment of dentinal sensitivity pain. Dent Today 2002;21(12):42-7.
[21] Petrou I, Heu R, Stranick M, et al. A breakthrough therapy for dentin hypersensitivity: How dental products containing 8% arginine and calcium carbonate work to deliver effective relief of sensitive teeth? J Clin Dent 2009;20(1):23-31.
[22] Reynolds EC. Calcium phosphate – based re-mineralization systems: scientific evidence? Aust Dent J 2008;53(3):268-73.