ORIGINAL RESEARCH

Novel Management of Hypersensitive Dentin Using Propolis-based Herbal Desensitizing Agents: An *In Vitro* Scanning Electron Microscopic Study

Sabari Murugesan[1], Prasanna Kumar[2], Bharath Naga Reddy[3], Karthikeyan Arumugam[4], Purushotham Mohankumar[5], Karthik Chandrasekaran[6]

Abstract

Aim: The objective of this *in vitro* study was to assess the efficacy of novel propolis-based varnish against the two conventional varnishes on quantitative and qualitative assessments of occlusion of dentin tubules and resistance to erosive and abrasive wears employing scanning electron microscope (SEM).

Methods: Thirty human premolars free from caries extracted due to orthodontic reasons were included in the study. Experimental group was done based on treatment received and divided into three groups. Group A: ClinProXT Varnish ($n = 10$), Group B: MI Varnish ($n = 10$), and Group C: Propolis Varnish ($n = 10$) were applied. Teeth were cleaned and decoronation of crown was done with dentin disks. Dentin specimens of dimension $4 \times 4 \times 2$ mm were prepared and subjected to finishing and polishing. The sample specimens were submersed in EDTA solution for a period of five minutes to open up the dentinal tubules. This was followed by treatment with varnishes and subjection to acidic-abrasive challenge. The specimens were analyzed with an image analyzer connected to SEM for the verification of the number of opened dentin tubules. The parameter assessed in SEM includes size, topography and surface characteristics of dentinal tubule were assessed. The obliteration potential of dentinal tubules was assessed with SEM images. Additionally, the dentin surface loss and resistance to acidic and abrasive wear were also evaluated with SEM. Data were analyzed with two-way analysis of variance (ANOVA) with post hoc Tukey's test.

Results: MI Varnish caused higher obliteration of dentin tubules followed by ClinproXT Varnish. Propolis Varnish showed the least obliteration of dentinal tubules among tested experimental groups. After acidic-abrasive challenge, Propolis Varnish was found to be more efficient with less material loss among the experimental groups tested. There was an insignificant difference among the MI Varnish and ClinProXT Varnish groups.

Conclusion: Simulation of hypersensitive lesions mimicking the clinical scenario was a challenging task in this *in vitro* study. All varnishes tested in the study had good efficacy in the management of dentin hypersensitivity (DH). Propolis-based varnish had good resistance to material loss after subjection to acidic-abrasive challenge among the tested materials. The casein phosphopeptide (CPP)-amorphous calcium phosphate (ACP)-based MI Varnish had good efficacy to obliterate the dentinal tubules among the tested materials. It was prudent to select the varnishes with good long-term efficacy to survive in the clinical scenario which still remains a challenging task for the clinicians.

Clinical significance: The stability of the varnish plays a vital role in maintenance of its long-term efficacy. The chemical nature along with the ability of the material to interact with the substrate plays a major role in management of DH.

Keywords: Casein phosphate, Dental varnish, Dentinal tubules, Propolis, Scanning electron microscope, Tricalcium phosphate.

The Journal of Contemporary Dental Practice (2021): 10.5005/jp-journals-10024-3154

Introduction

Dentin hypersensitivity is defined as a painful response of the exposed dentin to various stimuli such as thermal, evaporative, tactile, osmotic, and chemical stimuli. It is a recurrent condition associated with discomfort and pain and defers the patient from maintaining adequate oral hygiene. Breach in protection offered by enamel or cemental layer or in combination with the following triggering factors such as acidogenic diets, destructive habits, improper toothbrushing techniques, and noncarious lesions plays a major role in pathogenesis of hypersensitive lesions.[1]

Dentin hypersensitivity occurs in a phasic manner and begins with lesion localization and initiation that was best explained by hydrodynamic theory proposed by Branstrom. It was evidenced by him that the maneuver that causes a reduction in a fluid flow toward the dentinal tubules had higher efficacy in the management of hypersensitive lesions.[2]

The material-related factors that play a vital role in the management of dentin hypersensitivity (DH) include the type of material, composition, reaction potential to the substrate, contact

[1]Department of Restorative Dental Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia

[2,4]Department of Conservative Dentistry and Endodontics, Sri Venkateshwara Dental College, Puducherry, India

[3]Department of Conservative Dentistry and Endodontics, Adhiparasakthi Dental College and Hospital, Chennai, Tamil Nadu, India

[5]Department of Conservative Dentistry and Endodontics, Sathyabama Dental College and Hospital, Chennai, Tamil Nadu, India

[6]Department of Prosthodontics, Adhiparasakthi Dental College and Hospital, Chennai, Tamil Nadu, India

Corresponding Author: Bharath Naga Reddy, Department of Conservative Dentistry and Endodontics, Adhiparasakthi Dental College and Hospital, Chennai, Tamil Nadu, India, Phone: +91 9444792260, e-mail: drbharathendo999@gmail.com

How to cite this article: Murugesan S, Kumar P, Reddy BN, *et al.* Novel Management of Hypersensitive Dentin Using Propolis-based Herbal Desensitizing Agents: An *In Vitro* Scanning Electron Microscopic Study. J Contemp Dent Pract 2021;22(9):1030–1034.

Source of support: Nil

Conflict of interest: None

time, surface tension, substantivity, and release of material to the surrounding media.

The treatment strategies advocated for the management of DH in the literature include desensitization of nerve endings, formation of a mechanical barrier on the dentin surface, induction of dentinal tubule obliteration by the formation of crystalline material, and iontophoresis. The component of materials presents in varnish and the mode of delivery of material has an impact on the outcome of DH management.[3]

The parameters that govern the outcome of DH can be categorized as material-related and clinical factors. The mechanism which reduces the interaction between dentinal tubules and external stimuli plays an vital role in the management of hypersensitive lesions.

The efficacy of materials in the management of DH reason depends upon (a) capability and type of protective layer formed on the dentinal tubules (b) type and size of crystalline material formed inside the dentinal tubules, and (c) stability of varnish materials on subjection to acidic-abrasive challenge mimicking the clinical scenario. The film thickness and consistency of the material employed play a major role in the management of DH.

The disadvantages associated with the traditional material have made us to explore the efficacy of herbal-based materials in the management of DH.

Propolis-based varnishes were originally extracted from honeybees. It has been explored for its biological and antimicrobial activity. Ethanol Propolis extract acts as its main ingredient (45–65%), carboxymethyl cellulose, acetic acid, and fluoride serve as minor ingredients.[4]

The MI is a novel biomimetic varnish that delivers bioavailable calcium and phosphate ions into the saliva. It contains 5% NaF and a novel combination of casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP). The casein peptide has a potential to easily bind to teeth. It improves the stability of ACP and aids in the remineralization of tooth substrate.[5]

There is less literature evidence on the assessment of effectiveness of herbal-based Propolis Varnish on the dentin surface protection. This was evaluated by the assessment of residual material thickness formed on the dentin substrate. Mitra et al. evidenced that ClinproXT Varnish had less efficacy to protect the dentinal tubules after the acidic-abrasive challenge.[6]

The objective of this *in vitro* study was to assess the efficacy of novel propolis-based materials against the two conventional varnishes namely ClinProXT- and MI-based varnishes on the occlusion of dentin tubules and resistance exhibited to the acidic and abrasive wear employing scanning electron microscope (SEM).

The null hypotheses tested were the following: (1) the obliteration potential of the varnishes tested remains the same after the application of varnish, (2) the obliteration capacities of the varnishes tested are equal after subjection to acidic-abrasive challenge, and (3) the dentin protection ability does not vary among the tested varnishes.

Materials and Methods

This study was carried out in the Department of Conservative Dentistry and Endodontics after the approval from the Ethical Committee. Thirty human premolars free from caries extracted due to orthodontic reasons were considered as a major inclusion criterion. This study followed a complete randomized design with the categorization of tested materials into three groups based on the treatment received with a sample size of 10 specimens for each group. The efficacy of three desensitizing agents namely resin modified GIC-based ClinProXT Varnish, propolis-based varnish (Y beePharma), CPP-ACP-based MI Varnish [3M] were analyzed in this *in vitro* study.

Sample Preparation

The teeth were subjected to cleaning and disinfection protocols employing pumice and Gracey curettes. Decoronation of teeth was done using water-cooled diamond disks. Human dentin samples of 4 mm width, 4 mm breadth, and 2 mm depth were subjected to final finishing and polishing protocols.

Treatment Protocol

Initial step in the study was to subject the specimens to EDTA solution. This was followed by the application of varnish as per the manufacturer's instruction. The specimens were covered with adhesive tape to prevent the premature contact of varnishes.

A central window of 5 mm width and 3 mm breadth was left uncovered to control the delivery of varnish into a specific area.

Erosive Challenges of Protocols

The experimental groups were subjected to acidic-abrasive challenge proposed by Scaramucci et al.[7] The aidic challenge was stimulated with 0.3% citric acid for the duration of 2 minutes and exposed to periodicity of four times a day. The specimens were placed in artificial saliva for a duration of 1-hour period to resemble the oral environment. Three-body abrasive challenges were done with standardized protocols employing an electronic toothbrush and dentifrice twice daily for a period of 30 seconds.

Scanning Electron Microscopic Evaluation

The status and the patency of dentinal tubules were assessed at three different levels. Initial assessment was done after EDTA application to qualitatively evaluate the status of dentinal tubule morphology at 2000 × magnification in the center of each specimen as represented in Figure 1. Second assessment was done after the application of varnish appreciated by the layer of material on the dentinal tubules. The obliteration of dentinal tubule was also evaluated and represented in Figure 2. Final assessment was done after the acidic-abrasive challenge to check for dentin protection and obliteration

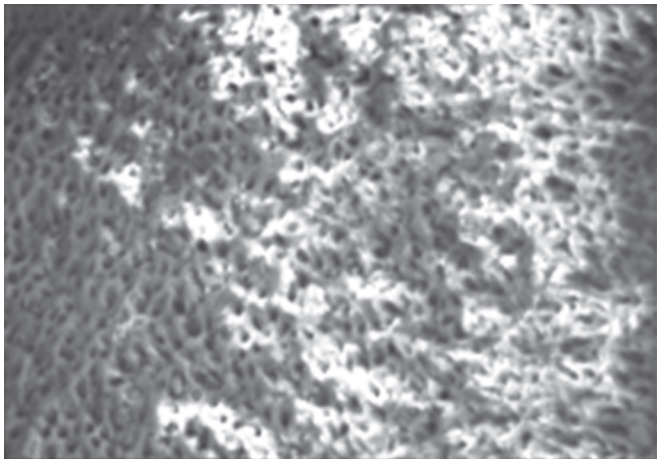

Fig. 1: Representation of the image at the magnification of 2000 × after pretreatment with EDTA reveals opening of dentinal tubules for Propolis Varnish group

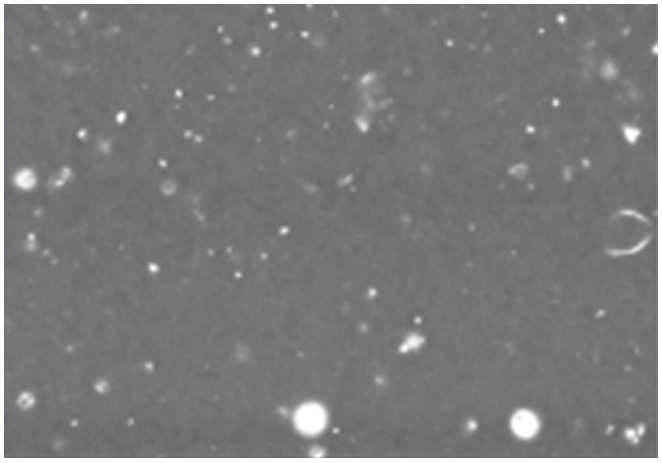

Fig. 2: Representation of the image at the magnification of 2000 × after application with Propolis Varnish group reveals the presence of dense material over the dentinal tubules

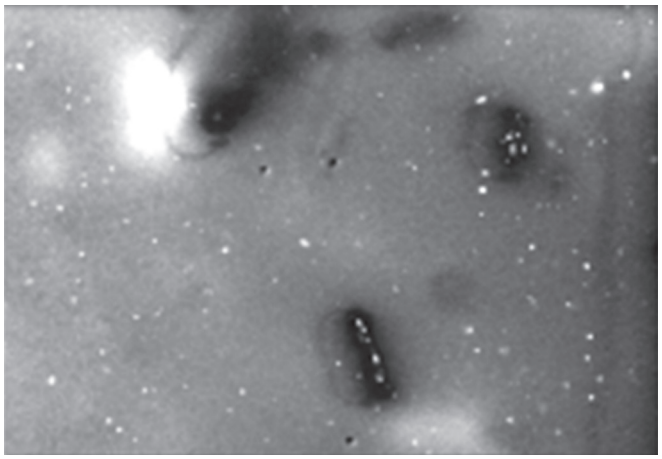

Fig. 3: Representation of the image at the magnification of 2000 × after subjection to acidic-abrasive challenge reveals patent dentinal tubules with less obliteration for Propolis Varnish group

Table 1: Descriptive analysis of study group in the study population ($N = 30$)

Study group	*Frequency*	*Percentages*
ClinProXT Varnish	10	33.33%
MI Varnish	10	33.33%
Propolis Varnish	10	33.33%

Table 2: Comparison of the mean after EDTA treatment across the study groups ($N = 30$)

Study group	*Mean ± SD after EDTA treatment*	*Mean difference*	*95% CI*		*p value*
			Lower	*Upper*	
ClinProXT Varnish	11.24 ± 0.94		Baseline		
Propolis Varnish	12.05 ± 0.54	0.71	−1.23	−0.32	0.003
MI Varnish	12.29 ± 0.62	1.05	−1.73	−0.38	0.004

Table 3: Comparison of the mean after treatment across the study groups ($N = 30$)

Study group	*Mean ± SD after treatment*	*Mean difference*	*95% CI*		*p value*
			Lower	*Upper*	
Propolis Varnish	0.29 ± 0.03		Baseline		
MI Varnish	0.3 ± 0.03	0.01	−0.21	0.23	0.925
ClinProXT Varnish	2.7 ± 0.41	2.41	−2.63	−2.20	<0.001

Table 4: Comparison of the mean after cycling across the study groups ($N = 30$)

Study group	*Mean ± SD after cycling*	*Mean difference*	*95% CI*		*p value*
			Lower	*Upper*	
Propolis Varnish	2.44 ± 0.57		Baseline		
MI Varnish	5.99 ± 0.56	3.54	−3.98	−3.10	<0.001
ClinProXT Varnish	5.08 ± 0.24	2.64	−3.08	−2.19	<0.001

of dentinal tubule as represented in Figure 3. Additionally, tubule status was assessed with customized software connected to SEM.

Statistical Analysis

The study groups comprised of ClinProXT, MI, and Propolis varnishes were considered as primary explanatory variables. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. For normally distributed quantitative parameters, the mean values were compared between the study groups using the ANOVA (>2 groups). If a statistically significant difference was found in ANOVA, an appropriate post hoc test (LSD) was used to assess the statistical significance of pairwise comparisons. *p* value <0.05 was considered statistically significant.

Results

A total of 30 specimens were included for the experimental analysis. Table 1 represents the descriptive analysis of the study group. Ten (33.33%) samples were treated with Clinpro XT Varnish, while another 10 samples (33.33%) were treated with MI, and the remaining final 10 samples (33.33%) were treated with Propolis Varnish.

Table 2 denotes the comparative mean after EDTA treatment across the study groups ($N = 30$). The mean value was 11.24 ± 0.94 in Propolis Varnish, 12.05 ± 0.62 in MI, and 12.29 ± 0.6 in ClinProXT Varnish. The mean after EDTA application was statistically significant with *p* value <0. 001 in Propolis Varnish. There was no statistically significant mean difference efficacy between MI and Propolis Varnish with *p* value 0.496.

There is no statistically significant mean difference after EDTA treatment between MI and Propolis Varnish with *p* value 0.496.

Table 3 represents the comparative mean after treatment across the study groups ($N = 30$). The mean after treatment was 0.29 ± 0.03 in Propolis Varnish, 0.3 ± 0.03 in MI, and 2.7 ± 0.41 in ClinProXT Varnish. The mean after treatment was not statistically significant with *p* value 0.925 in MI and was statistically significant with *p* value <0.001 in Propolis Varnish. There is a statistically significant mean difference after treatment between MI and Propolis Varnish with *p* value <0.001.

Table 4 represents the comparative mean after cycling across the study groups ($N = 30$). The mean after cycling treatment was 2.44 ± 0.57 in Propolis Varnish, 5.99 ± 0.56 in MI, and 5.08 ± 0.24 in ClinProXT Varnish. The mean after cycling treatment was not found to be statistically significant in MI Varnish. The mean after cycling treatment was statistically significant with p value <0.001 in Propolis Varnish. There is a statistically significant mean difference after cycling treatment between MI and Propolis Varnish with p value <0.001.

Discussion

Management of hypersensitive dentin remains a nightmare for the restorative dentist due to involvement of tricky components in the selection of the materials. Due to the advances in material, chemistry has thrown us a light to identify novel strategies for the management of DH lesions.

Conventional varnishes used for the management of DH had some inherent deficiency in the formation of dentin-protective adhesive layer related to the viscoelastic behavior and the handling characteristics of materials without altering the substrate characteristics, especially after the acidic-abrasive challenge.

The aim of this *in vitro* study was to assess the efficacy of novel herbal-based varnish subjected to erosive/abrasive challenge. In the present study, all desensitization agents had the potential to form a mechanical barrier inside/over the dentinal tubules for a short-term period; as regards, the first null hypothesis was found to be applicable.

The results presented by the Propolis group reveal that it had good efficacy after subjection to acidic-abrasive challenge among the tested groups; as regards, the second hypothesis was not accepted.

Lan et al. stated that the obliteration capacity, dentin surface characteristics, and varnish protection ability play a vital role in the management of DH. The dentinal tubule opening was considered as an important assessment parameter to test the efficacy of varnishes after the application of therapeutic agents. Additionally, the resistance offered to the erosion-abrasive challenge was included as an evaluation parameter for the outcome of DH management.[8]

The efficacy of ClinProXT Varnish was evaluated employing an erosion-abrasion model for the assessment of dentin tubule occlusion and dentin protection offered by varnish and found that ClinProXT Varnish had good short-term efficacy while the long-term efficacy still remains a major challenge. The efficacy of ClinProXTV has been explained by the action of its component that interacts chemically with the inorganic component of the dentin and is found to be superior to the other varnishes tested by Garofalo et al.[9]

The strategies related to the management of DH were best explained by Pereira et al.[10] Canali et al. have evidenced that ClinProXT is a resin-modified glass incomer-based varnish that releases fluoride, calcium, and phosphate. It releases more amount of fluoride in the first 24 hours.[11]

The role of Propolis in the management of dental caries and its antimicrobial properties has been explored in the previous literature and found to be satisfactory, and it has intended us to test the management of hypersensitivity.

Propolis-based herbal varnish offers a therapeutic benefit with less adverse effects on the dentinal substrate. The lack of material loss by Propolis after exploration to acidic-abrasive challenge might be related to the existence of adhesive layer formed by the strong interaction between the Propolis and dentin substrate. Additionally, it promotes a physical barrier at the dentin surface.

Lynch et al. proposed that the presence of calcium glycerophosphate in ClinproXTV Varnish controls the remineralization potential by the release of calcium and fluoride to the surrounding medium in response to the acidic challenge that provides a unique action.[12]

Virupaxi et al. evaluated the release of fluoride in XTV for a period of 6 months and found that fluoride release was high in a week time, and the value dropped to almost half in a month time and attained stability over a 6-month period related to the saturation kinetics.[13]

Zhou et al. observed that the demineralization potential for XTV was due to the release of calcium and phosphate present inherently when subjected to an erosive challenge and also reduced the pace of demineralization.[14]

The other mechanism to reduce the hypersensitivity includes the formation of adhesive layer over the dentinal tubules and reducing the interaction between the stimulus and the substrate, and this property can be measured by counting the number of open dentinal tubules.

The thickness of adherent layer formed by the varnish depends predominantly on the type of material used along with flow-related and material-related parameters.

Another experimental varnish evaluated in this study was a biomimetic varnish which is based on CPP-ACP combination found to be effective in the obliteration of dentin tubules.

The biomimetic varnish used in the current study has good remineralization property which might be related to nanoapatite crystal formation and consistency of the material. This material had ideal viscoelastic properties. The crystalline material formed resisted the acidic challenge effectively which can be revealed by the presence of adherent material over the dentin surface in the SEM images.

Purra et al. revealed that the flavonoids are the main active components capable of stimulating reparative dentin formation. Propolis is shown to be capable of obliterating the dentinal tubules in the previous study. Immediate relief was attributed to the tubular sealing action of flavonoids and the persistent effect because of the stability of the products formed.[15]

The study proved that the propolis-based varnish had a good adherent layer which resisted the acidic challenge and thus was found to be more effective in the reduction of interaction between the substrate applied and acidic challenge. The obliteration capacity of this group was also evidenced in the SEM images.

Hussain et al. evaluated the efficacy of Propolis extracts on the post-bleaching hypersensitivity in an *in vivo* study and confirmed that the application of Propolis has been found to be beneficial in the reduction of dentin hypersensitivity.[16]

Koosik et al. analyzed the microhardness of remineralization agents and evidenced that MI-based varnish had the potential to increase the microhardness of enamel substrate and mineralization of incipient carious lesions.

In this study, MI Varnish was found to have a good obliteration potential when compared to the other varnishes tested in this study which acts predominantly by plugging the dentinal tubules and thus reducing dentin sensitivity.

There was not much difference found between MI Varnish and Propolis after erosive cycling. The major obstacles in the management of DH were due to controlling the interaction

between the dietary acids and the dentinal tubules which changes their surface characteristics that lead to a reduction in the efficacy of tested varnishes.

After subjection to acid-abrasion protocol, it was revealed that the dentin protection offered by propolis-based varnish was found to be more effective than MI Varnish and ClinProXT Varnish; additionally, it was also revealed that the obliteration of dentinal tubules was found to be higher in MI Varnish comparable to that of ClinProXT- and propolis-based varnishes.

Limitations of the Study

Simulation of clinical conditions remains challenging. The application of the desensitizer agents was done in accordance with the manufacturer's instructions. The variations in the presentation of results might be attributed to the difference in the quality of the adhesive layer formed by the experimental groups. Additionally, the viscoelastic behavior and handling characteristics have a major influence on the efficacy. The studies in future should be directed to analyze the chemical composition of the materials formed to throw light on understanding the long-term efficacy.

Conclusion

Simulation of hypersensitive lesions mimicking the clinical scenario was a challenging task in this *in vitro* study. All varnishes tested in the study had good efficacy in the management of DH. Propolis-based varnish had good resistance to material loss after subjection to acidic-abrasive challenge among the tested materials. The CPP-ACP-based MI Varnish had good efficacy to obliterate the dentinal tubules among the tested materials. It was prudent to select the varnishes with good long-term efficacy to survive in the clinical scenario which still remains a challenging task for the clinicians.

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