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Evaluation of Dentinal Tubule Occlusion Using Propolis and Chitosan under the Scanning Electron Microscope: An In-Vitro Study.

[1]Dr. Ashish Jain, Department of Conservative Dentistry and Endodontics, Reader, PMVIDS, Hyderabad, India.

[2]Dr. P. Karunakar, Department of Conservative Dentistry and Endodontics, HOD and Principal, PMVIDS, Hyderabad, India

[3]Dr. Raji viola Solomon, Department of Conservative Dentistry and Endodontics, Professor, PMVIDS, Hyderabad, India.

[4]Dr. Umrana faizuddin, Department of Conservative Dentistry and Endodontics, Professor, PMVIDS, Hyderabad, India.

[5]Dr. A. Sarika, Department of Conservative Dentistry and Endodontics, PG student, PMVIDS, Hyderabad, India.

[6]Dr. R. Deepika, Department of Conservative Dentistry and Endodontics, PG student, PMVIDS, Hyderabad, India.

Corresponding Author: Dr. A. Sarika, Department of Conservative Dentistry and Endodontics, PG student, PMVIDS, Hyderabad, India.

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Abstract

Aim: To evaluate the dentinal tubule occlusion using propolis and chitosan.

Methods and Material: In this study, 50 caries-free extracted molar teeth were selected and stored in artificial saliva. Later, dentinal disks were prepared from the samples of size 3*3 and divided into following groups, Control group (n=10); Group 1- Propolis(P) (n=10); Group 2-Chitosan(C) (n=10). Group 3-Propolis with citric acid (PA) (n=10); Group 4-Chitosan with citric acid (CA) (n=10). These samples were treated with respective agents after conditioning with phosphoric acid. Later, groups 3 and 4 were subjected to citric acid. All the samples were observed under a scanning electron microscope and the data collected was statistically analysed.

Statistical analysis and Results: A significant difference was evident between the groups ($p<0.001$), hence individual group comparison was done using Kruskal Wallis ANOVA with post-hoc Conover test showing that Chitosan with citric acid (CA) had shown greater dentinal tubule occlusion than other comparative groups.

Conclusions: According to this study, chitosan with citric acid (CA) has shown greater dentinal tubule occlusion than the chitosan(C) followed by propolis (P), propolis with citric acid (PA) and control. Thus chitosan with citric acid needs further research.

Keywords: Dentinal tubule occlusion, Hypersensitivity, Chitosan, Propolis.

Introduction

Dentinal hypersensitivity is a common clinical condition that presents as a short sharp pain which is evident in 8-30 % of the adults between 20-40 years of age .1 The causative factor is the loss of enamel or cementum due to caries, non-carious lesions, periodontal diseases and procedures, etc., leading to the exposure of dentinal tubules.2 Several hypotheses have been proposed to explain the mechanism of dentinal hypersensitivity.

The treatments modalities include in-office and home use products that aim to block the dentinal tubules or to desensitize the nerve endings. Occlusion of the patent dentinal tubules is one of the main strategies in treating dentin hypersensitivity and has been extensively used to study the efficacy of desensitizing products. The therapeutic materials in use are the various fluorides, potassium nitrate, calcium silicates, dental varnishes, lasers, etc.3 The natural products pose as an alternative to conventional materials.

In search of such natural alternatives, Propolis and Chitosan have gained popularity in the field of dentistry. Propolis is a natural substance that is referred to as substance in defense of hive. It is a Greek word where pro means in front of and polis means community. It is a yellow-brown substance which has been extracted by the honey bees from different parts of different plants.4 It possess many beneficial biological and pharmacological properties because it contains amino acids, minerals, vitamins A,B,E, phenols and aromatic compounds. It has anti-bacterial, anti-inflammatory and anti-oxidant properties. It improves the microhardness of the enamel surface and has a notable effect on dentinal hypersensitivity. 5

Chitosan is a natural polysaccharide prepared by the partial deacetylation of chitin, which is obtained from the shells of crustaceans. It is the second most abundant natural polysaccharide found in the exoskeleton of crustaceans such as crabs and shrimps. It is composed of copolymers of glucosamine and N-acetyl glucosamine with two free hydroxyl groups and one primary amino group for each C6 structure unit. It has a become popular therapeutic agent in the fields of medicine and dentistry due to its anti-microbial, regenerative, haemostatic and remineralizing properties apart from its use as a component in desensitizing agents .6

Both Propolis and Chitosan have promising antimicrobial and remineralizing action in addition to being biocompatible and naturally obtainable. Hence the interest of this research study undertaken was to assess their efficacy on occluding the dentinal tubules to minimize the dentinal hypersensitivity.

Material and Methods

Chitosan; propolis; citric acid; 37% phosphoric acid; artificial saliva; diamond disk and periodontal probe.1% chitosan is prepared by dissolving 10mg of chitosan powder [Lipid life sciences limited, Janak -puri ,New delhi-58] in 10 ml of acetic acid. 6% of citric acid is obtained from the biochemistry lab.

Methodology

50 caries free extracted molar teeth were collected from the department of oral and maxillofacial surgery extracted for periodontal and orthodontic reasons.

The teeth collected were made free of surface contaminants, and then the obtained specimens were decoronated at cementoenamel junction using a diamond disk(Superflex 910S) and the root portions were discarded. Standardization of the specimen was done by making the dentinal disc of 3*3 sections using a diamond disk. The coronal portions were cut parallel to the occlusal

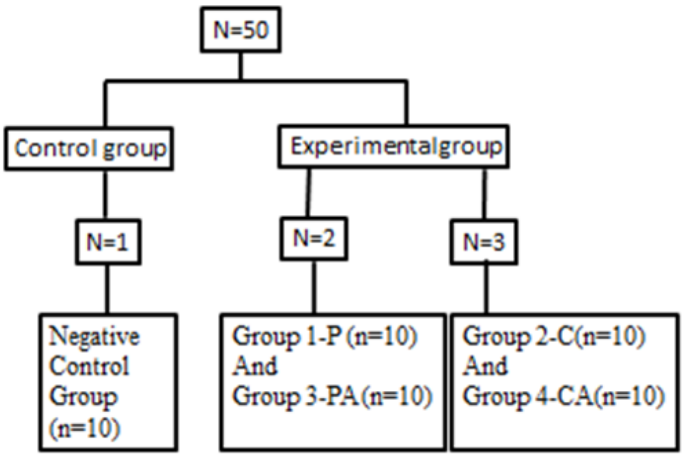
surface in order to expose the dentinal tubules mimicking the exposed dentinal tubules in case of hypersensitivity and another horizontal cut was made 3mm below the exposed dentinal surface**.** Then the specimens were stored in the artificial saliva.

All the samples were treated with 37% phosphoric acid (D-tech, Sakhi Chem Tech (I) Pvt Ltd.) for 30 seconds. Then the samples were randomly divided into three major groups. The **First major group** acts as a negative control group (n=10) and were stored in artificial saliva. The **Second major group** includes samples (n=20) that were treated with Propolis [HERB –PHARM .WILLIAMS OR 97544] by coating the dentinal disk using a micro-brush for 7 minutes and rinsed with water, then stored in artificial saliva. The **Third major group** includes samples (n=20) that were treated with chitosan (1% chitosan is prepared by dissolving 10mg of chitosan powder [Lipid life sciences limited, Janak -puri , New delhi-58] in 10 ml of acetic acid) by coating the dentinal disk using micro-brush for 7 minutes and rinsed with water, then stored in artificial saliva. The same process was carried out for seven days.

After one week (i.e., on the day of the examination), samples from the **Second major group** were randomly divided into two groups as, Group 1 (Propolis-P) and Group 3 (Propolis + citric acid-PA).

And samples from the **Third major group** were randomly divided into two groups, Group 2 (Chitosan-C) and Group 4 (Chitosan + citric acid-CA). Samples from groups 3and 4 were subjected to citric acid (obtained from the biochemistry lab) treatment by immersing the samples in a 6% citric acid solution for 2 minutes followed by rinsing with distilled water.

Then the samples were evaluated for dentinal tubule occlusion using a scanning electron microscope [JEOL,JSM-6390A].



Results

All the data analysis was done using MedCalc Version 14. A p-value of <0.05 was considered statistically significant.

1. Table:

	Group 1(P)		Group 2(C)		Group 3(PA)		Group 4(CA)		P-value	Post-hoc test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
all open	28.50	4.12	11.50	2.42	55.00	4.08	11.00	2.11	<0.001; Sig	3>1,2,4 1>2,4
partial	34.50	5.50	21.50	2.42	32.00	2.58	20.00	5.77	<0.001; Sig	1>2,4 3>2,4
complete	26.50	5.80	46.00	3.16	12.00	5.37	42.50	3.54	<0.001; Sig	2>4>1>3
precipitate	12.00	3.50	21.00	3.16	2.00	2.58	26.00	3.94	<0.001; Sig	4>2>1>3

Kruskal Wallis ANOVA with post-hoc Conover test

Group 1 –propolis **(P)** (fig: ii)

Group 2 – Chitosan **(C)** (fig: iii)

Group 3 – Propolis + citric acid **(PA)** (fig: iv)

Group 4 – Chitosan + citric acid **(CA)** (fig: v)

The Control group was not included in the analysis, as it was a negative control, where the samples were not treated. All the dentinal tubules were open under SEM (fig: i).

All open: There was an overall significant difference in the mean among the four groups. The Post-hoc test showed that group 3**(PA)** had significantly higher mean than groups 1**(P), 2(C) and 4(CA**). Similarly, group **1(P)** showed a higher mean than groups **2(C) and 4(CA).** No significant difference was seen (Tab: 1).

Partial: There was an overall significant difference in the mean among the four groups. The Post-hoc test showed that group **3(PA**) had significantly higher mean than groups **1(P), 2(C) and 4(CA).** Similarly, group **1(P)** showed a higher mean than groups **2(C) and 4(CA).** No significant difference was seen (Tab: 1).

Complete: There was an overall significant difference in the mean among the four groups. The Post-hoc test showed that **group 2(C)** had the highest mean followed by group **4(CA), group 1(P)** with the least being in **group 3(PA)** (Tab: 1).

Precipitate: There was an overall significant difference in the mean among the four groups. The Post-hoc test showed that **group 4(CA)** had the highest mean followed by **group 2(C), group 1(P)** with the least being in **group 3(PA)** (Tab: 1)**.**

Thus overall results show that chitosan with citric acid> chitosan> propolis>propolis with citric acid in dentinal tubule occlusion (Tab: 1).

Discussion

Dentinal hypersensitivity is a justifiable condition, considered as an enigma that is commonly encountered and needs to be correctly addressed. The etiology of dentinal hypersensitivity is an attribute of multifactorial mechanical and chemical causes with the most common factor being wasting diseases.

In this study, an attempt was made to compare different dentinal tubule occluding materials to provide a better desensitizing material that can be the best choice for empirical therapy until the definitive treatment is provided.

Two different desensitizing materials chosen for the comparison were propolis and chitosan, which are relatively new and biocompatible.

Propolis was chosen as a natural plant material of choice because of its ability to occlude the dentinal tubule by forming a precipitate over it due to the presence of high flavonoids. This aids in the prevention of hydraulic conductance of the dentinal tubules. 7The active compounds in propolis are distributed into several major classes: phenolic acids and their esters, flavonoids, terpenes, β steroids, aromatic aldehydes, alcohols, sesquiterpenes, naphthalene, stilbene derivatives of benzopyran, benzophenone, caffeic acid, cinnamic acid derivatives and benzoic acid. 7 Propolis has not only got antioxidant, heavy metal chelating properties but also antimicrobial, anti-inflammatory, immune-modulatory properties. It even has got a significant effect on wound healing and regeneration of tissues.

Chitosan has got wide variety of clinical applications. Here, its efficacy in hypersensitivity has been confirmed. Chitosan was copolymerized by N acetyl glucosamine and hydroxyl groups which are free and one amino group for each C6 structural unit. 8 The free amino group of chitosan was responsible for its interaction with the tooth.9 It even has hemostatic, fungi-static, antibacterial, antitumor and immuno-adjuvant properties. 10

In this study, chitosan has been employed because of its nanoparticle size and its potential positive charge which can interact with dentin. 11 Chitosan was available in various forms like microspheres, conjugates, capsules, nanoparticles, films, beads, tablets, etc depending on the use. (11, 12)

Chitosan, for its application over the dentinal tubule, needs a vehicle as it cannot be dissolved in water, bases or organic solvents. In order to make it into a stage that can be handled, it has been dissolved in an inorganic solvent like acetic acid. Thus in this study, we used acetic acid as an agent for delivering the particles into the dentinal tubule.

Dentinal tubule occlusion in this study has been evaluated under the scanning electron microscope of size 10 * 10

μm under 2500X resolution by sputter coating the samples with a thin layer of gold.

In this study, all the samples were subjected to a demineralization procedure by treating them with the phosphoric acid to mimic the open dentinal tubule condition, as in the case of hypersensitivity by removing the debris and slight demineralization of dentin.13

On the day of the examination, samples from major group two (N=2) and three (N=3) were divided into two groups each (P, PA, C, CA) respectively, in-order subject half sample from each major group to citric acid treatment. Citric acid (6%) treatment simulates the acidic environment of the oral cavity after the consumption of beverages to see the effect of ph variation on the retaining ability of these materials.14

Thus accordingly the second major group was divided into group1 and 3 and third major group was divided into 2and 4. Groups 3and 4 were subjected to citric acid treatment.

In this study, samples treated with propolis and chitosan had shown the dentinal tubule occlusion but the better results were obtained with that of chitosan with citric acid.

Dentinal tubule occlusion by propolis was due to the formation of a resinous layer due to its high flavonoid content.

Mahmoud *et al*. studied the effect of Propolis as a desensitizing agent and demonstrated that 85% of subjects were highly satisfied during the study period. 15 The presence of flavonoids in the propolis favours the reduction in the free radicals formation by binding to the heavy metal ions and further helps in process of catalyzation through full radicals.

Saber *et al*. conducted a study where he has used propolis as a direct pulp capping agent, with the evidence of inflammation ranging from mild to moderate at 2 and 4 weeks and partial dentinal bridge formation at 4 weeks beneath the pulp capping material. 16 This was due to flavonoids that would interact with the dentin and form the crystals.

Dentinal tubular occlusion by chitosan might be due to the interaction of positively charged chitosan with negatively charged collagen fibres and nanoparticle size.

In the later part of the experiment when half the samples from each major group, groups 3 and 4 were subjected to the citric acid treatment, citric acid-treated chitosan had shown better tubular occlusion than chitosan followed by propolis and citric acid-treated propolis.

This greater variation in the results after citric acid treatment was due to a varied type of interaction between the materials and the citric acid. Citric acid-treated propolis had shown less tubular occlusion due to removal of the resinous layer by the acid ph and citric acid-treated chitosan had shown better results interaction of the citric acid with that of chitosan. This is because the backbone chain of Chitosan has got the ability to cross-link with reactive –COOH groups of citric acid. 17

According to Arnaud et al, it was concluded that chitosan has got the greater effect on preventing the demineralization and little effect on remineralisation by assessing the phosphorous chemical analysis of the solutions and the microhardness of the samples and even the greater penetration of chitosan particles at the dentin-enamel junction using Optical coherence tomography (OCT). 18

According to kishen et al chitosan has got the protective mechanism against collagen degradation, where positively charged (NH groups) chitosan nanoparticles interact with the negatively charged (COO groups) collagen particles forming complexes that are more resistant to degradation. 19

Thus chitosan maintains the ph above the critical level by the interaction of free amino groups of chitosan with acids of dietary and cariogenic origin.20

The cross-linking of chitosan and saliva with the physical adsorption of chitosan onto saliva prevents acid erosion of the hydroxyapatite surface.

Phosphorylated chitosan nano complexes and amorphous calcium phosphate has got a significant capacity to remineralize the subsurface lesions of enamel than the fluoride treatment. 21Chitosan lactate which was a water-soluble form had shown better demineralization capacity under-simulated acid condition of acidogenic, dietary and bleaching. 22

Chitosan was even used as a chelating agent. Thus the multiple properties of the chitosan depend on the pH, ion particle and type of interaction.

According to Praveen et al chitosan helps in remineralisation and even acts as a chelating agent. These actions depend on the Ph of the solution, the chemical structure of the chitosan and type of ion that determines the type of interaction. Remineralisation might be due to covalent immobilization of chitosan on collagen, where calcium ions on the dentinal surface bind to functional phosphate groups of the chitosan molecule. This leads to the formation of a favourable surface for crystal nucleation structure. Chelating action might be due to the binding of chitosan to the metal ions. 23

Thus this function modulation of chitosan might be the reason for the wide variety of applications in dentistry and even in this study, it effects in dentinal tubule as shown better effect.

Conclusion

Within the limitations of the study chitosan with citric acid has shown better dentinal tubule occlusion in case of hypersensitivity. And its clinical application requires further studies.

Figure 1

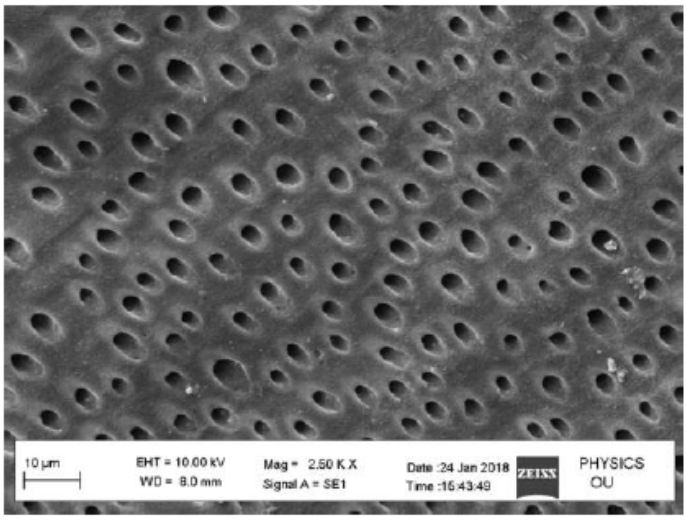


Figure 2

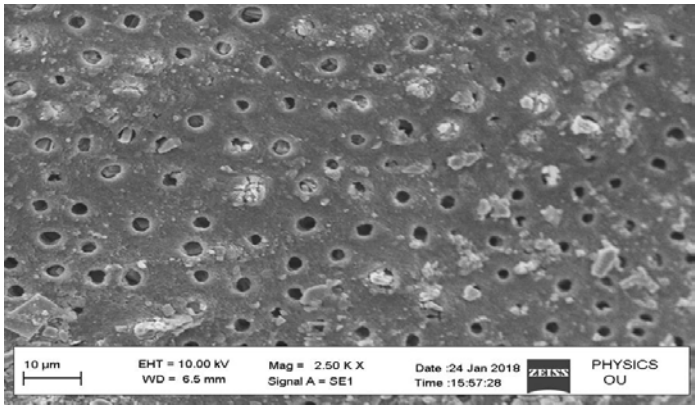


Figure 3

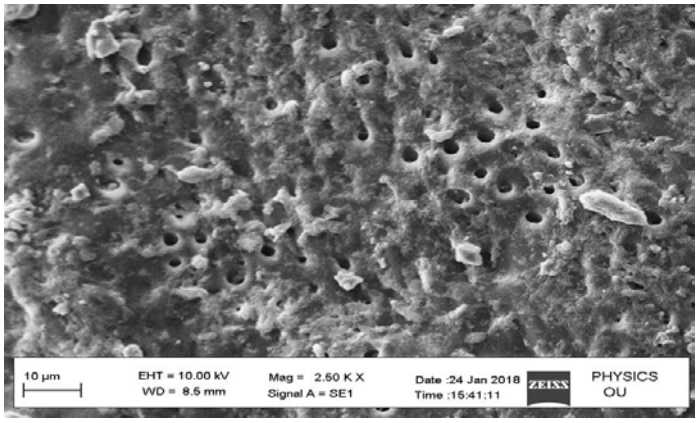


Figure 4

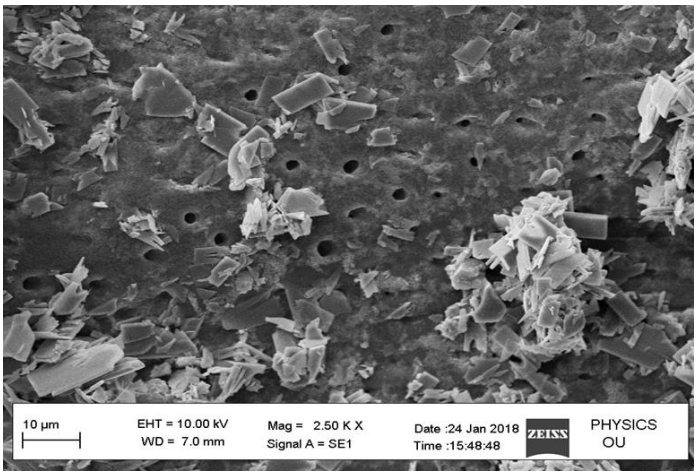
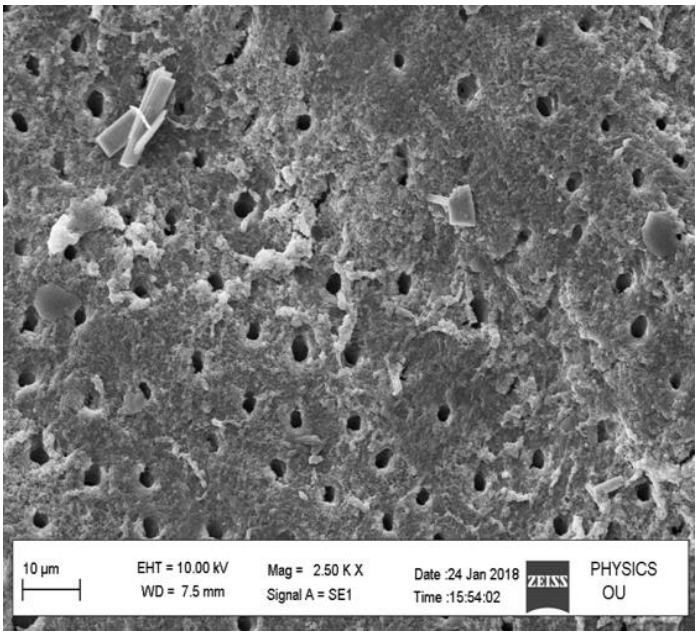


Figure 5



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