

Comparison of the Efficacy of Two Desensitizing Toothpastes in Occluding Dentinal Tubules under Artificial Saliva and Acid Conditions: An In-Vitro SEM Study

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ABSTRACT

Introduction: Dentin hypersensitivity, a prevalent clinical condition is typically treated with desensitizing toothpastes as the primary choice for relieving pain. Ideally, desensitizing toothpaste must minimize dentin permeability and sustain the tubule occlusion despite acid challenges and immersion in saliva.

Aim: To evaluate and compare the effectiveness of 5% NovaMin® dentifrice and 5% Potassium nitrate dentifrice in dentinal tubule occlusion after citric acid challenge and immersion in artificial saliva through scanning electron microscope (SEM).

Materials and Method: 36 samples of EDTA etched dentin discs prepared from extracted teeth were equally distributed in to three groups. Group 1 was brushed with 5% NovaMin® dentifrice and Group 2 with 5% Potassium nitrate dentifrice. Group 3 was brushed with Distilled water as a control. Each group was equally divided in to two subgroups to be treated with: 6 wt% citric acid or 24 hrs artificial saliva immersion. The samples underwent SEM analysis and images were subjected to Gutmann JL et al scoring method. The agreement between the scores of two examiners was assessed by Spearmans correlation coefficient. The data were analyzed using ANOVA and Tukey HSD test.

Results: Group 1 had showed better dentinal tubule occlusion after artificial saliva immersion and citric acid challenge when compared to other groups.

Conclusion: Hence 5% NovaMin® dentifrice represented an excellent approach to dentinal tubule occlusion on artificial saliva immersion and acid challenges compared to 5% potassium nitrate dentifrice.

Key words: Dentin hypersensitivity, Scanning electron microscope, desensitizing toothpaste, dentinal tubule occlusion, 5% NovaMin®, 5% Potassium nitrate.

INTRODUCTION

Dentin hypersensitivity (DH) is characterized by a short, sharp pain originating from exposed dentin in response to various stimuli. It is distinct from the sensitivity generated from other clinical causes, such as a cracked tooth, fractured restoration, dental caries, or microleakage.¹ DH is a common clinical condition that variably affects 1% to 98% of the worldwide population.² The incidence increases with age and reportedly peaks during the third and fourth decades of life.³ Dentin hypersensitivity can cause frequent discomfort during daily activities such as tooth brushing, drinking and eating thereby affecting quality of life and emotional well-being.⁴

Gysi (1900) was the first to propose that dentin hypersensitivity is caused by fluid movement in the exposed dentinal tubules that triggered pulpal nerves. Later this hypothesis was experimentally proven by Brannstrom et al which remains widely accepted as the “hydrodynamic theory”.⁵ Subsequently it was found that open dentinal tubules can act as a route for the diffusive transportation of bacterial components from the oral cavity to the pulp,

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potentially inducing localized pulpal inflammation.⁶

Various strategies have been implicated in the treatment of dentin hypersensitivity including lasers, ions and salts fluoride iontophoresis, dentin sealers, periodontal soft tissue grafting and homeopathic medications. But desensitizing toothpastes are preferred as initial treatment due to its convenience, affordability, and non-invasive nature. Majority of these toothpastes function either by occluding dentinal tubules or by blocking pulpal nerve response. In the tubular occlusion approach, the dentifrice forms a layer that mechanically occludes the dentinal tubules and prevents pulpal fluid flow, leading to pain relief.^{3,7} However exposure to dietary acids and saliva can potentially reverse the decrease in permeability induced by desensitizers. Ideal desensitizing toothpaste must not only minimize dentin permeability but also sustain the tubule occlusion despite acid challenges and immersion in saliva.⁸ Hence, it is essential to evaluate whether desensitizing agents could occlude dentinal tubules effectively under the circumstance similar to the oral environment. Thereby, our objective was to evaluate and compare the effectiveness of two commercially popular 5% NovaMin® dentifrice and 5% Potassium nitrate dentifrice in dentinal tubule occlusion after citric acid challenge and immersion in artificial saliva through scanning electron microscope (SEM).

Table 1: The correlation between tubule occlusion scores of two examiners analyzed by Spearman's Correlation Coefficient.

class	group	examiner1	r	p	N	examiner 2
AS	G1S	examiner1	r			1.000
			p			<.001 vhs
			N			6
	G2S	examiner1	r			.728
			p			.101
			N			6
	G3S	examiner1	r			1.000
			p			<.001 vhs
			N			6
CA	G1C	examiner1	r			.647
			p			.174
			N			6
	G2C	examiner1	r			.632
			p			.178
			N			6
	G3C	examiner1	r			1.000
			p			<.001 vhs
			N			6

AS- artificial saliva, CA - citric acid

MATERIALS AND METHOD

36 extracted normal premolars were collected from Yenepoya Dental College Karnataka, India with the approval from Yenepoya University Ethics Committee for the study. Teeth extracted for orthodontic purposes, where the crown and root remained intact without alteration during the extraction process, were considered for inclusion in the study. Conversely, teeth exhibiting developmental abnormalities, caries, restorations, fractures, associated with pulp and or periapical diseases or fluorosis were excluded from the study. Samples were cleaned thoroughly and stored in 0.5% thymol at 4°C no longer than a month prior to their use. Each dentin discs were prepared of thickness 1mm approximately above the cemento-enamel junction with diamond disk attached to straight handpiece. The occlusal surface of each dentin disc was sanded with 400-grit silicon carbide paper for 30 seconds to create a standard smear layer. These discs were dipped in 0.5M EDTA solution for 2 minutes to remove the smear layer. The etched discs were rinsed and kept wet. The specimens were equally distributed in to three groups each containing of twelve discs. Group 1 discs were brushed with 5% NovaMin® dentifrice for 2 minutes. Group 2 discs were brushed with 5% Potassium nitrate dentifrice for 2 minutes. Group 3 discs were brushed with Distilled water for 2 minutes were taken as Control. Each group was further divided in to two subgroups with six discs each. One of the subgroups were treated with 6wt% citric acid (pH 1.5) for 1 minute and rinsed in deionised water. Other subgroup stored in artificial saliva (pH 7.4) at 37°C for 24 hours and rinsed with deionised water. After artificial saliva immersion the Subgroups were labeled as G1S for 5% NovaMin®, G2S for 5% Potassium nitrate and G3S for Distilled water. Likewise, after Citric acid challenge the subgroups were labeled as G1C for 5% NovaMin®, G2C for 5% Potassium nitrate and G3C for Distilled water. The samples underwent SEM analysis at the Department of Metallurgy, National Institute of Technology Karnataka, Suratkal, Karnataka, India, utilizing the analytical microscope JEOL JSM-6380LA. Standardized SEM-micrographs were taken from the samples at 1000x magnification at 5 KV. SEM images were then evaluated and scored by two examiners in accordance with the Gutmann JL et al scoring method as following:

Score 1: Less than 25% of the tubules occluded.

Score 2: Between 25 and 50% of the tubules occluded.

Score 3: Between 50% and 75% of the tubules occluded.

Score 4: More than 75% of the tubules occluded.⁹

RESULTS

SEM images of all samples were evaluated and scored by two evaluators. Statistical analysis was performed using SPSS version 17. Spearman's correlation coefficient was used to assess the agreement between the two examiners. Significant correlation was observed between the scores of the two examiners across all groups (Table 1). The scores given by the examiners for G1S, G3S, and G3C samples exhibited a very highly significant correlation ($p < .001$). Since there was a strong agreement between the examiners, the values from examiner 1 were selected for ANOVA and Tukey HSD tests.

Analysis of variance (ANOVA) was used to test the



significance of the differences between the subgroups and the results were as follows:

After 24 hours of immersion in artificial saliva, G1S samples exhibited a mean score of 3.8333 with a standard deviation of 0.40825, yielding a p-value of <.001, indicating a very high level of statistical significance. Similarly, G2S samples showed a mean score of 2.8333 with a standard deviation of 0.75277, resulting in a p-value of <.001, also demonstrating a very high level of statistical significance. Likewise, G3S samples had a mean score of 1.1667 with a standard deviation of 0.40825, yielding a p-value of <.001, confirming a very high level of statistical significance as well (Table 2).

Following a 1-minute challenge in 6wt% citric acid, G1C samples exhibited a mean score of 2.5000 with a standard deviation of 0.54772, yielding a p-value of less than .001, indicating a very high statistical significance. Similarly, G2C samples displayed a mean score of 1.3333 with a standard deviation of 0.51642, also accompanied by a p-value of less than .001, showing very high statistical significance. G3C samples recorded a mean score of 1.0000 with a standard deviation of 0.0000, and a p-value of less than .001, demonstrating very high statistical significance as well (Table 3).

Tukey HSD test was used to compare the inter-subgroup differences and the results were as follows:

On comparing the subgroups after artificial saliva

immersion, there was a mean score difference of 1.16667 between G1S and G2S samples, demonstrating statistical significance (p-value 0.017). Similarly, the mean score difference between G1S and G3S samples was 2.66667, indicating very high statistical significance (p-value <.001). Additionally, when comparing G2S and G3S samples, a mean score difference of 1.66667 was observed, also showing very high statistical significance (p-value <.001) (Table 4). The mean scores of each subgroup after immersion in artificial saliva are illustrated in Graph 1.

When comparing the subgroups after citric acid challenge, a mean score difference of 1.66667 was observed between G1C and G2C, indicating very high statistical significance (p-value <.001). Similarly, the comparison between G1C and G3C also demonstrated very high statistical significance, with a mean score difference of 1.50000. However, when comparing G2C and G3C, the mean score difference of 0.33333 yielded a p-value of 0.402, which is not statistically significant (Table 5). The mean scores of each subgroup after citric acid challenge are depicted in Graph 2.

Based on the results, samples brushed with 5% NovaMin® dentifrice had more dentinal tubule occlusion after artificial saliva immersion and citric acid challenge when compared to 5% potassium nitrate containing dentifrice and Distilled Water. But most of the dentinal tubules were occluded by 5% NovaMin® dentifrice on artificial saliva immersion.

Table 2: Comparison among the subgroups in artificial saliva immersion by ANOVA test

Group	N	Mean	Std. Deviation	F	p
AS G1S	6	3.8333	.40825	36.3	<0.001 vhs
G2S	6	2.8333	.75277		
G3S	6	1.1667	.40825		

Table 3: Comparison among the subgroups in citric acid challenge by ANOVA test

Group	N	Mean	Std. Deviation	F	P
CA G1C	6	2.5000	.54772	19.71	<0.001 vhs
G2C	6	1.3333	.51640		
G3C	6	1.0000	.00000		

Table 4: Inter-subgroup comparison in artificial saliva immersion by Tukey HSD test

Class	(I) Group	(J) Group	Mean Difference (I-J)	P
AS	G1S	G2S	1.16667	0.017 sig
		G3S	2.66667	<0.001 vhs
	G2S	G3S	1.66667	<0.001 vhs

Table 5: Inter-subgroup comparison in citric acid challenge Tukey HSD test

Class	(I) Group	(J) Group	Mean Difference (I-J)	P
CA	G1C	G2C	1.16667	<0.001 vhs
		G3C	1.50000	<0.001 vhs
	G2C	G3C	.33333	.402

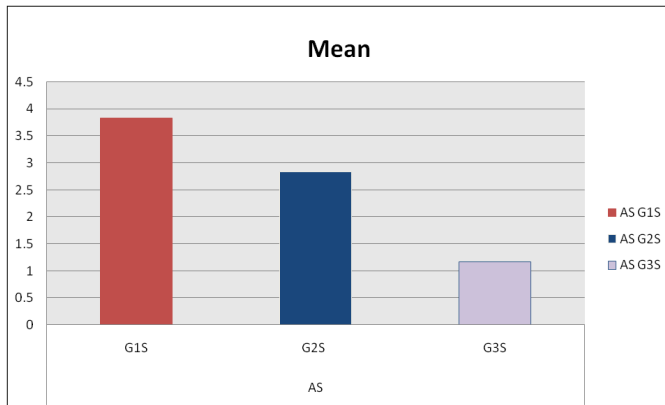


DISCUSSION

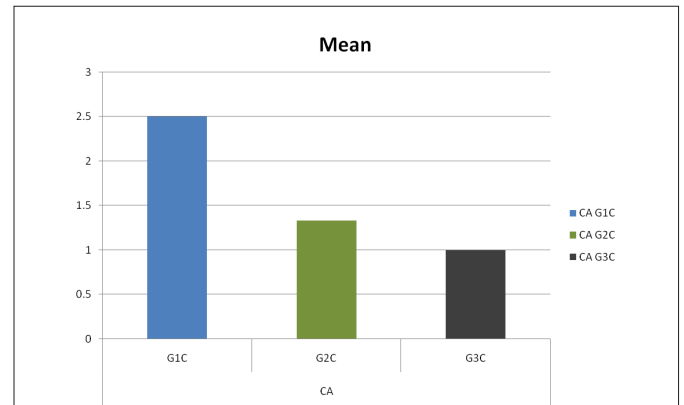
The present study was aimed at comparing the tubule occluding property of two widely used toothpastes, 5% NovaMin® and 5% potassium nitrate containing dentifrices. The evaluation was conducted following immersion in artificial saliva and citric acid as the data on such simulated challenges involving these two toothpastes were scarce. Our research revealed that 5% NovaMin® dentifrice had superior dentinal tubule occlusion after artificial saliva immersion when compared to 5% potassium nitrate dentifrice and Distilled Water. These results are coinciding with the findings of Wang Z et al. However even after citric acid challenge, 5% NovaMin® demonstrated better occlusive property compared to other groups, contradicting observations of Wang Z et al. According to their research, any substance capable of decreasing dentinal

fluid conductance by blocking the tubules can alleviate clinical symptoms of DH.⁸ Thus the present study substantiate that 5% NovaMin® can withstand saliva and acid conditions of oral environment thereby having better potential to relieve DH compared to 5% Potassium nitrate. Moreover our in-vitro findings align closely with many clinical studies, demonstrating NovaMin®'s superior pain relief efficacy over toothpaste containing 5% potassium nitrate.^{3,10-12} We believe these findings are a connecting bridge between two research paradigms, offering a clear insight into NovaMin®'s effectiveness in treating dentin hypersensitivity.

Initially, exposed dentinal tubules due to enamel or cemental loss are naturally occluded by a phenomenon called smear layer. These plugs consist of proteins and calcium phosphate precipitates derived from saliva, which help to



Graph 1 : Mean scores between the subgroups after artificial saliva immersion



Graph 2 : Mean scores between the subgroups after Citric Acid challenge

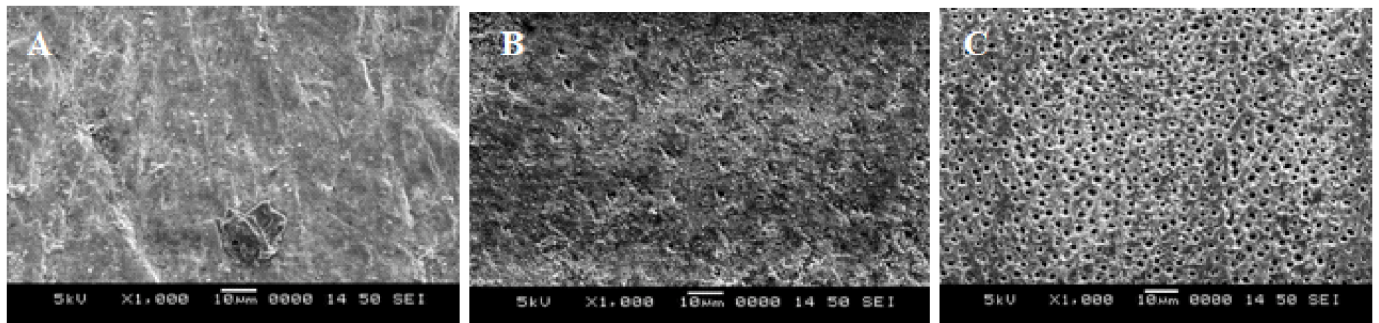


Fig. 1: Dentinal tubule occlusion after 24 hour artificial saliva immersion by A. 5% NovaMin® dentifrice, B. 5% Potassium nitrate dentifrice C. Distilled water (SEM, 1000X magnification)

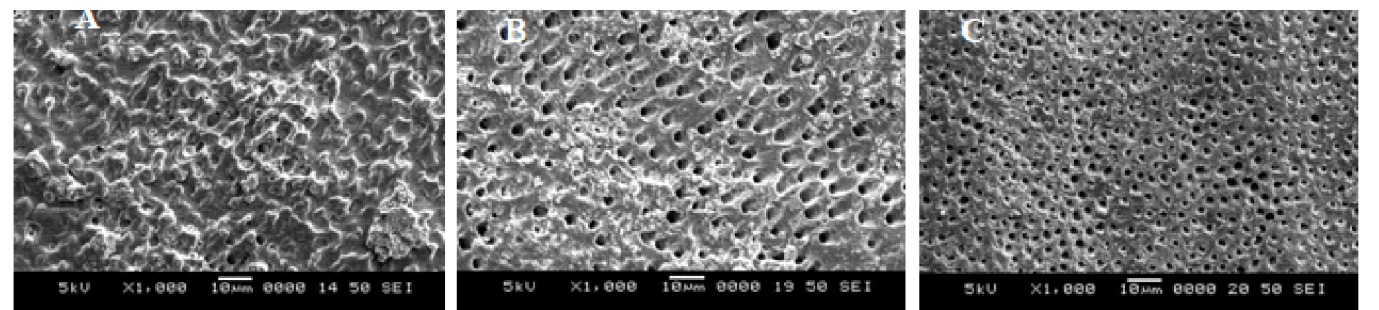


Fig. 2: Dentinal tubule occlusion after 6wt% Citric acid Challenge for 1 minute by A. 5% NovaMin® dentifrice, B. 5% Potassium nitrate dentifrice C. Distilled water (SEM, 1000X magnification)

reduce permeability. However, this process of tubule occlusion occurs gradually, and the plugs can be easily removed by dietary acids and physical abrasion making it ineffective and unreliable in DH relief.^{13,14} Distilled water samples in our study also showed occluded tubules of score less than 25% in saliva immersion, representing presence of smear debris probably derived from brushing. And Citric acid exposure showed more number of areas of open dentinal tubules. (Fig 1C and 2C) Dentifrice abrasives also possess similar ability to occlude tubules by forming smear layer that often occurs during tooth brushing, but this occlusion does not endure as well. Additionally, few authors have also mentioned about salivary enzymes could possibly deteriorate the tubule occlusion by dentifrices. Consequently recurrent pains occur, explaining the cyclic nature of DH.¹⁵⁻¹⁸

Researches have shown desensitizing toothpaste formulations that employ the tubule occlusion often contain inorganic solid fillers and organic polymers with adhesive properties to dentin. These components, along with mineral solids, create a protective layer on the dentin surface and plug the tubules within through diffusion into the porous dentin, physical adsorption, and chemical bonding, as well as electrostatic interactions. The coating layer and intra-tubular plugs are expected to adhere to the dentin and tubule walls and withstand salivary enzymatic and acidic erosion for at least the duration of two brushing sessions to ensure a sustained effect in preventing dentin hypersensitivity.^{3,8,17,19-23}

Over the last decade, numerous ultramicroscopic studies have been dedicated to explore the efficacy of NovaMin® in treating dentin hypersensitivity. NovaMin® is a bioactive glass; chemically known as Calcium sodium phosphosilicate. It reacts when exposed to aqueous media (water or saliva) and provides calcium and phosphate ions that form a hydroxy-carbonate apatite layer that is chemically similar to enamel and dentin. Thereby, NovaMin® has shown to be effective by physically occluding the dentinal tubules through development of this mineral layer. The bioactive glass particles bond with the collagen fibers exposed in dentin, creating a sealing layer that steadily releases calcium and phosphate ions.^{3,24} Our study had demonstrated that 5% NovaMin® toothpaste showed homogenous layer over dentin surface after 24 hrs saliva immersion achieving more than 75% of dentinal tubules occlusion with mean value of 3.8333 (Fig 1A). Although, there was a less occlusion score (mean 2.5000) after citric acid exposure, NovaMin® had retained better tubule occlusion compared to 5% Potassium nitrate and control which was statistically significant. The SEM image (Fig 2A) exhibited surface irregularities representing erosion patterns with some exposed dentinal tubules. Reviewing the literature, many well designed clinical trials also proved the effectiveness of 5% NovaMin® toothpaste over other desensitizing agents in the treatment of DH.²⁵⁻²⁹ Based on recent article, NovaMin® can trigger cellular migration and promote the proliferation and differentiation of pulp stem cells that in turn, can lead to the formation of reparative dentin.³⁰

Studies conducted in the past have established that potassium nitrate is a safe and effective method for dentin hypersensitivity relief.^{31,32} It is well known that potassium nitrate increases levels extracellular potassium ions there by depolarizing nerve impulse, thus preventing the conduction

of pain sensation. However, recent findings revealing tubule occlusion property of Potassium nitrate has elicited some uncertainty in its mechanism of action.³³⁻³⁵ Our study also have shown findings similar to these research, as samples brushed with 5% Potassium nitrate dentifrice have demonstrated significant tubule occlusion compared to control ranging from 25% to 75% (scores of 2 and 3) with mean score of 2.8333 under artificial saliva immersion. But, 6wt% Citric acid challenge has shown scores to a mean value of 1.333 representing occlusion lesser than 25% which was statistically insignificant compared to the control. In the present study, 5% Potassium nitrate dentifrice showed limited resistance to acid challenge exhibiting areas of open dentinal tubules. (Fig 1B and 2B).

Citric Acid is a common component of fruit and soft drinks widely used in previous investigations on enamel and dentine erosion mechanisms, hence citric acid was selected in this study as a post-treatment to simulate oral environment and test the resistance of desensitising toothpastes to acid challenge.^{8,13,36-39} In our study, crystal precipitate was retained over dentin surface sealing most of the tubules in the NovaMin® group even after citric acid challenge. On the contrary, lesser dentinal tubules were covered in 5% Potassium nitrate and distilled water samples after acid challenge. These denote that the constituents of 5% Potassium nitrate and distilled water may be dissolved by citric acid treatment.

Another study done by Gandolfi et al had immersed dentin treated specimens in artificial saliva for 10 minutes before acid challenge, while in the present study the post-treatments i.e. the Citric Acid treatment and Artificial Saliva immersion were applied separately to simplify interpretation.³⁸ In this study the samples were immersed in artificial saliva for 24 hours. Perhaps 24 hour was not long enough to evaluate the remineralization effect, because stable precipitates might not have formed in such a short period of time.

While we utilized an in-vitro methodology, one limitation was the inability to simulate oral health conditions. Also, our study did not include the provision of quantitative data on occlusion property and depth, nor identifying the composition of particles within dentinal tubules were incorporated – important aspects for evaluating the long-term efficacy of these products.

CONCLUSION

In our in-vitro study 5% NovaMin® dentifrice has represented an excellent approach to dentinal tubule occlusion on artificial saliva immersion and acid challenges compared to 5% potassium nitrate dentifrice. As this study was not designed to quantify tubule occlusion and the depth of occlusion and identifying composition of the particles in the dentinal tubules, further quantitative and functional studies with larger samples are required to increase the acid and saliva resistance of these toothpastes in order to prolong the occlusive effect in the treatment of dentin hypersensitivity.

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