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Evaluation of the Efficacy of Laser, Desensitizing Tooth Paste and Mouthwash on Obliteration of Dentinal Tubules: An in Vitro Scanning Electron Microscopic Study

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Original Article

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ABSTRACT

Background: The aim of this study is to evaluate and compare the efficacy of laser, desensitizing toothpaste, and desensitizing mouthwash on dentinal tubular obliteration using scanning electron microscopy (SEM).
Materials and Methods: Thirty extracted, sound, single-rooted mandibular premolars were selected. The enamel was removed with tungsten carbide fissure bur and crown dentin discs, with a thickness of 2 mm, which was cut perpendicular to the long axis of the tooth. Each specimen was etched with 37% phosphoric acid for 30seconds to expose the dentinal tubules. Specimens were again rinsed with distilled water and ultrasound to remove the residual smear layer for five minutes. Group I – Application of Arginine Calcium Carbonate containing toothpaste, Group II – Application of Potassium sorbate, and Cetyl Pyridinium Chloride containing mouthwash, Group III – Application of 810nm diode laser in noncontact mode. All samples were processed and examined under a scanning electron microscope (SEM) to compare the effectiveness of dentinal tubule obliteration.
Results: The mean values of percentages of dentinal tubule obliteration in Groups I, II, III were 2.20, 4.60, 1.10 respectively. Diode laser group (Group III) was found to be more effective in causing dentinal tubule obliteration followed by the toothpaste group (Group I) and mouthwash group (Group II). Conclusion: Within the limitations, the laser group was found to be more effective in dentinal tubule obliteration when compared to the desensitizing toothpaste group and the desensitizing mouthwash, which was statistically significant ($P<0.001$).

Keywords: Dentin Hypersensitivity, Diode Laser, Dentinal Tubule Obliteration, Scanning Electron Microscope, Arginine Calcium Carbonate, Cetyl Pyridinium Chloride.

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Introduction

The modern era is composed of sophisticated advances and technologies thereby reinforcing the awareness and the oral health status of the entire world. Because of the improved quality and standards of various dental treatment modalities and dental streams, there is a surge in the long life span of teeth in the oral cavity with an inverse relationship to caries and periodontal disease status.

Dentin is the main structural component of the tooth, mainly composed of 30,000-40,000 dentinal tubules, and is filled with dentinal fluid. The diameter of dentinal tubule increases at the pulpal region. Tapering from the inner to the outermost surface, they have a diameter of 2.5micrometer near the pulp, 1.2 micrometers near the mid-portion of dentin, 0.9 micrometers at the DEJ.[1] The term obliteration refers to total destruction or collapse and therefore dentinal tubule obliteration means utter destruction of tubules thereby leading to the opening of tubules. Dentine hypersensitivity (DH) is characterized by short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic, or chemical and which cannot be ascribed to any other form of dental defect or pathology. There is an increased prevalence of 25-46% among people between the ages of 18 to 70 years.[2]

The mechanism of dentin hypersensitivity can be explained under various theories like odontoblastic transduction theory, neural theory, and hydrodynamic theory. Among these many, the most widely accepted one is a hydrodynamic theory which was pioneered by Gysi and Brannstrom. According to this theory, due to odontoblastic destruction, the dentinal tubules are exposed and as a result, there is a diffusion of dentinal fluid, leading to stimulation of Aδ fibers resulting in dentinal hypersensitivity. According to this theory whenever there is a decrease in the dentinal tubule diameter, there will be a sudden decrease in the dentinal fluid movement thus a reduction in sensitivity.[3]

Dentinal tubule occlusion works by the external application of a physical or chemical agent and it forms an artificial layer on the outer surface of dentinal tubules by depositing an obliterating material on the dentin surface thereby reducing the tubule diameter, blocking the dentinal fluid diffusion, and cause a reduction in DH. The physical or chemical agents most commonly used are toothpaste, mouth wash, laser, dentin sealers, periodontal soft tissue grafting procedures. In blockage of nerve activity, potassium ions tend to concentrate in the interior of the dentinal tubules, causing a depolarization of the cellular membrane of the nerve terminal.[4]

Resolution of dentin hypersensitivity becomes a necessity, thus many treatment options like the application of diode laser, desensitizing toothpaste, mouth wash, etc were studied extensively on an individual basis. The comparative evaluation of all these three desensitizing agents within a framework will be meager. This conviction paved the way for conducting this study. It will surely be a critical juncture to determine the effectiveness of 3 different desensitizing agents in dentinal tubule obliteration. The oral environment being dynamic, the desensitizing agent has to withstand the challenges of salivary dissolution, prolonged contact with acidic foods, and/or forceful tooth brushing.[5]

Therefore the main aim of this study is to comparatively evaluate the efficacy of laser, desensitizing toothpaste, and mouthwash on dentinal tubule obliteration under a scanning electron microscope (SEM) and to find out a novel treatment alternative among the three in reducing dentinal hypersensitivity.

Materials and Methods

Informed consent was obtained from all participants. A total of thirty extracted, sound, single-rooted premolar teeth samples were collected from outpatients, Department of Oral and Maxillofacial Surgery. Ethical and research clearance was obtained from the institutional ethical and research committee.

Inclusion Criteria

1. Teeth obtained from individuals within the age group of 25 to 50 years.
2. Single rooted premolar teeth.

Exclusion Criteria

1. Decayed and fractured teeth.
2. Teeth with developmental anomalies.

The specimens assigned to the in vitro treatment group were rinsed in running tap water and cleaned off gross debris and then stored in sterile saline solution (NaCl 0.9% at 37°C for 24-48 hours). The enamel was then removed with plain cut tungsten carbide fissure bur. The crowns of each tooth were then resected at the level of Cemento Enamel Junction by using a double-sided carborandum disc bur to obtain a Crown dentin discs, at the level of a cervical region with a thickness of 2 mm, which was cut perpendicular to the long axis of the tooth. The dentin discs were then polished using a pumice and bristle brush. Specimens were washed in distilled water for 12 min and each specimen was etched with 37% phosphoric acid for 30seconds to expose the dentinal tubules. Specimens were again rinsed with distilled water and ultrasound to remove the residual smear layer for five minutes.

All samples were processed and examined under an analytical JEOL JSM 6390LA scanning electron microscope (SEM; CUSAT STIC, Cochin, Kerala, India) to compare the effectiveness of dentinal tubule obliteration.

Sampling Methods

Total 30 samples were randomly allocated into 3 separate groups , comprising of 10 samples in each.
Group 1 – Dentifrice containing 8% Arginine Calcium Carbonate (Colgate sensitive plus toothpaste ™)
Group 2- Mouthwash containing Potassium sorbate (Colgate Plax Gentle Care Mouthwash ™)
Group 3 – Diode laser (810 nm)

Experimental Groups and their Treatments

Group I

10 samples are applied with desensitizing toothpaste (arginine, calcium carbonate). Specimens were applied with arginine and calcium carbonate (ACC) containing desensitizing toothpaste. Pea-size amount was applied on the dentin surfaces with the help of a finger and kept for at least 2 minutes.

Group 11

10 samples were immersed in desensitizing mouthwash (Potassium sorbate). Specimens were immersed in desensitizing mouthwash for 30seconds and then rinsed in distilled water for a further 30seconds.

Group III

10 samples were exposed to a diode laser. Specimens were exposed with a soft tissue diode laser with 810nm; non -contact continuous mode on the region of exposed dentin, 1 mm away for 10seconds; output power - 0.8-1 W.

Scanning Electron Microscopy

All thirty specimens were air-dried. Each specimen was mounted on a metal stub. Samples were then sputter-coated with 25nm of gold for 10min and were examined under the SEM 1500X magnification [Figure 1]. Photomicrographs were taken using Scanning Electron Microscope of dentin discs at 1500X magnification to calculate the total number of tubules, number of open tubules, number of completely occluded tubules, and number of partially occluded tubules. The SEM analysis was performed by an independent examiner in order to avoid bias.

Statistical Analysis

The comparison between the groups was statistically analysed with ANOVA followed by Tukeys Post hoc test for multiple comparisons and it also observed a significant difference between the groups with $p<0.001$.

Results

Microscopic images were visually analysed based upon the patency of dentinal tubules as per the evaluation criteria.

Scanning electron microscopy scoring – **Davies et al., 2011**[6]

1. Occluded (100% of tubules occluded.)
2. Mostly occluded (50-100% of tubules occluded.)
3. Partially occluded (25-50% of tubules occluded.)
4. Mostly unoccluded (<25% of tubules occluded.)
5. Unoccluded (0% of tubules occluded.)

The highest mean completely occluded tubules was observed in Group 3 [Figure 3]. The highest mean partially occluded tubules was observed in Group 1 [Figure 1], highest mean unoccluded tubules was observed in Group 2 [Figure 2]. When mean complete occlusion/ partial occlusion / non-occlusion was compared between groups, all the results showed a statistically significant difference ($P<0.001$). We observed that there is a significant difference in mean indices between the groups with $p<0.001$. Significant mean values for the Toothpaste group, Mouthwash group, and Diode laser group were 2.20, 4.60, 1.10 respectively [Table 1]. When an intergroup comparison was done using post hoc test between the Laser-tooth paste group, the mean difference was found to be -1.1 and with the Laser-mouthwash group it was -3.5, the toothpaste-mouthwash group found to be -2.4 implies that laser group was more effective in causing dentinal tubule obliteration [Table 2].

Figure 1: Scanning Electron Microscope

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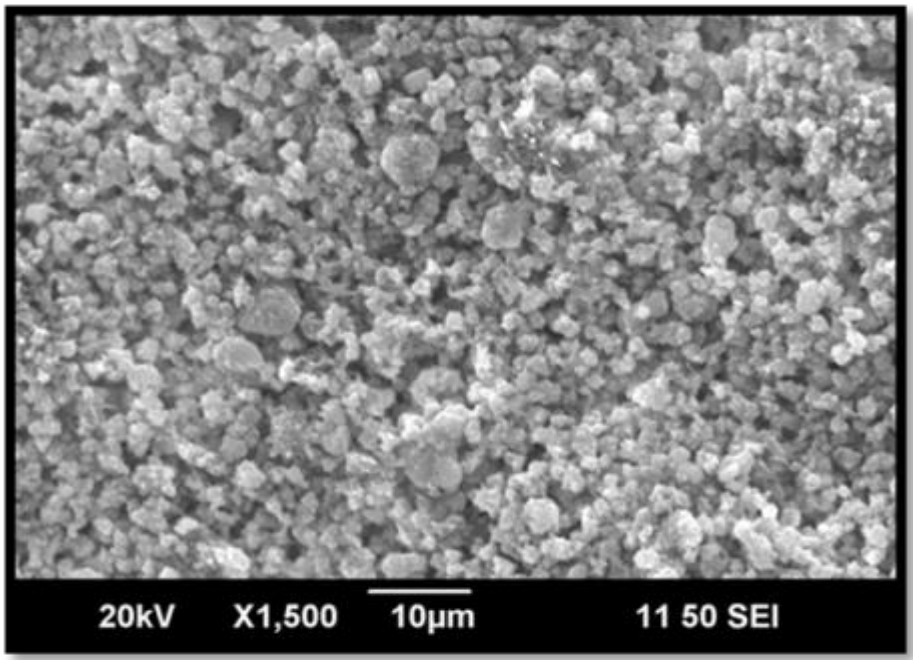

Figure 2: SEM image of Group 1

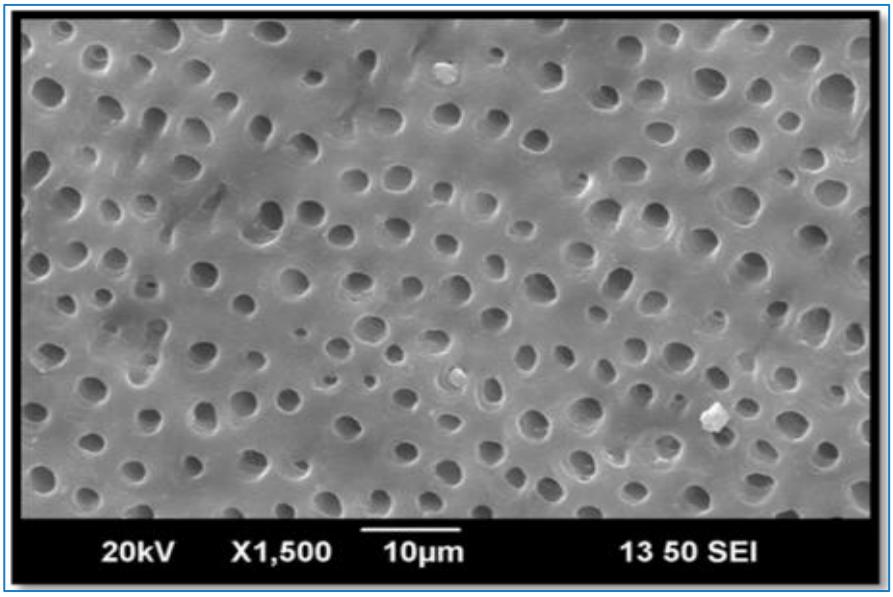

Figure 3: SEM image of Group II

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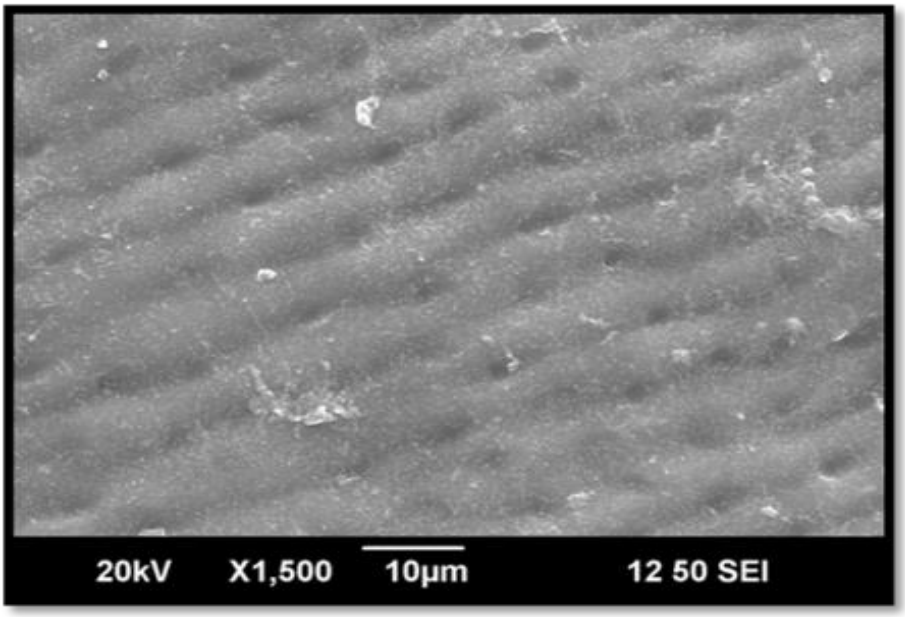

Figure 4: SEM Image of Group III

Groups	N	Mean	Std. Deviation	p-value
Laser Group	**10**	1.10	.316	
Mouthwash Group	10	4.60	.516	<0.001
Toothpaste Group	10	2.20	.422	

Table 1. Comparison of Indices between the Three Groups.

GROUPS		Mean Difference	Std. Error	p-value
Laser Group	Mouthwash	-3.500*	.191	<0.001
	Toothpaste	-1.100*	.191	<0.001
Mouthwash Group	Laser	3.500*	.191	<0.001
	Toothpaste	2.400*	.191	<0.001
Toothpaste Group	Laser	1.100*	.191	<0.001
	Mouthwash	-2.400*	.191	<0.001

Table 2. Tukeys Posthoc Test for Intergroup Comparison

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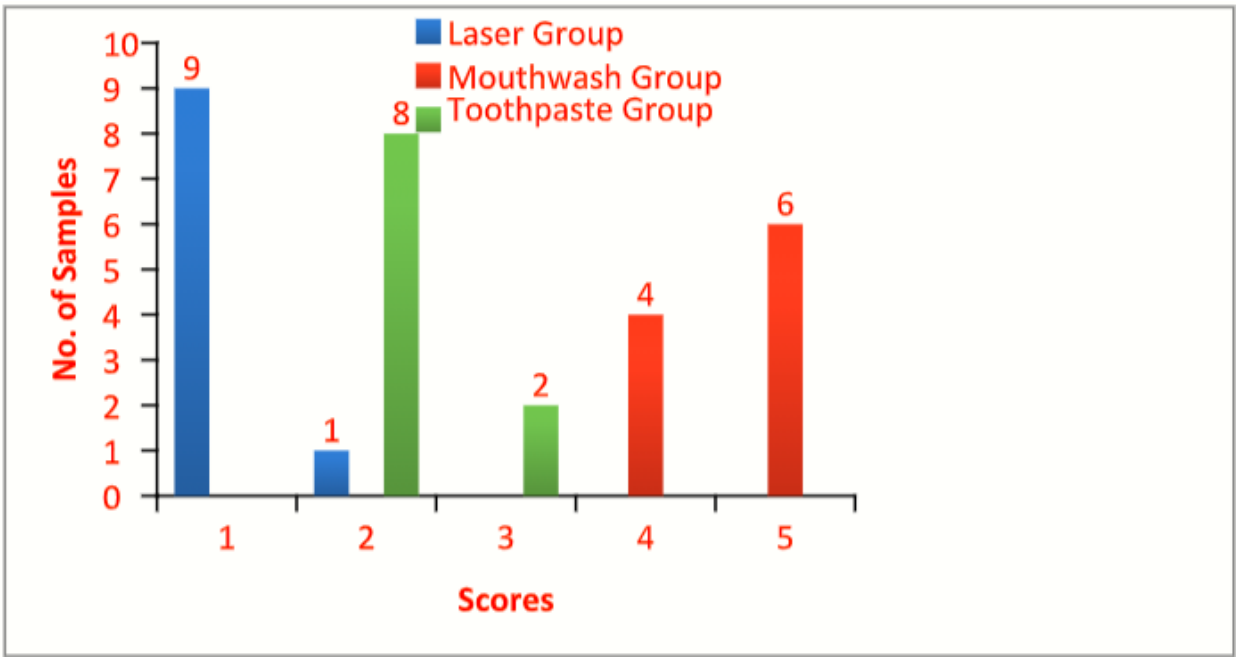

Graph 1: Graphical Presentation of Distribution of Scores in Each Group.

Discussion

Dentinal hypersensitivity is an enigma that is confronted by humankind for the time being. In particular, the main etiological factors are loss of enamel due to faulty tooth brushing and gingival recession. Due to the frequent and prolonged use, the patency of the dentinal tubules is lost and there occurs the opening up of the tubules.

Colgate Sensitive Plus™ is a novel desensitizing dentifrice, in which Arginine- Calcium Carbonate (ACC) content seals the open tubule by precipitating calcium ions and proteins. It was proposed that the soluble arginine bicarbonate (8%) adsorbed on the surface of the insoluble calcium carbonate particles, forming positively charged agglomerates that readily bind with the negatively charged dentine of the exposed tubule walls to form an occluding adhesive plug. The main advantage of this could be the natural occurrence of arginine in the saliva and at physiological pH arginine and calcium carbonate interact and bind to negatively charged dentin surface and dentinal tubules to plug and seal them.[7]

Davies et al. evaluated and compared the efficacy of a recently developed arginine-containing dentifrice, two established strontium-based products, and a fluoride control such as Colgate Sensitive ProRelief, Sensodyne Mint, Sensodyne Original and Colgate Cavity Protection. to occlude tubules when subjected to acid challenge by SEM study and observed that the strontium acetate- and arginine-containing pastes almost fully occluded tubules.[6]

Chen et al. compared the effectiveness of red propolis extract, calcium sodium phosphosilicate, and arginine-calcium carbonate in occluding dentine tubules on prepared dentin discs and concluded that ACC showed more occlusion following treatment.[8]

When compared to pastes and gels, mouth wash has become accepted as a compatible delivery vehicle because of its liquid nature, ease and simplicity, and by its ability to bypass the discomfort that occur while brushing

the sensitive dentin surface. Cetyl pyridinium chloride has shown a unique ability in inhibiting plaque formation.[9] Colgate Plax gentle care mouth wash is alcohol- free and its main ingredients include cetyl pyridinium chloride (CPC), potassium sorbate and the Potassium ions will result in nerve depolarization, reduces nerve excitation, pain sensation, and limits fluid movement.

Markowitz et al., described an arginine containing mouth wash (Pro- Argin™) and he compared it with the pre-existing potassium ions and he concluded that these potassium concentrations require several weeks in order to exert their desensitizing effect and can be used as an adjunct in the treatment of dentinal hypersensitivity.[10]

Diode lasers mainly function by interacting with the pulp thus leading to induction of photobiomodulating effect, which causes an increase in cellular metabolic activity of odontoblasts and intensifying tertiary dentine production, thus obliterating the dentinal tubules. Diode laser results in obliteration of dentinal tubules by melting peritubular and intertubular dentin. Diode laser exerts its desensitizing effect by in hibitting the depolarization of afferent fibres, and thus having a profound analgesic effect by depressing nervous signal transmission.[11]

Umana et al., evaluated the effect of diode lasers (810 and 980nm) at 0.8 and 1 W for 10sec in continuous mode on dentinal surfaces were able to seal the dentin tubules and can be considered harmless for pulp vitality, and effective in the treatment of dentinal hypersensitivity.[12]

Umberto et al. compared the effectiveness of Diode laser alone and with topical sodium fluoride gel (NaF) and found Diode laser to be more effective in reducing DH when used alone or in combination with NaF gel.[13]

In the present study, better dentinal tubule obliteration was in the toothpaste group than compared to mouthwash which was in accordance with the studies done by Sharda S et al,[14] Siladitya S et al,[4] George et al.[15]

Between diode laser and toothpaste, significant results are for diode laser which was in accordance with Reddy et al,[16] Dilsiz et al.[17]

From this present study, it was concluded that diode laser had found to be having highest dentinal tubule occlusion than compared to desensitizing mouthwash with the mean values 1.1 and 4.6 respectively. Many in vitro and in vivo studies were conducted till recent time by comparing various desensitizing agents. And this is the first of that kind of an in vitro study comparing the dentinal tubule obliteration among desensitizing toothpaste, mouthwash, and diode laser.

A laser is not an economical means for treatment of dentinal hypersensitivity and as well as not user friendly and requires expertise. But desensitizing toothpaste and mouthwash seems to be easily accessible and acceptable by the patients because of their easy mode of administration.

Conclusion

Dental professionals have a variety of regimens to manage patient dentinal hypersensitivity, including both in-office and patient applied products for home use. Home-care desensitizing products like desensitizing toothpaste appear to be the most simple, realistic, cost-effective, and practical means of treating patients with tooth hypersensitivity and should be the fundamental step in accustomed day –to –day management. In case of

persistence of severe hypersensitivity, it's time for us to bid farewell to the home care agents and have to eagerly opt for the in office products.

Hence from the results, it was concluded that laser (diode) can be considered as the best effective in-office treatment modality for dentin hypersensitivity. Both the desensitizing toothpaste and the mouthwash group revealed comparable effectiveness. of which, the mouthwash group can only be considered as an adjunct along with the first and third groups.

In a clinical situation, various parameters like brushing habits, salivary constituents, acidic pH may influence the tubule occlusion, so the results should be extrapolated with caution. Furthermore, a larger sample size of in vivo studies should be conducted to estabilish a healthy competition between these desensitizing agents in the oral environment.

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