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Comparative evaluation of efficacy of diode laser and two commercially available desensitizing toothpastes for dentinal tubular occlusion: A scanning electron microscope (SEM) study

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Abstract--Purpose: Dentinal hypersensitivity is a common clinical condition associated with exposed dentinal surfaces. Dentinal tubules can be entirely blocked after irradiation by 980-nm diode laser, with no significant morphological alterations after radiation. The use of toothpastes containing Potassium Nitrate and Calcium Hydroxyapatite reduces dentinal hypersensitivity. The main purpose of this study was to evaluate and compare the efficacy of Diode Laser and two Desensitizing Toothpastes for Dentinal Tubular Occlusion by Scanning Electron Microscopy (SEM). Methods: Twelve extracted premolars were divided into two halves each obtaining twenty-four dentin discs. Specimens were etched with 17% phosphoric acid to expose the tubules and were divided into four groups: i.e., Group A: Application of Diode Laser, Group B: Application of SHY-XT toothpaste, Group C: Application of Biomed toothpaste and Group D: Application of Distilled water. After treatment, quantitative analysis of occluded dentinal tubules was done by SEM analysis. Results: The mean values of total dentinal tubular occlusion in Groups A, B, C and D were 0.42, 0.86, 2.14, 4.07 respectively which was statistically significant ($p < 0.001^*$). Diode laser (Group A) yielded a significant occlusion of the dentinal tubules when compared to the two desensitizing toothpastes (Groups B and C). Conclusion: Among all the four groups, Diode laser (Group A) has shown more efficacy in occluding dentinal tubules when compared to desensitizing toothpastes which was statistically significant ($p < 0.001^*$).

Keywords--dentinal hypersensitivity, dentinal tubular occlusion, diode laser, scanning electron microscope.

Introduction

One of the most commonly presenting symptoms in dental practice is dentin hypersensitivity or dentinal hypersensitivity (DH). Dentinal hypersensitivity is defined as an abnormal response of the exposed vital dentin to thermal, chemical, or tactile stimuli. It is a frequent patient malady, most commonly affecting the premolar and canine facial surfaces. Recently irradiation of affected teeth with low-output power lasers known as Diode Laser have shown significant anti-inflammatory and analgesic effects.¹ Dentinal tubules can be entirely blocked after irradiation by 980-nm diode laser, with no significant morphological alterations of the pulp and odontoblasts after radiation.¹ The sealing of exposed dentinal tubules with melted and recrystallized dentin is caused by the thermal and occlusive effects of diode laser.² In 980nm diode laser the power inputs when used between 2 to 4 W in the continuous mode increases the absorption by the

dentin mineral content, resulting in its melting and crystalline arrangement.^{3,4,5} Low-power laser therapy is an appropriate treatment for the reduction of dentinal hypersensitivity, it promotes biomodulatory effect, increasing the cellular metabolic activity of the odontoblasts and obliterating the dentinal tubules with the production of tertiary dentin, which causes an analgesic effect and reduces inflammatory effect.⁶

The use of toothpastes containing Potassium Nitrate and Calcium Hydroxyapatite reduces dentinal hypersensitivity.⁷ A rational approach to the control of pain arising from exposed dentin would be to occlude or reduce the diameter of the exposed tubules. Occluding dentinal tubular agents can create a barrier by precipitating proteins calcium or phosphate ions on the surface or within the tubules. Therefore, main purpose of this study was to evaluate and compare the efficacy of diode laser and desensitizing toothpastes in occlusion of dentinal tubules and thus reduction in dentinal hypersensitivity, using scanning electron microscope (SEM).

Materials and Methods

Sample size

The study was conducted only after ethical clearance from the institutional scientific review committee. For this in-vitro study, 12 freshly extracted teeth (premolars) were collected from Oral Surgery Department, Bharati Vidyapeeth Dental college and Hospital, Navi Mumbai. Extracted teeth were chosen based on the below mentioned inclusion & exclusion criteria.

Inclusion criteria

The teeth which were extracted from individuals included maxillary and mandibular premolars not affected by caries and not fractured.

Exclusion criteria

Maxillary and mandibular premolars with dental caries, restorations, prosthesis and fractured maxillary and mandibular premolars.

Randomization

The extracted teeth were randomly divided into four groups to one of the treatment modalities:

1. Group A- 980 nm Diode laser: Biolase- Epic X laser system
2. Group B- SHY-XT toothpaste
3. Group C- Biomed sensitive natural toothpaste
4. Group D- Distilled water

Laser application guidelines

Extracted premolars were irradiated with 980nm Biolase diode laser (InGaAsP Semi-conductor diode at 4.0W/CW) 5 secs 5 times for each sample for once in a non-contact continuous mode keeping the laser tip 1 mm away from the prepared tooth surface to prevent contamination from dentin.

Methodology: The study design is structured below in Fig. 1.

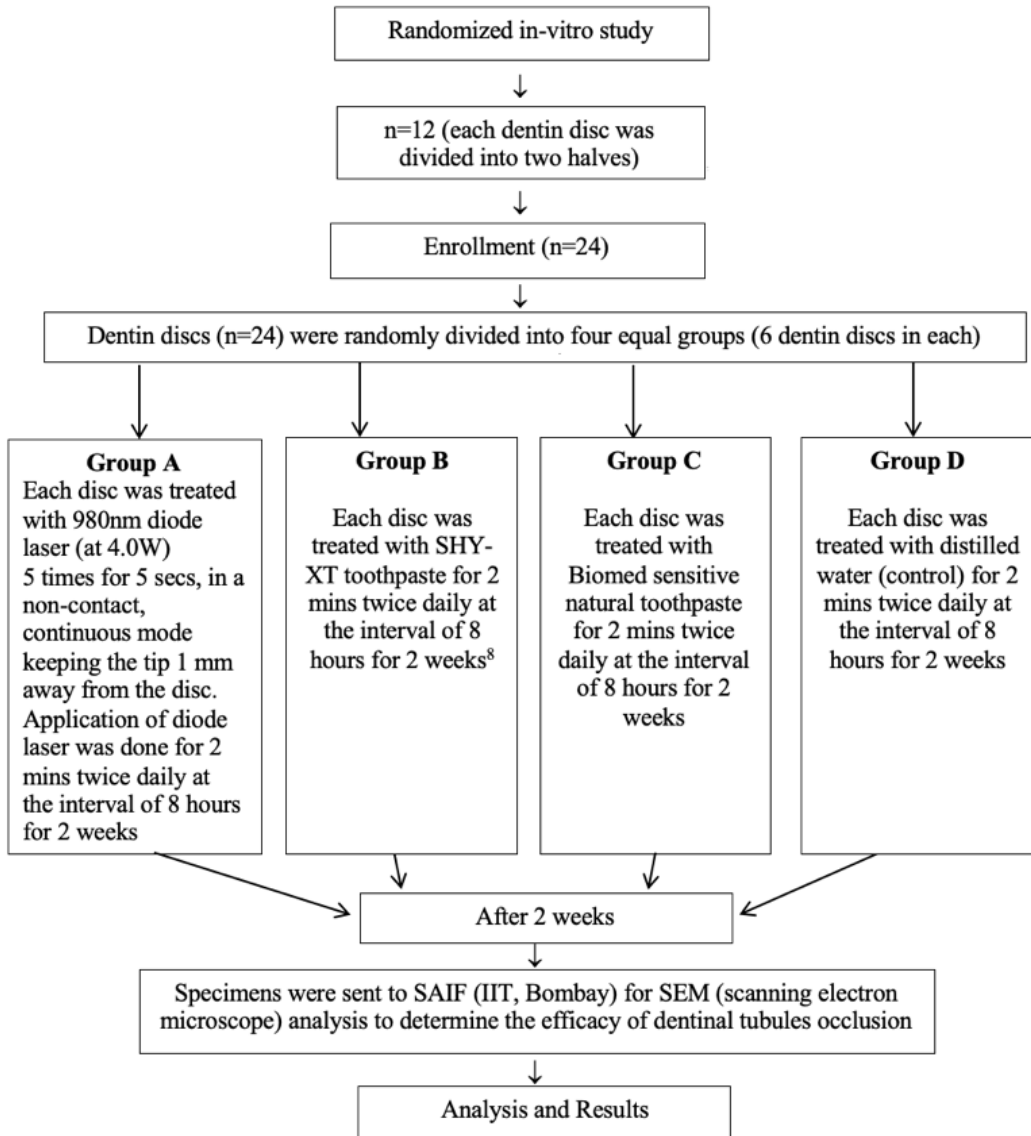


Fig.1: Study Design

- All the extracted teeth were stored in distilled water, followed by ultrasonic scaling and root planing to remove the debris and calculus.
- Enamel of each tooth was removed with a tungsten carbide fissure bur under continuous water spray.
- Every premolar was sliced to obtain dentin disc using the carborundum disc.
- Each dentin disc (n=12) was then divided into two halves using carborundum disc (Fig. 2)
- Total of 24 specimens were randomly and equally divided into four groups (three test groups i.e., Group A: Diode Laser, Group B: SHY-XT toothpaste and Group C: Biomed toothpaste and one control group i.e., Group C: Distilled water). Each group contained 6 dentin discs (Fig. 3).
- The average thickness of these dentin discs was 3 mm in dimension.
- All the specimens were then etched with 37% phosphoric acid for 2 mins to expose the dentinal tubules.
- Specimens were then rinsed with copious distilled water and kept in 10 ml artificial saliva (ICPA Wet Mouth 200ml) at pH 7 for 14 days which was changed after every 24 hours.⁸
- Acrylic stent was prepared to mount the dentin discs for the application of Diode Laser (980nm) and two Desensitizing Toothpastes (SHY-XT and BIOMED) (Fig. 4).
- Group A: Six dentin discs were treated with 980nm biolase diode laser (Fig. 18a) (InGaAsP Semi-conductor diode at 4.0W/CW) 5 secs 5 times for each sample for once in a non-contact continuous mode keeping the laser tip 1 mm away from the prepared tooth surface to prevent contamination from dentin.
- Group B: Six dentin discs were treated with SHY-XT toothpaste (Fig. 19a) and ultra-soft bristle toothbrush for 2 mins twice daily for 2 weeks.⁹
- Group C: Six dentin discs were treated with Biomed sensitive natural toothpaste and ultra-soft bristle toothbrush for 2 mins twice daily for 2 weeks. 980nm biolase diode laser.
- Group D: Six dentin discs were treated with distilled water (control group) for 2 mins twice daily for 2 weeks.
- After the application of diode laser, two commercially available desensitizing agents and distilled water, all the specimens were kept in an air-tight container immersed in 10ml of artificial saliva for 14 days which was changed after every 24 hours.
- These specimens were further sent to SAIF (IIT, BOMBAY) for Scanning Electron Microscopy (SEM) to determine the efficacy of dentinal tubular occlusion.



Fig. 2: Each dentin disc was divided into two halves to obtain 24 specimens



Fig. 3: Specimens divided into four groups for agent application

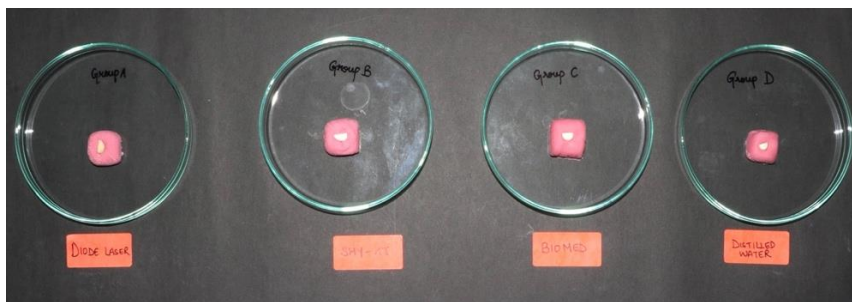


Fig. 4: Acrylic stent was prepared to mount the dentin discs for the application of different agents

Scanning electron microscope analysis

Specimens which were stored in artificial saliva after the application of Diode laser and two Desensitizing toothpastes were washed with distilled water and then dried under infrared lamp for 30 mins at 37°C. All the specimens were then mounted on the stubs after which they were stored in vacuum desiccator for another 20 mins; the specimens were sputter coated to aid conductivity. Placement of the specimens under SEM for the observation of dentinal tubular occlusion (Fig. 5).

Steps involved in SEM analysis are as follows;

1. Gold sputtering
2. Teeth mounted in SEM machine
3. Vacuumization
4. Image processing

Photomicrographs were taken from each specimen surface examined at 2000x magnifications under SEM. The surfaces of the specimens were visualized under SEM of magnification 2000x, and photographs of representative areas were obtained.

Clinical assessment parameters

1. Quantitative, measurement of diameters of patent dentinal tubules in each dentin disc model group.
2. Comparing the average of the diameter of patent dentinal tubules in each group.



Fig. 5: SEM equipment (Jeol JSM-7600F) for observation of dentinal tubular occlusion

Statistical analysis

Normality of numerical data was checked using Shapiro-Wilk test & was found that the data followed a normal curve; hence parametric tests have been used for comparisons. Inter group comparison (>2 groups) was done using one way ANOVA followed by pair wise comparison using post hoc test and statistical significance was calculated.

Results

The data provides complete description of the study parameters, and the results based on the statistical tests used for the analysis of variables of interest.

The mean values of dentinal tubular occlusion were compared between three test groups and one control group (Table 2) using one way ANOVA followed by pair wise comparison using post hoc test.

On SEM Evaluation

The microphotographic images from the SEM were quantitatively analysed for dentinal tubular occlusion/obliteration in each of the four groups using Image J software. All the evaluated treatments produced morphological changes in the dentin surfaces (Fig. 6-9). For Group A (DIODE LASER), the SEM images showed a wide occlusion of the dentinal tubules (Fig. 6) after the application of diode laser in a non-contact mode. In diode laser group, there was melting of dentin and complete occlusion of dentinal tubules, thus revealing efficient reduction in the diameter of dentinal tubules.

For Group B (SHY-XT Toothpaste), the SEM images showed reduction in the diameter of the dentinal tubules after the application of SHY-XT toothpaste. The image showed both partial and complete tubular occlusion of dentin (Fig. 7).

For Group C (BIOMED Toothpaste), the SEM images showed reduction in the diameter of the dentinal tubules after the application of Biomed toothpaste. The image revealed partial dentinal tubular occlusion with white crystal deposition of salts which was present in artificial saliva, thus showing minimum reduction in the diameter of dentinal tubules than the other two groups A and B (Fig. 6,7). For Group D (Distilled water), the SEM images showed a more homogeneous and uniform dentin surface with no dentinal tubular occlusion (Fig. 9) as compared to groups A, B and C (Fig. 6,7,8). Diode Laser group (Group A) showed better efficacy in dentinal tubular occlusion when compared to Group B, Group C and Group C.

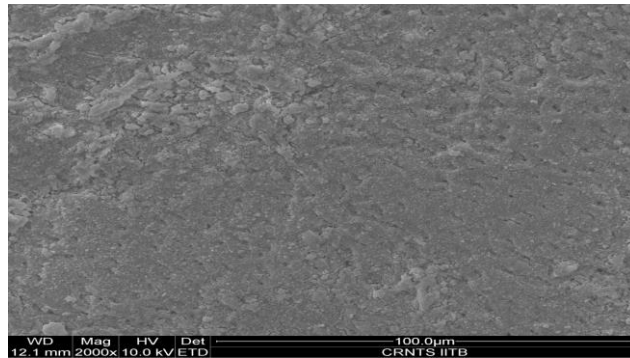


Fig. 6: SEM micrographs of Group A (Diode laser) dentin surface morphology at 2000x~ magnification showing complete dentinal tubular occlusion

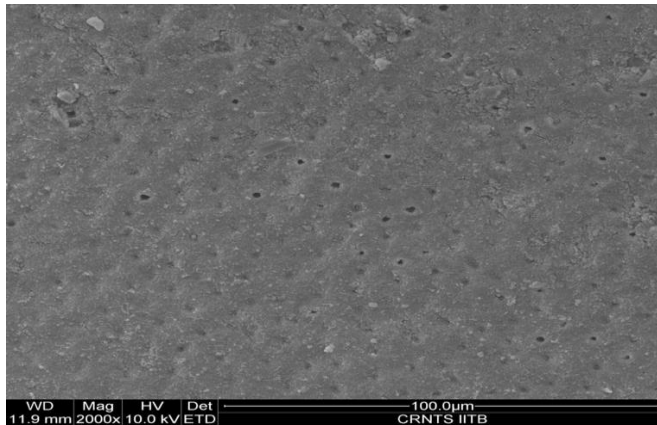


Fig. 7: SEM micrographs of Group B (SHY-XT toothpaste) dentin surface morphology at 2000x~ magnification showing both partial and complete dentinal tubular occlusion

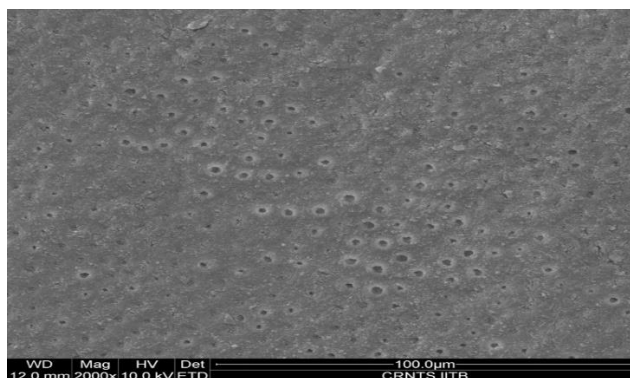


Fig. 8: SEM micrographs of Group C (BIOMED toothpaste) dentin surface morphology at 2000x~ magnification showing partial dentinal tubular occlusion with crystal deposition

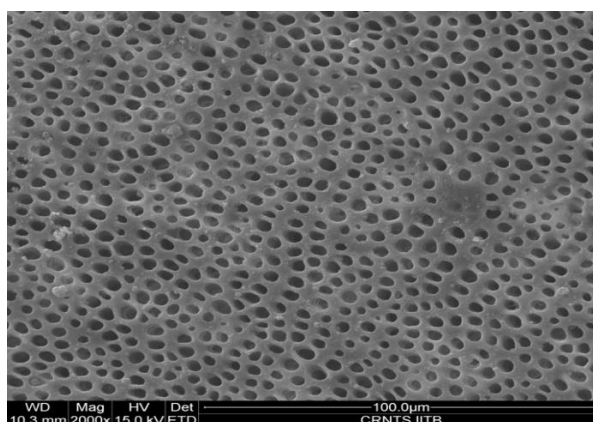


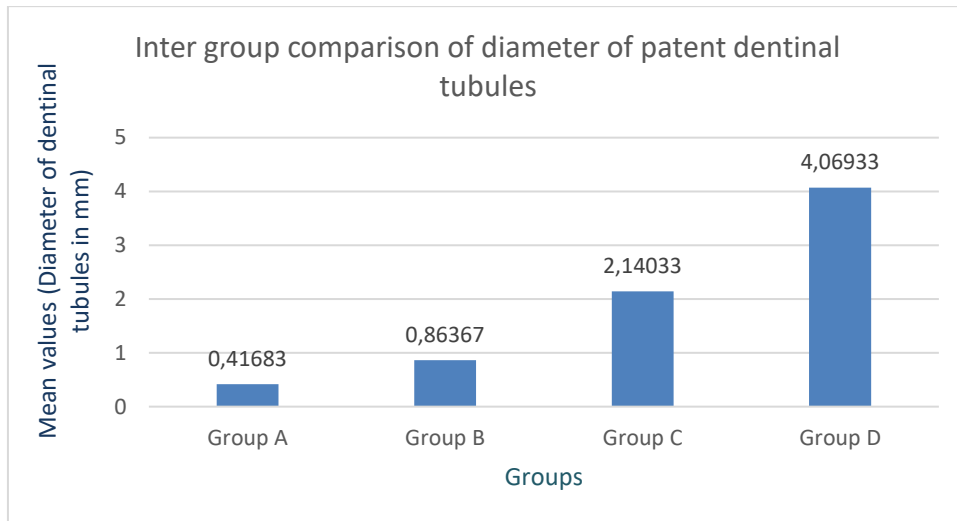
Fig. 9: SEM micrographs of Group D (DISTILLED WATER) dentin surface morphology at 2000x~ magnification showing no change in dentinal tubular occlusion.

One-way ANOVA test revealed a statistically highly significant difference amongst Groups A, B, C and D ($p < 0.001$) with higher values in Group D (Distilled water) and lowest in Group A (Diode laser) (Table 1). On inter-group comparison of diameter of patent dentinal tubules in each of the four groups (Table 1), the efficacy of dentinal tubular occlusion (reduction in the diameter of dentinal tubules) in control group (Group D) is least when compared to the test groups (Group A, Group B and Group C), therefore revealing that Group A (Diode laser) has the maximum efficacy in reduction of dentinal tubular diameter.

Group	One-way ANOVA test					p value of one way ANOVA
	N	Mean (M)	Std. Deviation	Std. Error	F value	
A	6	0.41683	0.119625	0.048837		

B	6	0.86367	0.248743	0.101549	186.632	0.001*
C	6	2.14033	0.167442	0.068358		
D	6	4.06933	0.490082	0.200075		

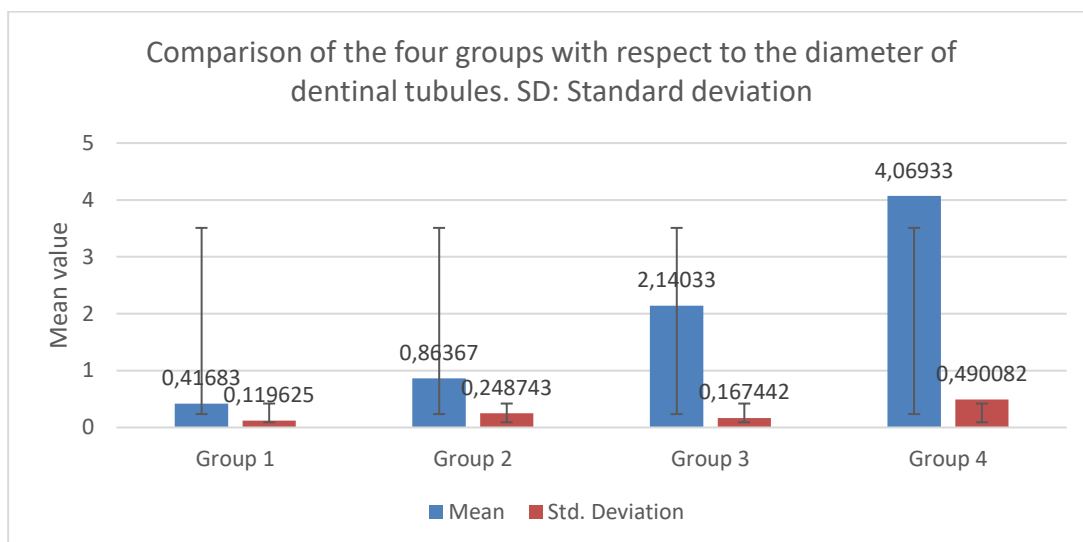
Table 1: Inter-group comparison of diameter of patent dental tubules



Graph 1: Inter group comparison of the four groups (A, B, C and D) with respect to the diameter of patent dental tubule

Comparison of the four groups (A, B, C and D) with respect to the diameter of dental tubules. SD: Standard deviation				
Mean±SD				
Groups	Group A	Group B	Group C	Group D
Mean±SD	0.42±0.12	0.86±0.25	2.14±0.17	4.07±0.49
p value	0.001*			

Table 2: Comparison of the four groups (A, B, C and D) with respect to the diameter of dental tubules. SD: Standard deviation



Graph 2: Comparison of the four groups (A, B, C and D) with respect to the mean diameter of dentinal tubules and standard deviation (SD)

Maximum efficacy of dentinal tubular occlusion was shown by Group A (Mean±SD: 0.42±0.12) i.e., the minimum diameter of dentinal tubules was seen in Diode laser group (Table 5, Graph 2), thus revealing a highly statistically significant difference amongst the four groups ($p < 0.001^*$).

Groups	Group	Pair wise comparison using Games-Howell Post Hoc Tests		
		Mean Difference	Std. Error	p value
A	B	-0.446833*	0.112682	0.021*
	C	-1.723500*	0.084011	0.001*
	D	-3.652500*	0.205949	0.001*
B	C	-1.276667*	0.122413	0.001*
	D	-3.205667*	0.224371	0.001*
C	D	-1.929000*	0.211431	0.001*

Table 3: Pair wise comparison using Games-Howell Post Hoc Tests

According to pair wise comparison using Games-Howell Post Hoc Tests, there was a statistically highly significant difference seen for the values among all the pairs of groups ($p < 0.01, 0.05$) (Table 3).

Among all the four groups, the efficacy of occlusion of dentinal tubules in Group A (Diode laser) was more when compared to the other three groups (Groups B, C, and D) and was statistically highly significant ($p < 0.001^*$). Diode Laser group (Group A) showed better efficacy when compared to Group B (SHY-XT) and Group C (Biomed) and hence, Group A (Diode laser) was statistically highly significant ($p < 0.001^*$).

Discussion

Dentinal hypersensitivity (DH) is defined as a “short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or disease.”¹⁰ The primary cause of DH is loss of enamel on the tooth crown and gum recession exposing the root, with subsequent loss of cementum. Enamel can be lost as a result of aggressive or incorrect toothbrushing, over-consumption of acidic food, and tooth grinding caused by stress and parafunctional behaviours.¹¹ The prevalence of DH varies from 45% to 57% and is between 60% and 98% in patients with periodontitis.¹²

The concept of tubular occlusion as a method to treat DH is a logical conclusion from the hydrodynamic theory. According to this theory, changes in the flow of the fluid in the dentinal tubules can trigger pain receptors present on nerve endings in the pulpal aspect to fire nerve impulses, thereby causing pain.¹³ In our study, Diode laser and two desensitising toothpastes SHY-XT and Biomed were used to evaluate and compare the efficacy of dentinal tubular occlusion using scanning electron microscope (SEM). The results of the present study demonstrated that all the three groups tested were effective in occluding the dentinal tubules after the application of Diode Laser and two desensitising toothpastes (SHY-XT and Biomed) for 2 mins twice daily at the interval of 8 hours for 2 weeks. Among all the four groups, the efficacy of occlusion of dentinal tubules in Group A (Diode laser) was more when compared to the other three groups (Groups B, C, and D).

Different mechanisms of action have been proposed for laser, its effect on the dentin and its effect on reducing DH. They include:¹⁴

1. Occlusion through coagulation of the proteins of the fluid inside the dentinal tubules
2. Occlusion of tubules through partial sub-melting
3. Discharging of internal tubular nerve

In this study, 4.0 W, 980-nm wavelength, diode laser (Epic-X Biolase) was used in a non-contact mode which was found to be effective in rapid sealing of exposed dentinal tubules without damaging the odontoblasts and pulp tissue. When the power inputs were increased from 4 to 6 W in the continuous mode, the energy of 980-nm Diode laser absorbed by the mineral content of dentin is increased, resulting in melting and crystalline arrangement leading to cracks in the dentin discs.^{15,16,17} The results of our study are contradictory to the study done by Ying L et al., wherein they used Diode Laser to evaluate the dentinal tubular occlusion. It was concluded that the Diode Laser of 980 nm when used in a contact mode did not occlude the dentinal tubules and lead to cracks in the dentin disc when checked under SEM.¹⁸

In addition, this type of therapy is highly acceptable to patients because its proper usage has no negative impacts. So far, there has been no report of adverse reactions or pulp damage in the studies. Thus, the use of laser in treatment of DH is both logical and acceptable.¹⁹ In Group A, it was noted that the diameter of the

dentinal tubules was least ($M=0.41683$) revealing complete occlusion of dentinal tubules. The obliteration of dentinal tubules was caused due to the melting of dentin after the use of diode laser (Group A). It can be concluded that Diode Laser (980nm) effectively occludes open dentinal tubules which could provide relief from pain due to DH. There was statistically significant difference in dentinal tubular occlusion for all the four groups ($p<0.001^*$).

In a systematic review and meta-analysis, published through the years 2000-2018, on the effects of laser therapy on treating DH, it has generally been claimed that laser therapy for the treatment of DH is preferred to other relevant local therapies.¹⁹ Sgolastra F. et al., conducted a meta-analysis to assess the efficacy of lasers in reducing DH based on the results he concluded that, Er:YAG, Nd:YAG, and GaAlAs lasers appear to be efficacious in reducing DH. However, given the high heterogeneity of the included studies, future randomized controlled clinical trials are needed to confirm the results.²⁰ Therefore, to the best of our knowledge, this is the first study till date, to evaluate and compare the efficacy of dentinal tubular occlusion amongst Diode Laser at 4.0 W power in a non-contact mode and two commercially available desensitizing toothpastes i.e., SHY-XT and Biomed.

SHY-XT used in this study has a rich composition of potassium nitrate, nano hydroxyapatite and fluoride. An active ingredient for depolarizing dentinal nerve fibres, potassium nitrate averts the pain caused due to sensitivity. Nano Hydroxyapatite works deeply to cork sensitivity by sealing the open dentinal tubules and forming a layer over dentin surface. SHY-XT toothpaste helps in relieving pain caused due to sensitivity and repairs damaged tooth enamel. In the present study, the SEM images showed reduction in the diameter of the dentinal tubules after the application of SHY-XT toothpaste. The microphotographic images from the SEM showed both partial and complete tubular occlusion of dentin.

In Group B (SHY-XT) the diameter of dentinal tubules was larger ($M=0.86367$) than Group A (Diode laser) showing lesser efficacy when compared to the Diode laser. The diameter of dentinal tubules was larger ($M=2.14033$) in Group C (Biomed) than Group B (SHY-XT), thus revealing lesser efficacy as compared to the Group A (Diode laser) and Group B (SHY-XT).

In a study done by Amaechi et al., it was concluded that toothpaste containing nano-HAP alone (10 or 15% nano-HAP) or supplemented with potassium nitrate (KNO_3) was effective in relieving DH symptoms when used at least twice daily.²¹ The effectiveness of nano-HAP to reduce DH is attributed to its ability to form a layer of mineral hydroxyapatite as well as hydroxyapatite plugs that occlude dentinal tubules.^{22,23,24,25} The results of this study also showed that nano-HAP and potassium nitrate (KNO_3) containing toothpaste (SHY-XT) occludes dentinal tubules. A new innovative technology based upon arginine and calcium carbonate (Biomed) has been developed and validated as a unique and highly effective treatment for dentin hypersensitivity. This innovative technology works by physically sealing dentin tubules with a plug that contains arginine, calcium carbonate, and phosphate. This plug, which is resistant to normal pulpal

pressures and to acid challenge, effectively reduces dentin fluid flow and, thereby, reduces sensitivity.²⁶

Biomed Sensitive toothpaste used in this study helps in enamel strengthening as well as reduction in dentinal sensitivity. Calcium Hydroxyapatite and L-Arginine strengthen and restore tooth enamel and reduce sensitivity. Farid Ayad et al., in a clinical study evaluated the efficacy of a toothpaste containing 8% arginine and calcium carbonate for immediate and lasting relief of DH to which he concluded that a desensitizing toothpaste containing 8% arginine and calcium carbonate, with or without fluoride, provides statistically significant reductions in DH when applied by a dental professional prior to a professional dental prophylaxis. The results also demonstrate that this desensitizing toothpaste provides statistically significant reductions in DH when used subsequently as an adjunct to routine twice daily tooth brushing.²⁷ In our present study, SEM analysis for Group C (Biomed) on dentin slices showed partial occlusion of dentinal tubules occlusion with white crystal deposition of inorganic salts which indicated that the efficacy of Biomed Sensitive was lesser than Diode laser and SHY-XT. In the control group D (distilled water), the SEM images showed a more homogeneous and uniform dentin surface with no changes in the diameter of the dentinal tubules when compared to the other three test groups (Groups A, B and C).

The results from the present clinical investigation clearly show that the two commercially available toothpastes i.e., SHY-XT containing nano-HAP and potassium nitrate and new fluoride free Biomed toothpaste containing L-arginine, calcium hydroxyapatite, were effective in treating dentin hypersensitivity when used twice daily for a period of 14 days (2 weeks). Diode Laser and both desensitizers occluded the tubules however, Diode Laser has shown highly statistically significant results ($p < 0.001^*$) in terms of complete tubular occlusion on initial application.

Amongst the four groups, Group A was most efficacious in the occlusion of dentinal tubules followed by Group B and then Group C. The results of our study were limited to physical findings of the change in the dentinal tubules and do not present in vivo differences that may result from the physiological effect of these desensitizing agents. In this study, it has been shown that diode laser and desensitizing toothpastes produce varying degrees of obliteration of tubules at initial application and hence could have differences in reduction in sensitivity based on the type and amount of blockage of tubules. All the three test groups produced varying degrees of tubular occlusion in the form of complete and partial occlusion amongst which diode laser showed statistically highly significant ($p < 0.001^*$) result revealing maximum obliteration of dentinal tubules. The experimental parameters chosen in this study were accurate which helped to determine the role of diode laser and desensitizing toothpastes in dentinal tubular occlusion.

Limitations of this study include a small sample size, no pre-treatment and post-treatment analysis of the dentinal tubular occlusion. More long-term follow-ups and studies in vivo are required before we can safely exclude the use of desensitizing agents completely and replace them with diode laser. Well-conducted clinical trials are needed to provide high-quality evidence able to guide

clinicians and patients when choosing the most appropriate treatments for DH. Therefore, correct diagnosis, proper treatment technique and treatment intervals plus sufficient dosage are all essential to obtain good results.

Conclusion

Diode laser (Group A) has shown more efficacy in occluding dentinal tubules when compared with two desensitizing toothpastes, i.e., SHY-XT toothpaste (Group B) and Biomed toothpaste (Group C) which was statistically significant. The mean diameter of the dentinal tubules was least in Group A (showing maximum occlusion) when compared to Group B and Group C. On inter-group comparison, Group A was most effective in occluding the dentinal tubules which showed highly statistically significant value ($p < 0.001^*$).

The 940nm diode laser on treatment of dentinal surface brought about a highly statistically significant reduction in dentinal tubular occlusion, hence diode laser was not only an efficacious treatment modality for reduction in dentinal hypersensitivity but also showed maximum reduction in the diameter of dentinal tubules when compared to the other two desensitising toothpastes. Therefore, Diode laser (980nm) treatment used at 4.0W in a non-contact, continuous mode on exposed dentinal tubules promises a non-invasive, pain-free and relatively safe treatment option with promising results.

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Conflict of interest: There are no conflicts of interest.

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