# A Comparative Evaluation of the Effectiveness of Three Different Modalities in Occluding Dentinal Tubules: An *In Vitro* Study

Anu A George<sup>1</sup>, Alex M Muruppel<sup>2</sup>, Sudeep S<sup>3</sup>, Dinesh N<sup>4</sup>, Sandeep Lal<sup>5</sup>

#### **A**BSTRACT

**Aim:** This *in vitro* study was designed to evaluate and compare the effectiveness of a dentifrice containing 8% arginine and calcium carbonate, diode laser (810 nm) and the combined use of diode laser (810 nm) and the same dentifrice in occluding dentinal tubules.

**Materials and methods:** Forty sectioned dentine discs were observed in this study. The Samples were randomly divided into 4 groups. In group A, a dentifrice containing arginine and calcium carbonate was applied. In group B, the specimens were subjected to diode laser (810 nm) irradiation. In group C, the same dentifrice was applied and the specimens were subjected to diode laser (810 nm) treatment. Group D was left untreated and served as the control. The specimens were observed under the scanning electron microscope. Then the photomicrographs of each specimen were taken which were uploaded into Photoshop 7°, and the diameters of the dentinal tubules of each specimen were measured and the data were analyzed statistically.

**Results:** The overall comparison between different treatment modalities using one-way analysis of variance (ANOVA) and multiple comparisons of two modalities assessed using post hoc Tukey method revealed that there is a highly significant difference (*p* value = 0.0001) in the dentinal tubule diameter and rate of occlusion in different treatment modalities.

**Conclusion:** The study concluded that all three treatment modalities caused significant occlusion of dentinal tubules. The combined use of desensitizing toothpaste and diode laser (810 nm) is the most effective, then comes the diode laser (810 nm) alone and finally the desensitizing toothpaste alone.

Clinical significance: This study suggests an effective treatment of dentinal hypersensitivity using diode laser and desensitizing toothpaste.

**Keywords:** Dentinal hypersensitivity, Desensitizing toothpaste, Diode laser, Tubule occlusion

The Journal of Contemporary Dental Practice (2019): 10.5005/jp-journals-10024-2538

#### Introduction

Dentinal hypersensitivity is a common, clinical condition that causes discomfort and sometimes severe pain, affecting 4–73% of the adult population in clinical dentistry.<sup>1-4</sup> It is caused by exposure of spots of dentinal tubules to the oral environment.<sup>5-12</sup>

It is typically experienced in an age range from 20–49 years, with a peak incidence between 30–39 years. The buccal cervical regions of the permanent teeth are the most commonly affected surfaces, with canine, premolar and incisor teeth being more frequently affected than molars. This problem is characterized by a short, sharp, and severe pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical, ceasing after their removal. 1,13

Dentinal sensitivity can be a great concern to the patient and dentist.<sup>2-4</sup> Furthermore, there is no unanimity of opinion about solutions proposed to reduce dentinal sensitivity.

Conventional therapy for hypersensitive teeth is based on using topically applied desensitizing agents, either professionally or at home. However, most treatments are not very effective and last for only a short while. Conventional desensitizing agents (professional pastes, kinds of toothpaste, mouthwashes) aim to obliterate the exposed dentinal tubules. 14-21

Induction of natural mineral formation by *in situ*—ew technologies, such as the Pro-Argin technology and NovaMin bioactive glass, are believed to bind to the exposed dentin surface and within the openings of the dentin tubules to mediate the formation of biological mineral. <sup>5,22,23</sup>

Laser desensitization is also a novel and efficient alternative for the immediate treatment of dentinal hypersensitivity. 24-28 Lasers are being investigated for the treatment of hypersensitivity

<sup>1</sup>Department of Prosthodontics, KMCT Dental College, Kozhikode, Kerala, India

<sup>2</sup>Department of Implantology, Azeezia College of Dental Science and Research, Kollam, Kerala, India

<sup>3,4</sup>Department of Prosthodontics, PMS College of Dental Science and Research, Thiruvananthapuram, Kerala, India

<sup>5</sup>Taluk Head Quarters Hospital, Kerala Health Services, Wayanad, Kerala, India **Corresponding Author:** Anu A George, Department of Prosthodontics, KMCT Dental College, Kozhikode, Kerala, India, Phone: +919446562968, e-mail: anusaara@gmail.com

**How to cite this article:** George AA, Muruppel AM, Sudeep S, Dinesh N, Lal S. A Comparative Evaluation of the Effectiveness of Three Different Modalities in Occluding Dentinal Tubules: An *In Vitro* Study. J Contemp Dent Pract 2019;20(4):454-459.

Source of support: Nil Conflict of interest: None

as a monotherapy or in combination with desensitizing agents incorporated in toothpaste in regular use by patients.<sup>17,29-31</sup>

Presently there is no standard protocol of treatment for the same. This study evaluates the effectiveness of a dentifrice containing 8% arginine and calcium carbonate, diode laser (810 nm) and combined use of diode laser (810 nm) and the same dentifrice in the treatment of dentinal hypersensitivity. It also compares the effectiveness of these three methods.

#### MATERIALS AND METHODS

This study was conducted in a laboratory setting in the Department of Prosthodontics, PMS College of Dental Science and Research,

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Thiruvananthapuram and in the SEM Lab, Sree Chitira Tirunal Institute of Medical Science and Technology, Thiruvananthapuram. Prior ethical clearance was obtained from the Institutional Ethics Committee, PMS College of Dental Science and Research, Golden Hills, Vattappara, Thiruvananthapuram (PMS/IEC/2012/04).

Forty dentin discs from extracted premolars free of caries used in the study were randomly allotted among the four groups given below. Teeth with cracked structures, carious lesions or restorations were excluded.

- · Group A which was treated with desensitizing toothpaste only
- Group B received treatment with GaAlAs diode laser (810 nm)
- Group C treated with both GaAlAs diode laser (810 nm) and Desensitizing toothpaste
- · Group D served as the control group

Briefly, the dentin discs were prepared to a thickness of 3mm by cutting perpendicular to the long axis of the tooth using a low-speed diamond blade disc.<sup>32</sup> The enamel was removed by a diamond disc at high speed under continuous water spray to prepare a dentin disc of average 6mm diameter. The specimens were then rinsed with a hot water spraying apparatus for 30 seconds and ultrasonically cleaned for 1 hour.<sup>16</sup> The specimens were then stored in phosphate buffer saline (PBS pH = 7).<sup>33</sup>

In group A, specimens were wetted with PBS buffer and thereafter dentifrice containing arginine and calcium carbonate was applied to the surface mixed with PBS and spread across the surface and left for 15 minutes at room temperature. The samples were then gently rinsed to ensure removal of the excess dentifrice. In group B, the specimens were subjected to diode laser (810 nm) irradiation at 1W for 10 sec, totaling 10J of energy, in continuous wave (CW), contact mode, using a non-initiated, 320µ tip, perpendicular to the surface, with scanning movements at 1 mm/second (Energy density with movement–305 J/cm²). In group C, a dentifrice containing arginine and calcium carbonate was applied and specimens were subjected to diode-laser (810 nm) treatment. Group D was left untreated and served as the Control. All the specimens were then transported to the lab in PBS buffer.

The specimens were fixed in 4% glutaraldehyde solution buffered with sodium cacodylate (pH = 7.4) for 18 hours. They were then dehydrated with a series of graded alcohol solutions. The samples were then covered in filter paper and subjected to critical

point drying for 20 minutes in liquid  $\mathrm{CO_2}$  and were sputter coated with gold in an ion sputtering device. The gold sputtered specimens were then placed in the vacuum chamber of the scanning electron microscope, and the dentinal tubule occlusion was evaluated by an experienced researcher who was blinded and unaware of the study groups per se, at 15 kV and 3000x magnification. Then the photomicrographs of each specimen were taken (Figs 1 to 4) for analyzing the occlusion of dentinal tubules.

The diameters of the dentinal tubules of each specimen were evaluated and measured from the photomicrographs using rectangular marquee tool of CS7 software, by an examiner who was also blinded to the sample allotment according to the group, (double-blinded). A pixel-based measurement was obtained, and thereafter it was converted into microns using the scale provided in the SEM photomicrographs as:

- $7\mu = 209 \text{ pixels}$
- 1 pixel =  $7 \div 209 \,\mu$
- $x \text{ pixels} = x \times 7/209 \,\mu$

## Statistical Analysis

The overall comparison between different treatment modalities was done using one way ANOVA. The multiple comparisons of different treatment modalities were done using post hoc Tukey HSD method. The entire test used was, and the p value was fixed at 5%. The data was tabulated on Microsoft Excel and analyzed using the software statistical package for social sciences (SPSS).

## RESULTS

In the overall comparison between different treatment modalities using One Way ANOVA (Table 1 and Graph 1) the mean diameters of dentinal tubules are:

- Group A: 1.12  $\mu$  (S.D = 0.13)
- *Group B*:  $0.51\mu$  (S.D = 0.095)
- Group C: 0.28μ (S.D = 0.055).
- Group D: 1.48  $\mu$  (S.D = 0.14) and F = 243.70 with p value of 0.0001 which revealed that there is a highly significant difference in the tubule diameter in different treatment modalities. The rate of occlusion of dentinal tubules (Table 2) is 24.32% in group A, 65.54% in group B and 81.1% in group C with the control value as the base value.

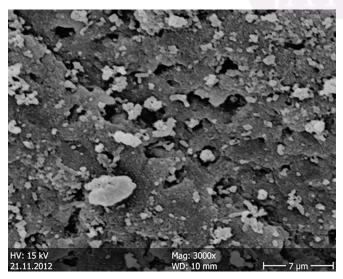
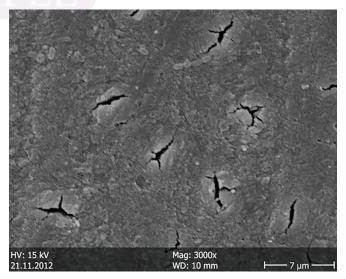
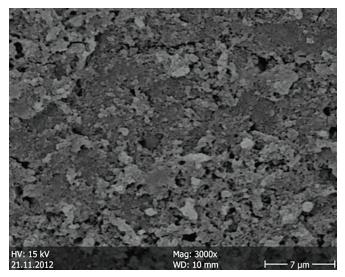


Fig. 1: SEM photomicrograph of the sample treated with desensitizing tooth paste



**Fig. 2:** SEM photomicrograph of the sample treated with diode laser (810 nm)



**Fig. 3:** SEM photomicrograph of the sample treated with diode laser (810nm) + desensitizing tooth paste

Multiple comparisons using post-hoc Tukey HSD method (Table 3) showed that the mean difference of tubule diameters on comparing groups A and B is 0.61 (p value = 0.0001) groups A and C is 0.84 (p value = 0.0001); groups A and D is -0.36 (p value = 0.0001); groups B and C is 0.23 (p value = 0.0001) and group C and D is -1.2 (p value = 0.0001). In all comparisons, the p values showed that the mean differences are statistically highly significant.

This showed that the combined use of desensitizing toothpaste and diode laser (810 nm) has the least tubule diameter suggesting that it is the best treatment modality in occluding dentinal tubules, the next better one is diode laser (810 nm) and finally the use of desensitizing toothpaste alone.

# **D**ISCUSSION

Dentinal hypersensitivity has a multifactorial etiology, and generally, more than one factor is found to be associated and active in this painful manifestation. The most widely accepted theory of

**Table 1:** Overall comparison between different treatment modalities using one-way ANOVA

Treatment	Tubule diameter in microns		Test applied		
modality-test group	mean	median	S.D	ANOVA	p value
Tooth paste– group A	1.12	1.15	0.13		
Laser–group B Tooth paste + laser	0.51	0.50	0.095	F= 243.70	0.0001 highly significant
Group C	0.28	0.27	0.055		
Control-group D	1.48	1.49	0.14		

Table 2: Rate of occlusion of dentinal tubules

Group	Rate of occlusion
Tooth paste-group A	[(1.48-1.12) ÷1.48]×100= 24.32%
Laser–group B	[(1.48-0.51) ÷1.48] ×100= 65.54%
Tooth paste + laser group C	$[(1.48-0.28) \div 1.48] \times 100 = 81.1\%$

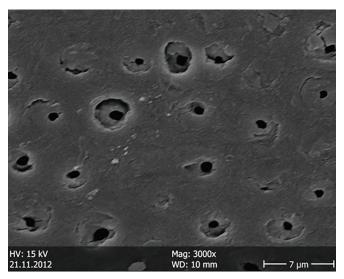
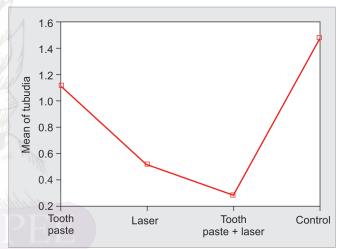


Fig. 4: SEM photomicrograph of the control sample

dentinal hypersensitivity is the hydrodynamic theory, which states that stimulus application induces pressure changes across dentin. As a result of the pressure changes, rapid shifts of fluids take place within the dentinal tubules, followed by the excitation of sensory



**Graph 1:** Line diagram showing overall comparison between different treatment modalities using one-way ANOVA

**Table 3:** Post-hoc Tukey comparison of different treatment modalities

Treatment modality–test group	Tubule diameter mean difference	p value
Tooth paste vs laser–group A VS group B	0.61	0.0001
Tooth paste vs tooth paste + laser		
Group A vs group C	0.84	0.0001
Tooth paste vs control -	-0.36	0.0001
Laser vs tooth paste + Laser		
Group B vs group C	0.23	0.0001
Laser vs control		
Group B vs group D	-0.97	0.0001
Tooth paste + laser vs control group C vs group D	-1.2	0.0001



nerves in the pulp/ dentin border. Therefore, either physically blocking the exposed dentinal tubules or reducing the excitability of the relevant sensory nerves would effectively alleviate dentinal hypersensitivity. 1,10,32

It is most commonly managed with the application of cavity varnishes, anti-inflammatory agents, tubular obturating procedures, or using dentine bonding agents and restorative resin.

Thus, as per the mechanism of action, the various treatment modalities for dentinal hypersensitivity include:

 Desensitization by blocking pulpal sensory nerves: It has been found that potassium ions (K<sup>+</sup>) in dentinal desensitizing preparations might act directly on intradental nerves by raising the concentration of (K+) in the extracellular fluids around these nerves sufficiently to prevent action potential generation through axonal accommodation.<sup>34</sup>

Desensitization by occluding the dentinal tubules and Desensitization by stimulation of the body's reparative mechanism by forming tertiary dentin.

Different noninvasive and reversible systems have been recommended for the treatment of this condition based upon their ability to occlude dentinal tubules.<sup>25</sup>

Lasers are found to have all three effects. Laser irradiation induces neural impairment including the slowing of conduction velocity (CV), decreasing compound action potential (CAP) amplitudes, suppression of response to noxious stimuli, suppression of pain-related neurotransmitters release, inhibition of enzyme activity, and morphological changes related to nerve conduction. 35 Thermal and occlusive effects of certain lasers cause the sealing of exposed dentinal tubules through melting and recrystallization of dentin.<sup>32</sup> Lee et al. in their study found that the dentin after Nd: YAG laser irradiation de monstrated a three-zone structure. The outer zone composed of hydroxyapatite and β tricalcium phosphate showed an orderly columnar structure. The intermediate zone was a homogenous glass phase. The inner zone was with grain growth and well-crystallized prism. All three zones showed microstructure without pores and voids. Thus the energy output with 100 mJ-10 pps-4 sec was found to reduce dentine permeability. <sup>36</sup> Furthermore the treatment of dentinal hypersensitivity by the Diode laser revealed that the laser interaction with the dental pulp causes a photobiomodulating effect, increasing the cellular metabolic activity of the odontoblast and obliterating the dentinal tubules with the intensification of tertiary dentin production.

This study embarked upon the probability of achieving tubule occlusion by using 3 different modalities namely using Desensitizing toothpaste containing arginine and calcium carbonate, a GaAlAs diode laser (810 nm, 1 W,10 J, 10 sec) irradiation alone and desensitizing toothpaste used in conjunction with the diode laser. The tubule occlusion was evaluated by scanning electron microscopy (SEM). The results fully define that lasers (diode laser 810 nm) has been an effective modality in conjunction with desensitizing toothpaste (mean tubule diameter = 0.28  $\mu$ , p value = 0.0001) and when used alone (mean tubule diameter = 0.51  $\mu$ , p value = 0.00001).

When compared to control samples (mean tubule diameter =  $1.48\,\mu$ ) the desensitizing toothpaste could achieve only nearly 1/4th of tubule occlusion (mean tubule diameter =  $1.12\,\mu$ , percentage occlusion = 24.32%). On the other hand, in group B when Laser was used alone significant and effective closure of dentinal tubules up to 65.54% was achieved.

In comparison, when the diode laser (810 nm) was used in tandem with toothpaste, nearly complete occlusion of 81.1% was achieved. This proves that diode laser (810 nm) is an effective

modality in the treatment of dentinal hypersensitivity through achieving tubule occlusion either when used alone or in conjunction with conventional methods.

When toothpaste was used alone there was only a marginal reduction (however significant reduction) in tubule diameter (24.3%) when compared to the other two methods. In stark contrast to this, when the laser was used alone there was a nearly 1/3rd reduction in tubule diameter (0.51  $\mu$ ) when compared to the control samples. And when diode laser was used in conjunction with toothpaste, there was nearly a 1/5th reduction in the tubule diameter (0.28  $\mu$ ).

Dilsiz evaluated and clinically compared the efficacy of desensitizer toothpaste alone and in combination with diode (GaAlAs) laser (100 mW for 25 seconds at 808 nm) in the management of dentin hypersensitivity. The immediate and late therapeutic effects of the diode laser were more evident compared with those of desensitizer toothpaste.

Lavender et al.<sup>33</sup> Yingying Fu et al.,<sup>22</sup> Que et al.<sup>23</sup> in their study showed that the treatment with the 8.0% arginine, a high cleaning calcium carbonate, and sodium monofluorophosphate dentifrice was highly effective in occluding dentin tubules which were confirmed by the present study.<sup>37-40</sup>

Lan et al. evaluated the combined occluding effect of sodium fluoride varnish and Nd.YAG laser irradiation on human dentinal tubules and found that over 90% of the dentinal tubule orifices were occluded.<sup>30</sup>

On the contrary, Gholami et al. in their study evaluating the occluding effects of Er.Cr.YSGG (P:0.25W,F:20Hz,Pd:140mS), Nd.YAG (P:1W, F:20Hz), CO2 (P:1W, Pd:50ms), and 810 nm Diode (P:2 W, Pd:30ms) lasers on dentinal tubules observed the highest reduction in mean tubule diameter with the Nd. YAG laser (53%). Tubular diameter reduction in all laser groups (p <0.05) was found to be statistically significant. However, Nd. YAG laser produces predominantly photothermal effects on teeth as compared to the Diode laser (810 nm) which has milder photothermal effects in comparison and can induce photochemical changes as well.

Sipahi et al. in their in vitro study demonstrated the dentinal tubule occluding effect of desensitizing laser (Er. Cr. YSGG Laser) treatment on dentin surfaces using environmental scanning electron microscopy (E-SEM). The tubule closure rates of 4 different irradiation modes were as follows: low potency/long time (74%) > high potency/short time (70%) > low potency/short time (51%) > high potency/long time (46%) > control (6%). It was demonstrated that desensitizing laser application was an effective treatment option for the occlusion of dentinal tubule apertures. These findings [low potency/short time (51%) and high potency/long time (46%)] are consistent with those of the present study (51.1%).

Pain and noxious stimuli are subjective responses that are highly variable from patient to patient according to the difference in pain threshold. Stimuli like thermal, tactile, osmotic, and electrical and exposure to air, can each elicit a sharp painful response in individuals with hypersensitive teeth. This paves the foundation for the different methods to measure dentinal hypersensitivity clinically. Tactile method ranges from the use of an explorer to devices that can quantify the probing pressure exerted usually along the cementoenamel junction. The thermal method involves the flow of air or metal probes applied in different ways. Electrical stimulus generally consists of progressive elevation of the magnitude of the stimulus until a sense of 'pre-pain' rather than pain is felt. Ideally, more than one method is needed in order to assess dentinal hypersensitivity. This study could evaluate it only from the viewpoint of tubule occlusion. A clinical and in vivo evaluation

of the patient's comfort or pain could give a more complete picture of the effectiveness of such treatment modalities.

Moreover, diode lasers, if not used in optimal parameters, can induce irreversible and potentially damaging thermal effects on the tooth. While the parameters used in this study did not induce any such effects<sup>27</sup> further investigation in this area is necessary. However, within such limitations, this study has undoubtedly established the effectiveness of diode laser (810 nm) when used alone or in combination with a desensitizing toothpaste containing arginine and calcium carbonate in the treatment of dentinal hypersensitivity.

#### Conclusion

Lasers are an effective modality in treating dentinal hypersensitivity by effecting tubule occlusion.

Desensitizing toothpaste containing 8% arginine and calcium carbonate has been found to significantly occlude dentinal tubules, but when used in conjunction with a diode laser (810 nm) it has been found to be the most effective.

Lasers have been found to be more effective in occluding dentinal tubules when used as a standalone modality even more than when the desensitizing toothpaste was used alone.

### **C**LINICAL SIGNIFICANCE

This study suggests an effective treatment of dentinal hypersensitivity using Diode Laser and desensitizing toothpaste.

This study is a thesis dissertation document submitted in fulfillment of Master of Dental Surgery (MDS) degree to the Kerala University of Health Sciences.

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