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RESEARCH ARTICLE

In vitro Analysis Comparing Efficacy of Lasers and Desensitizing Agents on Dentin Tubule Occlusion: A Scanning Electron Microscope Study

[1]Arulmozhi Nandakumar, [2]Vidyaa Hari Iyer

ABSTRACT

Dentinal hypersensitivity (DH), one of the commonest causes of dental pain, arises from exposure of dentinal tubules most commonly in the cervical region of the buccal and facial surfaces of the tooth in response to typically thermal, evaporative, tactile, osmotic or chemical stimulus. This study aims at evaluating the efficacy of Er,Cr:YSGG (dual lasers—hard and soft tissue) and diode lasers (soft tissue lasers) against clinically proven dentifrices prescribed by dentists, easily available over the counter, in treating DH. In 60 recently extracted human teeth, cervical cavities were prepared and etched with 17% EDTA to expose the tubules and eliminate smear layer. The teeth were further divided into groups: Er,Cr:YSGG laser, diode laser and commonly used dentifrices chosen on the basis of previous studies. After treatment, the teeth were subjected for scanning electron microscope (SEM) analysis on the same day for quantitative and qualitative analysis of the specimens. Quantitative analysis of the SEM images was done using image analysis software. Er,Cr:YSGG showed superior results in terms of number of tubules per millimeter square, tubule diameter and tubular area. Laser treatment of exposed dentinal tubules with Er,Cr:YSGG promises a noninvasive, pain free and relatively safe treatment option.

Keywords: Er,Cr:YSGG laser, Dentinal hypersensitivity, Scanning electron microscope, Dentinal tubule occlusion, Diode lasers, Pain-free dentistry.

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INTRODUCTION

Dentinal hypersensitivity (DH) is one of the commonest causes of pain encountered in regular dental practice. The prevalence of DH in the population is found to be at a peak in the age of 20 to 40 years,[1] more so in the buccocervical region of teeth due to branching of dentinal tubules at the dentinoenamel junction (DEJ).[2] It has been described as the brief acute pain that cannot be attributed to any other dental disease.[3,4] The stimulus evoking such a response is a cold stimulus and aggravated by mechanical trauma, such as brushing.[5-7]

Dentinal hypersensitivity, based on the causative factors, is best explained by Brännström's hydrodynamic theory.[8] This theory attempts to explain the etiopathogenesis of DH and localization of the lesion in the dentin brought about by exposure of dentinal tubules as a result of enamel loss (abrasion, erosion and abfraction) and/or loss of periodontal coverage (gingival recession) which are the prerequisite for DH.[9] This is further supported by the substantiation that hypersensitive dentin has numerous number as well as wider dentinal tubules as compared to normal dentin.[10,11] This causes increase fluid flow within the dentinal tubules and the pressure change stimulates Aδ fibers, generating a sharp shooting pain. The loss of smear layer by brushing and chemicals makes the tubule system permeable toward the pulp-lesion initiation.[12]

Thus, the treatment modalities to curb sensitivity have two distinct approaches namely, to occlude the dentinal tubules making them less permeable and to modify neural transmission. The success of desensitizing agents is directly proportional to its ability to seal or occlude the dentinal tubules and reduce the diameter of the opened tubules thereby decreasing the hydrodynamic pain transmission mechanism.[13] The scope of modification of causative factors in the management of dentinal hypersensitivity is one which is grossly overlooked.[14] The oral environment being dynamic, the desensitizing agent has to withstand the challenges of salivary dissolution, acid attack from microbes and food components as well as chemical, mechanical and thermal trauma to provide long-lasting pain relief for the patient.[15] All these warrant repeated application of dentifrices in order to reinforce their action which is lost by wear and tear. Thus, they fail to qualify under the criteria prescribed by Grossman as the requirement of a desensitizing agent.[16]

[1,2]Private Practitioner

[1]Department of Laser Dentistry, Smile Dental Clinic, Chennai Tamil Nadu, India

[2]Fellowship and Diplomate in Laser Dentistry, Smile Dental Clinic, Chennai, Tamil Nadu, India

Corresponding Author: Arulmozhi Nandakumar, Private Practitioner, Department of Laser Dentistry, Smile Dental Clinic, Chennai, Tamil Nadu, India, Phone: 9003273438 e-mail: docarulmozhi@gmail.com

Lasers, on the other hand, are a promising and upcoming treatment modality in management of DH. The action of lasers in DH depends on the laser wavelengths and parameters used.[17] The effect of laser as desensitizing agent is exemplified only when etiological factors are removed.[18] While low output lasers (He-Ne, diode, etc.) cause photobiomodulation in the dentin and bring about analgesia in the neural complex,[19] high output lasers (Nd:YAG, Er:YAG, Er,Cr:YSGG) cause thermal changes which encourages recrystallization of dentin to cause occlusion of the tubules and formation of a smear layer which is much more resistant in the oral environment.[20]

PROCEDURE

A total of 60 teeth (canines and incisors) extracted from adult patients from 18 to 50 years was collected. The inclusion criteria were that the teeth were devoid of bacterial infection, did not have any restorations and carious lesions and the patients had not used desensitizing dentifrices in the last 3 months, or subjected to periodontal surgery in the last 6 months.

The samples were divided into three groups: A, B and C; each containing 20 teeth (Table 1). Each group was further subdivided into two subgroups of 10 teeth: the Er,Cr:YSGG subgroup (Fig. 1) and the diode subgroup (Fig. 2). The teeth in group A were treated only with lasers (n = 20), group B with lasers and Sensodyne repair and protect (Glaxosmithkline group of companies, India) (n = 20) and group C with laser and Sensodyne rapid relief (Glaxosmithkline group of companies, India) (n = 20).

After disinfection, the teeth were labeled in separate containers and preserved in normal saline. The dentin disk model was used for the study,[21] the cervical region of the teeth was prepared using 2 mm round bur (BR-41, Mani Dia Burs), a box preparation of 2 × 2 × 2 mm was done. The teeth were etched using 17% EDTA (Desmear, Anabond, Stedman, India) to remove the smear layer within the prepared box. In groups B and C, the teeth were brushed for 6 minutes with desensitizing pastes followed by laser irradiation.

The teeth were washed with distilled water and were subjected to scanning electron microscopy (SEM) (S-3400N, Hitachi) (Fig. 3) analysis the same day after dehydration. Prior to SEM analysis, the samples were sputter coated with gold particles (Figs 4 and 5). Each tooth was examined at the cervical region under three magnifications of 2000×, 5000× and 10,000×, and five images were obtained under each magnification. The SEM images were quantitatively and qualitatively analyzed using Image J (Version 1.47, National Institute of Health, USA)

Table 1: Teeth were divided into three subgroups (A to C) further divided into subgroups (1 and 2)

	Group A (20)	*Group B (20)*	*Group C (20)*
Subgroup 1	Er,Cr:YSGG laser	Er,Cr:YSGG laser and Sensodyne repair and protect	Er,Cr:YSGG laser and Sensodyne rapid relief
Subgroup 2	Diode laser	Diode laser and Sensodyne repair and protect	Diode laser and Sensodyne rapid relief

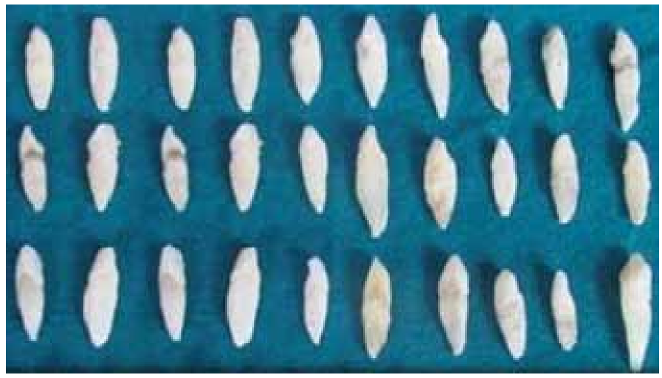

Fig. 1: Thirty teeth in the erbium subgroup

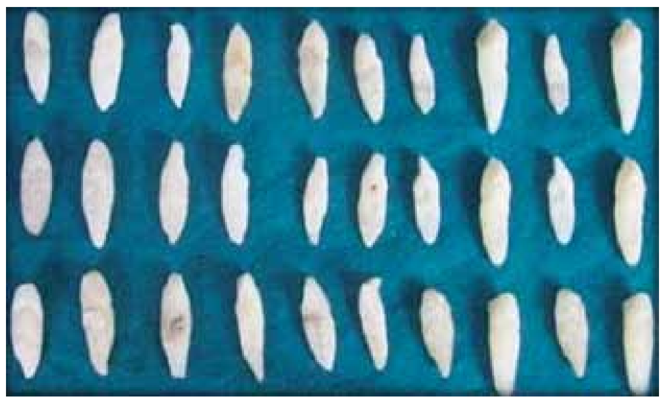

Fig. 2: Thirty teeth in the diode subgroup

Fig. 3: Scanning electron microscope

Fig. 4: Teeth mounted in SEM machine

Fig. 5: Sputtering of the teeth with gold prior to SEM analysis

Fig. 6: 940 nm diode laser (Ezlase, Biolase, USA)

Fig. 7: Diode laser parameters used

for number of tubules/mm^2 (×2,000 magnification) considering a total area of 40,000 μm^2, percentage of area occupied by opened tubules and the diameter of the tubules (× 5,000 magnification).

LASER EQUIPMENT

The laser device used was 940 nm diode laser (Ezlase, Biolase, USA) (Fig. 6) at the laser parameters of 1 W, continuous wave at 190 J for 15 seconds (Fig. 7) and 2780 nm Er,Cr:YSGG (Waterlase MD, Biolase, USA) (Fig. 8) at the laser parameters of 0.25 W, 50 Hz, 1% air and 1% water for 40 seconds (Fig. 9). Both the lasers were used in noncontact mode at about 1.5 mm away from the teeth. All laser safety precautions were used appropriately.

Fig. 8: 2780 nm Er,Cr:YSGG laser (Waterlase MD, Biolase, USA)

RESULTS

The microphotographic images from the SEM were qualitatively analyzed (Figs 10 to 15) and reveal completely or partially occluded tubules, with peritubular and intertubular dentin and surface cracks. The quality of smear layer formed and number and quality of tubules occluded were best for the group irradiated with only Er,Cr:YSGG, and least favorable for specimens irradiated with diode and Sensodyne rapid relief. The laser group showed alteration

Fig. 9: Erbium laser parameters used

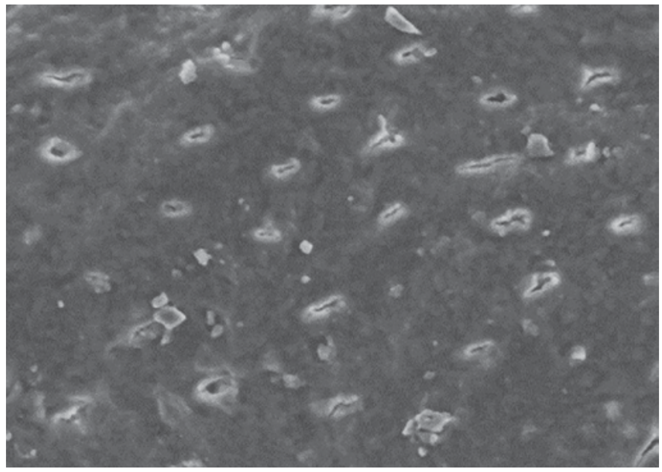

Fig. 10: Dentinal surface irradiated with Er,Cr:YSGG laser at 2000×

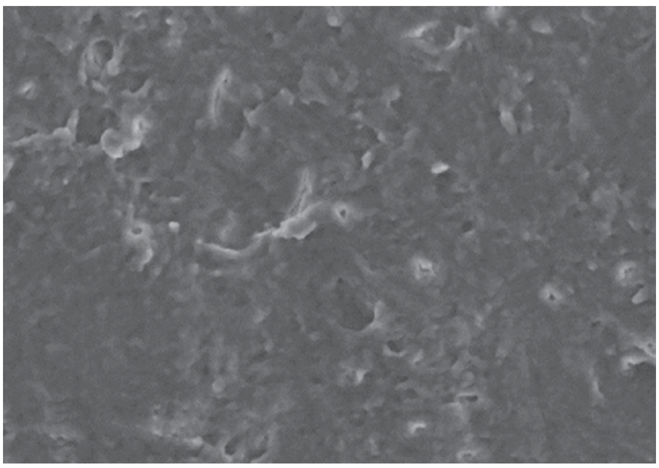

Fig. 11: Dentinal surface irradiated with diode laser at 2000×

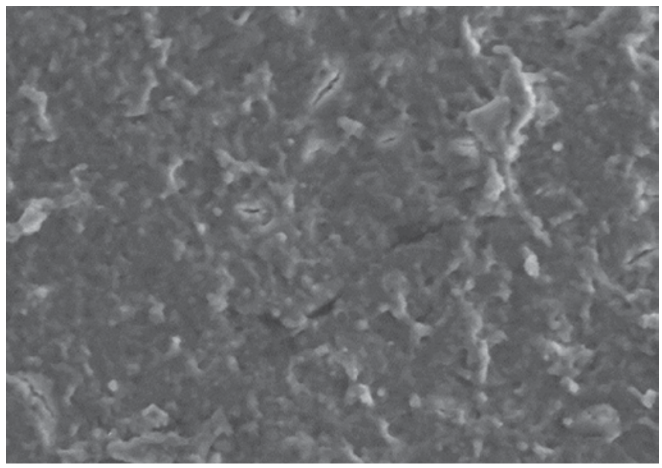

Fig. 12: Dentinal surface irradiated with Er,Cr:YSGG laser after brushing with Sensodyne repair and protect at 2000×

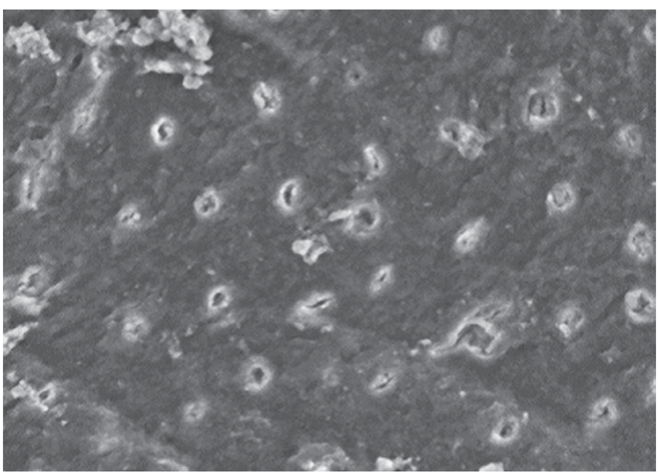

Fig. 13: Dentinal surface irradiated with diode laser after brushing with Sensodyne repair and protect at 2000×

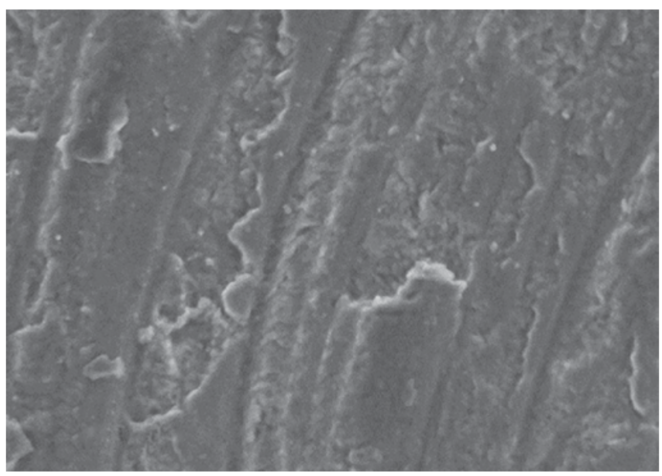

Fig. 14: Dentinal surface irradiated with Er,Cr:YSGG laser after brushing with Sensodyne rapid relief at 2000×

of dentin surface and sealing of the opened tubules. The Sensodyne repair and protect group revealed a hybrid layer along with alteration in root dentinal surface in teeth treated in conjugation with Er,Cr:YSGG. The Sensodyne rapid relief though blocked the opened tubules to a comparatively lesser extent no integration with dentin was observed. The number of open tubules was also more in these teeth.

Quantitative analysis, using Image J (Version 1.47) image processing software, was accessed for three criteria namely number of tubules/mm^2, percentage of area occupied

Table 2: Quantitative analysis of the laser irradiated tooth

Group	*Avg. percentage of open tubular area*	*Avg. number of tubules/mm²*	*Avg. diameter of the tubules (nm)*
Er,Cr:YSGG	8.62	5175	534
Diode	15.33	12675	741
Er,Cr:YSGG + repair and protect	17.50	10850	660
Diode + repair and protect	18.43	11150	717
Er,Cr:YSGG + rapid relief	21.56	18400	1.09
Diode + rapid relief	34.03	27225	1.13

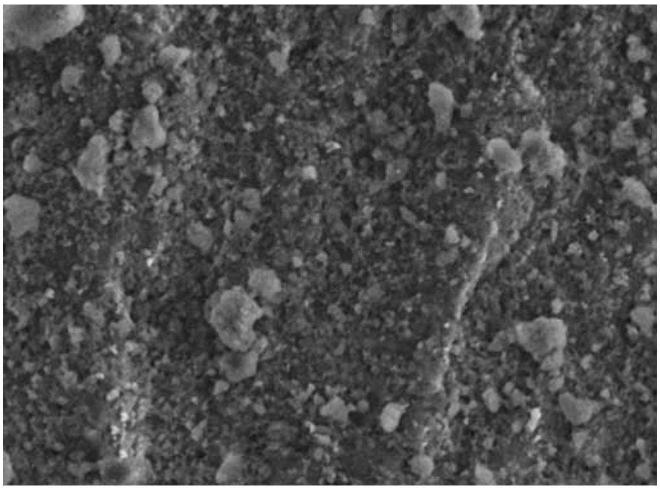

Fig. 15: Dentinal surface irradiated with diode laser after brushing with Sensodyne rapid relief at 2000×

by opened tubules and the diameter of the tubule (Table 2). The formula used to calculate the number of tubules was:

Number of tubules/mm^2 = counted number of tubules/ total area × 10^6

In this study, the number of tubules/mm^2 for the Er,Cr:YSGG (group A subgroup 1) was the lowest—5175 tubules as opposed to diode laser and Sensodyne rapid relief (group C subgroup 2)—27,225 tubules. The Er,Cr:YSGG group also showed minimum percentage of tubular area of 8.6% and smallest diameter of the occluded tubule was 534 nm.

DISCUSSION

The teeth most commonly affected by DH are canines, premolars, incisors and molars in the descending order, and, hence, these teeth were chosen for the study.[22,23] The box preparation was done at the cervical region as the number of dentinal tubules is more numerous in that region.[2]

The dentin disk model, comprising of small dentin disks prepared from extracted teeth, has been used to study hydraulic conductance in dentin. The same has been adapted in this study to measure tubule occlusion after examination under SEM.[24] The image was then digitalized and studied using image analysis software. This study was performed *in vitro* but, by including these dentin disks in intraoral appliances, the same can be studied simulating oral environment.[25]

Sensodyne rapid relief toothpaste containing strontium acetate proved to be superior to other desensitizing pastes in previous published studies;[26] hence, this was taken as a standard. The ability of strontium to deeply penetrate the hard tissues including dentin has contributed to its positive effects in hypersensitivity management.[27] Nevertheless, strontium reduces the permeability within tubules by physically blocking them, but fails to bind to dentin. This warrants repeated applications to reinforce its action and easy dissolution in oral acids.

Bioactive and biocompatible glasses have a capacity to bond with bone tissue and enhance its growth and repair.[28] These agents bond with the dentin after activation by the exposure to aqueous solutions. Exchange of sodium ions from the glass with hydrogen ions decreases the pH and aids in formation of a calcium-phosphate rich surface layer on the dentin.[29] These desensitizing agents in conjugation with lasers were claimed to penetrate the tubules for up to 10 mm in previous studies.[30] Sensodyne repair and protect, incorporating Novamin bioactive glass technology, was thus chosen for the study.[31]

Earlier studies have prescribed the repeated usage of desensitizing agents to ensure higher clinical effectiveness in dentinal tubule occlusion.[13] In this study, activating the dentifrices with Er,Cr:YSGG after brushing for 6 minutes and analyzing through SEM reveals diminished tubule diameter of 660 nm as opposed to 5-day application when using dentifrice alone.

The chromophore for Er,Cr:YSGG is water, hence, this laser is equally applicable for soft and hard tissue procedures. Studies using Er,Cr:YSGG laser for treatment of DH are limited. The output power recommended for usage in DH is 0.25 to 0.5 W.[32] Keeping in mind to use the lowest possible power settings to ensure minimum collateral damage 0.2 W was chosen. Results in this study were consistent with those conducted previously. Yilmaz et al also demonstrated that the single application of Er,Cr:YSGG is effective in the management of DH.[33]

The normal diameter of the dentinal tubule at the DEJ is 0.9 to 1 μm.[34] The diameter of the tubules in teeth irradiated with Er,Cr:YSGG showed diameter value of 534 nm. This could be explained by the surface alteration of peritubular dentin which causes occlusion of the dentinal tubules and result in reduction of their diameter. The tubules diameter in teeth irradiated with diode laser and sensodyne repair and protect show lesser diameter (717 nm) when compared to only diode irradiated teeth (741 nm). The calcium-phosphate like layer that binds to the dentin causes occlusion of the tubules and irradiation with diode cause additional thermal changes resulting in lesser diameter values when compared to only diode teeth.

The number of tubules/mm at the dentinoenamel junction is 20,000/mm^2.[34] In this study, Er,Cr:YSGG irradiated teeth had only 5,175 tubules/mm^2. This clearly demonstrates that laser causes complete occlusion of tubules thus decreasing the number of tubules/mm^2. Supporting these values, the percentage of area occupied by open tubules was least (8.6%) for the only Er,Cr:YSGG group.

Comparing within the Er,Cr:YSGG subgroup, laser irradiation alone was found to be superior compared to the combined usage of desensitizing pastes followed by laser irradiation. Previous studies have shown contradicting results. A study using GaAlAs laser—830 nm along with fluoridation concluded 20% increased efficacy than laser alone.[35] Earlier studies have compared diode, Nd:YAG lasers with topical application of fluorides and found them superior.[36] Research done with Er,Cr:YSGG are limited and hence results in this study were very promising and highlighted the use of only erbium lasers to be far superior to the use in conjuncture with desensitizing agents.

Within the limits of the present study on comparing the efficiency of erbium lasers *vs* diode on treatment of dentinal surface, Er,Cr:YSGG showed superior results in terms of number of tubules per millimeter square, tubule diameter and tubular area. Groups A, B and C showed partially occluded tubules with group C subgroup 2 (diode and rapid relief) having the most number of open tubules. Inappropriate use of lasers with high power settings, prolonged exposure times and contact modes could result in high risk of tissue damage, causing thermal damage to the dental tissues and adjacent bone.[37] This brings out the importance of meticulous documentation and follow-up of appropriate choice of laser wavelength and parameters to prove the efficacy of laser treatment in DH.

CONCLUSION

Laser treatment of exposed dentinal tubules with Er,Cr:YSGG promises a noninvasive, pain-free and relatively safe treatment option with promising repeatable results. More long-term follow-up and studies *in vivo* are required before we can safely exclude the use of desensitizing agents completely and replace them with erbium lasers.

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