

Effect of Nd: YAG laser on root surface smear layer and dentinal permeability – An invitro study

Dr Bindu R Nayar – MDS MPhil*, Dr. Divya P V—MDS**

* Professor and HOD Department of Periodontics Govt. Dental College Thiruvananthapuram Kerala India

** Asst. Professor Department of Periodontics Govt. Dental College Thiruvananthapuram
Kerala India

Corresponding Author: Dr Bindu R Nayar

Abstract

Background:

The present invitro study was conducted to find out the effect of continuous watt Nd:YAG laser radiation on root surface smear layer, dentinal tubule and dentinal permeability. Spectrophotometric dye recovery method is used to study the dentinal permeability and scanning microscopy is used to analyse the surface morphology

Materials and methods:

The tooth samples were irradiated with a Nd:YAG laser beam at various power and exposure levels. The morphologic changes produced in the smear layer by laser irradiation and its effect on dentinal tubules at different power levels and duration were studied under scanning electron microscope. Dentinal tubule sealing efficiency of laser irradiation at different power levels were compared quantitatively by spectrophotometric dye recovery method. In order to study the effect of enhanced absorption, cavity varnish is applied over the prepared dentin surface and all the experiments were repeated.

Results

It is noted that the mean, standard deviation and range of all lased samples shows a decreased dye penetration compared to controls. Also at higher power densities the mean concentration of dye penetration decreases. It is noted that at all power density and exposure duration studied except at power density of 1.04 w/mm² and an exposure duration of 7 sec, the reduction in dye penetration are statistically significant. It is also seen that at higher power densities the reduction in dye penetration was highly significant.

Conclusion: Laser treatment of exposed dentinal tubules with Nd:YAG laser promises a non invasive pain free and relatively safe treatment option with predictable results. More long term follow up and invivo studies are required before we can safely exclude the use of desensitising agents completely and replace them with lasers. Nd:YAG laser has a significant potential for the management of hypersensitivity provided further clinical studies with long term follow ups are oriented towards the multiparametrical variables in search of an optimum regime to exclude the use of other desensitising agents

Key words: Dentinal Hypersensitivity, Nd:YAG Laser

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I. Introduction

Dentinal hypersensitivity (DH) is an “enigma being frequently encountered” yet poorly understood. It is a short, sharp pain arising from exposed dentin in response to various thermal, evaporative, tactile, osmotic or chemical stimuli that cannot be attributed to any other dental disease^{1,2,3}

Dentinal pain is elicited by cold stimuli in almost 90% of patients. Dentinal hypersensitivity can arise through incorrect tooth brushing, gingival recession, inappropriate diet and so many factors.^{4,5} Several factors such as anatomic defects, gingival recession, periodontal treatment, attrition, erosion, abrasion are all considered as risk factors for DH. According to Porto JC and Cummins D at least one in seven patients suffers from some degree of dentinal hypersensitivity and the most affected areas are the cervical region of incisors and premolars often on the opposite side of dominant hand.^{2,3} The prevalence of DH in the population is found to be at a peak in the age of 20 to 40 years⁶ and more in the buccocervical region of teeth due to branching of dentinal tubules at the dentinoenamel junction (DEJ).⁷ In an attempt to explain DH a number of theories have been put forward. Currently, the most widely accepted theory is the hydrodynamic theory which states that rapid dentinal fluid flow serves as the final stimulus in activating intradental nociceptors for many different types of stimuli. The patency of the dentinal tubules is a prerequisite for the sensitivity of exposed dentin.^{1,3} It is claimed that 14.3% of all patients have some degree of sensitivity and a range of therapies has been derived to alleviate this

condition.^{8,9} In order to prevent pulpal damage and patient discomfort, on time diagnosis and treatment of DH are essential. The management of dentin hypersensitivity has a preventive as well as an interceptive aspect.

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. Management of DH involves the application of materials or devices that reduce the flow of dentinal fluid or lower the activity of dentinal neurons. Clinical interventions include resins, oxalate salts, fluoride releasing resins or varnishes, use of devices that burnish exposed dentin. Because of the absence of success in routine treatments for DH in the middle of 1980's the use of laser to decrease the level of DH was proposed. It was Albert Einstein who proposed the concept of stimulated insertion of radiant energy.¹⁰ This concept became the foundation for modern laser physics. The first laser was constructed by exciting a ruby rod with intense pulses of light from a flash lamp.¹¹

Laser is a acronym for light amplification by stimulated emission of radiation. It is an artificial single photon wavelength. Spontaneous emission of a photon by one atom stimulates the release of subsequent photon and so on. The wavelength of a released photon depends on the state of the electrons energy. The characteristics of a laser are defined by its wavelength.¹² After initial experiments with ruby laser^{13,14} other lasers such as He-Ne, GaAlAs (diode), CO₂, Nd:YAG and Er: YAG have been used by clinicians for treatment of DH.¹⁵ The first laser use for treatment of DH was reported by Matsumoto et al by using Nd: YAG and 780 nm- diode lasers.^{16,17} Lasers used for treatment of DH are divided into two groups-low output power or low-level lasers (He-Ne or diode lasers) and high output power lasers (argon, KTP, diode, Nd:YAG, Er:YAG, ErCr: YSGG and CO₂ lasers.¹⁸ 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 Depending on the optical properties of the tissue, laser light can have four different interactions with the target tissue. It can reflect, scatter, be absorbed or be transmitted to surrounding tissues. Laser effects are considered to be due to the effects of sealing the dentinal tubules, nerve analgesia, laser acupuncture or a placebo effect. Only the sealing effect is considered to be durable.²¹ Laser irradiation may exert a direct effect on the electrical activity of nerve fibers with in the dental pulp. Secondly, laser can modify the tubular structure of the dentin by melting and fusing the hard tissue or smear layer and subsequent sealing of the dentinal tubules.^{22,23}

Laser procedures are painless and the lesions treated by lasers heal faster. It produce bloodless field and improve visibility during procedures and may reduce the opportunity for blood borne contamination. Lasers might sterilize the operating site by vaporising the microorganisms and limit the trauma and damage to the adjacent tissues thereby minimizing postoperative swelling, pain and scarring. They are more readily accepted than traditional treatments and dental treatment out of far might be attracted to this new modality. With all these benefits they are not a part of day to day dental practice because of high cost of equipment, large size, need for complete knowledge regarding equipment, its use and safety. The effectiveness of laser irradiation largely depends on the physical characteristics of the laser beam including wavelength, power, pulse duration and energy density (J/cm²). Other factors include the optical properties of the irradiated tissue such as optical density, structure and maximum absorption.²⁴ Lasers may be used to alter the structure and chemistry of dentin surface.²⁵ Though laser study on enamel and dentin have been highlighted through numerous studies there is relative paucity on its effect relating to root surface preparation

In this scenario, the present invitro study is conducted to find out the effect of continuous watt Nd:YAG laser radiation on root surface smear layer, dentinal tubule and dentinal permeability. Spectrophotometric dye recovery method is used to study the dentinal permeability and scanning microscopy is used to analyse the surface morphology

II. Background

The tooth samples were irradiated with Nd:YAG laser beam at various power and exposure levels. The morphologic changes produced in the smear layer by laser irradiation and its effect on dentinal tubules at different power levels and duration were studied under scanning electron microscope. Dentinal tubule sealing efficiency of laser irradiation at different power levels were compared quantitatively by spectrophotometric dye recovery method. In order to study the effect of enhanced absorption, cavity varnish is applied over the prepared dentin surface and all the experiments were repeated. The detailed procedure adapted is given in flow chart 1.

Materials and Methods

The present study was an invitro study conducted in the Department of Periodontics Govt. Dental College Thiruvananthapuram and Biotechnology wing, SCTIMST, Poojappura, Thiruvananthapuram.

Equipment and Materials

The following equipments and materials were used for the study.

Equipments

- Nd:YAG laser
- Spectrophotometer
- Scanning Electron Microscope

Nd:YAG laser

First developed by Geusic in 1964, Nd:YAG laser is one of the most powerful surgical lasers. In this laser, energy levels of the element Neodymium (Nd) is used to get lasing action, the Yttrium Aluminium Garnet (YAG) crystal acting as the matrix to hold the neodymium atoms in place. The energy for the excitation of neodymium atoms are provided by krypton arc lamp. The light from the arc lamp is focused on the YAG crystal by suitable reflectors. The laser rod is placed in between two mirrors, one 100% reflective and the other partially transmitting. The laser output is obtained from the partially transmitting mirror end.

Schematic Representation of Nd:YAG LASER

Although the wavelength of the Nd:YAG laser beam is in the infrared region, it is of sufficiently short to be easily transmitted through an optical fiber made of quartz. The Nd:YAG laser beam is relatively poorly absorbed by the tissue inhomogeneities. So great is the scattering to the absorption ratio, the beam will be scattered many times before finally being absorbed. A beam which enters the surface at a given point can penetrate to tissue layers several millimeters below. In the case of CO₂ laser the wavelength 10.6µm is in the far infrared region of the electromagnetic spectrum where water and other tissue materials have appreciable absorption, resulting in lower penetration depth. But the disadvantage is that flexible optical fiber to guide the beam is not available.

In the present study a continuous wave 100 Watt Nd:YAG laser (Cooper Lasersonics USA, Model 6000) is used. The system is comprised of a movable console with large clear digital displays of operating parameters, and internal lasers mounted on a solid optical deck. The character of a Nd:YAG treatment beam such as power output, pulse duration, number of pulses and cumulative energy are selected on the front control panel.

The power level can be adjusted from 1-100 W at the treatment site, adjustable increments of 1 watt. The exposure timing is adjustable from 0.1-9.9 sec in interval of 0.1 sec or can be continuous. There is an aiming light which is He-Ne laser 5 mw projected to the sample position as the laser beam. The treatment beam is controlled by foot switch. The system is cooled by an external irrigation.

The Spectrophotometer

Hitachi model 220-Double beam spectrophotometer (Hitachi LTD Japan) was used for this study. It uses a high performance grating monochromator, 40w tungston lamp and dentrinum discharge tube as light sources and ultra high sensitivity photomultiplier tube as detector.

The basic principle behind spectrophotometry is that under suitable condition, if a coloured solution is illuminated with monochromatic light, its absorbance will be proportional to the concentration of the substance multiplied by the depth of the solution in the light path. Light passing through a curvette containing solution falls on light sensitive surface of the detector which generates the current proportional to the light intensity. The detector current is measured and a computer processes the data to give values in absorbance or transmittance.

Using a blank solution zero is adjusted in the spectrophotometer. The spectrophotometric reading of a standard solution is taken first and noted. The readings were taken for each of the samples of the test solution.

$$\text{Concentration of dye in test Solution} = \frac{\text{Spectrophotometric reading of test solution}}{\text{Spectrophotometric reading of Standard solution}} \times \text{Conc of dye in the standard}$$

Scanning Electron Microscope

Hitachi model S 2400 scanning electron microscope was used for the investigations. The resolution of the microscope is 4nm. The magnification can be adjusted from 20-3,00,000. The accelerating voltage ranges from 0.3-25kv. The display system consist of still image display unit with a built in memory.

Materials used for this study include

- 2% methylene blue solution
- 7% (HNO₃)Nitric acid
- A circular mask of 4.5mm diameter
- Nail polish
- Nail polish remover
- Methanol

Sample: Freshly extracted single rooted premolars for orthodontic purpose **Procedure**

Specimen Preparation and laser application

Teeth selected for the study were single root premolars (maxillary second, mandibular first and second premolars) extracted for orthodontic purpose. Immediately after extraction, specimens were rinsed free of blood and soft tissues. Calculus was removed using hand instruments following which they are cleansed with soap and water. Teeth were further subjected to examination using a magnifying lens to eliminate the possibility of developmental defects, cracks or spots. Care was taken to exclude root fractures or any anatomical abnormalities. In the specimen selected care was taken to ensure that at least one surface was relatively flat. The samples were stored in isotonic saline.

After removing cementum layer using airmotor handpiece and tapered fissure bur, two transverse cuts were made. The first 1mm above the cemento enamel junction and the second 6- 7 mm apical to the first. The specimens were of the size 7 x 7 x 4 mm. Just below the cemento enamel junction an area of flat root surface was selected as the site of laser treatment. In addition to sensitive measuring instruments and precise technique employed it is important to standardise the step of preparation of specimen. Area for determination of dye penetration was obtained by using a circular mask of diameter 4.5mm.

The laser beam is transmitted through a 600µm optical fiber and a focussing hand piece attached to the distal end of the fiber delivers the beam to the sample surface. With this arrangement a minimum spot size of 0.8mm is obtained at the focal plane of the hand piece. In the present experiments samples were placed 24cm away from the tip of the focussing hand piece to get a beam spot size of 7 mm. Angle of incidence is kept constant at zero degree. The laser power was set at 40w corresponding to a power density 1.04w/mm² and the exposure duration 3 sec, the foot switch is pressed to give the programmed irradiation to the sample surface. The experiments were repeated for powers 50w and 60w corresponding to 1.3w/mm² and 1.65w/mm² respectively and exposure duration of 3,5 and 7 sec.

The laser beam has the potential to be reflected and scattered causing eye damage. Eye protection for patients and dental personnel is of paramount importance. The affinity of Nd:YAG laser for pigmented tissue necessitate the use of safety glasses for eye protection because it can penetrate and damage retina. All the operators and by standers are provided with safety goggles during the time of experiments.

Spectrophotometric dye recovery method for the determination of dentinal permeability

For assessing dentinal permeability, spectrophotometric dye recovery method was used. Many techniques have been developed to evaluate the permeability. The use of organic dyes as tracers was one of the oldest and the most popular methods of detecting microleakage. However, a disadvantage of this method is that results are quantified by assigning numerals to specific points of penetration and it is somewhat proved that penetration cannot be clearly shown by the inspection of cross sections as is usually seen under dissecting microscope. Douglas and Zakariasen²⁶ have described a volumetric dye recovery method of assessing leakage invitro. This being a volumetric assessment of dye penetration, the error of linear assessment were overcome.

Powell et al²⁷ used radioactive tracers as the dye and auto radio graphs were taken of the cut surface of the specimens to detect the radioactive isotope. As in the dye studies, the autoradiographs also relied on qualitative judgment both for depth and path of tracer penetration.

The spectrophotometric dye recovery method of volumetric assessment of permeability was found to be highly accurate and easy to carry out. Even minute amount of leakage which might be clinically insignificant could be detected for a more correct comparison. Before taking each reading, blank was used for zero adjustment. This was done to control all variable which might affect the colour intensity of the solution other than the dye. The spectrophotometric readings were then due to the dye that had penetrated. Care was taken to prevent extraneous dye penetration by applying nail polish except a specified circular area.

After laser irradiation at different power levels and duration a spot size of 4.5 mm was selected just below the CEJ. Nail polish was applied all over the tooth surface excluding this area. Second layer of nail polish was applied after the initial layer dried up. The coated teeth were then immersed in 2% aqueous methylene blue solution. After 24 hrs teeth were recovered and rinsed in tap water (200mg-100ml) to remove the dye on the surface. Nail Polish coating was removed using nail polish remover, then cleaned with methanol, specimens were washed again and allowed to dry. In the group B samples, before applying laser irradiation, a thin layer of cavity varnish (Harvard-Lac) was applied using a camel hair brush. After irradiation varnish were removed

using methanol.

The specimen were dissolved in 5ml of 70% HNO₃ solution and spectrophotometric reading were taken. Methylene blue absorption peak is at wavelength 665nm. 70% HNO₃ being used as a blank, excludes all the variables which might affect the colour intensity of the solution except the dye. Spectrophotometric reading thus obtained were solely due to the dye present in the test solution. A standard solution was prepared by dissolving a known volume of dye (0.01 mg in 1 ml) in nitric acid and the corresponding reading were taken.

So using the formula

$$\text{Concentration of dye in test Solution} = \frac{\text{Spectrophotometric reading of test solution}}{\text{Spectrophotometric reading of Standard solution}} \times \text{Conc of dye in the standard}$$

Spectrophotometric reading of test solution = 0.139

Concentration of dye in the standard = 0.01 mg

$$\frac{X}{0.139} \times 0.01 = 0.07194 X \text{ mg}$$

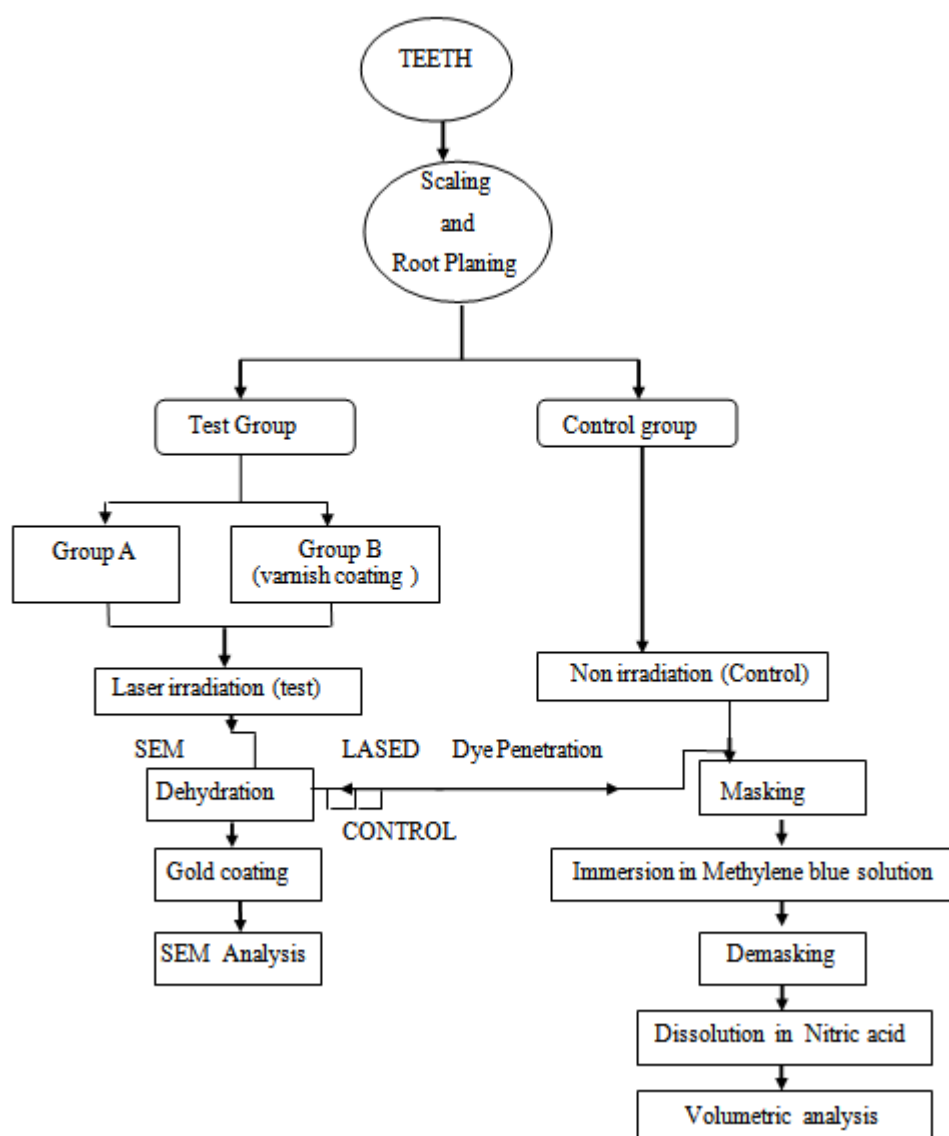
0.07194 X mg/ml dissolved in 5 ml of HNO₃. So 1ml of HNO₃ contains 0.07194 X × 5 mg of dye.

Group A & B were subdivided into eight subgroups of six specimen each. Control group comprised of 25 specimen from each group.

Scanning Electron Microscopic Analysis

Samples from each of the above subgroup were subjected to detailed scanning electron microscopic study. The tooth specimen were sectioned buccolingually and subjected to laser irradiation at specified power densities 1.04, 1.3 & 1.65 w/mm². The experiment was repeated for 3, 5 and 7 sec of irradiation time. Specimens were mounted on a brass stub using adhesive tape, following which they were dried in a vacuum evaporator for 2 hours and then coated with a thin layer of gold using an ion sputtering unit rendering the surface conductive. The brass stubs with coated samples were placed in the sample compartment of microscope. Micrographs were taken of the representative area. The effect of laser radiation on smear layer and dentinal tubule were subjected to detailed analysis. Because of the absence of qualitative data no attempts were made for statistical analysis in this group. Representative photo graphs obtained during scanning serve to deduce the results.

FLOW CHART(1) Showing procedure of experiments



Observations and Results

The tooth samples were irradiated with Nd:YAG laser beam at various power densities. The sealing efficiency of dentinal tubules by laser radiation were compared to controls (non irradiated group) quantitatively by spectrophotometric dye recovery method. The morphologic changes produced in the smear layer and dentinal tubules were assessed qualitatively by scanning electron microscopy.

In the present study the specimens were broadly divided into 2 groups (A & B). The group A samples had a bare mechanically prepared dentin surface. The group B samples had a thin layer of cavity varnish applied over the prepared surface of the dentin. The group B experiments enable to identify the effects of enhanced absorption of laser light. The varnish used is Harvard Lac.

The mean, standard deviation, and range of group A (test) and control specimens irradiated at power densities of 1.04 w/mm², 1.3 w/mm² and 1.65w/mm² with an exposure duration of 3,5,7 sec are given in table I. It is noted that all lased samples shows a decreased dye penetration compared to controls. Minimum amount of dye penetration is seen at power density 1.65 w/mm² at an exposure duration of 7 sec. This is fifty percentage of the value obtained for the control group(0.0083 mg). Further it is also noted that at higher power densities the mean concentration of dye penetration decreases. For a power density of 1.65 w/mm² with an exposure duration of 3,5,7 sec mean amount of dye penetration is 0.0035, 0.0019, 0.0014 mg respectively.

The mean, the standard deviation and range of group B (test) and control group samples are given in table II. It is noted that all lased samples shows a decrease in dye penetration when compared with control. Minimum amount of dye penetration is obtained at a power density of 1.65 w/mm² for an exposure duration of 5 sec. It is also noted that at higher power densities the mean concentration of dye penetration decreases. For a

power density of 1.65 w/mm² with an exposure duration of 3,5,7 sec mean amount of dye penetration is 0.0034mg 0.0008 mg and 0.0013mg respectively.

Group A and B with its subgroups were compared to control group. To evaluate optimum duration of lasing, statistical analysis was done using student ‘t test’ with the help of statistical software Epiinfo

Statistical analysis

Statistical comparison of group A with control is given in table III. It is shown that at all power density and exposure duration studied except at power density of 1.04 w/mm² and an exposure duration of 7 sec, the reduction in dye penetration is statistically significant. It is also seen that at higher power densities the reduction in dye penetration is highly significant.

Statistical comparison of group B with control is given in table IV. In this group also it is seen that at all power densities and exposure duration studied except at two power settings the reduction in dye penetration is statistically significant. As in group A, it is also seen that at higher power densities the reduction in dye penetration is highly significant. 't' value and 'p' value of group A and B samples are given in table III and IV respectively.

Table -I--Mean amount of dye penetration in Group A and control

| Power density w/mm ² | Duration | No. of samples | Amount of dye penetrated (mg) | |
|---------------------------------|----------|----------------|-------------------------------|---------------|
| | | | Mean ± SD | Range |
| 1.04 | 3 | 6 | 0.0038 ± 0.0017 | 0.0011-0.0054 |
| 1.04 | 5 | 6 | 0.0026 ± 0.0019 | 0.0004-0.0047 |
| 1.04 | 7 | 6 | 0.0058 ± 0.0019 | 0.0036-0.0079 |
| 1.3 | 5 | 6 | 0.0038 ± 0.0025 | 0.0007-0.0065 |
| 1.3 | 7 | 6 | 0.0051 ± 0.0017 | 0.0029-0.0068 |
| 1.65 | 3 | 6 | 0.0035 ± 0.0009 | 0.0018-0.0043 |
| 1.65 | 5 | 6 | 0.0019 ± 0.0014 | 0.0004-0.0043 |
| 1.65 | 7 | 6 | 0.0014 ± 0.0015 | 0.0004-0.0043 |
| 0- (Control) | 0 | 50 | 0.0083 ± 0.0032 | 0.0039-0.0165 |

TABLE-II Mean amount of dye penetration in Group B and control

| Power density w/mm ² | Duration | No. of samples | Amount of dye penetrated (mg) | |
|---------------------------------|----------|----------------|-------------------------------|----------------|
| | | | Mean ± SD | Range |
| 1.04 | 3 | 6 | 0.0011 ± 0.0012 | 0.0004-0.0032 |
| 1.04 | 5 | 6 | 0.0055 ± 0.0039 | 0.0002-0.0093 |
| 1.04 | 7 | 6 | 0.0070 ± 0.0021 | 0.0043-0.0097 |
| 1.3 | 5 | 6 | 0.0071 ± 0.0032 | 0.0036 -0.0108 |
| 1.3 | 7 | 6 | 0.0061 ± 0.0026 | 0.0036 -0.0086 |
| 1.65 | 3 | 6 | 0.0034 ± 0.0009 | 0.0018-0.0043 |
| 1.65 | 5 | 6 | 0.0008 ± 0.0007 | 0.0004-0.0018 |
| 1.65 | 7 | 6 | 0.0013 ± 0.0014 | 0.0004-0.0039 |
| 0- (Control) | 0 | 50 | 0.0083 ± 0.0032 | 0.0039-0.0165 |

Table III: Statistical comparison between Group A and Control

| Power density w/mm ² | Duration | 't' | P value | Remarks |
|---------------------------------|----------|------|---------|---------|
| 1.04 | 3 | 3.34 | <0.005 | S |
| 1.04 | 5 | 4.27 | <0.001 | S |
| 1.04 | 7 | 1.86 | >0.05 | NS |
| 1.3 | 5 | 3.34 | <0.005 | S |
| 1.3 | 7 | 2.42 | <0.05 | S |
| 1.65 | 3 | 3.48 | <0.001 | HS |
| 1.65 | 5 | 4.82 | <0.001 | HS |
| 1.65 | 7 | 5.15 | <0.001 | HS |

Table IV: Statistical comparison between Group B and Control

| Power density w/mm ² | Duration | 't' | P value | Remarks |
|---------------------------------|----------|------|---------|---------|
| 1.04 | 3 | 5.44 | <0.001 | S |
| 1.04 | 5 | 2.12 | <0.05 | S |
| 1.04 | 7 | 0.98 | >0.05 | NS |
| 1.3 | 5 | 0.91 | >0.05 | NS |
| 1.3 | 7 | 1.6 | >0.05 | NS |
| 1.65 | 3 | 3.69 | <0.001 | HS |
| 1.65 | 5 | 5.69 | <0.001 | HS |
| 1.65 | 7 | 5.32 | <0.001 | HS |

III. Discussion

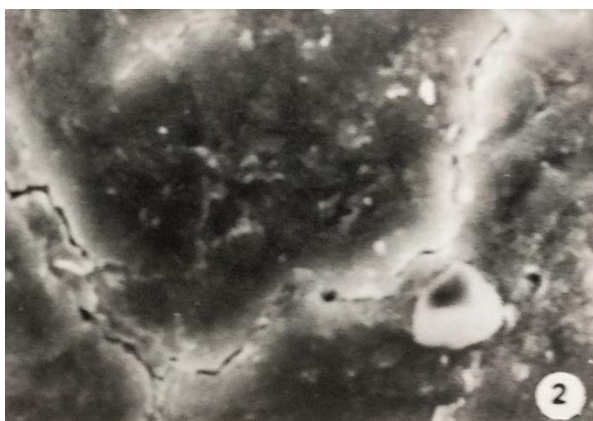
The present invitro study was conducted to investigate whether a continuous watt Nd:YAG laser could be used for removing the smear layer and closing the dentinal tubule and also attempted to find out optimum conditions at which the desired changes occur.

The morphologic changes induced by laser are primarily due to the absorption of light by the dentin or the smear layer. The absorbed light energy is converted into heat resulting in several physical changes occurring on the surface. Depending upon the amount and rate of heat generation at the surface, changes occurring may vary. The combined use of scanning electron microscopy and permeability measurements provide important information that are essential in evaluating the effects of laser on dentin.



(Fig 1)

Appearance of smear layer on dentin surface before exposure to laser energy (magnification X 2000)



(Fig 2)

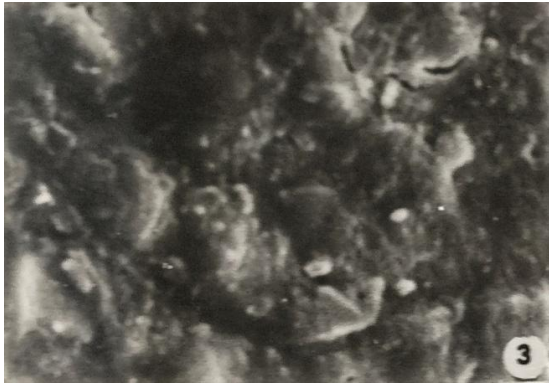
Laser treated area (at power density $1.04\text{W}/\text{mm}^2$ duration 3 sec) appears smoother than non lased areas, melting of smear layer (magnification X 3000)

The group A samples had a bare mechanically prepared dentin surface having a surface smear layer of 2-5 μm thickness. In the group B samples, a thin layer of cavity varnish was applied over the surface. This is to study whether an enhanced absorption of laser light change the surface morphology appreciably. Harvard-Lac cavity varnish is used for the study. Since this varnish is a proven biocompatible material in the oral environment, the varnish left over is not expected to create any harm to the surrounding hard and soft tissues. We have not selected highly absorbing coating like carbon black because the resultant damage to the dentinal surface is shown to be uncontrollable.

In the present study, it is found that in both the groups, the amount of dye penetration is less compared to controls. In group A, minimum amount of dye penetration is noted at power density $1.65\text{w}/\text{mm}^2$ at an exposure duration of 7 sec. It is noted that when power density is increased there is a gradual increase in permeability. But further increase in power densities resulted in reduction in dye permeability. The group B also shows a decreased dye penetration compared to controls. At lower power densities $1.04\text{ w}/\text{mm}^2$ for 3 and 5 sec the permeability is low. With increasing power densities there is a gradual increase in permeability but less than non lased samples. At higher power densities there is marked reduction in dye permeability as in the case of Group A.

Thus all the lased samples showed a decreased permeability than the non lased samples. But there is a specific pattern in the amount of decrease with respect to the power densities. At low power densities there is reduction in permeability, with increasing power densities there is gradual increase in permeability. On further increasing the power density the permeability again decreases. This pattern may be explained with the following physical model. At lower power densities, the decreased permeability is due to the fusion of smear layer and smear plugs that effectively close the dentinal tubule.

The scanning electron microscopic photographs shows the fusion of smear layer (Fig.3, 4,5).



(Fig 3,4)

Laser treated area (at power density $1.04\text{W}/\text{mm}^2$ duration 5 and 7 sec) shows most of the smear layer has been melted and resolidified on top of dentine surface (magnification X 3000)



(Fig 5)

Uniformly lasered dentine surface at an exposure duration of 1 sec and power density at $1.65\text{W}/\text{mm}^2$ (magnification X 2000)

With further increase in power density the partial or total loss of smear layers with the resultant crack formation, as is evident from scanning electron microscopic photographs (6,7,11,12), enhances the permeability.



(Fig 6)

Laser irradiation at power density $1.65\text{W}/\text{mm}^2$ and duration 3 sec shows removal of the smear layer and many dentinal tubules are open (magnification X 2000)



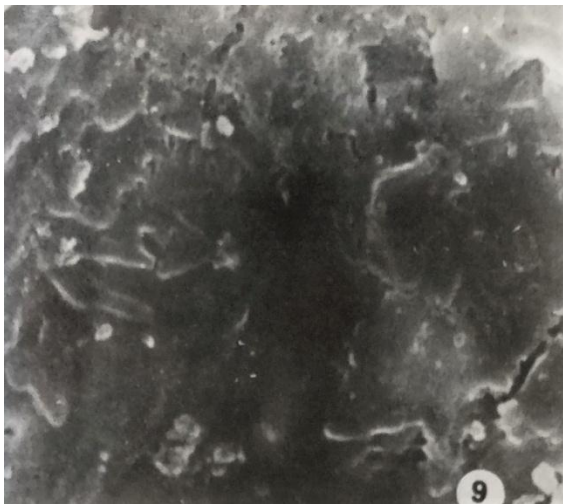
(Fig 7)

Diameter of dentinal tubule is less, almost without smear layer and increased number of microfracturing at power density $1.65\text{W}/\text{mm}^2$ and duration 5 sec (magnification X 2000)



(Fig 8)

Laser irradiation at power density 1.65W/mm^2 and duration 7 sec shows occluded dentinal tubules without smear layer (magnification X 2000)



(Fig 9)

Appearance of smear layer on dentin surface after applying a thin layer of cavity varnish (magnification X 5000)



(Fig 10)

Laser irradiation at power density 1.04W/mm^2 and duration 5 sec appears smoother. Smear layer and smear plugs covers the surface of dentinal tubule. (magnification X 3000)



(Fig11)

Laser irradiation at power density 1.04W/mm^2 and duration 7 sec where the smear layer has undergone surface changes (magnification X 3000)



(Fig 12)

Laser irradiation at power density $1.65\text{W}/\text{mm}^2$ and duration 1 sec and 3 sec shows removal of smear layer and smear plugs with patent dentinal tubules (magnification X 5000)

But at higher power densities sealing or occlusion of dentinal tubule occurs with marked reduction in permeability. The thickness of smear ranges from $2\text{-}15\mu\text{m}$, disruption of smear layer, actual melting and resolidification depends upon the magnitude of the power density. At lower power densities, the incident energy is absorbed by the smear layer or dentin surface resulting in fusion of smear layer and smear plugs that effectively close the dentinal tubule, leading to reduced permeability.

At intermediate power densities vaporization of water with accompanying microexplosions lead to partial or total loss of smear layer with resultant crater or crack formation and incompletely glazed surface that enhances the permeability.

At higher power densities there is formation of a completely glazed dentin surface with resultant decreased dye permeability. However, at the highest power densities the laser effectively strips away the smear layer, smear plugs and peritubular dentin with vapourization of water with in the dentin into steam thereby increasing dentinal permeability.

In the present study, transverse cuts were made at the cervical region as the number of dentinal tubules is more numerous in that region.²⁸ In an invitro study by Goharkhay et al, scanning electron microscopic and stain penetration tests revealed topographically incomplete closure of dentinal tubules with an inhomogenous dentin surface when irradiated with the Nd:YAG laser at 0.2 or 0.5 W, 10Hz with and without prior application of a stannous fluoride gel. Higher energy resulted in a greater number of closed tubules with an increased removal of dentin. It can be assumed that the dentin may be fused during Nd:YAG laser irradiation. Its effectiveness is probably due to an occlusion or narrowing of dentinal tubules, thereby blocking fluid flow across dentin^{29,30}. This infrared high power laser can easily interact with dental tissues. It is poorly absorbed by hard dental tissue but penetrates deep into irradiated tissues. Occlusion of exposed dentinal tubules may decrease the dentinal sensitivity level.³¹ Lan et al³² observed that most dentinal tubule orifices were occluded after the combined treatment of Nd:YAG laser with sodium fluoride varnish. Morphologic changes of the dentin surface with occluded dentinal tubules were also observed in a study by Naylor et al³³ and the absence of protrusive rods in the dentin surface was interpreted as sealing of the tubules.³⁴ The presence of a smear layer appears to have a strong influence on effective tubular occlusion possibly acting as a substrate with multiple nucleation sites, thus accelerating apatite formation.³⁵ In the present study, the Nd:YAG laser was shown to be

able to reduce tubular diameter via mechanical occlusion. Therefore the present study confirms other similar studies which have evaluated microstructural changes resulting from Nd: YAG laser exposure.^{36,37,38}

Nd: YAG laser can depress intradental nerve responses to dentin stimulation³⁹. Eleftherois-Terry R et al suggested that laser irradiation with 1w Nd: YAG is superior to other treatment modalities with regard to dentinal orifice occlusion⁴⁰. Liu et al in 1997 suggested that the sealing depth of Nd:YAG laser on human dentinal tubules at an energy of 30mJ/P with 10pp/s was found to be -4µm in the centre and 3µm at the margin of the lased surfaces⁴¹. An invitro study by Lan et al 1999⁴² reported a 90% occlusion of the exposed dentinal tubules by SEM. Gaspric et al 2001 found that craters and microcracks are possible side effects following Nd:YAG irradiation.⁴³ But in the study conducted by Reza Birang et al, no craters were observed in the Nd: YAG group while craters were observed in the Er:YAG group.⁴⁴ These craters may be caused by the density of the energy applied which is higher than the ablation threshold. According to Hargreaves et al 2002 the teeth most commonly affected by DH are canines, premolars, incisors and molars in the descending order^{45,46}

Watanabe et al 2003 evaluated the possibility of Er:YAG laser for DH using a normal laser probe, broom-type probe. The morphological change of dentinal tubules of bovine dentine plate after low power laser irradiation (5 or 10mJ/pulse, 10pps) or boiling was observed by SEM. Fifty teeth from 13 patients aged 31-54 years with complaint of DH were treated by laser irradiation at 25-35mJ/pulse, 10 pps using the broom-type probe. Clinical effect of laser irradiation was verified by the examination of sensitivity rate to cold water, air blow and mechanical stimuli of explorer before, immediately, after and 1,3,5 and 12 weeks after laser irradiation. The ratio of blockade and reduction of dentinal tubules after laser irradiation was 16-61%. Owing to vaporization of water in dentinal tubules, the accumulation of organic elements and degeneration or coagulation of them at the site of blockade and reduction were superficially described by SEM. Remarkable clinical improvement of DH by laser was obtained but recurrence was also partially detected thereby suggesting that low power irradiation of Er:YAG laser would be effective on the treatment of DH.⁴⁷ Lan et al 2004 proposed that the mechanism of Nd: YAG lasers effect on dentin is caused by thermal energy absorption and this thermal energy caused the hydroxyapatite crystals to melt partly or completely and finally the dentinal tubules were occluded. Aranha et al (2005) suggested the use of Nd:YAG at 1.5w, 15 Hz to decrease dentin permeability to about 19%.⁴⁸ They have also reported that Nd: YAG laser at 1w, 10Hz decreases permeability with a smaller effect.⁴⁹ Pro-Jen HSU et al (2006) evaluated the combined occluding effects of fluoride containing dentin desensitizer and Nd: YAG laser irradiation on human dentinal tubules. Scanning electron microscopy revealed that fluoridated dentinal tubule occluding agent (FDTOA) formed a fine crystalline deposit on the dentin surface. After soaking in 0.5M Vit.C solution for 3 hrs, the crystalline deposit of the FDTOA was completely dissolved. Furthermore, brushing of the teeth 3600 times removed most of the occluding agent. When the application of FDTOA was combined with Nd:YAG laser irradiation, the dentin melted and then recrystallized. The occluding agent was thus “burned into” the dentinal tubules, and could neither be dissolved by Vit. C solution nor removed by brushing. Therefore, they concluded that FDTOA combined with Nd:YAG laser irradiation burns the occluding agent into the dentinal tubules, there by resisting the effects of an acidic diet and brushing and increasing the duration of the desensitizing effect.⁵⁰ These studies are in accordance with the present invitro study.

Reza Birang in 2008 in an invitro study evaluated the changes of the dentin surface after Nd:YAG and Er:YAG laser irradiation using SEM. Fifteen freshly extracted mandibular molars were selected and 4 specimens with known dimensions (1x2x2mm) from the buccal surface and below CEJ of all the teeth were prepared to obtain a total of 60 specimens. These specimens were then divided into 4 groups. Group 1 act as the control and was not subjected to laser irradiation. Group 2 was subjected to Nd:YAG laser irradiation (0.5w, 50ml, 10Hz, 60S). Group 3 was irradiated by Nd:YAG laser (1w, 100ml, 10Hz, 60S). Group 4 was irradiated by Er:YAG laser (0.3w, 100ml, 3Hz, 60S.) After preparation and gold coating of specimens, they were observed under SEM. The number of open tubules showed significant differences between the Nd:YAG (1w) group and other groups, but no significant differences were observed between the Nd:YAG (0.5w), Er:YAG (0.3w) and control groups. The results of this study shows that Nd:YAG and Er:YAG laser irradiation can cause thermal effects such as decreased dentinal tubules diameter or occlusion. According to this study, Nd:YAG laser at 1W power is more effective than the Nd:YAG at 0.5w or Er:YAG in occluding tubules (Reza Birang 2008). Reza Birang(2008) showed that Nd:YAG laser (0.5w) in most cases caused a decrease in dentinal tubule diameter while the Nd: YAG laser (1w) additionally caused tubule occlusion and disappearance⁴⁶. Gholam Ali et al (2011) evaluated the occluding effects of Er:Cr :YSGG, 810nm diode, CO₂ and Nd: YAG lasers on dentinal tubules and found that the mean dentinal tubule entrance diameters for Er:Cr:YSGG; 810-nm diode, CO₂ and Nd: YAG were 1.73, 3.27, 2.10 and 1.64 microns respectively, compared with 3.52 microns before laser irradiation. This further indicates that Nd: YAG, Er:Cr : YSGG and CO₂ lasers through their ability to melt peritubular dentine, can occlude dentinal tubules partially or totally and therefore reduce patients hypersensitivity symptoms. The 810nm diode laser sealed tubules to a far lesser degree, with negligible effects on desensitization⁵¹

Eleftherios –Terry R in 2013 investigated the influence of bioglass, Nd:YAG laser irradiation and their combination on dentinal tubule occlusion. In this invitro study, they evaluated the efficacy of Novamin (NM) paste, Nd:YAG laser irradiation (L) at various power settings and their combination on Dentinal Orifice Occlusion (DOO). Forty nine human dentin surfaces were organized into seven equal groups: Groups A and B received NM followed by L (0.5 & 1W respectively), groups C and D received L (0.5 and 1 W respectively) followed by NM, group E received NM and groups F&G received L (0.5 & 1W respectively). Specimens were evaluated with regard to DOO under scanning electron microscope. Their results showed that treatment modality significantly affected DOO ($P = 0.012$). Groups B and G scored higher DOO than the rest which proves that Nd: YAG laser irradiation at 1 W alone or combined with NM is a superior method for producing DOO and can be an effective treatment modality for dentinal hypersensitivity (DH).⁴⁰ Meta-analysis by Sgolastra (2013) failed to recommend the clinical implication of Nd :YAG laser due to herterogeneity of various studies.⁵² Arulmozhi Nandakumar, Vidya Hari Iyer (2014) in an invitro analysis compared the efficacy of lasers and desensitising agents on dentinal tubule occlusion and found that laser treatment of exposed dentinal tubules with Er.Cr :YSGG promises a non invasive pain free and relatively safe treatment option.⁵³

Several clinical studies are also conducted using Nd:YAG laser. Matsumoto et al applied Nd:YAG laser to treat hypersensitivity for the first time and found to be 100% successful. White etal found that irradiation of dentin using Nd:YAG pulsed laser did not cause detrimental intrapulpal temperature rise.²⁵ Ciaramicoli et al evaluated the effect of Nd:YAG laser to treat DH and showed a statistically significant reduction of hypersensitivity in groups that received the treatment with Nd:YAG laser compared to control teeth.⁵⁴

IV. Conclusion

Lasers are constantly evolving and can be customised to meet dental requirements and could become a very useful tool for dental practioners. Moreover today's dental lasers are more reasonably priced, smaller, light weight, portable and to date several kinds of lasers have been developed in dentistry with multitude of wavelengths and pulse width. But all of them require optimisation of safety and effective power and duration for achieving satisfactory results without detrimental effects to manage hypersensitivity. Large multicentre trials with long term follow up is needed to substantiate the clinical benefits of Nd:YAG laser.

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