

Chemical Structural Alterations of Root Surface After Er: YAG Laser, Nd: YAG Laser Irradiation-An Infrared Spectroscopic Study

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Abstract:

AIM: To assess the chemical structural alterations of root surface by using Fourier Transform Infrared Spectroscopy followed by Er: YAG and Nd: YAG laser irradiation.

MATERIAL AND METHOD: Fifty five upper incisor teeth extracted from patients attending the outpatient Department of Periodontics, Tamilnadu Govt. Dental College and Hospital Chennai -3. 110 specimens of size 3mm x 4mm x 1mm were prepared. For evaluation fifty five samples were divided into three groups. Group A, B and C. Group A contain five samples which serve as non irradiated control. Group B and Group C further divided into five sub groups and each subgroup contains five samples. All the specimens within the subgroups of B and C irradiated with 100 mJ, 200 mJ, 300 mJ, 400 mJ, 500 mJ of Er:YAG laser and 211.66 J/cm², 423.33 J/cm², 635 J/cm², 846.66 J/cm² 1058.33 J/cm² of Nd:YAG laser respectively. For chemical analysis (FTIR spectroscopy) of root remaining fifty five samples were divided into Group D (non irradiated control), group E and F. All the sub groups of Group E and F were irradiated with same parameters used in Group B and Group C respectively. Chemical structural changes after laser irradiation were performed on Fourier Transform Infrared Spectroscopy instrument. The data obtained was statistically analyzed by one-way ANOVA multiple range test by Turkey- HSD and Mann Whitney tests.

RESULTS: Er: YAG laser at 100 mJ effectively remove smear layer without crater formation.. The Nd:YAG laser at the energy density of 211.66 J/cm² and 423.33J/cm² remove the smear layer at the energy density of 1058.33 J/cm² showed visible charring and FTIR spectroscopy profile showed reduction in peak height of major bands (Amide I, II III, OH group and phosphate) after 300 mJ of Er:YAG laser. The reduction in peak intensity observed only beyond the 846.66 J/cm² of Nd:YAG laser irradiation and new absorption band was noticed (2010 cm⁻¹ and 2017 cm⁻¹) at samples treated with 846.66 J/cm² and 1058.33 J/cm² of Nd: YAG laser.

CONCLUSION: Er:YAG at lower energy density effectively remove smear layer without production of toxic substance when compared to Nd:YAG laser.

Keywords- FTIR Spectroscopy, Nd.YAG Laser, Er.YAG Laser

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I. Introduction

Periodontal disease is one of the most prevalent afflictions worldwide. It has been referred in numerous historic writings beginning with "Ebers Papyrus" (circa; 1550 BC) and today appears to be as prevalent and severe. Ultimate goal of periodontal therapy is predictable regeneration of periodontium at the site of periodontitis and the major factor inhibiting periodontal regeneration appears to be the nature of affected root surface. Complete removal of the accretions from the root surface is one of the most important aspect of periodontal treatment. Conventional mechanical therapy has its limitations in removal of toxins from root surface and within the periodontal pocket¹. Many researchers have examined the effect of root conditioning after mechanical treatment, using chemical agents, which will remove the smear layer and expose collagen fibers and dentinal tubules, enhancing the histocompatibility and new connective tissue attachment with cementogenesis^{2,3}. Root conditioning agents with citric acid proved to be effective in removal of smear layer, but the acidic pH and demineralizing capacity of citric acid resulted in delayed wound healing, pulpal reaction

and bacterial penetration of the treated sites. Since then search was began for better physical or chemical agents that remove the smear layer without any adverse effects.

In 1964, Leon Goldman reported the laser application to a healing, living human tooth for the first time. Food and drug administration, USA approve the use of lasers for soft tissues surgeries since 1990 and periodontal surgeries since June 1999. The victory of Nd –YAG laser in the soft tissue surgery has inspired the thoughts of researchers to apply this technique for root planing. The success in Nd –YAG and ideal properties of Er –YAG laser has inspired the researchers to believe pretreating the roots with this technique would be promising factor in periodontal regeneration. The present invitro study deals in comparing the efficiency of Nd-YAG and Er-YAG laser treat on root planning using chemical compositional changes following different energy density of Nd-YAG and Er-YAG laser irradiation assessed with Fourier Transfer in Infrared Spectroscopy(FTIR).

II. Material And Methods

For this study subjects belonging to the both group were selected from the outpatient department of periodontics, Tamilnadu government dental college, Chennai. The age group of the selected patients ranged from 35-55 years. The inclusion criteria were patients with teeth with clinical probing depth of 6mm or more and Cal 5mm. Teeth with grade III mobility and the presence of anterior vital teeth on systemically healthy patients were included in the study. Exclusion criteria consisted of patients who had undergone periodontal therapy in the past 6 months and those with history of known systemic disease, pregnancy and lactating mother s and under any drug therapy. Patients with the habit of smoking, alcohol, and therapy were also excluded from the study. Teeth extracted for caries, orthodontic treatment purpose, impacted teeth, and non vital teeth were all excluded from the study.

110 specimens (3mm x4mm x1mm) were prepared from 55 periodontally involved upper incisors. For SEM study evaluation 55 samples were divided into 3 groups, group A,B,C. Group A contains 5 samples which serve as no irradiated control, Group B and C further divided into 5 subgroups and each subgroup contain the sample. All the specimens within the subgroups of B and C irradiated with 100nm to 500nm Er - Yag laser and 211.66 J/cm² to 1058.33J/cm² of Nd-YAG laser respectively. For chemical analysis (FTIR) of root, remaining 55 samples were irradiated with same parameters and in group B and group C respectively. Chemical and structural changes after laser irradiation were preformed on FTIR.

Control group

Under local anaesthesia upper anterior tooth having a probing depth of 6mm or more or CAL 5cm or more and which exhibited grade III mobility were extracted. Immediately after extraction the tooth was washed with normal saline to remove blood and loosely adhered tissue. The soft tissue and other debris on the root surface are removed with ultrasonic scaler and root planned with gracey curette (1-2). The teeth was stored in distilled water at 4°C until specimen preparation.

FOURIER TRANSFORM INFRARED SPECTROSCOPY STUDY

For the evaluation of chemical change 55 specimens (3mm x4mm x 1mm) were prepared with flexible diamond disc under copious cold distilled water coolant and samples were randomly divided into 3 groups. .Group D – control irradiated 5 specimens, Group E - irradiated with Er-Yag laser subgroups E1,E2,E3,E4,E5 Group F- irradiated with Nd –YAG laser, subgroups F1,F2,F3,F4,F5

LASER TREATMENT

Er-YAG andNd-YAG solid state laser DEKA –LASERS ITALY, were used. Er-YAG laser emitted light of 2940nm wavelength in a pulse mode (10 pulses / second, length of pulse was 250 nm), spot size of 6mm and light was conducted through a mirror system in a titanium articulated arm. The laser beam was found in the sample with the help of inbuilt He-Ne found in laser guide. The laser hand piece was continuously moved during the irradiation over the entire surface of the sample at the distance of 1.5cm that constant focal spot size.

All specimens were irradiated as follows

Er-YAG laser irradiation parameters

SUBGROUP	ENERGY	POWER
1	1W	100mJ
2	2W	200mJ
3	3W	300mJ
4	4W	400mJ
5	5W	500mJ

Table 1

Nd –YAG laser irradiation parameters

subgroup	Time of irradiation	Energy density
C1	20sec	211.66 J/cm ²
C2	40sec	423.33 J/cm ²
C3	60sec	635 J/cm ²
C4	80sec	846.66 J/cm ²
C5	100sec	1058.33 J/cm ²

Table 2

Nd-YAG laser irradiated light of 1064nm wave length in a pulse mode 1 pulse per second pulse length of 250 nm , spot size of 6mm, and light was conducted through optical fiber system . The delivery hand piece was continuously moved back and forth to cover the entire sample surface . All specimens were irradiated at the above mentioned parameters (table 2)

PREPERATION OF SPECIMEN FOR FTIR SPECTROSCOPY AFTER LASER IRRADIATION

All specimens both irradiated and non irradiated were stored in a dessicater at 4°C for 1 week prior to spectroscopic study.

55 specimen surfaces, 5 non irradiated , 25 irradiated with Er-YAG and 25 irradiated with Nd-YAG laser were scratched with scapel. 3mg of each scratched sample were mixed with KBr and pressed into disk with help of KBr press instrument according to the manufacturer . Infraredspectra were recorded on spectrometer from 4000cm⁻¹ to 400 cm⁻¹ and analysed with inbuilt software (Ominic)

III. Data Analysis

The first analysis consisted of comparison of spectra among the treatment types at locations where the formation of new bands and change in the height of each peak were observed . A quantitative analysis of the intensity of the chemical group left after each treatment was considered. However , direct quantitative comparison based on the height of each band peak was not possible between treatments, because the thickness of the scraped material in the pellet that influence the peak height could not be standardized in this present experiment. Thus the intensity height of the peak of the major band (OH <Amide I, Amide II, Amide III, phosphate and band was observed from each pellet using the spectrometer software.

The statistical package SPSSPC+ (Stastical package for social sciences , version 12) was used for stastititl analysis

The mean values were compared by one way ANOVA multiple range test by Turkey –HSD (Honestly significant difference) procedure was employed to identify the significant groups , if P- value in one way ANOV is significant . In the present study p<0.05 was considered as the level of significance. Mann Whitey test is used tocompare the observations of two samples.

IV. Results

All the specimens in the sub group B1 were irradiated with 100 mJ Er:YAG laser showed chalky appearance in naked eye

All specimens treated with 400mJ and 500 mJ of laser energy shows visible charring of the root surface

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) STUDY

FTIR spectra of non treated controls show five main bands hydroxyl , amide I ,amide II, amide III and phosphate and carbonate . the hydroxyl group absorption peeks were recorded between 3600 and 2400cm⁻¹ and the amide peeks were recorded between 1680-1200cm⁻¹

The amide peak were divided into amide I peak (between 1680 and 1600cm⁻¹ and amide II peak (between 1580-1480cm⁻¹) amide peak III (between 1300 and 1200₁ , the orthophosphate group was recorded in the absorption peek between 1030-1150cm⁻¹ . The carbonate band was present between 1560₁ 1410cm₁ and consequently an overlap of this band was present with amide bands was observed in this region

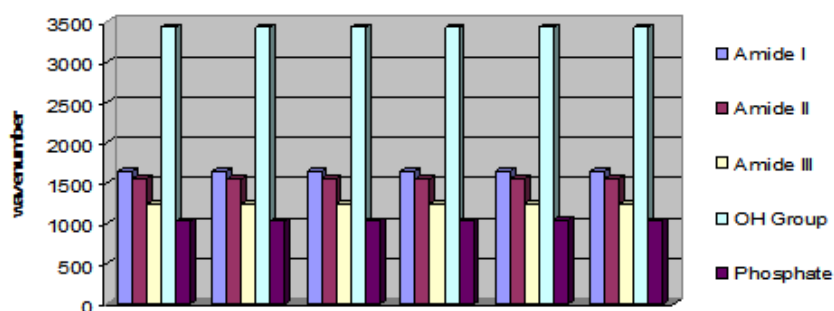


Image 1

– Mean values of infrared spectra Location of peak (Wave number) with control and different energy levels of Er:YAG laser irradiation

INTER GROUP COMPARISON – LOCATION OF THE PEAK (D VS E1- E5)

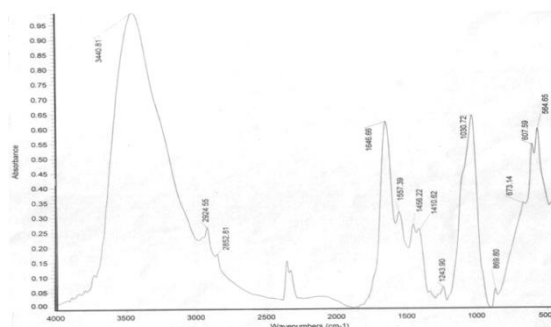
Mean and significant P Values of Infrared spectra (Location of the peak) between the control and Er: YAG lasers at different energy levels by Mann Whitney U test

Variable	Wave Number (cm ⁻¹) Control –D Mean ± SD	Mean (Wave number) Significance (P- Value)					
		Mean± SD	Er:YAG-100 mJ E1	Er:YAG-200 mJ E2	Er:YAG-300 mJ E3	Er:YAG-400 mJ E4	Er:YAG-500 mJ E5
Amide I	1647.40 ± 1.14	Mean± SD	1647.80 ± 1.92	1646.80 ± 0.837	1646 ± 2.348	1644.60 ± 2.60	1648.40 ± 3.209
		P - Value	0.914 - NS	0.381 - NS	0.140 - NS	0.017 - S	0.914 - NS
Amide II	1556.40 ± 1.140	Mean± SD	1556 ± 0.70	1556 ± 0.707	1555 ± 1.73	1554 ± 4.062	1555.40 ± 1.949
		P - Value	0.575 - NS	0.575 - NS	0.180 - NS	0.280 - NS	0.502 - NS
Amide III	1242.60 ± 1.140	Mean± SD	1240.60 ± 3.209	1242.20 ± 0.837	1242.80 ± 0.837	1242.80 ±	1243.20 ± 1.924
		P - Value	0.197 - NS	0.511 - NS	0.827 - NS	0.746 - NS	0.669 - NS
Hydroxyl Group(OH)	3437.40 ± 8.142	Mean± SD	3438 ± 6.745	3441 ± 1.225	3438.60 ± 6.025	3441.60 ± 0.894	3436.40 ± 10.90
		P - Value	0.748 - NS	0.511 - NS	0.667 - NS	0.159 - NS	0.830 - NS
Phosphate Group	1030.60 ± 0.894	Mean± SD	1030.80 ± 0.837	1030.40 ± 0.548	1031.80 ± 1.304	1033.80 ± 5.728	1332 ± 2.828
		P - Value	0.650 - NS	0.811 - NS	0.100 - NS	0.100 - NS	0.316 - NS

Table 3

Er:YAG treated samples

The profile of Er-YAG treated surface differed clearly from the control other than location of the peak. The samples in group E1 and E2 , which were irradiated with 100 and 200 mJ showed no significant reduction in peak height (amide I,II,III, hydroxyl (OH) and phosphate) whereas sudden decrease in intensity of amide I,II,III, hydroxyl were observed in other groups (E3,E4, and E5) . In all the subgroups there was no gross alteration of peak height of phosphate. No new bands were observed even at higher energy level.

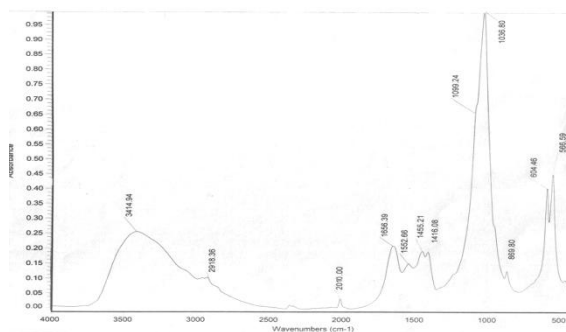


FTIR spectroscopy profile of Er:YAG (200mJ) laser treated root

Image 2

Nd:YAG laser treated samples

The infrared spectra of Nd:YAG treated sub groups showed gradual reduction in primary bands . The peak height of hydroxyl group decrease only beyond 846.66J/cm² of Nd: YAG laser irradiation whereas phosphate peak was unaltered .Theres was a new band observed at 2010cm⁻¹ in few spectra obtained from sample irradiated with 846.66J/cm² and 1058.33J/cm² Nd:YAG laser.



FTIR spectroscopy profile of Nd: YAG (1058.33/cm²) laser treated root

Image 3

V. Discussion

Periodontal disease is characterized by chronic inflammatory lesion and destruction of supportive periodontal tissue . Periodontally diseased root surfaces are contaminated with etiological agents such as endotoxin .Lasho DJ (1983) stated that scaling and root planning are integral part of periodontal treatment⁵ . Generally , a surface smear layer is seen on root planed surfaces. A smear layer may adversely affect the healing of periodontal tissues as it contains bacteria and inflammatory substances such as debris of infected cementum and calculus and endotoxins.

Conventional mechanical debridement using curette is still technically demanding and time consuming and ultrasonic scalers cause uncomfortable stress to the patient s from hypersensitivity , noise and vibration. The complete removal of bacterial deposits and their toxins from the root surfaces and within the periodontal pockets is not necessarily achieved conventional , mechanical therapy. In addition , access to areas such as furcations, concavities , grooves and distal sites of molars is limited.

Many researchers have examined the effects of root conditioning after mechanical debridement , using chemical agents such as tetracycline , citric acid (ph 1.0) Miller PD (1983) , fibronectin and ethydiamine tetraacetic acid (EDTA)⁶ . Root conditioning has been shown to remove the smear layer , and to expose collagen fibers and dentinal tubules , enhancing the histocompatibility and new connective tissue attachment with cementogenesis. However , bacterial tubules of citric acid treated teeth resulting in exacerbation of inflammatory pulp response.

Therefore development of novel systems for scaling and root planning, as well as further improvement of currently used chemical methods of smear layer removal is required.

Lasers were first employed in dentistry in hard tissue treatments , such as caries removal and cavity preparation. As a substitute for mechanical cutting and drilling. As lasers can achieve excellent tissue ablation with strong bactericidal and detoxification effects , they are one of the most promising new technical modalities

for non surgical periodontal treatment. Another advantage of lasers is that they can reach inaccessible areas which cannot be reached by mechanical instrumentation. The adjunctive or alternative use of lasers with

Conventional tools may facilitate treatment and has the potential to improve healing. Hence lasers has been selected in this study to evaluate the efficacy of smear layer removal, root biomodification

Many researchers have investigated the effects of various lasers such as argon, CO₂, Nd:YAG and ER:YAG on dental hard tissues. The CO₂ laser (10,600nm) produces severe thermal damage, melting and carbonization when applied to hard tissues hence its use is limited to soft tissues procedure and hence not taken for this study.

The Er:YAG laser was introduced by Zharikov et al, as a solid state laser that generates a light with a wavelength of 2940 nm⁷. The absorption coefficient of water of the Er:YAG laser is higher than of the other laser, hence Er:YAG laser is well absorbed by all biological tissues that contain water molecules. This laser is indicated not only for the treatment of soft tissues but also for ablation of hard tissues. The excellent ablation effect of the Er:YAG laser of both soft and hard tissues has received a lot of attention in the field of periodontal therapy. The Er:YAG LASER does not cause carbonization of the irradiated root surface.

In this study Er:YAG laser with minimal power of 100mJ per pulse was used because of the available minimal energy density of the laser instrument and the energy level coincides with Schoop et al and Gaspiric and Skaleric study^{8,9}.

VI. Fourier Transform Infrared Spectroscopy (Ftir)

When applying lasers for hard tissue ablation, thermal side effects have been major problem. Local increase in tissue temperature may break weak bonds while energetically rich chemical bonds might be broken at higher temperature. Biocompatibility of the root surfaces following laser irradiation might therefore be decreased due to an inadequate removal of contaminating substances and due to thermal denaturation of the surface matrix protein. Denaturation of extracellular matrix bound growth factors (IGF-1, FGF-1 & FGF2) and extracellular matrix components involved in periodontal regeneration may impair healing process. This can be assessed by infrared spectroscopy.

Since FTIR Spectroscopy is non destructive, provides precise measurement without external calibration and increases speed, it is preferred over other methods.

FTIR spectroscopy profile of control and all laser treated samples showed five major bands attributed to proteins namely amide I,II,III, hydroxyl, phosphate. The location of the bands (Wave number cm⁻¹) coincide with Sasaki et al, Gaspire B, Skaleric and Spencer^{15,16} P, Cobb CM studies

The samples irradiated with 100 and 200 mJ of Er: YAG laser showed no significant reduction in peak height particularly for organic compounds (amide and hydroxyl group) and all orthophosphate bands were unaltered even at higher energy density of 500mJ which coincides with Sasaki et al study¹⁴

The samples treated above 200 mJ showed significant reduction in amide and hydroxyl group. The orthophosphate bands were nearly equal in visually charred specimens treated with 400mJ, 500mJ. This clearly indicated Er:YAG laser does not alter inorganic substances of the root. No new bands attributed to toxic substances observed even at maximal energy

The samples treated beyond 846.66 J/cm² of Nd: YAG laser irradiation showed reduction in peak height particularly for the inorganic compounds (amide and hydroxyl) which coincides with Spencer et al study.

In this study new absorption band was noticed (2010cm⁻¹ and 2017 cm⁻¹) in samples treated with 846.66J/cm² and 1058.33J/cm² of Nd:YAG laser coincides with earlier studies. The absorption at 2010 cm⁻¹ is tentatively assigned to ammonium. The appearance of the ammonium band may be attributable to the breakdown of protein. Such surface contamination could potentially affect cell viability and cell attachment

Considering the result of our study the Er: YAG laser¹² may perform a preferential ablation of organic component compared to the inorganic component during irradiation. A selective ablation of protein might be useful for removing toxic enzymes and bacterial protein antigens. During the periodontal treatment. However the actual effect of this event should be thoroughly investigated.

The FTIR technique is useful for analyzing chemical structural changes on hard tissues^{11,13}. The number of spectroscopic techniques presently available is immense with a number of advantages or disadvantages during analysis of chemical structure of dental tissue. Among the disadvantages of FTIR spectroscopy technique artifacts, such as potential alteration of the chemical structure by grinding procedure and mutilation of the surface during sampling the treated sites are cited

Our results of this preliminary study indicate that Er: YAG laser at 100 mJ and NdYAg laser at the energy density of 211.66J/cm² and 423.33 J/cm² remove the smear layer without altering chemical structure of the underlying cementum and dentin.

VII. Conclusion

Further in vivo studies are to be done focusing an increase in sample size with laser instrument capable of generating minimal energy with special delivery tips and calibrated device to standardize the angle and constant laser exposure on the sample. Exact chemical alteration of lased root surface can be assessed with Fourier transform infrared Photoacoustic spectroscopy instrument to overcome the chemical structural alteration during sample preparation also needed .

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