Original Article

Morphological and Chemical Alterations of Root Surface after Er:YAG laser, Nd:YAG Laser Irradiation: A Scanning Electron Microscopic and Infrared Spectroscopy Study

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Abstract

Aims and Objectives: This study aimed to evaluate the efficacy of Nd:YAG and Er:YAG lasers in removing the smear layer and to study the morphological and chemical alterations of the root surface using scanning electron microscopy (SEM) and infrared (IR) spectroscopy. **Material and Methods:** Fifty-five extracted upper incisor teeth were collected and 110 specimens of size 3 mm × 4 mm × 1 mm were prepared. For SEM evaluation, these samples were divided into six groups: A, B, and C. Group A comprised five samples that served as control. Groups B and C were further divided into five subgroups and each subgroup comprised five samples. All the specimens within the subgroups of B and C irradiated with 100, 200, 300, 400, and 500 mJ of Er:YAG laser and 211.66, 423.33, 635, 846.66, and 1058.33 J/cm^2 of Nd:YAG laser, respectively. The morphological changes of the laser-treated sites were observed qualitatively using an arbitrary scale under SEM. The data obtained were statistically analyzed by one-way analysis of variance (ANOVA) multiple range test by Turkey's honestly significant difference and Mann–Whitney *U* test. In chemical structural changes, Group D comprised five samples that served as nonirradiated control and Groups E and F were irradiated with the same aforementioned parameter and evaluated using Fourier-transform infrared spectroscopy. **Results:** Er:YAG laser at 100 mJ effectively removed smear layer without any crater formation. The Nd:YAG laser removed the smear layer at the energy density of 211.66 J/cm^2 and 423.33J/cm^2. The energy density of 1058.33 J/cm^2 showed visible charring and deep crater with increased area of melted and resolidified minerals in SEM. In the chemical changes, IR spectroscopy graph showed the reduction in peak intensity beyond 846.66 J/cm^2 of and new absorption band was noticed (2010 cm^{-1} and 2017 cm^{-1}) at samples treated with 846.66 and 1058.33 J/cm^2 of Nd:YAG laser. **Conclusion:** Er:YAG laser at lower energy density effectively removed smear layer without production of toxic substance as compared with Nd:YAG laser. Thus, Er:YAG laser can be used as an effective root biomodification agent.

Keywords: *Laser, ND: YAG laser, scanning electron microscopy, YAG laser*

Introduction

The ultimate goal of periodontal therapy is predictable regeneration of periodontium at the site of periodontitis. Conventional mechanical

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therapy has its limitations in removal of toxins from root surface and within the periodontal pockets.[1] Moore *et al.*[2] and Polson *et al.*[3] 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. Root conditioning agents, such as 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.

This *in vitro* study deals with comparing the efficiency of Nd–YAG and Er–YAG laser treat on root planning using scanning electron microscopy (SEM). We aimed to evaluate the morphological and chemical structural alterations in root surface and removal of smear layer by using Er:YAG and Nd:YAG lasers by SEM.

Materials and Methods

In this prospective study, 55 extracted upper incisor teeth were collected from patients in the age group of 35–55 years.

The inclusion criteria of the study were as follows:

- Clinical probing depth of 6 mm or more
- CAL of 5 mm or more.
- Teeth with Grade III mobility

The exclusion criteria of the study were as follows:

- Patients who had undergone periodontal therapy in the past six months
- Patients with history of known systemic disease
- Patients with the habit of smoking and alcohol
- Teeth extracted for caries, orthodontic treatment purpose, impacted teeth, and nonvital teeth

In total, 110 specimens (3 mm × 4 mm × 1 mm) were prepared from 55 upper incisors. For SEM evaluation, 55 samples were divided into three groups: A, B, and C. Group A comprised five samples that served as irradiated control. Groups B and C were divided into five subgroups. All the specimens within the subgroups of B and C were irradiated with 100–500 nm Er–YAG laser [Table 1] and 211.66 J/cm^2 to 1058.33 J/cm^2 of Nd–YAG laser, respectively [Table 2]. The morphological changes of the laser-treated sites were observed by SEM. Group D comprised five samples that served as irradiated control. Groups E and F were further divided into five subgroups. All the specimens within the subgroups of E and F were irradiated the same parameters as Groups B and C, respectively. The chemical changes of the laser-treated sites were observed by Fourier-transform infrared spectroscopy (FTIR) spectroscopy.[21]

Specimen Preparation

The soft tissue and other debris on the root surface are removed with ultrasonic scaler and root planned with gracey curette (1–2).[4] The teeth were stored in distilled water at 4°C until specimen preparation.

The evaluation of morphological changes in 55 specimens (3 mm × 4 mm × 1 mm) were prepared with flexible diamond disk under copious cold distilled water coolant and were stored in distilled water at 4°C until laser treatment. They were randomly divided into three groups: Group A—control nonirradiated five specimens; Group B—irradiated with Er–YAG laser, subgroups B1, B2, B3,B4, and B5; Group C—irradiated with Nd–YAG laser subgroups C1, C2, C3, C4, and C5.

Laser Treatment

Er–YAG and Nd–YAG solid-state lasers (DEKA Laser, Florence, Italy) were used. Er–YAG laser emitted light of 2940 nm wavelength in a pulse mode (10 pulses/s; length of pulse = 250 nm), spot size of 6 mm, 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 continually moved during the irradiation over the entire surface of the sample at the distance of 1.5 cm that constantfocal spot size.

Nd–YAG laser (1064 nm wavelength) in a pulse mode 1 pulse/s pulse length of 250 nm, spot size of 6 mm, 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 aforementioned parameters [Table 2].

Table 1: Er–Yag laser irradiation parameters

Subgroup	Energy (W)	Power (mJ)
1	1	100
2	2	200
3	3	300
4	4	400
5	5	500

Table 2: Nd–Yag laser irradiation parameters

Subgroup	Time of irradiation (s)	Energy density (J/cm^2)
C1	20	211.66
C2	40	423.33
C3	60	635
C4	80	846.66
C5	100	1058.33

Preparation Of The Specimen For Scanning Electron Microscopy

The specimens were fixed with a freshly prepared 2.5% gluteraldehyde in 0.2-M phosphate buffer (7.5) at room temperature for 2h and 30min and washed thrice with phosphate bur for 10min each. The specimens were dehydrated in graded series with aqueous ethanol (50%, 70%, 80%, 95%, and 100%) for 10min at each concentration. These specimens were then air dried and were mounted in SEM stubs and sputter coated with approximately 200 Å of platinum using a spiller coater for SEM viewing using SEM operated at accelerated voltage of 15–20kV.

Preparation Of The Specimen For Fourier Transform Infrared Spectroscopy

All specimens for FTIR study, both irradiated and nonirradiated, were stored in a dessicater at 4°C for one week prior to FTIR study. In total, 55 specimen surfaces, 5 non-irradiated, 25 irradiated with Er–YAG, and 25 irradiated with Nd–YAG laser were scrapped with scalpel. 3mg of each scrapped sample were mixed with potassium bromide (KBr) powder and formed into disk with help of KBr disk-forming instrument supplied by the manufacturer. Infrared spectra were recorded on spectrometer from 4000 cm^{-1} to 400 cm^{-1}. OMNIC software Waltham, MA USA was used to analysis the spectroscopy data.

Data Analysis

The Statistical Package for the Social Sciences software version 12, IBM Corporation NY USA was used for statistical analysis.

The mean values were compared by one-way analysis of variance (ANOVA) multiple range test by the mean values were compared by one-way analysis of variance (ANOVA) multiple range test. Turkey's honestly significant difference procedure was employed to identify the significant groups, if *P*-value in one-way ANOVA is significant. The Mann–Whitey *U* test is used to compare the observations of two samples.

Results

All control SEM specimens showed no alterations [Table 3]. All the specimens in the subgroup B1 irradiated with 100 mJ Er:YAG laser showed chalky appearance in the naked eye and SEM observation at ×200 magnification showed irregular roughness and loss of smear layer [Figure 1 and Table 4]. Specimens treated with 300 mJ of Er:YAG showed irregular sharp-pointed crater ×200 with notch-edged border [Figure 2]. All specimens treated with 400 and 500 mJ of laser energy showed visible charring of the root surface.

Table 3: Group A (control): morphological changes score

Specimen no.	Morphological changes score
S1	1
S2	1
S3	1
S4	1
S5	1

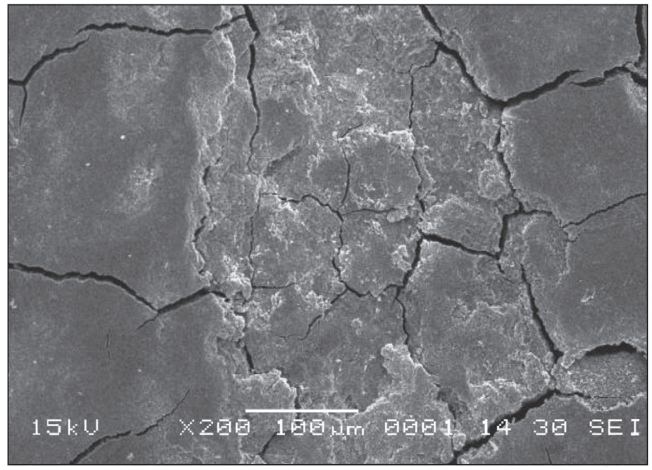

Figure 1: Irregular roughness subgroup (B1) Er:YAG 100 mJ magnification ×200

Table 4: Group B: Er:YAG laser morphological changes score

Specimen no.	B1 100 mJ	B2 200 mJ	B3 300 mJ	B4 400 mJ	B5 500 mJ
S1	3	3	3	6	7
S2	3	3	4	6	7
S3	2	3	5	7	7
S4	3	4	5	7	7
S5	2	3	5	7	7

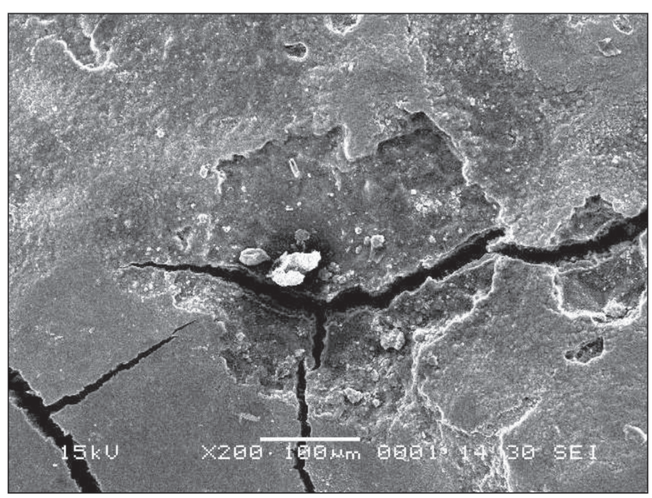

Figure 2: Irregular sharp-pointed craters Er:YAG 300 mJ at magnification ×200

The SEM observation at ×200 showed deep crater, loss of cementum, and visible dentinal tubules orifice. The specimens in the subgroup C1 treated with 211.66 J/cm^2 of Nd:YAG laser showed mild superficial scratch-like alteration [Figure 3 and Table 5]in few samples and absence of smear layer in rest of the samples. The SEM observation of subgroup C2 (423.33 J/cm^2) showed an increased number of scratches [Figure 4] and loss of smear layer and subgroup C3 (635 J/cm^2) revealed irregular roughness in surface.

All the specimens of C4 treated with energy density (846.66 J/cm^2) showed deep craters at × 200 [Figure 5] Higher magnification showed typical melting and re-solidification of mineral.

Root surface specimens treated at 1058.33 J/cm^2 showed visible charring in some areas and deep crater with increased area of resolidified minerals resulting in closing of dentinal tubules in SEM. Peripheral areas of specimens show patent opening of dentinal tubules [Figure 6].

FTIR observation comprised locations, formation of new bands, and change in the height of each peak. Thus, the intensity height of the peak of the major band (OH, Amide I, Amide II, Amide III, and phosphate) was analysed using the spectrometer software.

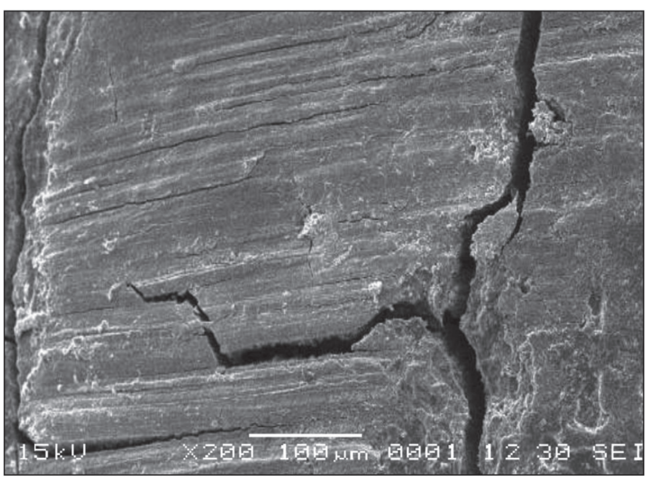

Figure 3: Superficial scratches Nd:YAG 211.66 J/cm^2 at magnification ×200

Discussion

Periodontal disease is characterized by chronic inflammatory lesion and destruction of supportive periodontal tissue. Hence, the primary goal of periodontal therapy strives to remove bacterial deposits and halt the progression of disease with scaling and root planning as an integral part of treating periodontitis.[5] However, complete removal of bacterial deposits and their toxins from the root surfaces, furcations, and

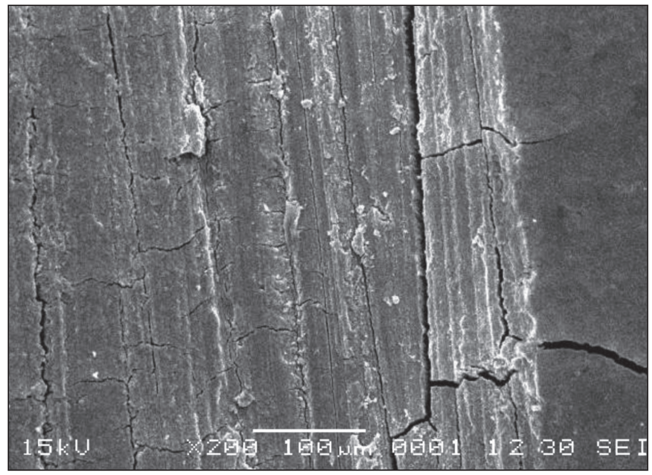

Figure 4: Increased scratches Nd:YAG 423.33 J/cm^2 at magnification ×200

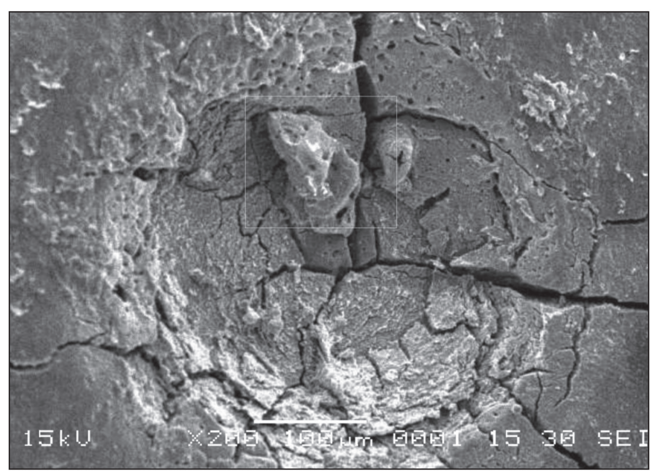

Figure 5: Deep craters with exposed dentin Nd:YAG 1058.33 J/cm^2 at magnification × 200

Table 5: Group C: Nd:YAG laser morphological changes score

Specimen no.	C1 211.66 J/cm ^{2}	C2 423.33 J/cm ^{2}	C3 635 J/cm ^{2}	C4 846.66 J/cm ^{2}	C5 1058.33 J/cm ^{2}
S1	2	2	3	5	7
S2	2	2	3	5	7
S3	2	3	2	6	7
S4	2	3	3	6	7
S5	3	2	3	6	7

within the periodontal pockets cannot be achieved by conventional mechanical therapy while leaving behind smear layer on root-planed surfaces. A smear layer may adversely affect the healing of periodontal tissues as it comprises bacteria and inflammatory substances such as debris of infected cementum and calculus and endotoxins.

Miller *et al.*[6] examined the effects of root conditioning after mechanical debridement, using chemical agents such as tetracycline, citric acid, fibronectin, and ethylenediaminetetraacetic acid. Root conditioning has been shown to remove the smear layer, and to expose collagen fibers and dentinal tubules, thereby enhancing the histocompatibility and new connective tissue attachment with cementogenesis.

Attention has been paid to the clinical applicability of lasers as one of the most promising new technical modalities for nonsurgical periodontal treatment. Aoki *et al.*[14] investigated the effects of various lasers such as argon, CO_2, Nd:YAG,[15,17] and Er:YAG on dental hard tissues. The CO_2 laser (10,600 nm) produces severe thermal damage, melting, and carbonization when applied to hard tissues and hence its use is limited to soft-tissue procedure and not been taken for this study. As Er:YAG laser[7] and Nd:YAG[18] laser achieve excellent hard- and soft-tissue ablation with strong bactericidal and detoxification effects, these lasers have been selected in this study to evaluate the efficacy of smear layer removal and root bio modification.[16,19]

This prospective study compared the efficacy of Nd:YAG and Er:YAG lasers in removing the smear layer as well as analyzed the morphological alterations of root surface using SEM and chemical structural alteration using infrared spectroscopy following different powers of Nd:YAG and Er:YAG laser irradiation. Sample size was determined by statistician and primary outcome of Er:YAG laser with minimal power of 100 mJ per pulse was used because of the available minimal energy density of the laser instrument and the energy level coincides with Gaspirc and Skaleric's[8] and Schoop *et al.*'s[9] study.[18]

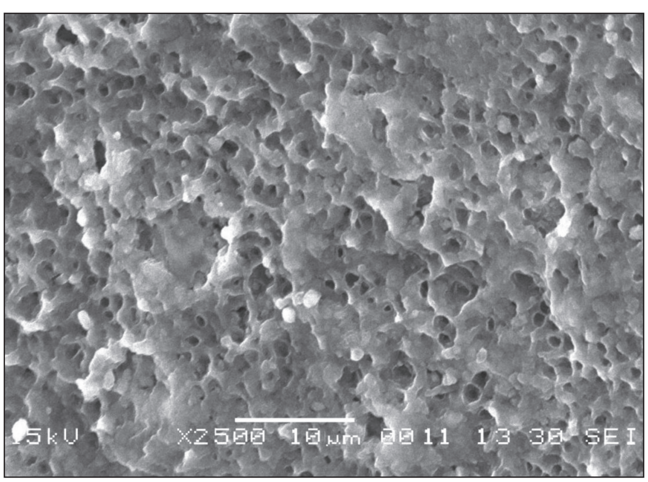

Figure 6: Nd:YAG 1058.33 J/cm² at magnification ×2500 opening of dentinal tubules

In this study, samples treated with Er:YAG laser at lower energy (100 mJ) effectively removed the smear layer [Table 6] with irregular roughness of the cementum, which correlates with the results obtained from the study of Frank.[10] These results can be attributed to its wavelength (2940 nm), which is well absorbed by hard tissues comprising water because the peak is close to the absorption coefficient of water. Hence, Er:YAG laser proves to be efficient in removal of subgingival calculus as well as the superficial layers of contaminated cementum without carbonization of irradiated root surface

The specimens treated with 211.66 and 423.33 J/cm² of Nd:YAG showed mild-to-increased superficial scratch-like alteration and absence of smear layer [Table 7]. These morphological alterations are in line with Wilder-Smith and Arrastia's[11] study. Specimens treated with higher energy density (1058.33 J/cm²) showed both exposure of dentinal tubules in peripheral areas and closure with resolidified mineral in central area, which are in accordance with Koichi *et al.*'s[12] study.

Secondary outcome of this preliminary study indicated that Er:YAG laser at 100 mJ and Nd:YAG[13] laser at

Table 6: Intragroup comparison (B1–B2, B2–B3, B3–B4, and B4–B5)

Subgroup compared	Morphological changes score Mean ± SD	Significance (*P* value)
Er:YAG 100 mJ/cm² (B1)	2.60 ± 0.55	0.469 NS
Er:YAG 200 mJ/cm² (B2)	3.20 ± 0.45	
Er:YAG 200 mJ/cm² (B2)	3.20 ± 0.45	0.023 S
Er:YAG 300 mJ/cm² (B3)	4.40± 0.894	
Er:YAG 300 mJ/cm² (B3)	4.40± 0.894	0.000 HS
Er:YAG 400 mJ/cm² (B4)	6.60 ± 0.55	
Er:YAG 400 mJ/cm² (B4)	6.60 ± 0.55	0.795 NS
Er:YAG 500 mJ/cm_2 (B5)	7.00 ± 0.000	

S = significant (<0.05), NS = not significant (>0.05)

Table 7: Intragroup comparison (C1–C2, C2–C3, C3–C4, and C4–C5)

Subgroup compared	Mean ± SD	Significance (*P* value)
Nd:YAG 211.66 J/cm² (C1)	2.20 ± 0.45	1.000 NS
Nd:YAG 423.33 J/cm² (C2)	2.40 ± 0.547	
Nd:YAG 423.33 J/cm² (C2)	2.40 ± 0.547	0.207 NS
Nd:YAG 635 J/cm² (C3)	2.80 ± 0.45	
Nd:YAG 635 J/cm² (C3)	2.80 ± 0.45	0.000 HS
Nd:YAG 846.66 J/cm² (C4)	5.60 ± 0.55	
Nd:YAG 846.66 J/cm² (C4)	5.60 ± 0.55	0.000 HS
Nd:YAG 1058.33 J/cm² (C5)	7.00 ± 0.00	

S = significant (<0.05), NS = not significant (>0.05)

Table 8: Intergroup comparison Er: YAG vs. Nd: YAG (Groups B1–B5 vs C1–C5)

Group compared	Morphological changes score Mean ± SD	Significance (*P* value)
Er:YAG 100 mJ (B1)	2.60 ± 0.55	0.041 S
Nd:YAG 211.66 J/cm² (C1)	2.20 ± 0.45	
Er:YAG 200 mJ (B2)	3.20 ± 0.45	0.004 S
Nd:YAG 423.33 J/cm² (C2)	2.40 ± 0.547	
Er:YAG 300 mJ (B3)	4.40 ± 0.894	0.018 S
Nd:YAG 635 J/cm² (C3)	2.80 ± 0.45	
Er:YAG 400 mJ (B4)	6.60 ± 0.55	0.031 S
Nd:YAG 846.66 J/cm² (C4)	5.60 ± 0.55	
Er:YAG 500 mJ (B5)	7.00 ± 0.000	1.000 NS
Nd:YAG 1058.33 J/cm² (C5)	7.00 ± 0.000	

S = significant (<0.05), NS = not significant (>0.05)

the energy density of 211.66 J/cm² and 423 .33 J/cm² efficiently removed the smear layer without altering chemical structure of the underlying cementum and dentin [Table 8 and Graph 2]. Removing smear layer by hard-tissue laser with different settings showed a positive pathway for regeneration and removal of smear layer using Nd:YAG is not so significant.

Infrared Spectroscopy

FTIR spectroscopy data of control and all laser-treated samples showed five major bands related to proteins, namely Amides I, II, III, hydroxyl, and phosphate [Table 9]. The location of the bands (wave number cm^{-1}) coincides with Sasaki *et al.*;[22] Gaspirc and Skaleric;[8] and Spencer *et al.*'s[20,21,24] studies.

The samples irradiated with 100 and 200 mJ of Er:YAG laser showed no significant decrease in peak height for organic compounds [Figure 7] (amide and hydroxyl group) and all orthophosphate bands remained same even at higher energy density of 500 mJ, which are similar to the findings of Sasaki *et al.*'s[22] study. The samples treated above 200 mJ showed marked reduction in the amide and hydroxyl groups [Figure 8]. The orthophosphate bands were nearly same in visually charred specimens treated with 400 and 500 mJ. This clearly indicated that Er:YAG laser does not alter inorganic substances of the root.

The specimens treated beyond 846.66 J/cm² of Nd:YAG laser irradiation showed a decrease in peak

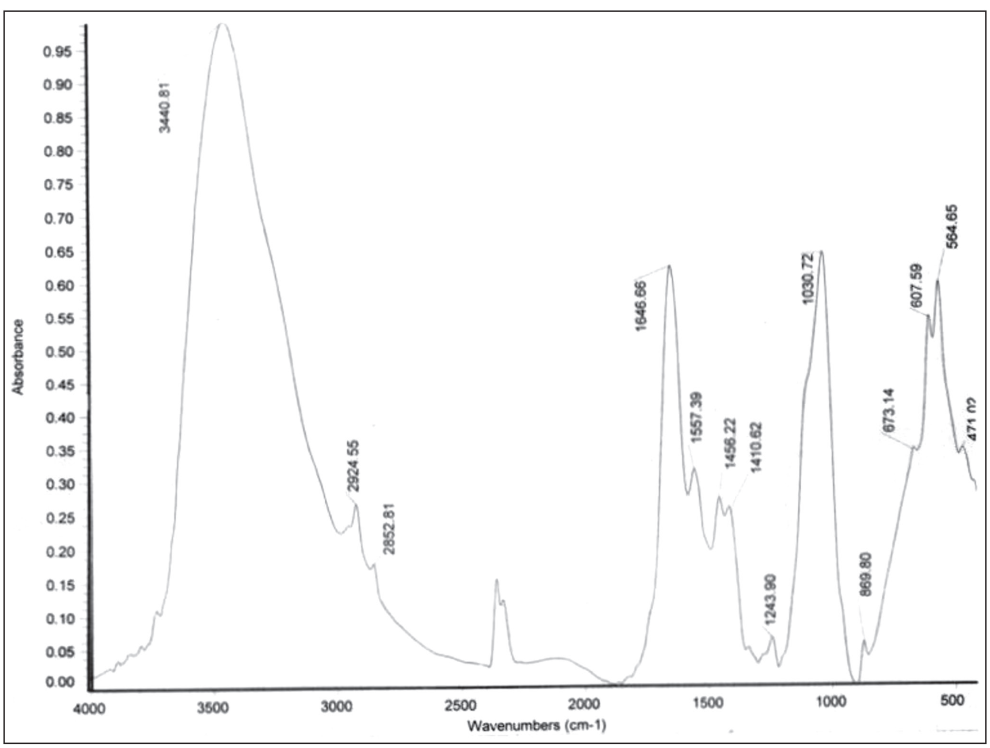

Figure 7: FTIR spectroscopy profile of Er:YAG (200 mJ) laser-treated root

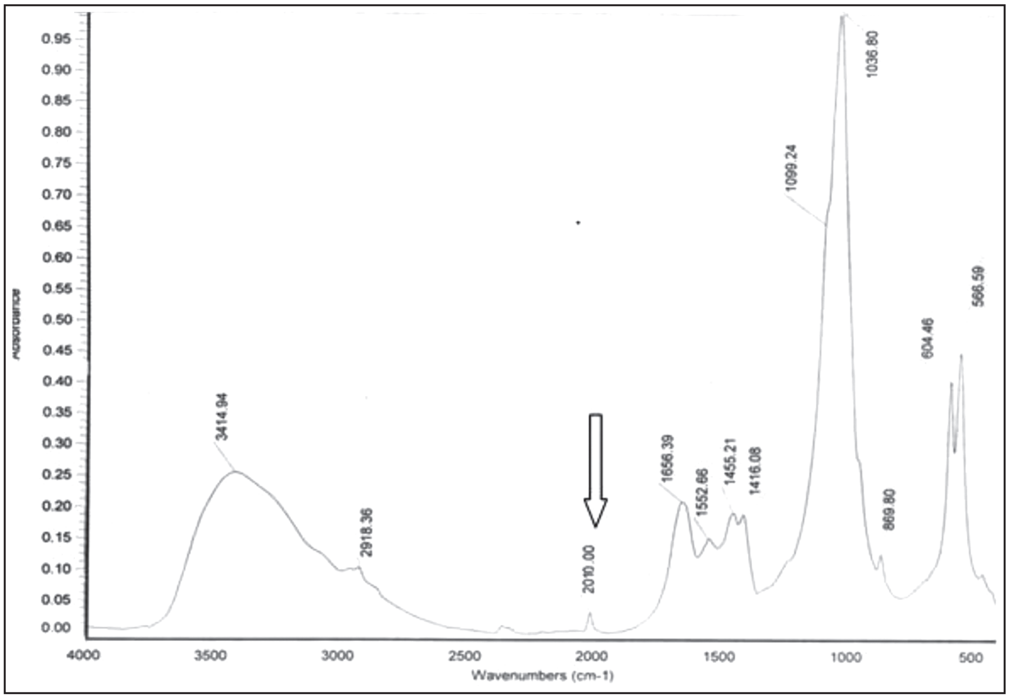

Figure 8: FTIR spectroscopy profile of Nd:YAG (1058.33 J/cm^2) laser-treated root

Table 9: Intergroup comparison—location of the peak (D vs F1–F5)

Variable	Wave number (cm^{-1}) Control –D	Mean (wave number) significance (*P* value)					
	Mean ± SD	Mean ± SD	Nd:YAG 211 J/cm²F1	Nd:YAG 423.33 J/cm²F2	Nd:YAG 635 J/cm²F3	Nd:YAG 846.66 J/cm²F4	Nd:YAG 1058.33 J/cm²F5
Amide I	1647.40 ± 1.14	Mean ± SD	1650.60 ± 3.578	1649 ± 6.245	1649.80 ± 4.086	1647 ± 3.240	1649 ± 4.243
		P value	0.090 NS	0.523 NS	0.435 NS	1.000 NS	0.915 NS
Amide II	1556.40 ± 1.140	Mean ± SD	1555.00 ± 2.828	1556.60 ± 2.510	1553.80 ± 3.347	1555.80 ± 1.095	1555.20 ± 1.924
		P value	0.502 NS	0.585 NS	0.084 NS	0.502 NS	0.329 NS
Amide III	1242.60 ± 1.140	Mean ± SD	1242.20 ± 1.304	1243.80 ± 1.304	1242 ± 1.581	1241 ± 1.414	1242.80 ± 1.304
		P value	0 .590 NS	0.180 NS	0.522 NS	0.081 NS	0.746 NS
Hydroxyl group (OH)	3437.40 ± 8.142	Mean ± SD	3439.20 ± 6.301	3438.80 ± 5.586	3438.20 ± 7.396	3442 ± 1.225	3435.20 ± 11.862
		P value	0.341 NS	0.667 NS	0.456 NS	0.110 NS	0.743 NS
Phosphate group	1030.60 ± 0.894	Mean ± SD	1031 ± 0.707	1031.20 ± 0.837	1032.80 ± 3.033	1034 ± 4.637	1032.20 ± 2.280
		P value	0.371 NS	0.268 NS	0.095 NS	0.053 NS	0.154 NS

height for the inorganic compounds [Figure 8] (amide and hydroxyl).[20,23] New absorption band was noticed (2010 cm^{-1} and 2017 cm^{-1}) [Figure 8] in specimens treated with 846.66 J/cm^2 and 1058.33 J/cm^2 of Nd:YAG laser. The absorption at 2010 cm^{-1} is tentatively indicated to ammonium. The presence of the ammonium band[25] shows the breakdown of protein.

This preliminary study result data showed that Er:YAG laser at 100 mJ [Table 8] and Nd:YAG [Table 8] laser at the energy density of 211.66 J/cm^2 and 423 .33 J/cm^2 removed the smear layer without altering underlying chemical structure of the cementum and dentin.

Conclusion

In conclusion, further *in vivo* studies are to be carried out focusing an increase in sample size with laser instrument capable of generating minimal energy levels with special delivery tips and calibrated device to standardize the angle and constant laser exposure on the sample.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Author contributions

Dr. R. Karthikeyan: Study conception, data collection, data acquisition and analysis. Dr. Pradeep Kumar Yadalam: Data analysis, manuscript writing. Dr. A.J Anand: Manuscript writing and corresponding. Dr. Kamalakannan Padmanabhan: Data analysis, manuscript writing. Dr. G. Sivaram: Data analysis, manuscript writing.

Ethical policy and institutional review board statement

In vitro Study – not applicable.

Patient declaration of consent

In vitro study – not applicable.

Data availability statement

In vitro study – not applicable.

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