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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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/cm² 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/cm² and 423.33 J/cm². The energy density of 1058.33 J/cm² 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/cm² of and new absorption band was noticed (2010 cm⁻¹ and 2017 cm⁻¹) at samples treated with 846.66 and 1058.33 J/cm² 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
therapy has its limitations in removal of toxins from root surface and within the periodontal pockets. Moore et al. and Polson et al. 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 treatment 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: 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 constant focal 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].

**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). 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.

**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/cm²) |
|----------|-------------------------|------------------------|
| 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 2 h and 30 min and washed thrice with phosphate buffer for 10 min each. The specimens were dehydrated in graded series with aqueous ethanol (50%, 70%, 80%, 95%, and 100%) for 10 min 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 sputer coater for SEM viewing using SEM operated at accelerated voltage of 15–20 kV.

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. 3 mg 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.

| 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         |
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/cm² 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/cm²) showed an increased number of scratches [Figure 4] and loss of smear layer and subgroup C3 (635 J/cm²) revealed irregular roughness in surface.

All the specimens of C4 treated with energy density (846.66 J/cm²) 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/cm² 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.

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

| Specimen no. | C1 211.66 J/cm² | C2 423.33 J/cm² | C3 635 J/cm² | C4 846.66 J/cm² | C5 1058.33 J/cm² |
|--------------|----------------|----------------|-------------|----------------|-----------------|
| 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               |

**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. However, complete removal of bacterial deposits and their toxins from the root surfaces, furcations, and
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.\cite{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.\cite{14} investigated the effects of various lasers such as argon, CO₂, Nd:YAG,\cite{15,17} and Er:YAG on dental hard tissues. The CO₂ 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\cite{7} and Nd:YAG\cite{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.\cite{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 Gasparic and Skaleric’s\cite{8} and Schoop et al.’s\cite{9} study.\cite{18}

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.\cite{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\cite{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\cite{12} study.

Secondary outcome of this preliminary study indicated that Er:YAG laser at 100 mJ and Nd:YAG\cite{13} laser at

![Figure 6: Nd:YAG 1058.33 J/cm² at magnification ×2500 opening of dentinal tubules](image-url)

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 | 0.023 S |
| Er:YAG 300 mJ/cm² (B3) | 4.40 ± 0.894 | 0.000 HS |
| Er:YAG 400 mJ/cm² (B4) | 6.60 ± 0.55 | 0.795 NS |
| Er:YAG 500 mJ/cm² (B5) | 7.00 ± 0.000 | |

S = significant (<0.05), NS = not significant (>0.05)
Karthikeyan, et al.: Morphological and chemical alterations of Nd:YAG and Er:YAG treated root surface

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., Gaspirc and Skaleric; and Spencer et al.’s 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 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

| Table 7: Intrigroup comparison (C1–C2, C2–C3, C3–C4, and C4–C5) |
|---------------------------------|-----------------|-----------------|
| Subgroup compared              | Morphological changes score | 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 ± 0.54     | 0.207 NS        |
| Nd:YAG 635 J/cm² (C3)          | 2.80 ± 0.45     |                |
| Nd:YAG 846.66 J/cm² (C4)       | 5.60 ± 0.55     |                |
| 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 | Significance (P value) |
| Er:YAG 100 mJ (B1)           | 2.60 ± 0.35     | 0.041 S         |
| Nd:YAG 211.66 J/cm² (C1)     | 2.20 ± 0.45     | 0.207 NS        |
| Er:YAG 200 mJ (B2)           | 3.20 ± 0.45     | 0.004 S         |
| Nd:YAG 423.33 J/cm² (C2)     | 2.40 ± 0.54     |                |
| Er:YAG 300 mJ (B3)           | 4.40 ± 0.894    |                |
| Nd:YAG 635 J/cm² (C3)        | 2.80 ± 0.45     | 0.018 S         |
| Er:YAG 400 mJ (B4)           | 6.60 ± 0.55     |                |
| Nd:YAG 846.66 J/cm² (C4)     | 5.60 ± 0.55     | 0.031 S         |
| 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)

Figure 7: FTIR spectroscopy profile of Er:YAG (200 mJ) laser-treated root
height for the inorganic compounds [Figure 8] (amide and hydroxyl).

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 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.

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

| Variable      | Wave number (cm$^{-1}$) Control –D | Mean ± SD | Nd:YAG 211 J/cm$^2$F1 | Mean ± SD | Nd:YAG 423.33 J/cm$^2$F2 | Mean ± SD | Nd:YAG 635 J/cm$^2$F3 | Mean ± SD | Nd:YAG 846.66 J/cm$^2$F4 | Mean ± SD | Nd:YAG 1058.33 J/cm$^2$F5 | Mean ± SD |
|---------------|------------------------------------|-----------|-----------------------|-----------|--------------------------|-----------|-----------------------|-----------|--------------------------|-----------|--------------------------|-----------|
| 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 ± 1.14                      | 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 ± 1.14                      | 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 ± 8.14                | 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                 |

## 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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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.

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In vitro study – not applicable.

PATIENT DECLARATION OF CONSENT
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In vitro study – not applicable.

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