

Analysis of Er:YAG Lased Dentin Using Attenuated Total Reflectance Fourier Transform Infrared and X-ray Diffraction Techniques

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The purpose of this study was to investigate the chemical characteristics of dentin after Er:YAG laser irradiation using various output energies with or without water irrigation. Analysis was carried out by means of attenuated total reflectance Fourier transform infrared spectroscopy (FT-IR/ATR) and X-ray diffraction (XRD). Furthermore, the relative infrared peak intensities of dentin specimens were compared statistically. Results showed that Er:YAG laser with an output energy of 100 mJ/pulse with water irrigation did not cause any detectable change in dentin. However, a higher energy output or the absence of water irrigation affected the organic portion of dentin. With XRD, no obvious phase changes were observed between the XRD pattern of the control (non-irradiated) dentin powder and those after Er:YAG irradiation—regardless of Er:YAG laser output energy or dehydration condition. It was suggested that the intrinsic water content of dentin— together with extrinsic water irrigation— were important factors to achieving the desired outcome of dentin ablation by Er:YAG laser.

Keywords: Er:YAG laser, Fourier transform infrared spectroscopy (FT-IR/ATR), X-ray diffraction

INTRODUCTION

Different types of lasers have been applied in dentistry for cavity preparation and caries removal, however, the use of some types of laser like CO₂ and Nd:YAG lasers for cavity preparation has long been plagued by undesired effects, such as damage of hard tissue and necrosis of tooth pulp. These effects are a result of excess heat production during laser treatment¹⁻³.

Erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser— with a wavelength of 2.94 μm — can remove dental caries together with sound enamel and dentin⁴. This laser is efficiently absorbed by the intrinsic water and hydroxy group of apatite mineral in dental hard tissues. The heating expands and builds up pressure until a micro-explosion occurs, and then a small portion of tissue is removed. This phenomenon is called thermally induced mechanical ablation⁵.

Er:YAG laser was found to cause no significant effect on the tensile bond strength of a self-etching system to enamel⁶. Moreover, Er:YAG laser showed less penetration in dentin and had less effects on the ultimate tensile bond strength of dentin when compared to diode laser or CO₂ laser⁷. At this juncture, it must be pointed out that although available literature has reported on various aspects and parameters about Er:YAG laser, it remains to be clarified whether the application of Er:YAG laser is really effective in improving adhesion to bonded restora-

tions⁸.

A previous research work⁹ showed that Er:YAG laser treatment could induce some chemical changes on treated dentin surface, depending on the duration time of Er:YAG laser pulse. It was also suggested that there is a need to develop a new class of restorative materials with optimal interaction properties with laser-treated dentin surface⁹. To develop such restorative materials and to determine the most suitable parameters for Er:YAG laser application on dentin, an extensive research work should be carried out analyzing the chemical compounds and changes in crystalline structure of dentin after Er:YAG laser irradiation.

The attenuated total reflectance Fourier transform infrared (FT-IR/ATR) spectroscopy technique is often applied for the chemical characterization of restorative materials¹⁰⁻¹³ and dentin¹⁴, since it is an effective nondestructive technique. Besides, X-ray diffraction (XRD) is considered as one of the most important tools for investigating the crystalline structure of solid materials¹⁵. The purpose of this study, therefore, was to investigate the chemical characteristics of dentin after Er:YAG laser irradiation, using various output energies and under wet or dehydrated condition, by means of FT-IR/ATR and XRD.

Table 1 Er:YAG irradiation conditions on dentin

Condition	Hydrated specimens				Dehydrated specimens		
	Control	Group I	Group II	Group III	Group IV	Group V	Group VI
Laser output power (mJ)	-	100	200	250	100	200	250
Water irrigation	-	+	+	+	-	-	-

MATERIALS AND METHODS

Dentin tablets preparation

Ten freshly extracted, non-carious, third molars were used in this study. They were hand-scaled, cleaned, and stored in saline solution at 4 °C. Disks of 3 mm thickness were obtained from mid-coronal dentin by cutting parallel to the occlusal surface of the teeth using a water-cooled diamond saw (1600 Microtome, Leitz, Wetzlar, Germany). The enamel of each disk was removed using a plain-cut tungsten carbide fissure bur. Each disk was then cut into two halves: one half was dehydrated in ascending grades of ethanol (25%, 50%, 75% for 20 minutes, 95% for 30 minutes, and 100% for 60 minutes)¹⁶, and then left to dry in a desiccator. The other half was stored in distilled water.

All specimens were crushed and then pulverized into powder using a mortar and pestle. Hydrated and dehydrated dentin powders were set apart and labeled, and then each powder was pressed into a plastic mold under a load of 245 N for 30 seconds to form 15 tablet-shaped specimens of hydrated and dehydrated dentin with a diameter of 10 mm and a thickness of 5 mm. For FTIR/ATR analysis, the hydrated dentin tablets were distributed randomly into Groups I, II, and III, while the dehydrated dentin tablets were distributed randomly into Groups IV, V, and VI, with five tablets in each group.

Laser treatment of dentin tablets

The dentin tablets were subjected to laser treatment using an Er:YAG laser system (Elfine 400, Osada, Tokyo, Japan) with wavelength of 2.94 μm , pulse energy of 10 - 400 mJ, pulse frequency of 1 - 25 pps, and pulse duration of 300 μsec . The contact probe had a sapphire tip of 0.63 mm diameter, and the contact tip was applied perpendicular to the dentin tablets in a sweeping motion with the lased dentin spots overlapping each other. This was to ensure effective application of Er:YAG laser to all parts of each lased dentin tablet. Irradiation conditions of the dentin tablets are summarized in Table 1. Any signs of thermal damage, *i.e.*, carbonization¹⁷ or change of dentin powder color, were recorded by the operator.

FT-IR/ATR analysis

In-order to obtain the Infrared spectra of the Er:YAG irradiated and the non irradiated (control) dentin powders, a small amount of the dentin powder was collected before the Er:YAG irradiation and served as the control. As for the Er:YAG irradiated specimens a very small amount of the remaining irradiated dentin tablet was scraped gently using the tip of a scalpel blade to avoid contaminating the sample with non-irradiated dentin powder particles¹⁸.

Infrared spectra of the control and Er:YAG laser irradiated dentin powders mentioned above were obtained using a FT-IR spectrometer (FTIR-8300, Shimadzu, Kyoto, Japan). This was done by pressing the collected dentin powder onto the face of a diamond of an ATR attachment (DuraSamplIR II Smiths Detection, Danbury, CT, USA). Spectra were obtained under the following conditions: multiple reflections, 650 - 4,000 cm^{-1} range, 4 cm^{-1} resolution, and entrance angle of 45 °.

Spectra of the samples after Er:YAG laser irradiation were checked for appearance of new bands and changes in the height of band peaks. For each group of powder, the intensity peak of amide I that represents one of the organic contents peaks was compared to the carbonate intensity peak which is one of the inorganic components of dentin (*i.e.*, amide I/carbonate intensity ratio), and the carbonate peak will be compared to the phosphate peak intensity which is one of the major inorganic components of dentin (*i.e.*, carbonate/phosphate intensity ratio). These two intensity ratios were observed to identify possible changes that might take place in the chemical structure of dentin after Er:YAG laser irradiation.

XRD analysis

Crystalline phases of dentin powder before (control) and after Er:YAG irradiation were examined using an X-ray diffractometer (RAD IIA, Rigaku Denki, Tokyo, Japan) with CuK α radiation and Ni filter. Scanning range was 20 ° to 50 °, with a scanning speed of 4 ° per minute.

Statistical analysis

A comparison was made among the average intensity ratios of amide I/carbonate and carbonate/phosphate

before and after Er:YAG irradiation. All data were analyzed using one way ANOVA, and Dunnett's t-test was used to compare the control group (non-irradiated dentin) against all the other Er:YAG laser-irradiated groups ($p \leq 0.05$).

RESULTS

FT-IR/ATR analysis

The infrared spectrum of Er:YAG laser-irradiated dentin powder showed no extra peak formation at all irradiation conditions used in this study when compared to the control (non-irradiated) dentin powder. The IR spectrum of the control (non-irradiated dentin) is shown in Fig. 1(a): orthophosphate group peak which is one of the major inorganic components of dentin was observed at $1,000 \text{ cm}^{-1}$ (between $1,030$ and $1,150 \text{ cm}^{-1}$), amide I peak at $1,650 \text{ cm}^{-1}$ (between $1,680$ and $1,600 \text{ cm}^{-1}$), amide II peak at $1,550 \text{ cm}^{-1}$ (between $1,580$ and $1,480 \text{ cm}^{-1}$), amide III peak at $1,250 \text{ cm}^{-1}$ (between $1,300$ and $1,200 \text{ cm}^{-1}$), carbonate peak which is one of the minor inorganic components of dentin was observed at $1,400 \text{ cm}^{-1}$ (between $1,560$ and $1,410 \text{ cm}^{-1}$), and hydroxy group (OH) peak at $3,400 \text{ cm}^{-1}$ (between $3,600$ and $2,400 \text{ cm}^{-1}$). The IR spectra of Er:YAG laser-irradiated hydrated dentin powders of Groups I, II, and III are shown in Fig. 1(b). Amide I peak was well defined in specimens of Group I (Fig. 1(b)), while relative intensity peaks of amide I and II decreased slightly in specimens of Groups II and III (Fig. 1(b)).

Figure 1(c) shows the IR spectra of Er:YAG laser-irradiated dehydrated dentin powders of Groups IV, V, and VI. The relative intensity peak of amide I slightly decreased in Group IV, *versus* a significant decrease in the relative intensity of amide I peak in Group V. Group VI showed a more accentuated decrease in amide I peak, with a severe depletion in the relative intensities of amide II and III. Signs of thermal damage, *i.e.*, carbonization, started to appear on the irradiated dentin powder of Group IV, and increased gradually on the irradiated dentin powders of Groups V and VI.

Ratios of the peak intensities of the different dentin components amide I/carbonate and carbonate/phosphate were compared among the control and test groups to evaluate the effects of Er:YAG laser irradiation conditions on dentin, as shown in Fig. 2. One-way ANOVA and Dunnett's test revealed that there were no statistically significant differences among the ratios of the peak intensity of carbonate/phosphate, although there were some deviations in the observed results which might be attributed to variation in the degree of mineralization of the samples used. However, the ratios of amide I/carbonate for Groups V and VI were significantly lower than that of control.

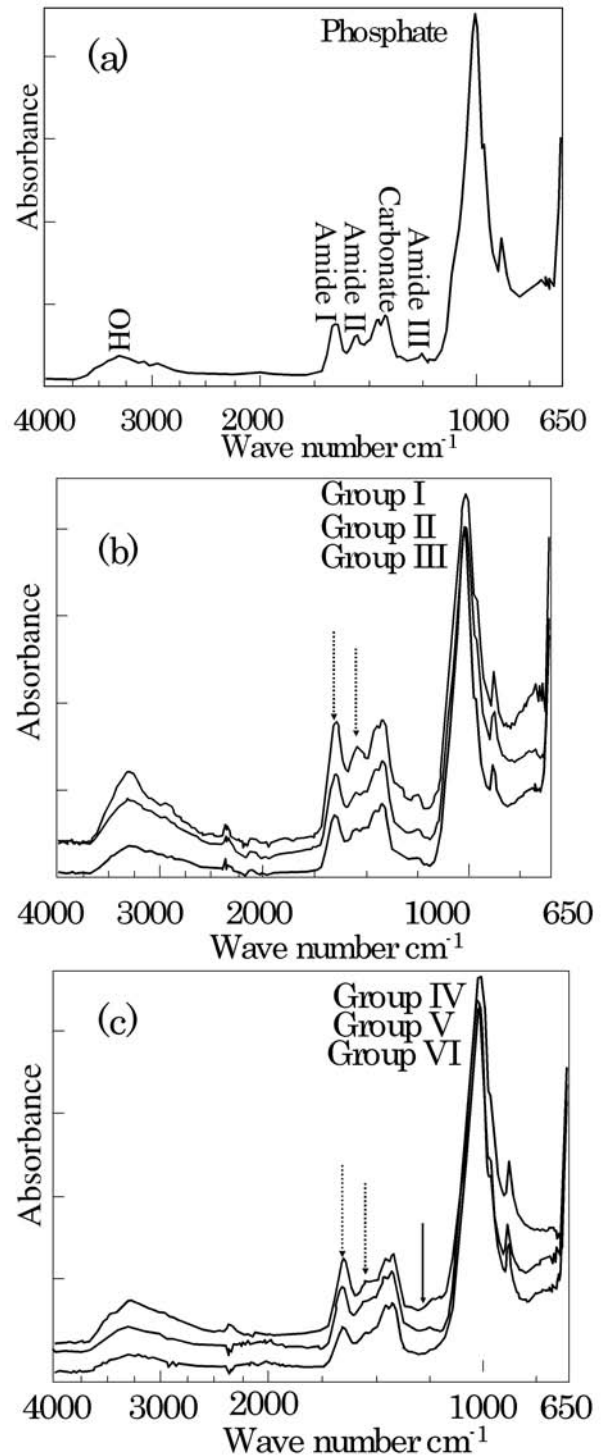


Fig. 1 FTIR spectroscopy profiles of: (a) Control; (b) Groups I, II, and III; (c) Groups IV, V, and VI. Dotted arrows show weakening of the relative intensities of Amide I and II peaks, while solid arrow shows disappearance of Amide III peak especially in Group VI.

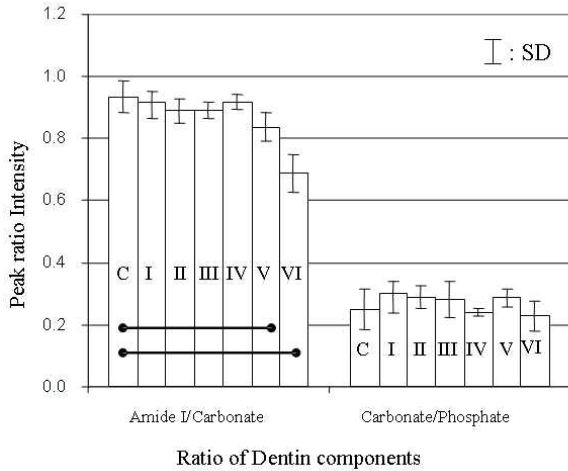


Fig. 2 Peak intensity ratios of Amide I to Carbonate (Amide I/Carbonate) and Carbonate to Phosphate (Carbonate/Phosphate), where: c - Control; I - Group I; II - Group II; III - Group III; IV - Group IV; V - Group V; and VI - Group VI. Connected columns are significantly different statistically ($p < 0.05$).

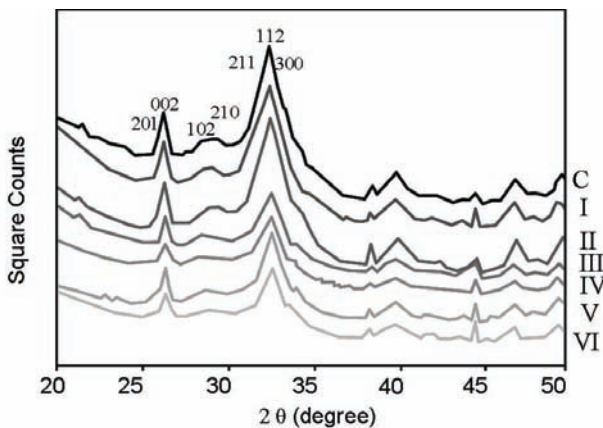


Fig. 3 X-ray diffraction patterns of: c - Control; I - Group I; II - Group II; III - Group III; IV - Group IV; V - Group V; and VI - Group VI.

XRD analysis

Figure 3 shows the XRD patterns of control (non-irradiated) and Er:YAG laser-irradiated dentin powders. Apatite peaks of (002) and (112) lattice planes were located at $2\theta = 25.8^\circ$ and $2\theta = 32.2^\circ$ respectively. After irradiation, there were minor changes in the peaks (38.1° ; 40° ; 44.4° ; 46.5° ; 49.5°), together with a very slight shifting of the (112) peak. However, the XRD patterns of $\text{-Ca}_3(\text{PO}_4)_2$ were not clearly observed among the XRD patterns of all specimens regardless of Er:YAG laser output energy used or dehydration condition.

DISCUSSION

The complementary use of FT-IR/ATR with XRD technique in the present study yielded important

information on the chemical interactions occurring in dentin structure when irradiated with Er:YAG laser. The FT-IR/ATR technique can provide information on the organic and inorganic molecular structures of laser-irradiated hard tooth structure¹⁹, while XRD is the most important tool for characterizing changes in the solid phase¹⁵. With increase in output energy and when water irrigation was not used, intensities of the organic peaks of dentin weakened. However, no obvious phase changes were noticed between the XRD pattern of control dentin powder and those after Er:YAG laser irradiation.

With FT-IR/ATR technique, the infrared beam of the spectrometer penetrates a very short distance beyond the diamond on which the specimen is pressed. This penetrating beam is called the evanescent wave and its penetration depth typically reaches a few micrometers. Since the intensity of the infrared beam is reduced (attenuated) by the tested sample, it is necessary to ensure the close adaptation between sample and diamond ATR of the spectrometer²⁰, which presented a difficulty in the current experiment, since Er:YAG laser specimens possess a highly rough surface. However, since FT-IR/ATR can easily provide the infrared spectra of powdered samples, dentin powder was alternatively tested in this experiment. Against this background, dentin powder was compressed into tablets to prevent the powder from rising up or scattering during Er:YAG laser irradiation. In this manner, it was easy to scrap off the remaining Er:YAG laser-irradiated dentin powder for FT-IR/ATR analysis.

Moreover, it was previously reported that the size and roughness of test particles influenced the peak heights of the infrared spectra²¹, and since it was difficult to standardize the size of test particles in the current study, direct quantitative comparison based on the height of each peak was not reliable. Against this background, a relative comparison between peak ratios was carried out instead.

Infrared spectra of specimens in Group I did not show obvious signs of thermal damage hence there were no statistically significant differences in their peak ratios compared to the control group. These findings could be explained by the fact that the heat induced in the tissue by Er:YAG laser was not high due to the relatively low output energy, coupled with the use of a water-cooling spray in Group I²². These findings agreed with those of Sasaki *et al.*¹⁸, whereby it was reported that the infrared spectra of dentin irradiated by Er:YAG laser using an output energy of 40 mJ/pulse were similar to those of non-irradiated dentin. Further, the results of the present study agreed with those of Shigetani *et al.*²³, whereby laser scanning microscope observation revealed that the appropriate laser output for dentin caries removal was 100 mJ/pulse.

When the output energy of Er:YAG laser was increased, intensity of amide II peak in Groups II and III weakened. These results complemented the micro-Raman spectroscopy findings of a previous study⁹⁾, whereby it was reported that dentin collagen components were modified when Er:YAG laser was used at 350 mJ/pulse coupled with short pulse duration and water irrigation.

For groups without water irrigation, *i.e.*, Groups IV, V, and VI, signs of thermal damage such as carbonization of dentin powder increased gradually with increase in output energy, thereby suggesting an increase in the temperature of dentin powder in these groups. Moreover, the increase of output energy affected the organic dentin portion whereby this effect was observed as a slight decrease of amide I, II, and III peaks. It should be noted that amide I peak might be overlapped by OH peak at 1630 cm⁻¹²⁴⁾. However, control dehydrated samples in this experiment did not show weakening of the peak intensity at 1,650, but rather at 3,400 cm⁻¹. Further, previous research work²⁵⁾ showed that amide III peak of bovine dentin was affected by an increase in the output energy of Er:YAG laser irradiation and with no water irrigation.

Changes detected in the organic components in this experiment might be explained as follows. Absorbed Er:YAG laser radiant energy in the hydroxyapatite crystals of dentin increased because of the absence of water irrigation. As a result, dentin temperature might have been raised higher than 60 ° a temperature at which collagen transforms from highly ordered helices to amorphous gelatin²⁶⁾.

Previous research work²⁷⁾ showed that using of Er:YAG laser with parameters of 30 mJ/pulse and 10 pps the root temperature increased under dry condition to 66.5 °, while that under wet condition remained at 28.6 °. Moreover, it was reported that the calcified collagen molecules in dentin might break down at approximately 175 °, however changes in collagen structure, which might occur with such heating and dehydration, can be reverted to natural conformation after rehydration provided that the surface temperature did not increase beyond 175 °²⁸⁾. The increased temperature together with the dehydrated condition of dentin might have been the cause of the more detectable changes in the organic component of specimens in groups IV, V and VI compared to groups I, II and III, that were using the same output energies with water irrigation.

Statistical analysis of the peak ratios showed a significant decrease of amide I/carbonate peak ratio in Groups V and VI compared to the control (non-irradiated dentin). This decrease was probably not due to the increase of carbonate content, but rather due to the decrease of amide content¹⁸⁾. Moreover,

none of the groups showed a statistically significant difference in peak ratio of carbonate/phosphate compared to the control group. Therefore, it was speculated that heat effect induced by Er:YAG laser in Groups V and VI was high enough to cause a significant decrease in the amide I component of dentin. However, this occurred without affecting the carbonate band which has been reported to show significant loss from dentin at 700 ° and totally vanish from dentin at 1,000 °²⁹⁾.

It has been suggested that correlation between the results of such *in vitro* experiments and the results obtained *in vivo* needs discerning interpretation and discrete comparison. This is because when dentin tissue is irradiated *in vivo*, the maximum temperature rise in the superficial and sub-superficial layers would be lower than that during *in vitro* irradiation. This lower temperature arises from the large thermal dissipation that occurs in the tissue when the tooth is whole and inserted in the oral cavity. By inference, the effects observed in this work which were a function of the temperature produced by laser irradiation would happen in the same form but with a lower intensity *in vivo*, assuming that a major thermal dissipation occurs *in vivo*²⁵⁾.

X-ray diffraction analysis was used in this study to detect any phase change in the crystalline structure of dentin. The selected range of observation was from 20 ° to 50 °, and which included the strong peaks of 002, 211, 112, 300, and 202, as well as the weaker 201, 102, and 210 reflections of hydroxyapatite crystals. However, due to the poorly crystalline structure of dentin apatite, only the peaks of 002 and 211 were clearly identified and used to compare the phase changes of dentin before and after Er:YAG irradiation¹⁵⁾. There were minor changes in peaks (38.1 °, 40 °, 44.4 °, 46.5 °, 49.5 °) after irradiation, together with a very slight shifting of (112) peak. These changes might be attributed to the rough Er:YAG laser surface of the samples, which might have caused such minor changes in peak positions.

However, there was no clear change in the crystalline structure of dentin after Er:YAG irradiation in any group. These results were in agreement with the IR results that no detectable change in inorganic peak ratio was found for any group. Carbonated hydroxyapatite in dentin changes into the less soluble hydroxyapatite at 350 °. It recrystallizes to -Ca₃(PO₄) from 650 ° to 1,100 °, and if heated above 1,100 ° recrystallization into -Ca₃(PO₄) will occur^{30,31)}. In the present study, the peaks of -Ca₃(PO₄) were not clearly observed. Therefore, it could be suggested that the temperature of Er:YAG laser did not reach 650 °. As a result, the crystalline structure of dentin was stable in all the test groups.

CONCLUSIONS

Within the limitations of this *in vitro* study, it was concluded that the intrinsic water content of dentin together with extrinsic water irrigation played an important role in achieving the desired outcome of Er:YAG ablation to coronal dentin during conservative tooth treatment. The laser output of 100 mJ/pulse with water irrigation did not cause any detectable chemical change on the remaining irradiated dentin. On this score, these irradiations conditions of 100 mJ/pulse laser output with water irrigation were recommended to be used for dentin ablation by Er:YAG laser.

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