Mid-Infrared Spectroscopy Analysis of the Effects of Erbium, Chromium:Yattrium-Scandium-Gallium-Garnet (Er,Cr:YSGG) Laser Irradiation on Bone Mineral and Organic Components

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The effects of varying the energy density of a high-intensity erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser on the mineral and organic components of bone tissue were evaluated using Fourier transform infrared spectroscopy. Bone samples obtained from the tibias of rabbits were irradiated with five energy densities (3, 6, 8, 12, and 15 J/cm^2), and the effects on the carbonate to phosphate ratio and in the organic components were compared with those of nonirradiated samples. The increased temperature during the laser irradiation was also measured using infrared thermography to relate the observed spectral changes to the laser thermal effects. The analyses of the infrared spectra suggests that the irradiation with Er,Cr:YSGG promoted changes in bone tissue in both the mineral and organic components that depend on the laser energy density, pointing to the importance of using the proper energy density in clinical procedures.

Index Headings: **Fourier transform infrared spectroscopy; FT-IR; Mineralized tissue; Bone; Temperature; Erbium, chromium: yttrium-scandium-gallium-garnet laser; Er,Cr:YSGG laser.**

INTRODUCTION

The use of lasers in clinical procedures has increased since its first applications with the development of the ruby laser by Maiman in 1960.[1] Today laser-based instruments are used in a wide variety of specialties, such as dermatology, ophthalmology, and dentistry.[2–4] In dentistry, infrared erbium lasers, such as the erbium-doped yttrium aluminum garnet (Er:YAG) laser (2.94 μm) and erbium, chromium: yattrium-scandium-gallium-garnet (Er,Cr:YSGG) laser (2.78 μm) stand out, due to the higher absorption of their wavelength by the main components of the mineralized tissues. When we compare the two erbium lasers, the Er,Cr:YSGG laser has the advantage of providing clean and precise cuts due to the strong laser energy interaction with O–H bonds of both water and hydroxyapatite.[5] For this reason, this wavelength can be used in several clinical procedures, such as caries removal, hard tissue cutting, and osteotomy.[6]

There is a strong interest in using these lasers for bone cutting because they present advantages, such as better homeostasis and precision, in material removal compared to commonly used tools.[7] However, to assure a safe and efficient laser application, avoiding unnecessary thermal damage, it is necessary to know the chemical effects of the laser irradiation on tissues. Especially, knowledge about how the laser energy density affects the tissue is essential for a safe procedure because the use of inappropriate parameters can cause morphological and chemical damage that will affect tissue healing.[8]

Mid-infrared spectroscopy is a technique by which the chemical structure of an analyzed material can be identified; it also allows the semi-quantitative analysis of the components of the material.[9] It has been already shown that Fourier transform infrared (FT-IR) spectroscopy can be used to evaluate the effects of high-intensity laser irradiation on dentin and enamel tissues,[10–12] and the technique has also been applied to the study of bone properties[13] and pathologies.[14,15] Nevertheless, there are only a few studies in which bone tissues have been characterized after laser irradiation.[8,16] The available studies did not made a deep analysis of the FT-IR spectra obtained; furthermore, they evaluated only one single irradiation condition. Therefore, the chemical effects of Er,Cr:YSGG laser irradiation on bone tissue at different energy densities are still unknown.

The aim of this study is to use FT-IR spectrometry to evaluate the effects of different energy densities of the Er,Cr:YSGG laser on the mineral and the organic components of bone tissue and relate them to the temperature increase promoted by laser irradiation.

MATERIAL AND METHODS

Sample Preparation. This study was approved by the Animal Ethics Committee of the Instituto de Pesquisas Energéticas e Nucleares (IPEN, 6/CEPA-IPEN/SP). For this study, 43 bone slabs ($8 \times 5 \times 0.1$ mm) were obtained from the tibias of adult male New Zealand rabbits (age 10 months) using a high-speed dental drill. After cleaning the bone slabs with deionized water in ultrasound for 10 min, we polished the surfaces of the bone slabs with silicon carbide abrasive paper (#1200; Buehler) to obtain a parallel and planar surface. After that, the samples were kept in a +3 °C environment with a relative humidity of 90% until the beginning of the

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TABLE I. Experimental groups used in the present study.

Group	Laser energy density (J/cm^2)	Mean power (W)	Mean energy per pulse (mJ)
1	3	0.25	10
2	6	0.50	20
3	8	0.75	26
4	12	1.00	40
5	15	1.25	50

experiments. We used 18 samples for the FT-IR spectroscopy analysis and the remaining 25 slabs for the temperature analysis during irradiations.

Sample Irradiation and Experimental Groups. The irradiation was performed using an Er,Cr:YSGG laser (Waterlase; Biolase, Inc.), which emits at a wavelength of 2.78 μm; the repetition rate was 20 Hz, and the pulse width was approximately 140 μs. A sapphire S75 tip (Biolase, Inc.) with a length of 400 μm and diameter of 750 μm was used without air or water coolant. During the irradiation, the samples were positioned 1 mm away from the laser tip and were moved using a high-precision motorized translator (ESP300; Newport Corporation) adjusted to a speed of 13 mm/s. The distance between each irradiation line was 600 μm to ensure uniform irradiation.

For the temperature measurements, five experimental groups were defined ($n = 5$) according to the laser energy density used, described in Table I. The calculation of the energy density was performed for the area of the spot size on sample surface of 0.376 mm at a $1/e^2$ intensity level. For this analysis, only the edge of the samples was irradiated to analyze the maximum temperature generated during each pulse.

For the FT-IR measurements, we used the same five experimental groups presented in Table I ($n = 3$); we also included an unlasered control group ($n = 3$) for comparative analysis. For this analysis, the entire surface of the samples was irradiated.

Temperature Analysis. To relate the changes we observed in the spectroscopy analysis to the temperature increase, we measured the temperature of each of the experimental groups in Table I during laser irradiation ($n = 5$).

The temperature analyses were performed using a thermographic camera (ThermaCam FLIR SC3000 Systems). The infrared images were recorded at rates of 300 Hz. The temperature in the laboratory remained constant at 20 °C throughout the entire experiment. For the thermal camera adjustment, the emissivity of the bone was considered to be 0.91. The images were acquired and analyzed using the manufacturer software (ThermaCAM Research; FLIR System).

For the images acquisition, the samples were positioned in the camera focus (at 0.1 m away from the camera). Laser irradiation was positioned perpendicularly to the sample of the bone surface, tangentially to the lateral plane of sample, to analyze the temperature increase on the surface and depth of the sample. To assure irradiation at only the edge of each sample, the position of the laser tip was fixed and the samples were moved under the laser beam. On the infrared image, we determined the maximum temperature for each sample, and the data were compared using an analysis of variance (ANOVA) and Tukey's test, at 5% significance level.

Fourier Transform Infrared Spectrometry. A Nicolet 6700 FT-IR spectrometer (ThermoFisher) with a Smart Orbit Attenuated Total Reflection (ATR) accessory (ThermoFisher) equipped with a diamond crystal as the internal reflection element was used. The ATR accessory used in the study has an incident angle of 42°, and the diamond crystal area is 2.25 mm^2. The detector we used was a deuterated triglycine sulfate pyroelectric bolometer.

The region between 4000 and 580 cm^{-1}, which corresponds to the absorption bands of the most of the organic and inorganic compounds in bone tissue, was analyzed.[9] It was acquired with a single spectrum per specimen, and each spectrum was collected as an average of 80 scans at a resolution of 4 cm^{-1}. Each experimental group contained three specimens; therefore, in the study for the six experimental groups for the spectral analyses (the five groups in Table I plus the control group), we collected a total of 18 spectra.

Sample Spectral Analysis. The spectral analysis was performed immediately after laser irradiation to avoid the rehydration of the samples.[17] Each spectrum was analyzed individually; as part of the analysis, we calculated the ratio of the area of the carbonate band (between 850 and 890 cm^{-1}) to the area of phosphate band (900–1200 cm^{-1}) (e.g., the carbonate to phosphate proportion). We also calculated the ratio between the band areas of amide I (1585–1720 cm^{-1}) and phosphate (900–1200 cm^{-1}), between the band areas of amide II (1500–1585 cm^{-1}) and phosphate, and between the band areas of amide III (1210–1280 cm^{-1}) and phosphate, which are related to the proportions of collagen and hydroxyapatite. The full width at half-maximum (FWHM) of both phosphate bands (around 1010 cm^{-1} and around 600 cm^{-1}) were also evaluated. These results were calculated using OriginPro 8.0 software (Origin Lab Corporation, Northampton, MA).

We analyzed the data obtained statistically using ANOVA and Tukey's test at 5% significance level and power analysis of 80%. The statistical analysis was performed considering the undamaged group (the 0 J/cm^2 group) as the reference because this group had no compositional changes during the experiments.

RESULTS AND DISCUSSION

The use of infrared spectroscopy to characterize biological material has increased in the last 20 years.[18] The reasons for its popularity are the large number of organic and inorganic components that have absorption bands in the infrared region and the relatively easy preparation that the samples require.[9] Especially for the mineralized tissues, the attenuated total internal reflection (ATR) technique is particularly useful because the sample thickness is not a concern, as it is in other methods.[19] However, the ATR technique can be used to analyze only a few micrometers in depth, so the information obtained is just about the surface of the sample.[18]

TABLE II. Infrared bands of the untreated bone tissue.

Component	Band position (cm^{-1})
Water (H_2O) adsorbed (antisymmetric stretch)	3450
H_2O or amide A	3300
H_2O ($\nu_1 + 2\nu_2$)	3212
Amide B	3083
C–H (stretch)	2950
	2853
Amide I (C=O stretch)	1642
H_2O adsorbed (H–O–H bending ν_2)	
Amide II	1553
Carbonate (antisymmetric stretch $\nu_{3)}$	1409
	1445
Amide III	1240
Phosphate (antisymmetric stretch $\nu_{3)}$	1003
	1100
Carbonate (antisymmetric deformation ν_2)	871
Phosphate (symmetric stretch $\nu_{1)}$	957
Structural OH (libration)	676
Phosphate(antisymmetric deformation ν_4)	595

The lower penetration analysis possible using the ATR technique was an important point considered in this study, especially in the analysis of the irradiated samples. Under certain conditions, the laser irradiation promoted the removal of bone material, causing a roughening of the sample surfaces. This change in the samples surface might affect the contact between the samples and the ATR crystal, resulting in spectra with a lower intensity and more noise than in the nonirradiated group.

The effect of irradiation is not exactly the same in all sample surfaces because it also depends on energy profile of the laser beam. Thus, in the ATR analyses, we observed the average effect caused by laser irradiation in the sample.

Table II reports the positions of the infrared bands observed in the untreated bone samples. The bands were found at positions close to the ones reported in literature;[20–22] the small differences observed were expected because the band positions in ATR spectra differ from those in transmission spectra due to the effect of anomalous dispersion.[18] For this reason, when infrared bands found in different studies are compared, small differences in their positions are considered acceptable.

Bone tissue is composed of an inorganic matrix mainly of carbonated hydroxyapatite and an organic matrix mainly of collagen type I.[23] This composition is very similar to other mineralized tissues of the human body, such as dentin and enamel, with differences in the proportion of the matrices: while bone has 25 wt% organic matrix, dentin and enamel have 20 and 1.5 wt%, respectively.[24] Despite this difference, it is possible to find a spectral similarity among the infrared bands of the three materials that makes the comparisons of these tissues possible.

The Er,Cr:YSGG laser is an infrared pulsed laser developed for dental applications. Using this laser, it is possible to remove enamel, dentin, bone, and carious tissues with minimal amounts of thermal disruption to the residual tissue when the correct energy density has been adjusted for each biological condition to be treated. The effects of laser irradiation on biological tissues depend on these adjusted parameters, such as energy density, repetition rate, mean power, and the presence (or absence) of an air–water spray to refrigerate the tissue during the procedure.[25] The removal of material using erbium lasers occurs by thermal ablation; in this process, the absorption of laser irradiation by firmly bounded water molecules in the hard tissue leads to an increase in temperature that promotes their micro-explosions. During the explosion of the water, heat and the irradiated material are expelled, causing tissue removal.[26] The ablation process occurs due to the high absorption of the Er,Cr:YSGG laser wavelength by the main components of the mineralized tissues:[26] the hydroxyl (OH^-) groups in hydroxyapatite strongly absorb at wavelength of 2.78 μm (~3600 cm^{-1}) and the absorption coefficient of water reaches 5160 cm^{-1} at this wavelength.[27,28]

To ablate any material using laser irradiation, the energy density must be adjusted so that it is above the ablation threshold.[29] Therefore, in a clinical application it is important to know the ablation threshold to assure effective material removal and to minimize the thermal damage that can be promoted by the generation and propagation of heat in the tissue.[16] The literature has shown that the use of energy densities below the ablation threshold can cause thermal damage, such as micro-cracks in the surface, which can be unacceptable in a clinical application.[30] The ablation threshold for Er,Cr:YSGG laser is between 2.69 and 3.66 J/cm^2 in dentin.[29] Because bone has a higher content of organic components (25%) than dentin (20%), we assume that a lower energy density is needed to promote the ablation of bone. Therefore, considering the composition of bone and the results obtained in this study, we expect that the ablation threshold of the bone is below 3.6 J/cm^2; however, further studies are necessary to determine the actual value.

It has been reported in the literature that bone tissue heated to 50 °C for 1 min or to 47 °C for 5 min lost its functionality.[31] Therefore, an increase in temperature might promote irreversible damage to biological tissues, and for this reason, it is important that the laser parameters be adjusted correctly to control for increases in temperature.

To better understand the changes promoted by Er,Cr:YSGG laser irradiation in bone tissues, we measured the maximum increased temperature for each irradiation condition used in this study (Fig. 1). We observed that irradiation with 3 J/cm^2 promoted an increase in temperature to 96 ± 12 °C and with 6 J/cm^2 an increase in temperature to 213 ± 17 °C. The samples irradiated with 8, 12, and 15 J/cm^2 reached temperatures of 301 ± 23, 284 ± 17, and 303 ± 7 °C, respectively.

For energy densities ranging from 3 to 8 J/cm^2, it is therefore possible to suggest a positive relation between an increase in the energy density and the increased temperature due to laser irradiation; above 8 J/cm^2, this positive relation was not observed. Because the thermographic camera measures the average increased temperature, it is not possible to ensure that the

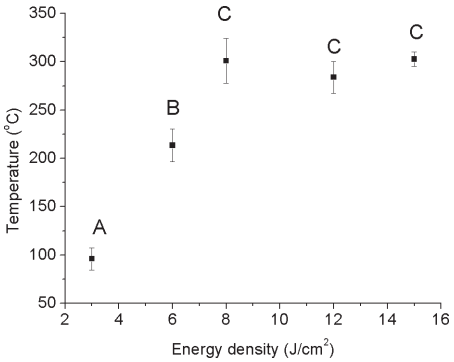

FIG. 1. The maximum temperature increase caused by the five irradiation energy densities used in this study. Bars denote standard deviations. Letters indicate statistically significant differences according to the Tukey test.

recorded temperature is the maximum that the laser could promote.

Of the parameters adjusted in this study, the energy density of 3 J/cm^2 was the only one that did not promote the removal of material. All the other parameters we tested caused ablation of the bone tissue, and the reason that there was some removal of material must be considered. We suspect that the removed material reached higher temperature levels than the surface of the samples because the temperature increase promoted micro-explosions. These explosions release thermal energy and may explain why the relation between the sample temperature and the laser energy changes for energy densities above 8 J/cm^2.

It is important to note that the increased temperatures are related to the irradiation condition used and that any alteration in this condition might result in changes in these temperature values. Kilinc et al.[32] observed an increase of almost 9 °C when enamel surfaces were irradiated using the Er,Cr:YSGG laser with 15.5 J/cm^2 and using air and water coolants; to compare, Fried et al.[33] reported a temperature of 800 °C in enamel surfaces when irradiating them using a Er,Cr:YSGG laser at 18 J/cm^2 without using an air or water coolant.

Another important point to be considered is that the irradiations were performed in a single pulse, without using an air–water spray, so a direct comparison of the observed results with clinical applications of the Er,Cr:YSGG laser is not possible. Nevertheless, the results represent an extreme condition of the laser, and its effects might indicate a secure threshold for a future clinical application.

Figure 2 shows three ranges of infrared spectra of the experimental groups in this study: 3800–2650 cm^{-1}, 1750–1175 cm^{-1}, and 1175–560 cm^{-1}. In all of them, it is possible to identify the same bands observed in the spectra of the nonirradiated samples. No significant differences in the positions of bands were observed, indicating that Er,Cr:YSGG laser irradiation did not promote a total degeneration of the bone components when used under the conditions of this study. Similar results were reported using the Er,Cr:YSGG laser to irradiate enamel, at energy densities similar to those used in the present study, using FT-IR and FT-Raman techniques.[10,12,34] However, the intensities of these bands do vary according to the energy density applied, and the intensities of all the bands are decreased compared to the nonirradiated bone, suggesting that laser irradiation can affect the structure of bone in a direct relation to the energy density. This behavior has been confirmed by previous studies in which laser irradiation was shown to affect the structure of enamel and dentin depending on the energy density used.[12,35]

To evaluate the effects of Er,Cr:YSGG laser irradiation on the inorganic components of bone tissue, we calculated the carbonate to phosphate ratio because carbonate radicals can substitute for OH^- and hydrogen phosphate (HPO_4^{2-}) ions linked to the hydroxyapatite molecule[36] and this ratio can give us an idea of the changes promoted in the inorganic material.[37,38] For the analysis of the area under the infrared bands, the phosphate band is commonly used to normalize the FT-IR spectra[39,40] because phosphate is the component of hard tissues that is most stable with increases in temperature.

The carbonate to phosphate ratio shows a decreasing trend with energy density (Fig. 3); however, there is no associated change in the mineral crystallinity. Although no statistical differences were observed among the groups, all samples irradiated with energy densities equal to or higher than 6 J/cm^2 presented a tendency to have a smaller carbonate to phosphate ratio than the control group; this suggests that Er,Cr:YSGG laser irradiation with energy densities higher than 3 J/cm^2 could affect the carbonate to phosphate ratio. The group irradiated at 3 J/cm^2 did not present this trend. The literature has reported that the reduction of the carbonate content in enamel and dentin starts at temperatures

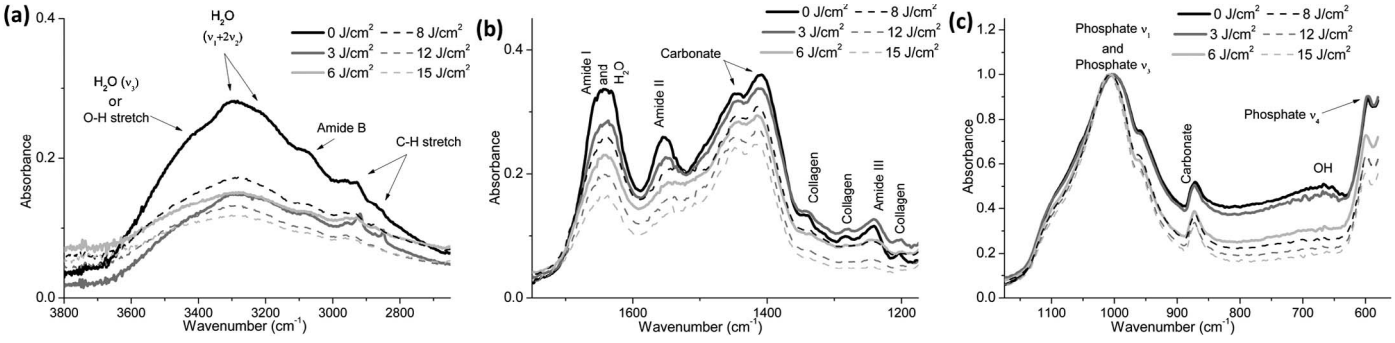

FIG. 2. Mean infrared spectra of samples from the six groups analyzed in three ranges. (**a**) Range 3800–2650 cm^{-1}. (**b**) Range 1810–1220 cm^{-1}. (**c**) Range 1180–550 cm^{-1}.

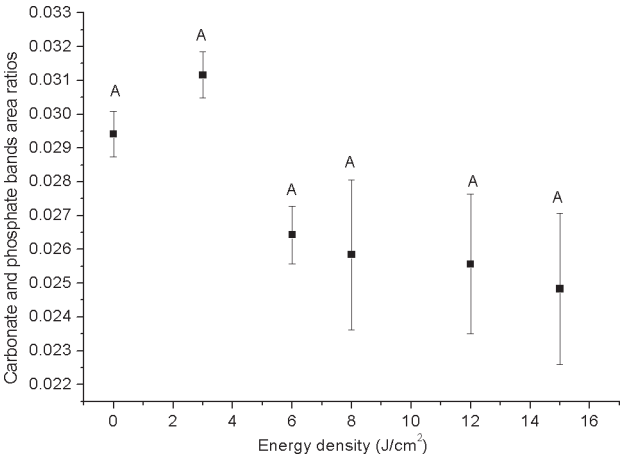

FIG. 3. Means of the carbonate to phosphate ratios obtained from the bone samples irradiated with different laser energy densities. Bars denote standard deviations. Letters indicate statistically significant differences according to the Tukey test.

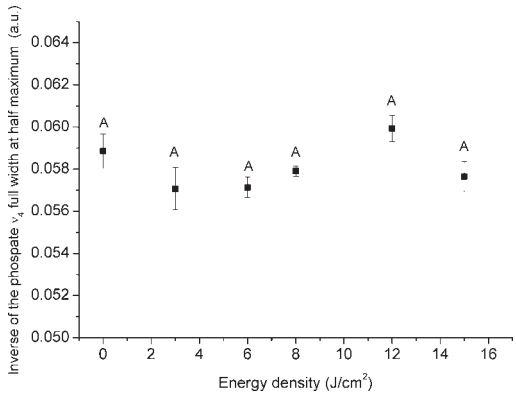

FIG. 4. Inverse of the FWHM mean values of the ν_4 phosphate band (around 600 cm^{-1}) obtained from the bone samples irradiated with different laser energy densities. Bars denote standard deviations. Letters indicate statistically significant differences according to the Tukey test.

above 100 °C.[40] In bone, it was reported that carbon dioxide is lost in the temperature range 200–600 °C when heated in a muffle furnace.[41] The observed trend is in line with the temperature measurements because it was observed in temperature above 200 °C even when energy densities higher than 3 J/cm^2 were used. The temperature of the samples irradiated at 3 J/cm^2 was nearly 100 °C, which is probably not high enough to promote carbonate reduction.

Literature studies[10,12] show that irradiating enamel with a Er,Cr:YSGG laser at energy densities between 7.5 and 14 J/cm^2 causes a decrease in the carbonate ratio of enamel. The removal of carbonate results in a decrease on the carbonate to phosphate ratio, which agrees with the trend of the findings of the present study. However, for bone tissue, a previous study[41] observed an increase of the phosphate band intensity when bone samples were submitted to temperatures of 850 °C in a muffle furnace for 1 h. Based on the results of our temperature analysis, the irradiation of the samples under the conditions we used did not cause temperatures higher than 350 °C; therefore, we can consider that the decreasing trend in the carbonate to phosphate ratio observed in the present study to be an effect of the reduction of carbonate due to the increased temperature promoted by laser irradiation.

For a better understanding of the changes promoted by laser irradiation in the mineral content of the bone samples, we calculated the FWHM values of the phosphate band located around 600 cm^{-1}. Other studies[42] have shown that this parameter is inversely related to the crystallinity of the mineralized tissues. The FWHM mean values of the ν_4 phosphate band (around 600 cm^{-1}) obtained from the bone samples irradiated at different energy densities are shown in Fig. 4. No statistically significant difference was observed in the results, suggesting that the energy density values used in this study were not high enough to cause changes in the crystallinity of the bone samples.

Literature studies have reported that the thermal effect produced by the Er,Cr:YSGG laser irradiation can interfere with the crystalline structure of mineralized tissues,[12,43] such as changing the lattice parameters of hydroxyapatite or causing the formation of new compounds, such as tricalcium phosphate and tetracalcium phosphate.[43,44] Changes in the crystalline structure of mineralized tissues caused by laser irradiation are dependent on the increase in the surface temperature. Studies of laser-irradiated or oven-heated enamel and dentin showed that new crystallographic phases are formed around 800 °C and that α-tricalcium phosphate and tetracalcium phosphate are formed near 1100 °C.[45–47] For bone tissue, the formation of new phosphate phases starts at temperatures greater than 775 °C[48] in a way similar to enamel or dentin. Based on the reported temperature values and the measured temperature values, it seems that the laser irradiation at the energy densities studied here are not high enough to promote crystalline changes in bone. In this way, the results of FT-IR analysis are in line with the observed increased temperature.

To explore other effects of Er,Cr:YSGG laser irradiation on the mineral content of the bone samples, we calculated the FWHM values of the phosphate band (around 1010 cm^{-1}) for the irradiated bone samples. Studies using Raman spectroscopy reported that a decreasing bandwidth of the ν_1 mode of the phosphate band at 960 cm^{-1} indicates the higher crystallinity of bone.[49] In the infrared spectrum, the ν_1 mode of the phosphate band overlaps the ν_3 phosphate (most intense vibration mode) and other vibration modes, which are related with nonstoichiometric, poorly crystalline apatite. Because of this mode overlap, during the analysis of this band we were not able to dissociate the influence of crystalline size from the lattice imperfections,[42] and we could not directly relate the FWHM of the phosphate band around 1010 cm^{-1} to the crystallinity or maturity of the bone samples. However, considering that the infrared peak widths are related to intermolecular interaction,[50] the changes observed in FWHM of the this phosphate band could be related to changes in the atomic order of the hydroxyapatite.[51]

The FWHM mean values of the phosphate band around 1010 cm^{-1} are shown in Fig. 5. We observe that the FWHM of the phosphate band is statistically lower in all

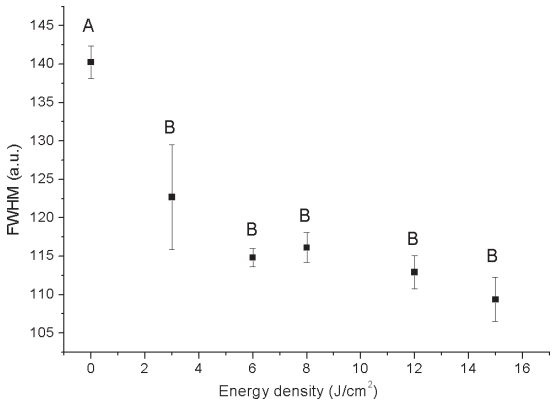

FIG. 5. Means of the FWHM of phosphate band (around 1010 cm^{-1}) width obtained from the bone samples irradiated with different laser energy densities. Bars denote standard deviations. Letters indicate statistically significant differences according to the Tukey test.

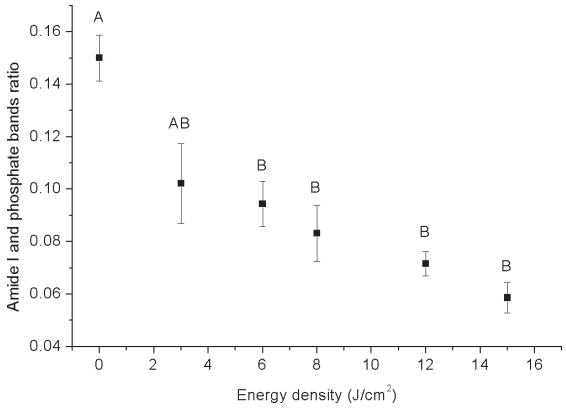

FIG. 6. Means of the amide I to phosphate ratios obtained from the bone samples irradiated with different laser energy densities. Bars denote standard deviations. Letters indicate significant statistical differences according to the Tukey test.

irradiated groups compared to the untreated group, which suggests a change in the mineral structure of the bone samples after laser irradiation that is not related to crystallinity. Additional studies are necessary to understand the exact changes in the mineral content of bone due to laser irradiation.

For the bone inorganic matrix, the obtained results suggest that the Er,Cr:YSGG laser irradiation under these conditions promoted changes in the carbonate content and seems to affect the intermolecular interaction of the bone mineral part. However, it was not possible to detect changes directly related to the crystallinity of the sample. These results agree with the increased temperature observed on samples during irradiation, showing that the FT-IR spectroscopy can be used to detect the thermal effects promoted by Er,Cr:YSGG laser irradiation on the mineral matrix of bone samples.

To analyze the effects promoted by Er,Cr:YSGG laser irradiation on the organic matrix of bone tissue, we calculated the ratio between the area under the bands of phosphate (900–1200 cm^{-1}) and amide I (1585–1720 cm^{-1}) because literature studies have shown that this ratio is related to the proportion of collagen and hydroxyapatite.[23] Figure 6 shows the average ratios of the amide I and phosphate band areas obtained from the bone samples irradiated at different laser energy densities. We can observe a significant decrease in the amide I band area with the increase of the laser energy density used, indicating the degradation of the organic matrix.

In the infrared spectroscopy, one vibration mode of water band at 1640 cm^{-1} overlaps with the amide I band,[52] which can cause a misinterpretation if this band is the only one used to analyze the effects of the laser in organic components. The ablation process promoted by the Er,Cr:YSGG laser occurs as a consequence of a micro-explosion caused by the heating of the water molecules.[26] Because the amide I and water bands overlap, it is difficult to know whether the changes observed in Fig. 6 were caused by a decrease of water or amide I (i.e., it is not possible to ensure the exact contribution of each component in the band located at 1640 cm^{-1}).

In the same way, we analyzed the ratio between the areas under the bands of amide II and phosphate (900–1200 cm^{-1}) and the ratio between the areas under the bands of amide III and phosphate to confirm that the laser irradiation alters the organic components of the bone tissues. Figure 7 shows the average values of the amide II to phosphate and amide III to phosphate ratios. As in the mineral to matrix ratios, the results of amide II/phosphate and amide III/phosphate ratios show that the proportion of organic components significantly decreased with the increase in the energy density used. A previous study using an infrared laser on dentin suggested that laser irradiation affected the organic content of that mineralized tissue,[8] and studies of dental tissues have shown that the loss of organic material is proportional to the laser energy density used and probably also related to the thermal effect.[12,21]

We observed that the organic components of the bone decrease significantly with the increase of laser energy density on bone. Because the laser irradiation cause an increase in the temperature of the tissue[21] and the amides are more susceptible to temperature variation than is phosphate,[21] the observed changes probably occur due to a decrease in the amount of organic components with the increase in the laser energy density used, which reinforces the increased temperature observed. In temperatures up to 195 °C, the removal of absorbed water and moisture takes place, while up to 550 °C, the complete removal of the organic components of bone takes place.[48]

The degradation of organic compounds was observed in all the irradiated groups; however, a statistical difference between the control and irradiated groups was observed only when the energy density was greater than 6 J/cm^2, with the exception of the amide III band analysis. The degradation of the organic matrix started at 175–200 °C,[21] and the temperature study showed that samples reached temperatures higher than 200 °C when irradiated at 6 J/cm^2 or higher. Thus, the degradation of the organic components is in line with the observed increased temperature.

The FT-IR results suggest that the organic degradation takes place in groups irradiated at 3 J/cm^2 because the amount of components in this group was always smaller

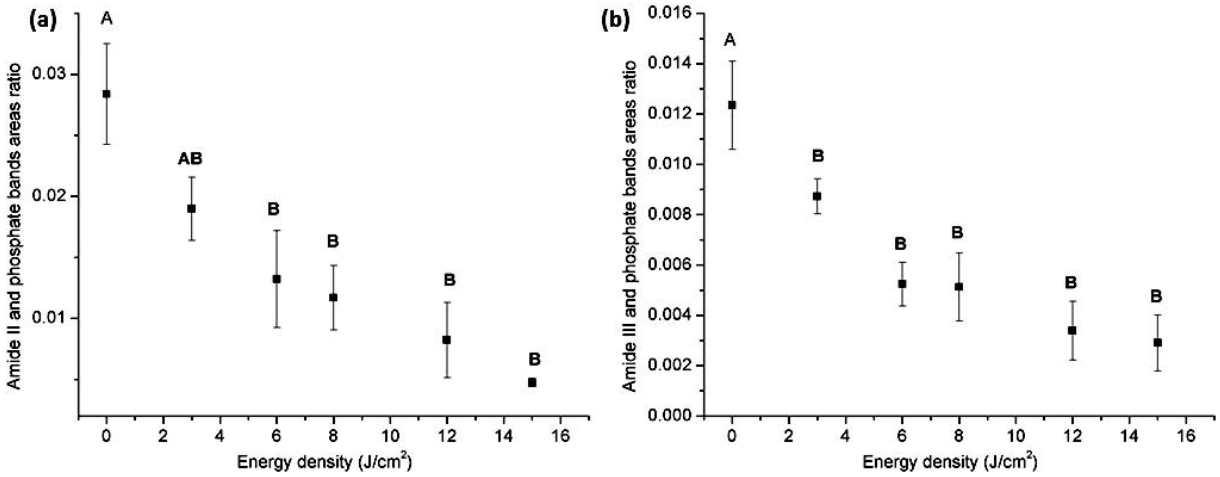

FIG. 7. Means of the band ratios obtained from the bone samples irradiated with different laser energy densities. (**a**) Amide II/phosphate area. (**b**) Amide III/phosphate area. Bars denote standard deviations. Letters indicate significant statistical differences according to the Tukey test.

than in the control group. Studies using FT-IR to analyze dentin subjected to temperature increases have shown that changes in collagen can be detected at a temperature of 100 °C.[21] Thus, considering that our samples irradiated at 3 J/cm^2 reached a temperature of 100 °C, we can expect a decrease in the collagen content.

Despite the thermal damage to both mineral and organic components that can be promoted using an Er,Cr:YSGG laser, in vivo studies using the infrared laser to perform small cuts in mineralized tissues have shown that the healing process looks similar to or better than cuts performed using a usual cutting tool.[53] A relevant result for the clinical application of the Er,Cr:YSGG laser is that a total degradation of the organic components was not observed even when critical clinical laser parameters were used because the organic matrix is directly related to the quality and speed of the bone-healing process. Therefore, the results observed in this study highlight the importance of choosing the correct energy density in clinical procedures to minimize thermal damage and to assure better healing.

CONCLUSION

Irradiation using the Er,Cr:YSGG laser increases the temperature of bone surface; the amount of the increase depends on the energy density used. Irradiation at 3 J/cm^2 promotes an increased temperature of approximately 100 °C, while 6 J/cm^2 promotes increased temperatures up to 215 °C; 8, 12, and 15 J/cm^2 promoted increased temperature up to 300 °C. The compositional analysis using FT-IR spectroscopy revealed that laser irradiation promotes changes in the carbonate content and affects the intermolecular interaction from mineral part of bone. However, it was not possible to detect changes directly related to the samples' crystallinity. Moreover, the proportion of organic components (amides) in the bone samples significantly decreased with the increase in energy density, which points out the importance of using the proper energy density in clinical procedures to avoid thermal or chemical damage to the tissue caused by laser irradiation.

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