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# ATR-FTIR spectroscopy to study the effects of laser irradiation in bone tissue

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**Abstract.** The use of lasers on bone cut can provide a series of advantages for both the surgeon and the patient. However, for a safe and efficient application it is necessary to know the exact effects that the laser causes in the bone tissue. The aim of this work was to study the effects of Er,Cr:YSGG irradiation in bone tissue using the ATR-FTIR technique. Pieces of tibia rabbit bone were divided in six groups with three samples per group. In one of the groups the samples did not undergo any treatment; in the others the samples were laser irradiated with different energy densities. The infrared spectra acquisition was made using an ATR accessory. For a semi-quantitative analysis, the area under each band was calculated and normalized by the phosphate band area of the same spectrum. The results showed a gradual material loss as the energy density increased in the bands of water, amide I, and carbonate, amide II, amide II and collagen. This is probably caused by the temperature rise due to laser irradiation. These results are the first steps in testing the Er,Cr:YSGG laser efficacy as a cutting tool, a pivotal aspect of its consolidation in clinical procedures.

Keywords: FTIR, bone, laser

## 1. Background

The use of laser in dental application has been increasing over the years [2]. In particular, the infrared lasers can be useful in several applications, since they are strongly absorbed by water and hydroxyapatite [12], two of the main components of the body mineralized tissues, like enamel, dentine and bone [9].

The Er,Cr:YSGG laser (2780 nm) already proved to be effective to cut teeth and bones [2,17]. The cut process of this laser happens by thermal ablation. In this process, the water molecules of tissue absorb the laser energy, increasing the temperature. The water boils and there is an increase of pressure inside the tissue, causing a micro-explosion that removes the material [10]. However, for an efficient and safe application it is necessary to know the exact effects that the laser irradiation promotes in tissue.

The Fourier transform infrared spectroscopy (FTIR) is able to analyze compositional and structural changes of mineralized tissues [8]. The technique has already been successfully used to study differences that laser irradiation promotes in dentine, enamel and bone [4,7,14,18]. It was observed that the

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laser can affect the mineralized tissues according to the irradiation conditions, especially the organic compounds [4,18].

## 2. Objective

This work aims to establish the Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy technique for the characterization of natural and irradiated osseous tissue, and to verify the possible chemical and structural changes caused by different irradiation conditions.

## 3. Methods

This study was approved by the by Animal Ethics Committee of IPEN (6/CEPA-IPEN/SP). Pieces of tibia rabbit bone were cut in pieces of $8 \times 5$ mm, and polished until a thickness of 100 μm. The samples were divided into six groups with three samples per group. In one of the groups, the samples did not undergo any treatment; in the others, the samples were irradiated with different energy densities using Er,Cr:YSGG laser (Waterlase®, Biolase, USA), an infrared pulsed laser with wavelength of 2780 nm and frequency of 20 Hz. The laser tip used was the S75 (diameter of 750 μm), and refrigeration of no kind was employed.

To avoid pulse overlap, the samples were moved during the irradiation with a velocity of 13 mm/s. The laser energy density used in each group was: 3 J/cm$^2$, 6 J/cm$^2$, 8 J/cm$^2$, 12 J/cm$^2$, 14 J/cm$^2$; non-irradiated sample was used as a control group.

The infrared spectra acquisition was made using an ATR accessory (Smart Orbit, Thermo, EUA) linked to a FTIR spectrometer (Nicolet 6700, Thermo, EUA). This ATR have a diamond crystal as the internal reflection element. The spectra range analyzed was from 4000 to 550 cm$^{-1}$, with a resolution of 4 cm$^{-1}$ and 80 scans per spectra. This range was chosen because it is where the organics components are mainly found [16].

## 4. Results

A bone spectrum of a non-irradiated sample is showed in Fig. 1 with the major bands identified. It was possible to observe the main infrared bands reported in literature [11,15]. The same bands were detected in the samples irradiated with the Er,Cr:YSGG laser, indicating that the densities energy used did not promote total degradation in any bone component. Comparing the average spectrum of each group, it was possible to observe that the laser irradiation did not promote any significant difference in bands' position and width. The phosphate band width is related with the crystallinity of the material [13]. The results suggest that the bone tissue did not suffer significant changes in their crystallinity after the laser irradiation.

For a semi-quantitative analysis, in each spectrum the area under the band of interest was calculated and normalized by the phosphate area band (1030 cm$^{-1}$). The phosphate band was used because it is the most stable band with the increase of temperature [3,13]. A temperature increase was expected, since the Er,Cr:YSGG laser cuts mineralized tissues by the thermal ablation process [10]. The bands analyzed in this study represent the main organics components of the bone [8]: water (3600–2400 cm$^{-1}$), amide I (1642 cm$^{-1}$), amide II (1553 cm$^{-1}$), amide III (1240 cm$^{-1}$) and collagen (1023 cm$^{-1}$). The carbonate (1409 cm$^{-1}$) was also analyzed, because this band overlaps with bands of the amides [6].

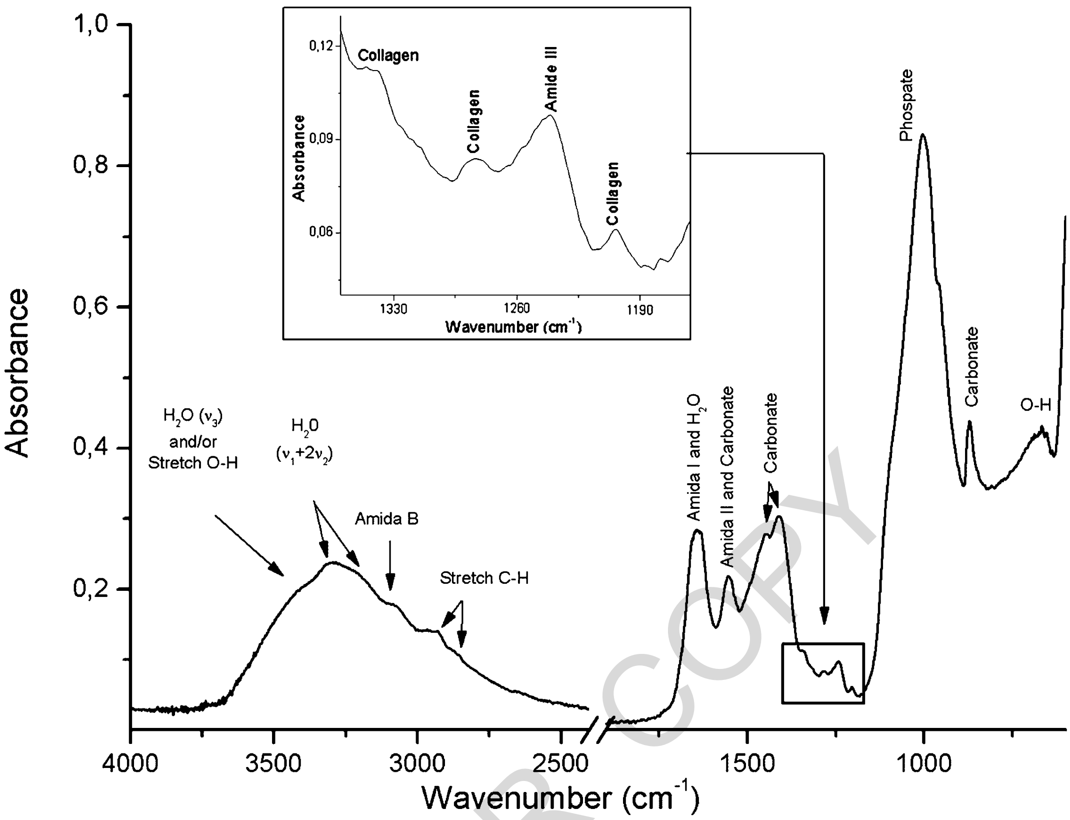


Fig. 1. ATR-FTIR bone spectrum.



The results can be seen in Fig. 2 and show a gradual material loss as the energy density increases. To compare the different groups, it was used ANOVA, $p < 0.05$. As to water and amide I, it was possible to observe significant differences between the non-irradiated group and the irradiated groups. There were not statistical differences between groups irradiated with different energy densities. However, it was possible to see a gradual decrease of the components proportion alongside with the increase of the laser energy density, indicating that they may be related.

For the amide II, amide III, collagen and carbonate components, the reduction was statistically different between the control group and the groups irradiated with energy density equal or greater than 6 J/cm$^2$. It was not observed any statistical difference between the control group and the group irradiated with 3 J/cm$^2$, indicating that this energy density is not high enough to cause a decrease in these contents. No significant change was observed between the irradiated groups, but an inverse relationship between the laser energy density and the components amount was also observed.

In thermal ablation processes, the material is removed due to a micro explosion caused by the water evaporation [10], so it was expected that the water would be the first component affected by the laser irradiation, even when the smaller energy density was used (3 J/cm$^2$). The amide I band also suffered a significant decrease when the samples were irradiated with the smallest energy density, while the other amides were not significantly affected. It is possible that the changes observed for the amide I band are related to the water loss. Water presents vibrational modes in the same range where amide I band is observed and their bands overlap [4,14]. Since no other amide band was affected by 3 J/cm$^2$, it is

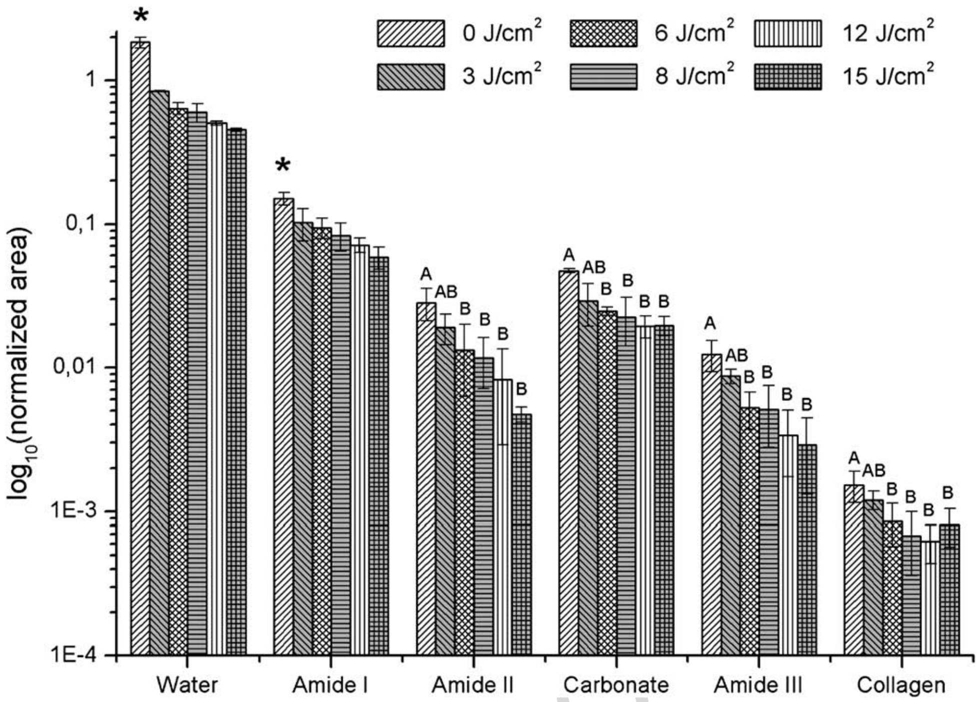


Fig. 2. Normalized area of the bone spectrum. Same symbols (*/A/B) show that the data are not significant different. The log scale was used in order to be possible to present different components in the same graph.

possible that the observed decrease in the amide I band in the irradiated samples with this energy density happened, in fact, because the water band is affected; not because of the degradation of amide I.

The degradation of organic components with laser irradiation had already been observed for enamel and dentine using infrared spectroscopy [5,18]. Similar changes were observed in studies that only heat mineralized samples [13], indicating that the alterations observed in the laser irradiation samples were consequence of the increase in temperature.

The temperature of the material removed from the tissues by thermal ablation with the Er,Cr:YSGG laser is almost 800°C [1]. The remaining material did not suffer the same increase in temperature, but probably, it reached an increase of approximately 175°C in the samples irradiated with more than 4 J/cm$^2$, since it was observed a decrease of organic components (amide II, amide III and collagen) [3].

Despite the fact that no significant changes in the organic components have been observed between irradiated groups, it was noticed a tendency that, the higher the laser energy density used during the irradiation, the higher was the organic component loss. So it is necessary to be very careful on the selection of the laser energy density on clinical procedures, since although a higher laser energy density is faster in material removal, it may cause a higher increase of temperature, potentially promoting irreversible damages in the tissue.

## 5. Conclusion

It was possible to use the ATR-FTIR technique to observe chemical changes in bone tissue caused by laser irradiation. The changes observed seem to be related with the increase of temperature promoted by the irradiation. These results are the first steps in testing the Er,Cr:YSGG laser efficacy as a cutting tool, an essential aspect of its consolidation in clinical procedures.

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