Detection of chemical changes in bone after irradiation with Er,Cr:YSGG laser

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ABSTRACT

The use of laser for bone cutting can be more advantageous than the use of drill. However, for a safe clinical application, it is necessary to know the effects of laser irradiation on bone tissues. In this study, the Fourier Transform Infrared spectroscopy (FTIR) was used to verify the molecular and compositional changes promoted by laser irradiation on bone tissue. Bone slabs were obtained from rabbit's tibia and analyzed using ATR-FTIR. After the initial analysis, the samples were irradiated using a pulsed Er,Cr:YSGG laser (2780nm), and analyzed one more time. In order to verify changes due to laser irradiation, the area under phosphate (1300-900cm^{-1}), amides (1680–1200cm^{-1}), water (3600–2400cm^{-1}), and carbonate (around 870cm^{-1} and between 1600-1300cm^{-1}) bands were calculated, and normalized by phosphate band area (1300–900cm^{-1}). It was observed that Er,Cr:YSGG irradiation promoted a significant decrease in the content of water and amides I and III at irradiated bone, evidencing that laser procedure caused an evaporation of the organic content and changed the collagen structure, suggesting that these changes may interfere with the healing process. In this way, these changes should be considered in a clinical application of laser irradiation in surgeries.

Keywords: Er,Cr:YSGG, infrared spectroscopy, hard tissues

1. INTRODUCTION

The use of high-speed drills is a common procedure in medical and dental surgeries for cutting mineralized tissues, such as bone and teeth. If used without proper refrigeration, these instruments can cause thermal damages and release of particles to the environment[1]. In this way, the use of laser irradiation as a surgical tool propitiates better visualization of surgical field due to its homeostatic effect and the possibility of removal of tissues in areas with limited access, apart from minimizing mechanical damages[1,2]. For surgical application, Er:YAG (λ=2.94 μm) and Er,Cr:YSGG (λ=2.78 μm) lasers are the most frequent in clinical procedures[5,6] due to their higher absorption by both water and hydroxyapatite[3,4], which are the main components of mineralized tissues.

Considering that erbium lasers interact with mineralized tissues by ablative process, there is a fundamental interest in investigating the thermal effects of laser irradiation on the chemical composition of these tissues, in order to understand how it can influence on healing process[7]. For this purpose, infrared spectroscopy analysis is a promissory technique, being firstly applied for bone analysis in the last century[8]. The Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR) spectroscopy is a technique that allows to measure changes in chemical composition of the material, in a very thin layer of biological tissues with good accuracy[7].

The objective of this study was to verify the compositional differences between non-irradiated and Er,Cr:YSGG laser irradiated bones using ATR-FTIR spectroscopy.

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2. METHODOLOGY

2.1 Samples Preparation

After approval by Animal Ethics Committee of IPEN (6/CEPA-IPEN/SP), 1.0 x 0.5 cm bone slabs were obtained from the diaphysis region of femurs of male New Zealand white rabbits. The rabbits were 1-2 years old and weighted from 2 to 3 kg. Bone slabs were sanded and polished in both sizes using carbide papers until getting 100 µm thicknesses. After that, all samples were kept in humid environment under refrigeration until the beginning of the experiments. The analyses and irradiation were made in periosteun.

2.2 ATR-FTIR

The compositional analysis was performed, using the Attenuated Total Reflectance - Fourier Transform Infrared spectroscopy (ATR-FTIR), in two different times: immediately before and immediately after sample irradiation. The ATR-FTIR spectra of each sample were obtained with 2.0 cm-1 resolution, on a Thermo Nicolet Smart Orbit spectrometer equipment, with a DTGS detector using a diamond crystal (45^o angle of incidence) as the internal reflection element. The size area of the data acquisition of the ATR-FTIR spectra is $1.5mm^2$ which corresponds to the size of diamond crystal of ATR acessory.

Each spectrum was obtained with 80 scans in the range of 4000 to 600 cm^{-1}. The environment temperature and humidity were controlled and maintained at 20 ^oC and 30%, respectively. Each obtained spectrum had a background spectra subtracted during acquisition. The OMNIC Spectra Software was used for acquisition and storage of data.

2.3 Laser Irradiation

Samples were irradiated using an Er,Cr:YSGG hydrokinetic laser device (Millenium, Biolase Inc., San Clemente, CA, USA), with 2.78 µm wavelength, 140 µs pulse duration, 20 Hz repetition rate. In this study, samples were irradiated with energy density of 6.06 J/cm^2, using the S75 sapphire tip (750 µm diameter and 6 mm length). Laser handpiece was positioned in optical supports a standardized distance of 1 mm from samples, and laser irradiation was done scanning all area of bone surfaces. During irradiation, samples were positioned at a motorized translation stage (Newport, Irvine, CA, USA), adjusted to a speed of 13 mm/s in order to avoid non-irradiated areas or overlapping of focused areas.

2.4 Data Analysis

All obtained spectra were constructed in the Origin 8.0 software (Northampton, MA, USA) for analysis. The bands considered for this study were: phosphate (1300–900 cm^{-1}), Amide I (1680-1600 cm^{-1}), Amide II (1580-1480 cm^{-1}), Amide III (1200-1300 cm^{-1}), water (3600–2400 cm^{-1}), the ν_2 vibration mode of carbonate (around 870 cm^{-1}) and the superposition of the stretching ν_3 and bending ν_4 vibration mode of carbonate (between 1600-1300 cm^{-1})[9,10]. Considering the spectra range obtained in this study, it was possible to evidence two vibrations mode of carbonate. This occurred because a molecule can have more than one vibration mode, and, for this reason, it absorbs radiation in more than one wavenumber. So, it is possible to identify the same component in more than one region of the spectra.

The center positions and the widths of each band were measured and the areas under the considered bands were calculated after normalization by the area of phosphate band (1300–900 cm^{-1}) for semi-quantitative comparison between non-irradiated and irradiated groups. The statistical analysis was performed with the same software, using the paired sample T test at 5% significance level

3. RESULTS

The Figure 1 shows a measured ATR-FTIR spectrum of non-irradiated bone tissue, in which it can be evidenced all bands considered for this study.

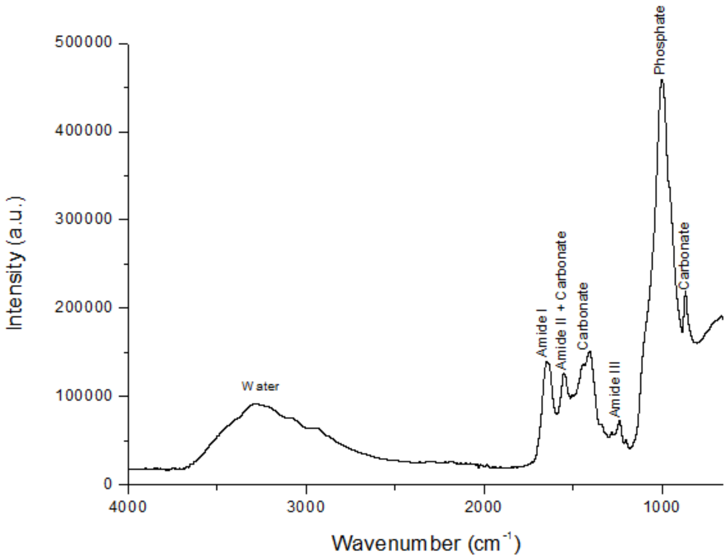

Figure 1. Measured infrared bone spectrum evidencing the bands analyzed in this study.

A visual comparison of the non-irradiated and irradiated bones can be made by normalizing the spectra with phosphate band intensity (Figure 2).

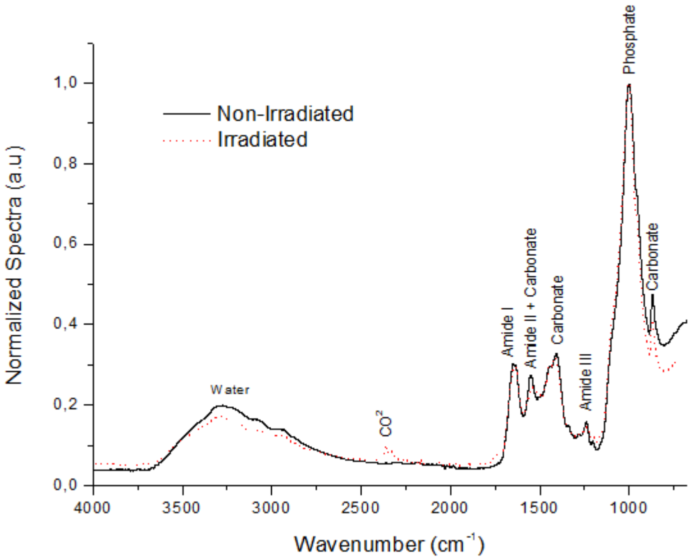

Figure 2. Visual comparison of the three non-irradiated and irradiated samples spectra normalized by phosphate band intensity.

Between the changes promoted by laser irradiation on bone tissue, it can be observed a significant change in the width of Amide II+Carbonate band (1600–1500 cm^{-1}) after laser irradiation, which can be seen in Figure 3.

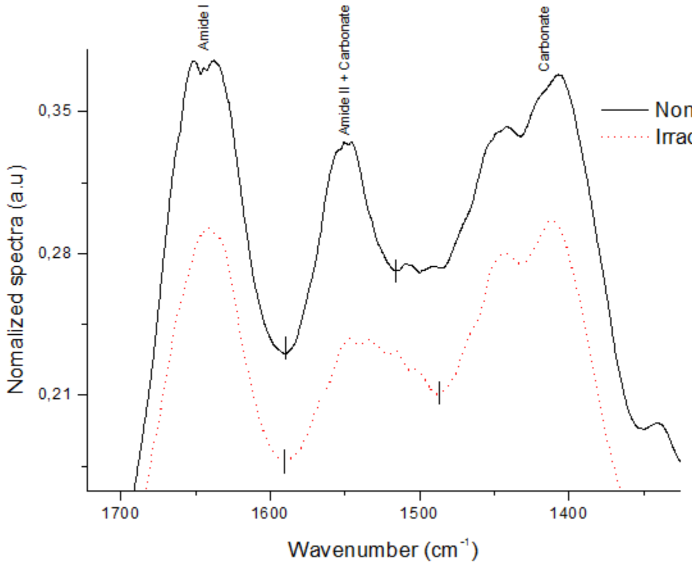

Figure 3. Width changes of Amide II+Carbonate band.

The Figure 4 evidences the differences in areas under the normalized bands between non-irradiated and irradiated bones. It is possible to observe that Er,Cr:YSGG laser irradiation significantly decreased the content of water, amide I and amide III contents. However, it was not possible to observe significant change on content of carbonate and amide II after laser irradiation in comparison with non-irradiated samples.

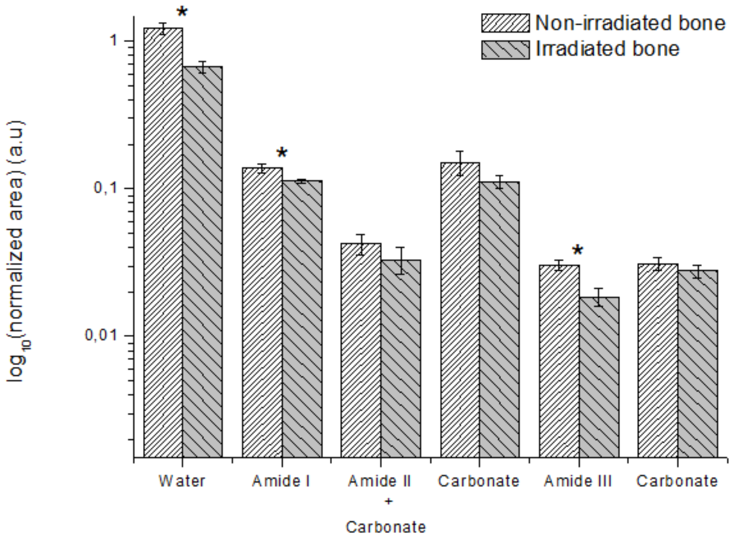

Figure 4. Differences between ratios areas of non-irradiated and irradiated spectra areas. The log of y-axis was calculated due to the differences in scales. The * indicates statistically significant differences between unlased and lased group

4. DISCUSSION

Since the first successful application on hard tissues[11], laser irradiation is nowadays widely used in surgeries of cartilage, bones and teeth, offering benefits for the practitioner, making possible the realization of procedures with better visualization, minimally invasive and with low thermal or mechanic damages[12]; and also benefits for the patient, assuring faster and more comfortable post-operative period.

Considering these extensive use of laser irradiation in surgery, it is important to know the chemical changes occurred on irradiated tissue, in order to evaluate whether laser parameter used can effect the healing process.

The present study demonstrated that bone irradiation with Er,Cr:YSGG laser, in radiances higher than the ablation threshold, promoted significant chemical changes. This was expected because the laser beam wavelength in 2.78 μm is strongly absorbed by both water and hydroxyapatite[6,13], which are the main constituents of bone tissue[14]. In this way, erbium laser energy is highly absorbed by this tissue, being transformed into heat and promoting a temperature increase on the surface, which depends on the parameter used, such as energy density and water coolant. The change in surface temperature during laser irradiation is reported to be the main responsible for the chemical modification of hard tissues[9], such as previously described in dentin and enamel[10,15,16]. Considering that bone has similar constituents than enamel and dentin, it is expected that infrared laser irradiation causes some similar chemical changes in this tissue.

Taking into account the laser parameter that can be used in a clinical application, some observations are essential. If infrared laser irradiation is performed with energy density higher than ablation threshold, thermal ablation process occurs[17], which is responsible for cutting of hard tissues. It means that the energy absorbed by water is enough to boil, causing increased pressure within the tissue and creating a small explosion that propitiates the removal of material and energy release[18,19]. In mineralized hard tissues, where thermal ablation occurs, the surface of tissue can reach 800 °C and this temperature decreases significantly with increasing depth[5]. The material that suffered the highest increment of temperature was removed and the remaining material did not reach the necessary temperature for ablation, but still suffered a temperature increase, that could be enough or not for inducing chemical changes in this remaining tissue. Hence, by analyzing the surface of ablated tissue, we are analyzing material that had a temperature increase less than 800 °C. It is important to notice that, during laser irradiation, the tissue temperature is not homogenous, but it depends on the depth and the region of irradiation.

In both non-irradiated and irradiated bone samples, it was possible to verify the presence of all the considered components for this study (water, amide, carbonate and phosphate), and the intensity of the bands of these substances changed with the analyzed sample. For this reason, it was necessary to normalize all obtained spectra in order to obtain a semi-quantitative comparison of non-irradiated and irradiated samples.

The comparison of the ratios of areas bands of non-irradiated and irradiated bone samples shows a significant reduction of all organic components (amide I and amide II) and water in ablated bone after irradiation. This fact indicates that the remaining irradiated tissue was exposed to a temperature large enough to cause denaturation of collagen, indicates by the loss in the content of amide I and amide III, and the tissue dehydration (water evaporation), which corresponds to the previous literature findings for enamel[10,20]. These losses are expected since both boiling water and collagen denaturation occur in temperatures higher than 100 °C[15,16]. These losses suggest that the remaining surface after ablation probably reached at least 100 °C.

Analyzing all the ATR-FTIR spectra obtained in this study at the same graph is not adequate in this case, because the intensity does not correspond to the absolute amount of the material, but it is related with the reflection of the samples. In this way, the reflection varies from sample to sample and depends slightly on the analyzed area on the sample. For this reason, the only direct comparative analysis that could be done between the non-irradiated and irradiated bone spectra were the widths and the center peak of each band. For a semi-quantitative comparison, it is necessary to normalize the spectra with a specific band area. Then, the normalization was made using the phosphate area band, once it is the more stable component of the bone when thermal increase occurs[21,22]. The band area corresponds to the amount of its component in the analyzed tissue.

The band width is related to material crystallinity[23]; in this way, the detection of the increase in width of AmideII+Carbonate band suggests a modification of bone crystallinity under irradiation. The Amide II + Carbonate band is explicitly considered because both components have vibrational states with the same intensities, which makes it impossible to analyze them separately[9].

Considering the findings of this study, we can observe that Er,Cr:YSGG laser cutting is able to alter the chemical composition of bone. Previous literature studies reported that bone cutting with CO_2 and Nd:YAG lasers promoted a significant delay in the healing process, probably due to the extensive thermal effects promoted[2,24]. Regarding this aspect, the use of erbium lasers became popular since these lasers promote less temperature increments and higher cutting efficiency mainly when used with proper water cooling, besides affecting a thin layer of the irradiated surface[25]. In this way, it was demonstrated that the non-hazardous thermal effects of Er:YAG and Er,Cr:YSGG lasers can propitiate a delay in bone healing[26], or a faster process when compared to conventional burs[27], or a faster initial bone healing[28] or even do not interfere with the bone-healing process[25]. As we can see, there is no consensus on literature regarding this aspect, mainly due to the fact that the previous studies use different laser wavelengths, parameters and conditions. In this way, it is necessary to know how laser irradiation can affect chemically this process and, for that, several parameters and conditions should be evaluated.

Concerning the use of Er,Cr:YSGG laser for cutting bones, it was previously demonstrated a delay in the first week of the healing process after irradiation[2]. Besides using lower energy density than the above mentioned study, the results of this work suggest that the chemical changes induced by laser irradiation can be the responsible for this delay. If we consider the results obtained by using the Er,Cr:YSGG laser in other calcified tissues, such as enamel or dentin, it is possible to evidence that the chemical changes induced on irradiated tissue increase with the increment of the energy density[15,16]. In this way, we can suggest that the initial delay on healing process observed by the above mentioned study could be a result of even more chemical changes induced on irradiated bone.

The present study corroborated the safety of using this Er,Cr:YSGG laser parameter for surgery purpose, since it was not observed any signal of necrosis, carbonization or other thermal damages in irradiated bones. In this way, this low energy-density parameter used can be an alternative for surgeries. However, further studies are necessary to evaluate the effect of several laser parameters on bone composition and healing including histology, in order to determine the most appropriate condition for a safe and satisfactory clinical application

5. CONCLUSION

The ATR-FTIR is an efficient technique to detect chemical differences promoted by the Er,Cr:YSGG laser ablation on bone tissue. The compositional changes observed by this technique confirm that the temperature increase in tissue during the ablation process affects the chemical composition of bone, which can interfere with the healing process In this way, the chemical changes induced by laser irradiation, should be considered in a future clinical application of laser irradiation in surgeries.

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