Biochemical Evaluation of Bone Submitted to Ionizing Radiation by ATR-FTIR Spectroscopy

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Abstract: FTIR spectroscopy associated with PC-LDA was able to discriminate bone samples receiving different ionizing radiation doses (0,01 kGy, 1 kGy, 15 kGy), showing potential to the use of phosphate vibrational modes as a dose marker.

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1. Introduction

Ionizing radiation is used as an important treatment and diagnostic method for several diseases [1]. Additionally, it can cause biochemical changes on the biological tissues and consequently, disturb the healing and mechanical properties of irradiated tissues. In order to improve information for dose decision and minimize side effects, it is necessary to determine the chemical changes promoted by ionizing radiations.

Fourier transform Infrared Spectroscopy (FTIR) provides biochemical information on a sample exposed to infrared radiation.

In this work, we used infrared spectroscopy to evaluate the molecular changes in bone matrix caused by different doses of ionizing radiation and assess the potential of Principal Component – Linear Discriminant Analysis(PC-LDA) [2] to classify the non-irradiated spectra against bone irradiated with different doses.

2. Material and Methods

30 fragments of bone were obtained from bovine femur diaphysis. Samples were cut in 1 cm x 1 cm x 1 mm, which were polished and stored properly in refrigerated environment.

Irradiation of samples was performed with a Cobalt-60 Gammacell Irradiator source at doses of 0,01 kGy, 1 kGy and 15 kGy, whereas the fragments exposed to dose of 15 kGy was irradiated in a multipurpose irradiator of Cobalt-60.

ATR-FTIR measurements, in the range of 4000 to 400 cm^{-1}, with 4 cm^{-1} of spectral resolution, were recorded using an Attenuated Total Reflectance (Smart Orbit, Thermo Scientific, Waltham, MA, USA) accessory coupled to a Fourier transform infrared spectrometer (Thermo Nicolet 6700, Waltham, MA, USA) system. For each spectrum, 100 scans were co-added and the vector normalization was applied. In the sequence, Savitzky-Golay filter with a polynomial of second order in a eleven-points window was applied to the second derivative of absorbance, Then Principal Component – Linear Discriminant Analysis (PC-LDA) was performed in order to evaluate phosphate changes in each groups and the area under the phosphate band (900 – 1300 cm^{-1}) was calculated [3-4].

3. Results and Discussion

Figure 1 shows the spectral average of each group. The Figure 1A provides spectral range corresponding to the fingerprint region (900 – 1800 cm^{-1}).

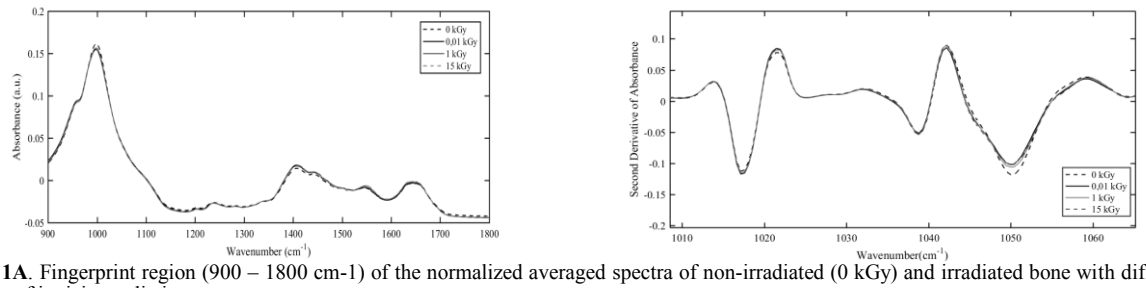

Fig. **1A**. Fingerprint region (900 – 1800 cm-1) of the normalized averaged spectra of non-irradiated (0 kGy) and irradiated bone with different doses of ionizing radiation.
1B. Second derivative of averaged spectral data of non-irradiated (0 kGy) and irradiated bone with different doses of ionizing radiation.

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The Figure 1A exhibit no apparent pattern between each group, however it was possible to increase the level of spectral detail evaluating the second derivative of absorbance with Savitzky-Golay filter as shown in Figure 1B. The amplitude of second derivative of the bands related to phosphate content change in all groups in comparison with the non-irradiated. This finding can be related to the recovery of bone regeneration due to biochemical effects provided by different doses.

A higher level of detail in the spectra was obtained using PC-LDA and the phosphate area under its bands as shown in Figure 2.

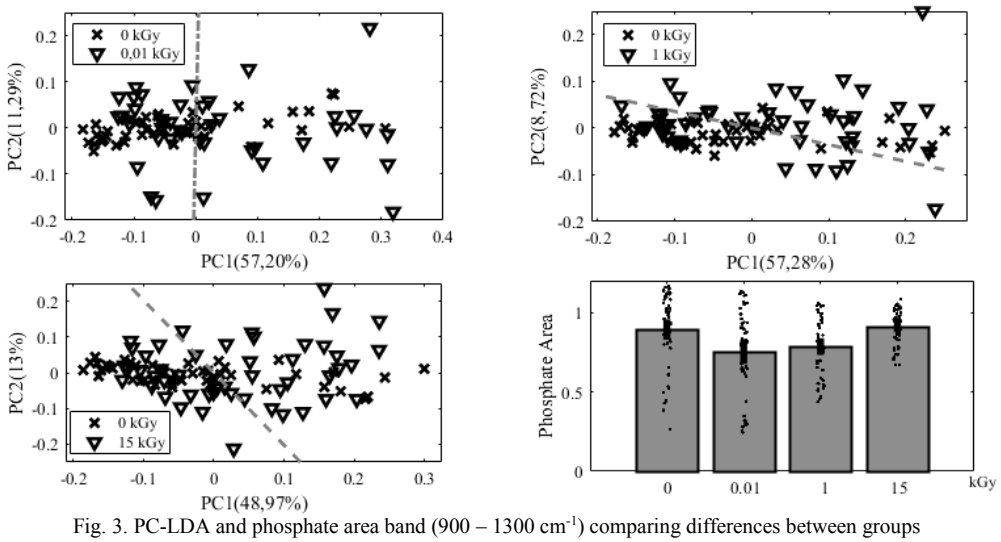

Fig. 3. PC-LDA and phosphate area band (900 – 1300 cm^{-1}) comparing differences between groups

In PC-LDA showed in Figure 2, it is possible to observe the distinction according to dose changing. These differences between the groups reported in phosphate agree with the second derivative spectra as both studies indicate a phosphate recovery.

According to the results observed in the present study, it is possible to conclude that infrared spectroscopy has the potential to contribute to describe chemical changes promoted by ionizing radiations. The multivariate technique PC-LDA when associated to second derivative spectra and area band measurement has proven to be effective to enhance spectral differences between non-ionizing and ionizing radiation in bone matrix.

4. References

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5. Acknowledgments

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