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Biochemical evaluation of bone submitted to ionizing radiation using ATR-FTIR spectroscopy associated to cluster analysis

Derly Augusto Dias[1]*, Marcelo Noronha Veloso[1], Pedro Arthur Augusto de Castro[1], Cassio Aparecido Lima[1], Denise Maria Zezell[1]

[1] Center for Lasers and Applications-(IPEN – CNEN/SP),
Av. Professor Lineu Prestes 2242
05508-000 São Paulo-SP
zezel@usp.br

ABSTRACT

Ionizing radiation is often used in medicine for radiodiagnostic exams, radiotherapy and sterilization of allografts bone. The interaction of gamma radiation with bone matrix is dose-dependent and induce changes in collagen fibers and, consequently, in the dynamic-mechanical properties of bone. Defining these biochemical events and the effects of radiation dose on tissue could result in optimized irradiation protocols as a function of temperature as well as understanding the effects of radiation on the bone from patients submitted to radiotherapy. In this sense, this study aimed to evaluate molecular changes in bone matrix submitted to different doses of ionizing radiation. For this, 30 fragments of bone obtained from bovine femur diaphysis were irradiated with gamma radiation (0.01 kGy, 0.1kGy, 1kGy and 15kGy) and submitted to Fourier transform Infrared spectroscopy (FTIR). Spectra were collected in the mid-infrared range in Attenuated Total Reflectance (ATR) sampling mode and submitted to Hierarchical Cluster Analysis (HCA) to evaluate the similarity level between spectral data structures. Irradiated groups were compared with non-irradiated bone and the results showed an increase in the values of classification accuracy according to the increase of radiation dose, allowing us to conclude that molecular changes are induced in both organic and inorganic bone content. Furthermore, we also concluded that FTIR spectroscopy associated with HCA is a good method to analyze the changes in bone tissue submitted to ionizing radiation.

1. INTRODUCTION

Gamma radiation promotes changes at molecular level by interacting with the biological tissue and according to the dose and irradiation conditions, changes in collagen fibers are induced and, therefore, in the mechanical and biological properties of bone. Sample irradiation in wet environment causes release of free radicals via radiolysis of water molecules that induces cross-linking reactions in collagen molecules. Considering that bone strength is determined by a combination of bone size, shape, and material properties[1][2][3], define the biochemical events and the effect of radiation dose on bone tissue could result in optimized irradiation protocols as a function of temperature as well as understanding the effects of radiation on the bone from patients submitted to radiotherapy.

Biological molecular bonds with an electric dipole moment that can change by atomic displacement due to natural vibrations are infrared active and therefore are quantitatively measured by infrared spectroscopy, which have succeeded in characterizing biological materials providing important information on the biochemistry of the analyzed tissue. The position, intensity and width of a vibrational band obtained with infrared spectroscopy can be used for monitoring a particular functional group or a particular molecule in different conditions [4].

In the study of mineralized tissues, infrared spectroscopy has been used to evaluate the molecular structure of bone, enamel and dentine. Specifically in the case of bone tissue, the infrared spectroscopy provides information about their mineral content, crystallinity and maturity of collagen and other important information for the maintenance of the functions of this tissue. Furthermore, many of the infrared spectrum characteristics are associated with age and the maturity of the tissue and can be used to study the process of bone repair and diseases such as osteoporosis[5]. In this way, the aim of this study was to evaluate the molecular and mechanical changes in bone matrix caused by different doses of ionizing radiation and evaluate the ability of Hierarchical Cluster Analysis (HCA) to classify the non-irradiated spectra against bone irradiated with different doses.

2. MATERIAL AND METHODS

2.1. Processing of bones

30 fragments of bone were obtained from bovine femur diaphysis. After removing the soft tissue attached to it, the bone was cutted in 1 cm x 1 cm x 1 mm samples, which were polished and stored in refrigerated environment.

2.2 Irradiation

Irradiation of samples was performed at the Centro de Tecnologia das Radiações (CTR) at Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEM/SP) with a Cobalt-60 Gammacell Irradiator source at doses of 0.01 kGy, 0.1kGy and 1kGy, whereas the fragments exposed to dose of 15kGy was irradiated in a multipurpose irradiator of Cobalt-60.

2.3 ATR-FTIR Spectroscopy

ATR-FTIR measurements, in the range 4000–400 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. The samples were pressed into the diamond crystal of ATR with a standardized pressure using a manometer. FTIR spectrometer was fitted with a deuterated triglycine sulfate (DTGS) detector (Thermo Scientific). For each spectrum, 100 scans were co-added and the spectrum obtained for each sample represents the averaged from 10 replicates measured in each sample. The Thermo Scientific system was controlled with Omnic software (Thermo Scientific). Spectra were vector normalized and submitted to Hierarchical Cluster Analysis (HCA) as an unsupervised classification technique in order to evaluate the similarity level between spectral data structures. The similarity of different clusters was defined by euclidian distance and calculated by Ward's method using software Minitab 17 (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

ATR-FTIR spectroscopy was used to compare bone fragments non-irraditated with tissue irradiated with different doses of ionizing radiation. Figure 1 shows the spectra from 400–1800 cm^{-1} (fingerprint region), which provides information of vibrational modes associated with organic and inorganic bone content.

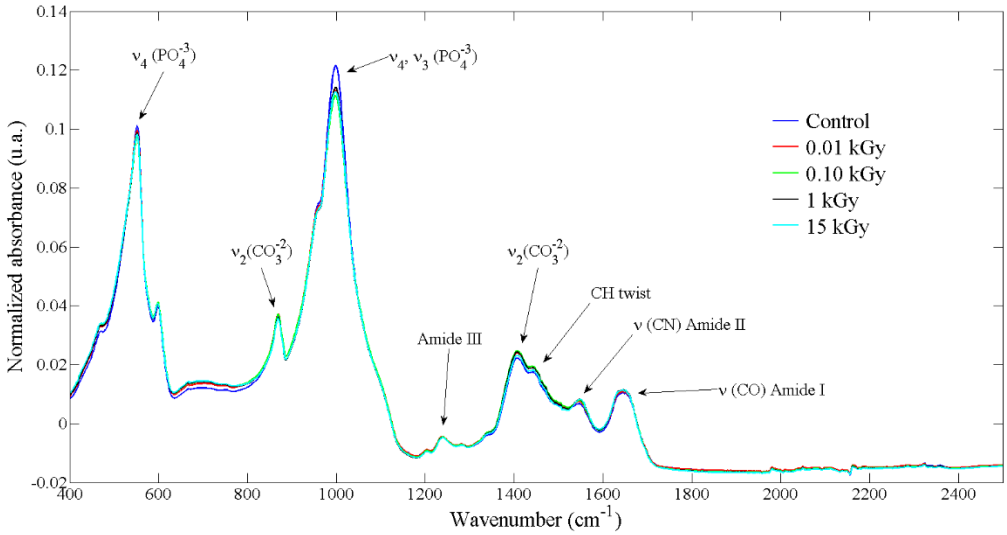

Figure 1. Averaged spectra for non-irradiated bone (Control) and irradiated bone with different dosses of ionizing radiation

As depicted in Figure 1, significant alterations in the absorption of the vibrational modes was not observed. However, it is noticeable that the tissue-sample analysis is much more complex than the simple explanation of the features of single component vibration. In a biological system the effect of each structure may interact with the others and result in the amplification or reduction of a specific signature. In this sense, spectra obtained for each group were submitted to Hierarchical Cluster Analysis (HCA) against the spectra from non-irradiated group aiming to obtain a pattern between data analyzed.

The dendrogram showed in Figure 2 classify spectra from non-irradiated and bone irradiated with 0.01 kGy dose into two groups. Samples listed 1-50 correspond to the spectra obtained for the non-irradiated bone, whereas those listed as 51-100 represent the spectra obtained for bone irradiated. The way that the data are distributed in the groups is shown on the abscissa axis and the distance of spectra within the same cluster is shown on the ordinate axis.

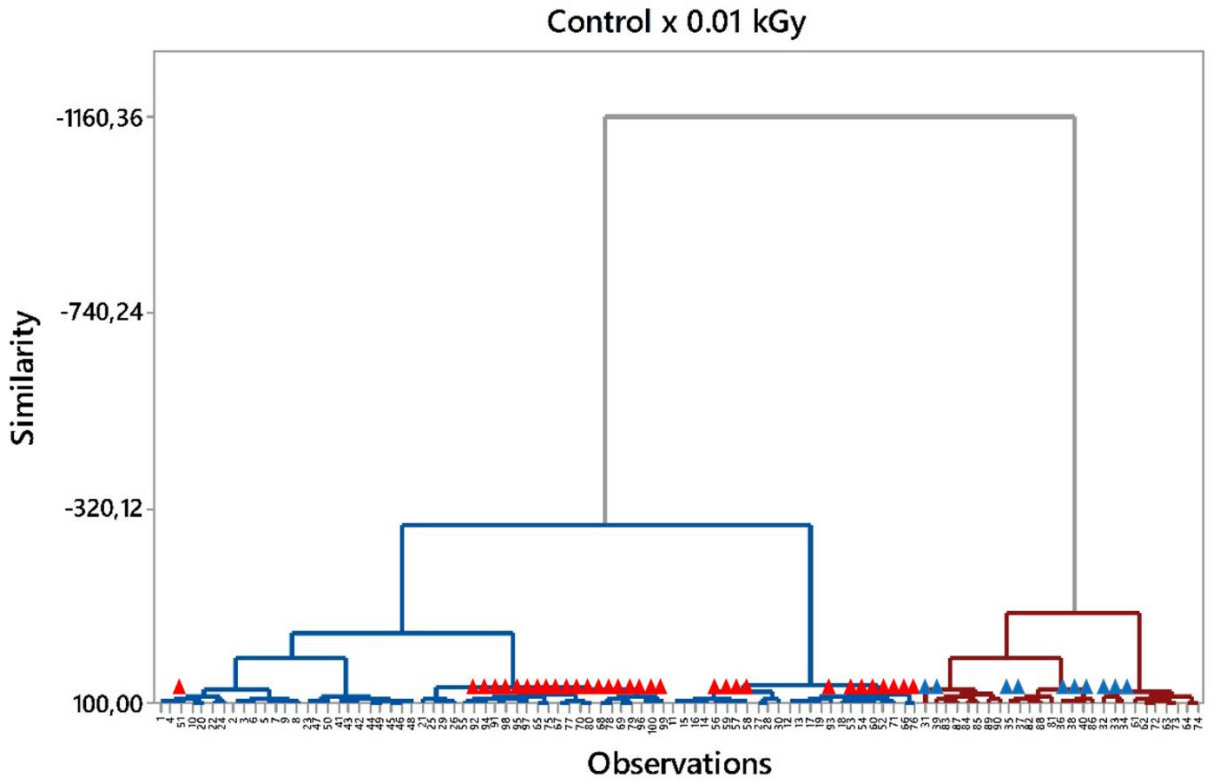

Figure 2. Classification of the Control spectra and bone irradiated with 0.01 kGy into two categories. Cluster blue represents non-irradiated spectra and red cluster depicts irradiated group. Triangles in blue depict non-irradiated spectra in the irradiated group, whereas red triangles represent the irradiated spectra in the non-irradiated group.

Considering the distribution of the data into the clusters obtained with HCA, the accuracy, sensitivity and specificity of classification was calculated as shown in Table 1.

Table 1. Distribution of the dataset in groups for the calculus of the accuracy of clustering classification.

	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Spectral data	19	40	10	31	60%	38%	80%

For the calculus, we considered the true positive as the irradiated spectra in the irradiated group; true negative as the non-irradiated spectra in the non-irradiated group; the false positive as non-irradiated spectra in the irradiated group; and false negative as irradiated spectra in the non-irradiated group. The triangles in blue depict false positive data, whereas

red triangles represent the false negative. The accuracy obtained for the classification of all dataset was 60%, sensitivity of 38%, and specificity 80%.

Figures 3, 4 and 5 represent the classification obtained HCA for non-irradiated bone against 0.1, 1 and 15 kGy doses.

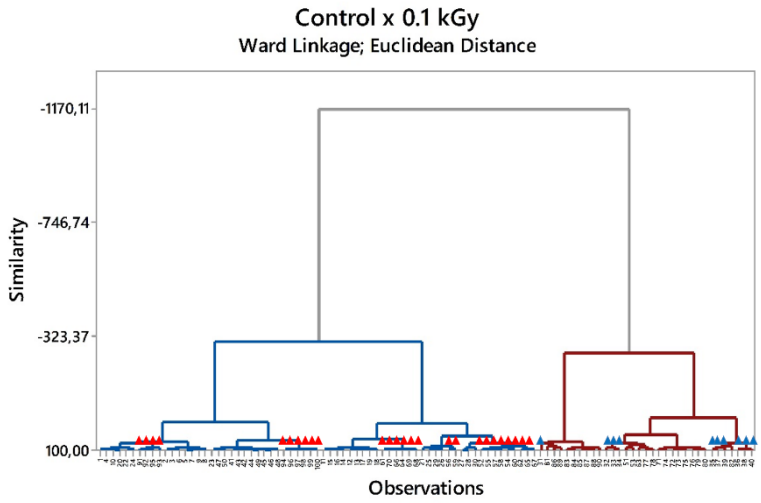

Figure 3. Classification of the Control spectra and bone irradiated with 0.1 kGy into two categories. Cluster blue represents non-irradiated spectra and red cluster depicts irradiated group. Triangles in blue depict non-irradiated spectra in the irradiated group, whereas red triangles represent the irradiated spectra in the non-irradiated group.

Table 2. Distribution of the dataset in groups for the calculus of the accuracy of clustering classification.

	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Spectral data	24	40	10	26	64%	48%	80%

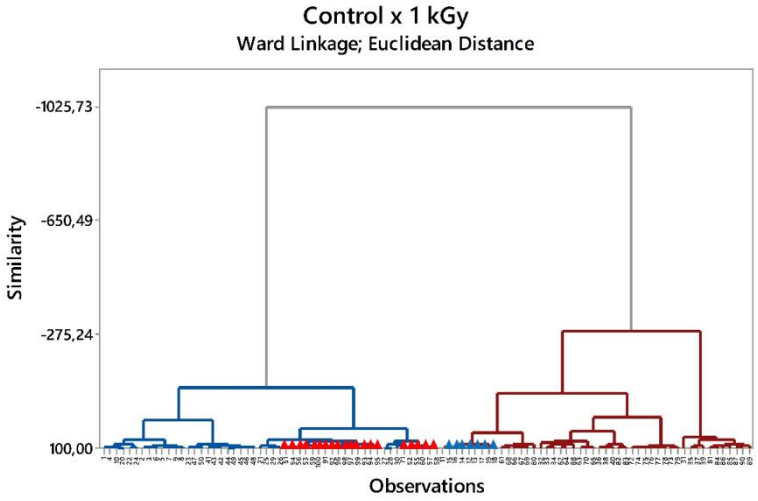

Figure 4. Classification of the Control spectra and bone irradiated with 1 kGy into two categories. Cluster blue represents non-irradiated spectra and red cluster depicts irradiated group. Triangles in blue depict non-irradiated spectra in the irradiated group, whereas red triangles represent the irradiated spectra in the non-irradiated group.

Table 3. Distribution of the dataset in groups for the calculus of the accuracy of clustering classification.

	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Spectral data	30	43	7	20	73%	60%	86%

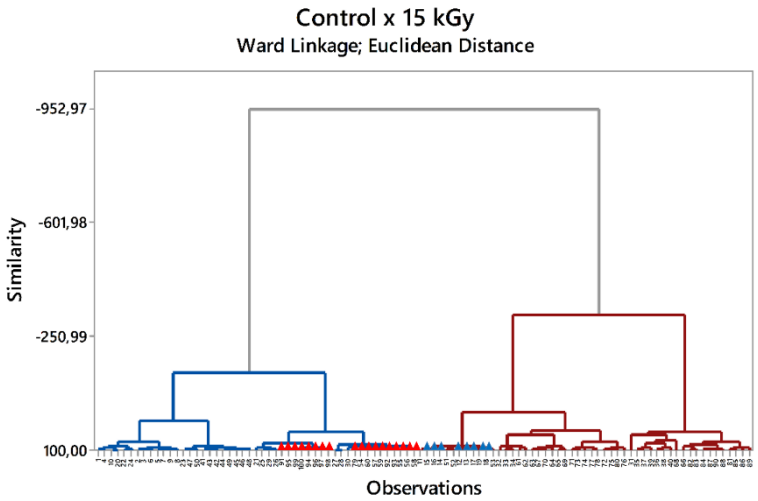

Figure 5. Classification of the Control spectra and bone irradiated with 15 kGy into two categories. Cluster blue represents non-irradiated spectra and red cluster depicts irradiated group. Triangles in blue depict non-irradiated spectra in the irradiated group, whereas red triangles represent the irradiated spectra in the non-irradiated group.

Table 4. Distribution of the dataset in groups for the calculus of the accuracy of clustering classification.

	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Spectral data	32	43	7	18	75%	64%	86%

Considering the accuracy values presented in Tables 2, 3 and 4, it is possible to identify an increase in the accuracy values, which is proportional to the radiation dose. The ability of classification obtained with HCA depends of similarity level between data analyzed. Thus, it is possible to conclude that the level of similarity between the irradiated and non-irradiated bone increases with radiation dose due to the biochemical effects provided by higher doses.

4. CONCLUSIONS

The current study showed that classification accuracy obtained with HCA increase with radiation dose (60% for bone irradiated with 0.01 kGy; 64% for 0.1 kGy irradiated samples; 73% for 1 kGy and 75% for samples irradiated with 15 kGy). In this way, we conclude that ATR-FTIR spectroscopy associated with HCA is a good method to evaluate the biochemical changes promoted by ionizing radiation in bone matrix.

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