2017 International Nuclear Atlantic Conference - INAC 2017
Belo Horizonte, MG, Brazil, October 22-27, 2017
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN

Agência Brasileira do ISBN
ISBN 978-85-99141-07-6
9 788599 141076

EFFECTS OF GAMMA IRRADIATION ON MICROHARDNESS AND FOURIER TRANSFORM INFRARED SPECTROSCOPY OF BOVINE BONE

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ABSTRACT

The skeletal systems with the structural arrangement of the bone are very important for load distribution, mechanical resistance and vital organs protection. The bone structure is multiphase and composed of organic, inorganic (mineral) compounds and water. Gamma radiation is an ionizing radiation that comes from gamma radiation sources or X-ray generator is commonly used in health establishments such as radio diagnostic exams, radiotherapy and sterilization of allograft. The characterization of the irradiated bone tissue can be is an important tool to study of the components that are affected and how much each dose of ionizing radiation can alter its mechanical properties. This information will be very important in *in vitro* and *ex vivo* studies where sterilization of the bone material is necessary and may still be useful in understanding the effects on the bone tissue of patients undergoing short-term radiotherapy. For this, 110 samples of bovine femur diaphysis were randomized into 11 groups: G1– untreated (control); G2 to G11 were submitted to gamma irradiation (60Co Gammacel). Samples were polished before irradiation and submitted to a Knoop Microhardness Test to determine the hardness of bovine bone and Fourier transform Infrared spectroscopy (FTIR) to biochemical characterization. Spectra were collected in the mid-infrared range in Attenuated Total Reflectance (ATR) sampling mode associated whit PCA multivariate technique to evaluate the molecular changes in bone matrix. It was observed that hardness was not altered by gamma irradiation and FTIR spectroscopy associated with PCA is a good method to analyze the changes in bone tissue submitted to ionizing radiation.

1. INTRODUCTION

Bone carries important functions in the body such as locomotion, support and protection for soft tissues and vital organs, storage of calcium and phosphate and bone marrow housing. The most abundant protein in the human body is collagen, this represents about 30% of the body's proteins and provides structural integrity of the tissue and ductility to tissues and organs [1,2,3]. The bone structure is multiphase and composed of organic, inorganic (mineral) compounds and water, the combination of these compounds provide the hardness and elasticity necessary for the bone tissue to perform its functions in the body.

It is known that no international consensus has been found on the optimal radiation dose to be applied to bone tissue due to the wide range of individual variables and decisions involving tissue banks. This radiation is capable of impair or alter the material properties of bone because of the collagen degradation. The damage of collagen integrity primarily results from the reduction in cross-link density and the fragmentation of collagen by the free radicals produced through the radiolysis of water due to indirect effects of ionizing radiation on tissues. It is concluded that the characterization of the irradiated bone tissue can be an important tool to understand which components are affected and how different doses of ionizing radiation alter their molecular structure. This information can be used in in-vitro and ex-vivo studies, where there is a need for sterilization of the bone material; and also useful in understanding the short-term effects on bone tissue of patients undergoing radiotherapy treatments.[4]

Ionizing radiation is used in industry for provide energy, in medicine is used for radiotherapy, radiodiagnostic exams and sterilization of tissues and surgical materials. Radiation have effects on cells and microorganisms depending on the effects of wave-length, dose rate and exposure time. Irradiation of the particles with gamma rays or X-rays does not induce materials or products to turn into a radioactive form.[5]

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. Microhardness is an important mechanical technique to measure hardness. In this work we used Knoop Microhardness Tester (Shimadzu HMV-2000, Japan) for determination of the hardness or the resistance of the material to penetration, non-irradiated and post-irradiation. In this way, the aim of this study was to evaluate the mechanical changes in bone matrix caused by different doses of ionizing radiation.

2. MATERIAL AND METHODS

2.1. Processing of bones

An *in vitro* study involved 110 (1cm x 1cm x 2mm) samples of bovine femur diaphysis which were polished, after being cut. After remove the soft tissue attached to the bone the samples were obtained, these samples were randomly distributed in eleven experimental groups (n=10): G1- bovine bone without treatment, G2 to G11 were submitted to gamma irradiation (^{60}Co Gammacel) 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-CNEN/SP) with a Cobalt-60 Gammacell Irradiator source at independent doses of 0.002 kGy, 0.004kGy, 0.07 kGy, 1kGy, 10 kGy, 15 kGy, 25kGy, 35kGy, 50kGy and 60kGy, all groups were irradiated in a humid environment and maintained under the same storage conditions.

2.3. Microhardness

Microhardness test were performed by a Knoop Hardness Tester (Shimadzu HMV-2000, Japan) which uses a pyramidal diamond for indentations. It was used a constant load of 490,3mN (compatible with sample resilience) applied perpendicularly on the surface of sample for 15s. Five indentations were made in the central region of the sample with a distance of 200μm between each of them.

2.4. Statistical Analysis

The means and standard deviations assessed in the microhardness tests were calculated, and one way analysis of variance (ANOVA) was performed (GraphPad Prism 6.0; California,USA). In cases of statistical significance $p < 0.05$, Kruskal Wallis' multiple comparison was used to determine which groups differed from each other.

2.5. 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. FTIR spectrometer was fitted with a deuterated triglycine sulfate (DTGS) detector (Thermo Scientific). The Thermo Scientific system was controlled with Omnic software (Thermo Scientific). Spectra were vector normalized and submitted to Principal component analysis (PCA).

2.6. Principal Component Analysis

PCA analysis can provide the most significant variance between the spectra groups**.** This technique reduces the dimensionality of the measurement matrix with the goal to represent the data using smaller number of factors or principal components (PCs).[6]

3. RESULTS AND DISCUSSION

3.1 Microhardness

There was no statistically significant difference in the irradiated groups when compared to the control group. The dose of 25 kGy, more used for sterilization of bone tissue, did not present a statistically significant difference in an experimental mechanical test. Microhardness values are showed in Figure 1.

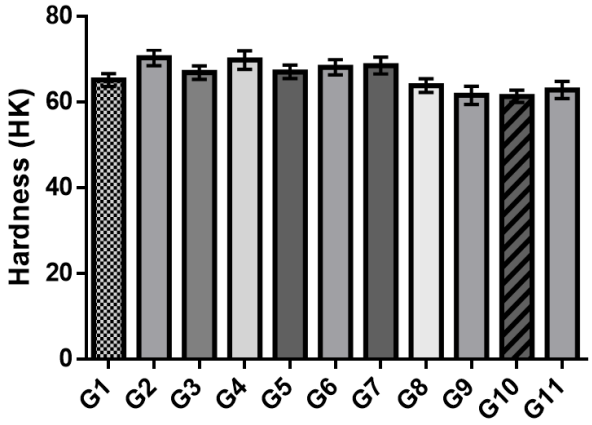

Figure 1 - Microhardness results

3.2 PCA

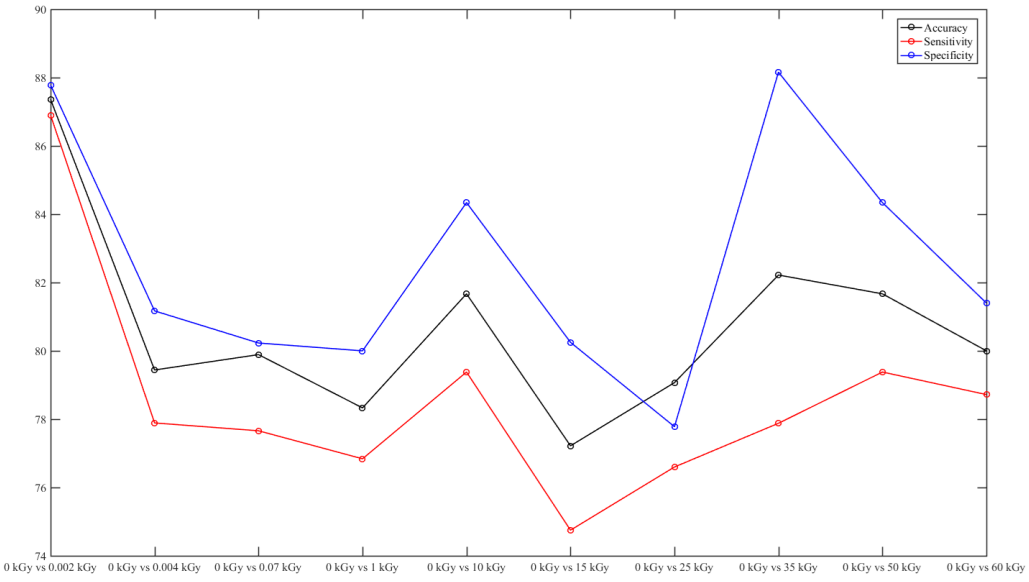

Figure 2 – PCA Performance compared

Accuracy, sensitivity and specificity are shown in Figure 2. All the irradiated groups were compared with the control group, the groups of 0.002 kGy, 10 kGy and 35 kGy are distinguishable as its shown in figure 3.

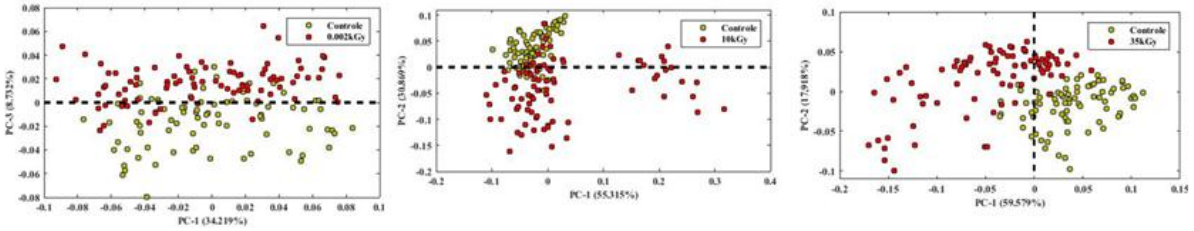

Figure 3 – score plot of the groups irradiated with 0002 kGy, 10 kGy and 35kGy, compared to the control group.

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All the irradiated groups presented differences in relation to the control group. These differences demonstrate the effectiveness of the FTIR associated with the PCA technique

4. CONCLUSIONS

The microhardness analysis did not present a significant statistical difference between the irradiated and control groups, showing that ionizing radiation did not affect the mechanical structure of the samples, on a micro scale. The results of FTIR with the PCA technique were effective in separating all groups, especially those irradiated with doses of 0.002 kGy, 10 kGy and 35 kGy. We conclude that ATR-FTIR spectroscopy associated with PCA is a good method to evaluate the biochemical changes promoted by ionizing radiation in bone matrix.

ACKNOWLEDGMENTS

This work was supported by CNPq/INCT 465763/2014-6, PQ 312397/2013-5 and CAPES/PROCAD 88881.068505/2014-01.

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