In vitro evaluation of ionizing radiation effects in bone tissue by FTIR spectroscopy

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1-ABSTRACT

We verified the changes promoted by ionizing radiation in bone tissue using FTIR. Samples of bovine bone were irradiated using Cobalt-60 with 0.01kGy, 0.1kGy, 1kGy, 15kGy and 75kGy. The effects of ionizing irradiation on chemical structure of bone, were studied considering the sub-bands of amide I, the crystallinity index and relation of organic and inorganic materials. ATR-FTIR spectroscopy showed changes in organic components and in hydroxyapatite crystals organization. High correlation with statistical significance was observed between (amideIII+collagen)/ $\nu_{1,}\nu_3PO_4$, crystallinity and mechanical properties of the samples.

Keywords: Ionizing radiation, bone, FTIR spectrocopy

2- INTRODUCTION

Ionizing radiation is frequently used in Medicine, such as radiodiagnostic exams, radiotherapy, and sterilization of halograft. It breaks polypeptidic chains, causing the release of free radicals by radiolysis of water, and may change the material mechanical properties. In the specific case of bone tissue, studies report that ionizing radiation induces changes in collagen molecules and reduces the density of intermolecular crosslinks. The aim of this study was to verify the changes promoted by different doses of ionizing radiation in bone tissue using FTIR. Samples of bovine bone were irradiated using Cobalt-60 with five different doses: 0.01kGy, 0.1kGy, 1kGy, 15kGy and 75kGy. To study the effects of ionizing irradiation on the chemical structure of the bone, the sub-bands of amide I, the crystallinity index and relation of organic and inorganic materials, were analysed.. To verify whether the chemical. It was possible to evaluate the effects of different doses of ionizing radiation in bone tissue. With ATR-FTIR spectroscopy, it was possible to observe changes in the organic components and in the hydroxyapatite crystals organization. High correlation with statistical significance was observed between (amide III+collagen)/ $\nu_{1,}\nu_3PO_4^{3-}$ and 1/FHWM, which is inversely proportional to the crystallinity degree.

3- MATERIAL AND METHODS

FTIR spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a characterization technique which uses the interaction of electromagnetic radiation with matter for physical and chemical information on the material . This technique is able to provide quantitative information on the maturity of collagen in bones , specifically the relationship between two of the main enzymatic cross-linking : The pyridoline (Pir) that are part of group of mature collagen cross-links and dihidroxinorleucina (DHLNL) , part the group of collagen crosslinks immature . These connections have absorption 1660 cm $^{-1}$and 1690 cm $^{-1}$(Pyr and DHLNL , respectively) . The relative percentage area ratio of the two sub-bands provides a semiquantitative measure of the profile cross-linking in collagen matrix and is related to the abundance of

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Biophotonics South America, edited by Cristina Kurachi, Katarina Svanberg, Bruce J. Tromberg, Vanderlei Salvador Bagnato, Proc. of SPIE Vol. 9531, 953118 · © 2015 SPIE
CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2181096

these cross links collagen in mineralized tissue [1].The analysis of the material according to the techniques described were made in the laboratory Biophotonics Laser and Applications Center IPEN . The spectrometer used was an accessory of Attenuated Total Reflection - ATR (Smart Orbit, Thermo Inc.,USA) coupled to a spectrometer in infrared Fourier transform - FTIR (Nicolet 6700, Thermo Inc., USA).

Bones processing

The femur diaphysis were used , and the epiphysis will be discarded. Were selected 5 bone fragments for analysis in a spectroscope . Cattle bones were cleaned to reduce its initial contamination and remove the soft tissues associated with bone. Later , they were packed and stored in a freezer at -20 ° C. The cuts were made with the aid of a cutter (Accuton - 5 , Struers, Ballerup , Denmark). Finally, bone samples were ground to a suitable thickness for analysis. The procedure adopted was to radiate 5 fragments with doses of 0,01kGy , 0,1kGy , 1 kGy , and 15kGy 75kGy each. Thus, 10 spectra for each sample will be collected by ATR- FTIR.

Irradiation

Bone samples were analyzed with fractionated doses of gamma radiation 0,01kGy , 0,1kGy , 1 kGy 15kGy and 75kGy , or were subjected to irradiation with successive doses of 0.01kGy , 0.9kGy 0.9 kGy , 14kGy and 60kGy . The irradiation 0.01kGy , and 0.09kGy 0.9kGy were performed in irradiator Cobalt-60 type Gammacell 1.43kGy and irradiation with a dose of 14kGy and 60kGy were performed in multipurpose irradiator Cobalt-60 , with a rate 6kGy dose/h . All radiations were performed at the Radiation Technology Centre (CTR) of IPEN - CNEN / SP.

Analysis by ATR- FTIR spectroscopy

The samples for spectroscopic analysis by the technique of attenuated total reflection (ATR) were cut with dimensions of approximately 7mm x 7mm x 1mm (with area similar to ATR crystal area) , and subsequently sanded with sandpaper Microcut tm S Abrasive Discs (Buehler , Lake Bluff , II , USA) 2500 and 4000 granulation successively. Ten spectra of each sample were collected every stage. The equipment used for analysis was a FTIR spectrometer (Nicolet 6700 , Thermo Scientific®, Madison, WI , USA) with a DTGS -TEC detector and an enhancement of Attenuated Total Reflection - ATR (Smart Orbit , Thermo Scientific®, Madison, WI , USA) coupled for the ATR - FTIR analysis .

Adjustment of amide I sub-band

One way to identify changes in collagen is used the ratio of the areas of the sub- amide I bands located at 1660cm $^{-1}$and 1690cm $^{-1}$. Ratio reported in the literature by the corresponding ratio of cross-links of collagen from mature and immature bone. These positions are assigned to collagen crosslinks pyridinoline (crosslinked immature) and dihydroxynorleucina (mature cross-links) , which formulas are presented in Figure 1. [1][2][3][4]

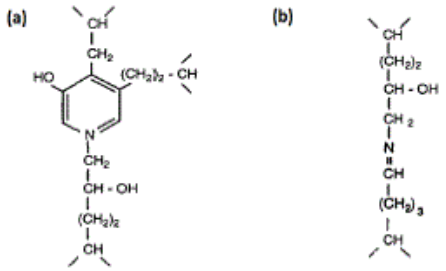

Figure 1: molecular structure of the crosslink pirinolina (a) and dihydroxy - norleucine (b) [05]

Crystallinity Index

The crystallinity index (CI) is calculated according to:

$$CI = \frac{A_{604} + B_{564}}{C_{590}}$$

to perform this procedure is necessary to draw a base line in the spectrum (400 cm^{-1} to 635 cm^{-1}) (Figure 2) . The lower bandwidth of the two vibrational modes $_4PO_4^{-3}$ located at 550 cm^{-1}(A) and 600cm^{-1}(B) will be the lowest intensity I_{588} cm^{-1}(C) , the higher the crystallinity index [6] [7] [8] [9] [10] .

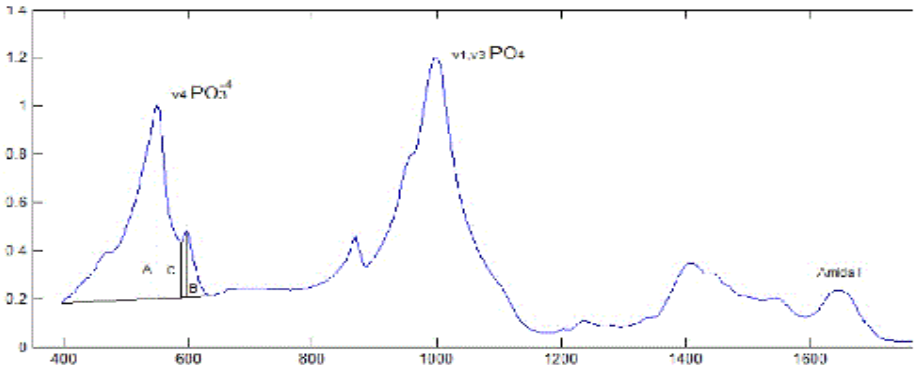

Figure 2: Bone Spectrum non- irradiated

FHWM analysis of the band $v_1PO_4^3$

Analysis width at half height of $v_1PO_4^3$ phosphate band. Evaluates roughly the size and perfection of hydroxyapatite crystals . The ratio is used to assess crystallinity is = 1 / (FHWM) [11] , [12] , indicating that the bandwidth is inversely proportional to the organization and integrity of the crystals.

Bands of the areas of Amida reason III / phosphate

The ratio of the areas of the bands amide collagen III + and phosphate , is calculated by the ratio of the integrated area of the Amide III band (1270-1210cm^{-1}) plus the two bands corresponding to collagen structure (1296 to 1269 cm^{-1}) and (1213-1180cm^{-1}) by the integrated phosphate band area (1180-916cm^{-1}).

4- RESULTS

Adjustment of amide I sub-band

Observed an increase in the ratio of sub bands in the first group we react to the control group . The graph shows a more marked increase in 1kGy where reason has its maximum value. The curve begins to decrease in 15kGy and 75kGy reaches a value close to the original.[Figure 3]

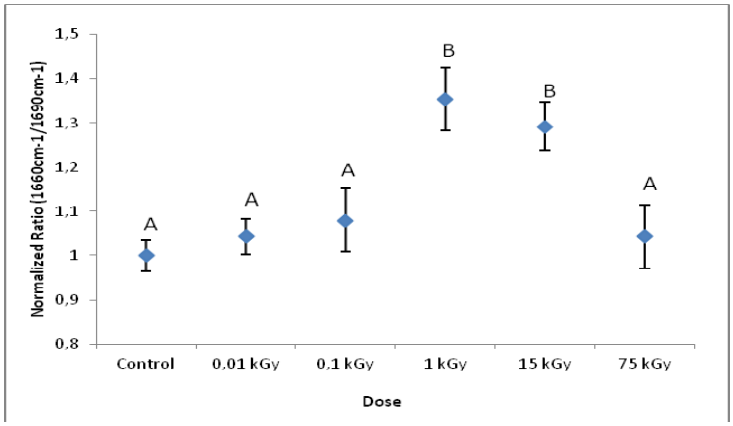

Figure 3: Evolution of the normalized ratio of amide sub- bands I, pyridinoline and DHLNL .

Crystallinity analysis

In Figure 4 , in the analysis of 1/ FWHM , the 0,01kGy group showed a decrease in the control group . From this group the values remain below the control group , but exhibit a gradual increase . In 75kGy the value exceeds the control group.

The Crystallinity Index (CI) values decay gradually in the control group up to level but low in 0.1kGy . From this group, the IC has increased up to 15 kGy , reaching its peak in 75kGy whose close relative values are the technique of 1 / FWHM .

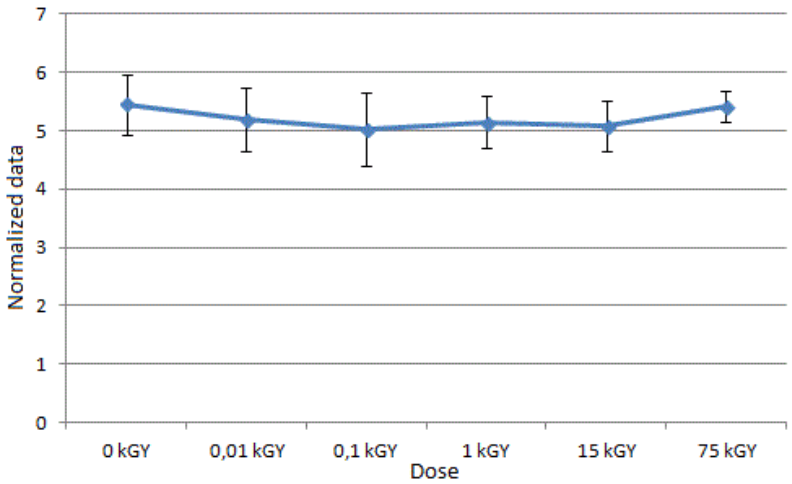

Figure 4: Comparison of standardized data crystalline Index and 1 / FWHM of □□PO_4^{3-} band.

Bands of the areas of Phosphate/ Amide I

The relationship $\nu_1,\nu_3 PO_4^{3-}$/amide I presents progressive decrease up 1kGy where it reaches its lowest value , growing slightly in 15kGy , and manteining until 75KGy , as shown in Figure 5.

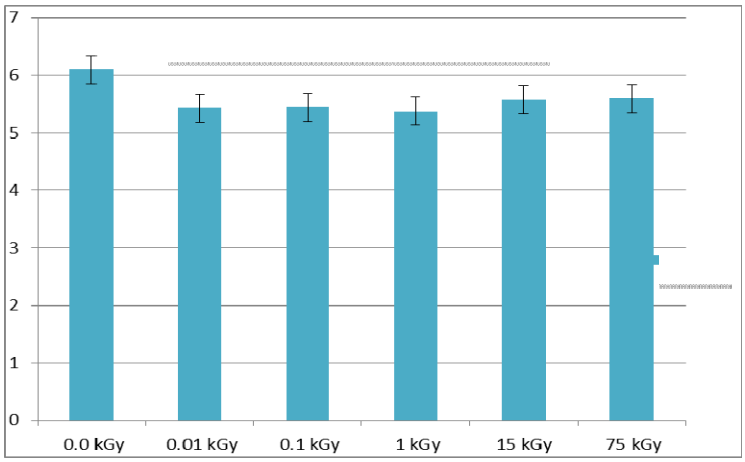

Figure 5: Normalized data of phosphate in relation to the main components of bone organic material.

5- DISCUSSION

The crystallinity index seems to be an important information to compare the effects of radiation on bone tissue as a function of irradiation dose. Taking into account that a patient in radiotherapy treatment receives about 0.08 kGy, in fractionated doses, the damage (mechanical fragility) caused by short-term ionizing radiation can be severe if the patient suffers a fall or go through some kind of dental treatment, for example.

According to the literature the ratio of phosphate to amide I band is inversely related to bone mineral density. The increase in this ratio can be attributed to an increase in the chances of bone fracture.

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