Bone as a biomarker for detecting low dose of ionizing radiation: a pilot study

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Abstract: FTIR spectroscopy was able to discriminate bone samples receiving low-dose ionizing radiation doses (0.002 kGy, 0.004 kGy, 0.007 kGy), offering valuable insights in view of the understanding of radiation dose response in biological systems. © 2022 The Authors

1. Introduction

The use of ionizing radiation is an important treatment and diagnostic methodology for many diseases [1]. Besides causing biochemical changes in the biological tissues, it can also interfere with the healing and mechanical properties of the irradiated tissues. Identifying the chemical changes caused by ionizing radiation is essential in order to improve the decision-making process and to minimize side effects. Thus, Fourier transform Infrared Spectroscopy (FTIR) can provide valuable insight into the biochemical composition of a material exposed to ionizing radiation.

To address this question, we performed infrared spectroscopy to evaluate the molecular changes in bone matrix caused by low-doses of ionizing radiation using the amplitude of the second derivative for calculation.

2. Material and Methods

Irradiation of bone samples was performed with a Cobalt-60 Gammacell Irradiator source at doses of 0.002 kGy, 0.004 kGy and 0.007 kGy. Using five bovine femurs, bone samples were assessed by performing 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. Every spectrum, in the range of 4000 to 400 cm⁻¹, with 4 cm⁻¹ of spectral resolution, 100 scans were co-added and vector normalization was conducted. In the following step, after the second derivative of absorbance, a Savitzky-Golay filter was applied using a second order polynomial in an eleven-point window [2]. The amplitude of the second derivative was separated for further ANOVA multiple comparison using Graph Pad Prism.

3. Results and Discussion

Bone FTIR spectra from low doses were analyzed to identify potential differences between groups. For this purpose, the second derivative of the FTIR spectrum (Figure 1.B) was used to examine all hidden and overlapped peaks



Fig. 1. Sample figure with preferred style for labeling parts.

As shown in Figure 1.A, when comparing the FTIR spectra of low-doses and control bone samples, no significant differences were found in peak shifts in the examined spectral region. Yet, there is a distinct tendency to differ groups according to peak intensity among which could be better visualized by using the second derivative. As shown in Figure 1.B, the second derivative emphasizes the differences in the average band intensity between groups such as 871, 956 and 1000 cm⁻¹. Based on these peaks, the amplitude of the second derivative was considered for further statistical analysis, as illustrated in Figure 2.

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Fig. 2. The "n.s" indicates no statistical difference. The asterisks presence represent the statistical significance (* p<0.05, ** p<0.01,*** p<0.001) that analysis was evaluated with the ANOVA multiple comparison (p<0.05).

The amplitude of the second derivative allowed the semi-quantitative evaluation by ANOVA multiple comparison test. In all comparisons with the control group, with the exception of 871 cm⁻¹, there were statistically significant differences. These statistical differences may be linked to the fact that irradiated bones are susceptible to side effects, affecting the activity of cells and causing direct effects on repopulation of the bone [3].

4. Conclusion

Research in dose-response provides many opportunities through FTIR spectroscopy. These pilot studies are intended to advance the understanding of dose response with regards to the effects of ionizing radiation on biological systems, showing the possibility to use the proposed protocol as a biomarker for ionizing radiation.

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