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SR-FTIR microspectroscopy coupled with multivariate data analysis in study of interaction between radiation and living matter

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All living organisms are continually exposed to radiation through natural sources such as cosmic rays and terrestrial sources including radon thorium and uranium. Radiation has found its application in cancer treatment and diagnosis. It has direct effects on tissues and cells. The most sensitive molecule in terms of affecting cell function is DNA due to its sensitivity to damage and because the genetic information is stored here [1]. The processes at the cellular and molecular level are the basis for the macroscopic effects of ionising radiation on whole organisms. The aim of presented research is to study the effects of how radiation interacts with living cells and radio-biologically relevant molecules.

The current practice for investigating the effect of ionising radiation on cells is to use biochemical assays such as the comet assay [2], Gamma H2AX foci [3] and/or the micronuclei assay [2]. However, the assay itself may affect the biological samples leading to changes in their structure due to non–physiological chemical substances and complex preparation procedures [2-4]. Moreover, most biochemical methods are not intended to study the effect of radiation at the single cell level. Therefore, there is a need for complementary techniques to confirm cellular damage arising from radiation exposure. SR–FTIR (Synchrotron Radiation Fourier Transform Infrared) microspectroscopy has become potential analytical method in single cells studies [5] on a molecular level. A growing amount of literature data demonstrates their usefulness in studies of the conformational aspects of lipids, nucleic acids and other biomolecules. It is known also that SR-FTIR method is a sensitive tool, that can be applied to DNA damage study at chemical bond level [5]. FTIR microspectroscopy is useful as well for cellular membrane damage investigation.

In presented study the SR-FTIR microspectroscopy with subsequent multivariate analysis was applied to investigate radiation damage to nucleic acids, proteins and lipids and to monitor the cellular response to radiation exposure. Those methods were also applied successfully in study of non targeted effects such as bystander and sun-light influence.

Cellular response to proton exposure

SR-FTIR spectra of single human prostate adenocarcinoma PC3 cells, irradiated with a defined number of 2MeV protons generated by a Cracow proton microbeam along with non-irradiated control cells, were analysed using multivariate methods. A number of different Principal Component Analysis (PCA) models were tested and the spectral ranges associated with nucleic acids, proteins and lipids were analysed separately. The most important PCA results are Scores Plots and Loadings Factors. The Scores Plots represent the spectra in multidimensional space of principal components (PC-1, PC-2, PC-3….), explaining some percentage of total variance. They can be used to detect sample patterns and group them accordingly. This analysis separates the data into their principal components. Loadings Plots indicate, which variables in the data set are responsible for a clustering.

Sample results for nucleic acids spectral range (1030 cm^{-1} – 1300 cm^{-1}) are presented in Fig. 1. Score Plot (Fig. 1a) have shown two clusters of spectra collected from cells irradiated with 400 protons (open triangles) and non-irradiated ones (black points). Loadings Plot (Fig 1b) shows, which variables are responsible for the greatest degree of separation.

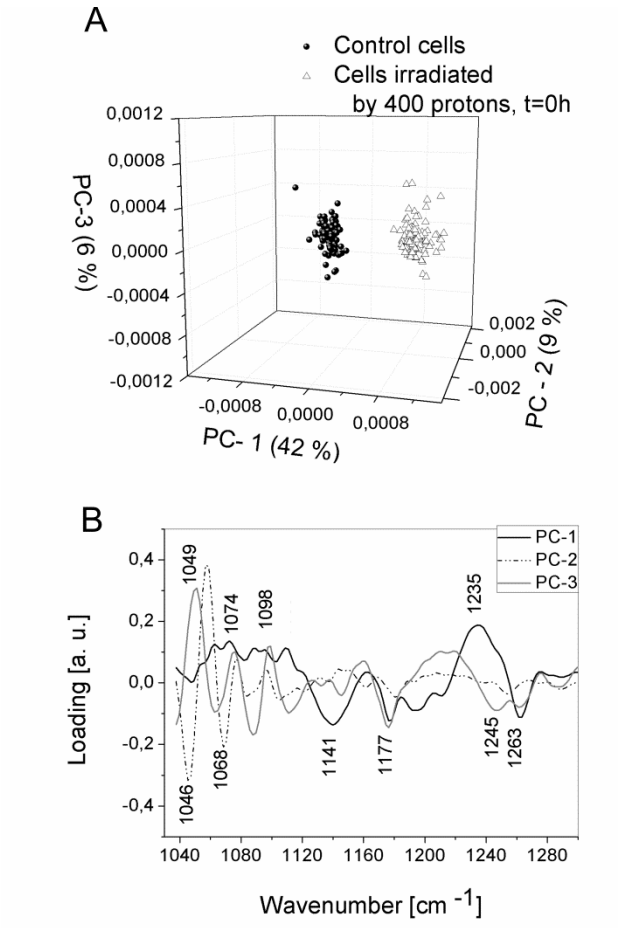

Figure 1 The results of PCA applied to 2 groups of spectra (control and irradiated by 400 of 2MeV protons) in the nucleic

acids spectral range showing a three dimensional Scores Plot (A) along with the corresponding Loadings Plot (B).

The results show a dose dependent shift of the O–P–O asymmetric stretching mode from 1235 cm^{-1} to 1245 cm^{-1}, consistent with local disorder in the B-DNA conformation along with a change in intensity of the O–P–O symmetric stretching band at 1098 cm^{-1} indicative of chromatin fragmentation - the natural consequence of a high number of DNA Double Strand Breaks (DSBs). 2D mapping collected at IRENI (Infrared Environmental Imaging) beamline of characteristic functional groups at the diffraction limit has showed evidence of lipid deposition and chromatin condensation in cells exposed to protons indicative of cell apoptosis following irradiation (Fig. 2).

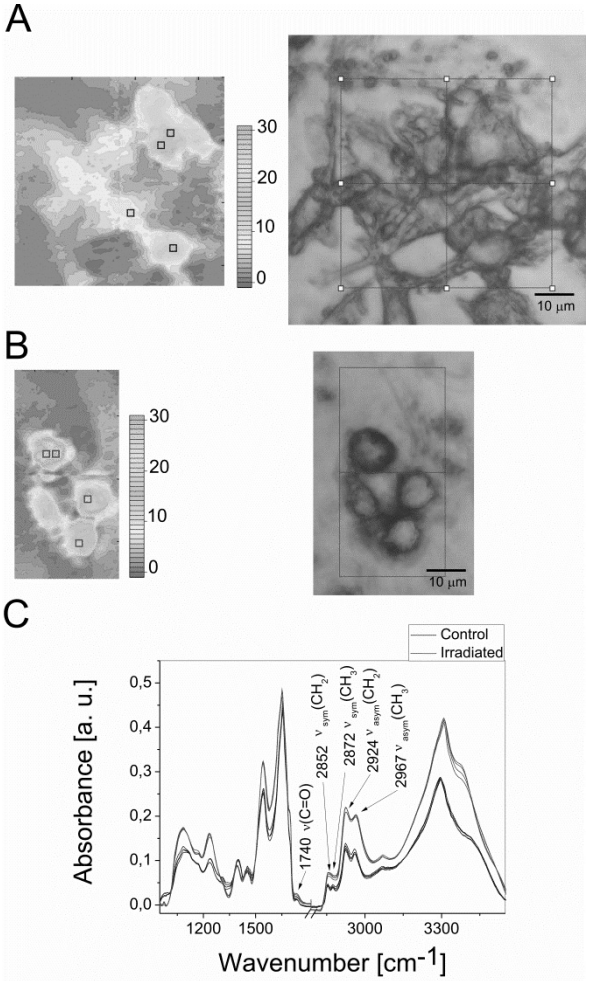

Figure 2 The distribution of CH_2, CH_3 stretching modes mainly from lipids bands (the integrated area of spectral range 3000 cm^{-1}-2800 cm^{-1}) together with corresponding microscopic images of control (A) and irradiated cells (B) and extracted averaged spectra from marked areas (C).

Bystander Effect

Synchrotron Radiation - Fourier Transform Infrared (SR-FTIR) microscopy coupled with multivariate data analysis was used as an independent modality to monitor the cellular bystander effect. Single, living prostate cancer PC3 cells were irradiated with various numbers of protons, ranging from 50-2000 with an energy of either 1 MeV or 2 MeV using a proton microprobe [5, 6, 7]. SR-FTIR spectra of cells fixed 24 h after exposure to protons and non-irradiated neighboring cells were recorded. Spectral differences were observed in both the directly targeted and non-irradiated neighboring cells (bystander cells) and included changes in the DNA backbone and nucleic bases along with changes in protein secondary structure. Principal Component Analysis (PCA) was applied to investigate the variance in the entire data set. Neighboring cells which spectra were clustered together with irradiated ones were considered as bystander cells. The percentage of bystander cells versus the applied number of protons with two different proton energies (1 MeV and 2 MeV) was calculated. It was found that out of all the applied doses, 400 protons at 2 MeV was the most effective in causing significant macromolecular perturbation in PC3 cells. [6]

"Sun light" influence on living cells

The response after laboratory "Sun light" exposure of COLO–679 cell line was successfully studied by SR–FTIR with subsequent multivariate data analysis. Living cells and isolated cellular nuclei after appropriate incubation time post irradiation, were investigated. The dose dependent intensity decrease of the C=O stretching mode at 1713 cm^{-1} is caused by base–pair damage such as purine, pyrimidine dimers formation or 6–4 lesions. The shift of O–P–O asymmetric stretching from 1230 cm^{-1} to 1240 cm^{-1} is associated with a partial conformation change from B–DNA to A–DNA. Additionally, an intensity increase in the Amide II band at 1549 cm^{-1} was observed following UV radiation exposure. This effect is possibly related to DNA repair, because the enzymes involved in DNA repair are mainly proteins and an increase in the amount of protein would result in an increase the intensity of Amide II band in the FTIR spectrum. This conclusion is confirmed by the observed intensity decrease of O–P–O symmetric and asymmetric stretching mode (1088 cm^{-1} and 1230 cm^{-1} – 1240 cm^{-1}) for cells irradiated for 4 minutes and 40 minutes, indicating that after radiation exposure the cells are stopped in G1 phase during the DNA repair stage. A nuclei isolation is recommended in study of the response to radiation using SR–FTIR. The spectral profiles of isolated nuclei and living cells are similar, however in nuclei spectra the DNA bands are more clearly defined and include the O–P–O stretching at 1088 cm^{-1}, 1170 cm^{-1}, 1230 cm^{-1} and C–O stretching at 970 cm^{-1} as well as base stacking mode: C=O stretching in purines and pyrimidines rings at 1713 cm^{-1}. [8]

The results presented herein enhance the knowledge about the interaction between radiation and living matter in a cell population, single cells, single extracted cellular nuclei, double stranded DNA and other radiobiologically relevant molecule levels, which has important implications in radiation effects protection and the treatment of tumors.

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