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Potential for biomedical applications of Positron Annihilation Lifetime Spectroscopy (PALS)

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Abstract. Positron Annihilation Lifetime Spectroscopy (PALS) allows examining structure of materials in nano and sub-nanometer scale. This technique is based on the lifetime and intensity of ortho-positronium atoms in free volumes of given structures. It is mostly used for studies in material sciences, but it can also be used for in vivo imaging of the cell morphology as proposed in [1], [2]. Cancer cells are characterized by an altered macro structure in comparison to normal cells, thus the main objective of these studies is to compare if these differences can be detected on sub-nanometer level with PALS technique. First studies on standard PAL spectrometers conducted by Jean [3],[4] and J-PET collaboration [5], [6], give promising results showing differences between normal and cancer tissues.

This perspective will allow for simultaneous determination of early and advanced stages of carcinogenesis, by observing changes in biomechanical parameters between normal and tumour cells, and standard PET examination, which can be performed with the Jagiellonian Positron Emission Tomograph (J-PET), a multi-purpose detector used for investigations with positronium atoms in life-sciences as well as for development of medical diagnostics. Results of first PALS measurement of cardiac myxoma, with J-PET detector is presented in this paper. Obtained o-Ps lifetime for tumor tissue is equal to 2.03(01)[ns] and its intensity 25.7(1)%.

INTRODUCTION

Proposed investigation mainly concerns the studies of the cellular organization and structure at nano and sub-nanometer level and its relation to the cell morphology and signalling. Correlations between cellular processes such as proliferative activity and proapoptotic sensitivity (activity) and Positron Annihilation Lifetime Spectroscopy (PALS) parameters can be determined. Different living normal and cancer cells and tissues are being investigated in order to connect PALS parameters to carcinogenesis and metastatic processes.

In our studies we test the hypothesis [1], [2] that cancer cells differ in their sub-nanostructure, molecular interactions or activity, which are specific for cancer from normal ones. These changed molecular interactions may influence their molecular properties that can be detected as the changed PALS signal. Such detailed research in this area have not been conducted so far, mostly due to the limitations of experimental techniques. Currently used in cell biology methods with high resolution such as Confocal Microscopy [7] or Scanning Electron Microscopy [8], require usage of fluorescent labels or fixed and dehydrated samples, thus all these studies can be performed only in vitro and can't be applied for in vivo patient diagnostic. Observed changes in biomechanical parameters between normal and tumor cells can be correlated with parameters obtained from PALS technique. Combining this technique with J-PET detector [9], [10], [11] would make possible in vivo studies of human cells and tissues in nanometer scale and could be used as additional diagnostic parameter.

DIFFERENCE BETWEEN STRUCTURE AND METABOLISM IN NORMAL AND CANCER CELLS

Cells and tissues are inherently very complex in their structure. Main research in cell biology and biophysics aims to explain and understand how living processes, which occur on the cellular level are regulated in different spatial

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and temporal domains. The main challenge in these studies is that the living objects are built of different cellular and molecular structures and chemical compounds, which function depend on many environmental and intracellular factors.

Cancer cells differ from normal ones by their structure and organelles functionality. These cells are irregular in shape, have a reduced amount of cytoplasm, a bigger and often multiplied nuclei. Organelles such as the Golgi apparatus, mitochondria or endoplasmic reticulum become disorganized or have impaired function [12]. Even though main factors responsible for DNA mutations leading to cancer have been broadly studied over the last few years, there is slight evidence about the molecular structure of the cell, their ultrastructure and the difference between normal cells and cancer cells in proliferation, protein and lipid synthesis and intracellular transport and how it can related to positronium lifetime.

Cancer cells can be divided into two types: benign and malignant. Benign cells have a similarity to their stem cell and are well differentiated, and therefore tumors are progressing slowly as an encysted change. These tumors do not form metastases. Malignant tumors have less differentiated cells and the ability to infiltrate surrounding tissues and cause metastasis [13].

Cardiac myxoma

Cardiac myxoma is very rare heart disease with an overall incidence of about 0.5 per million per year but accounts for approximately 50-75% of benign and about half of the all primary cardiac neoplasms. The tumor usually arises from the endocardium into the cardiac chamber. About 75% of cardiac myxomas are located in the left atrium, and 15-25% in the right atrium. The cells which form the tumor are considered to be remnants of subendocardial vasoformative reserve cells or multipotential primitive mesenchymal cells that persist as embryonal residues during septation of the heart. The precursor mesenchymal cells can differentiate into endothelial and epithelial cells, smoothmuscle cells, angioblasts, fibroblasts, cartilage cells, and myoblasts forming myxoma tumor. The only method of treatment of cardiac myxoma is radical surgical tumor excision using extracorporeal circulation [14].

POSITRON ANNIHILATION LIFETIME SPECTROSCOPY

Positronium is a hydrogen like atom and, at the same time anti-atom. It is build out of electron and its anti-particle positron. Its bounding energy is equal to 6.8 eV and its diameter amounts to about 0.2 nm, and hence it is sufficiently small to be trapped in the volumes of lower electron density, so called free volumes between molecules in various living and non-living matter. The smaller are the free voids between molecules and atoms the shorter the o-Ps lifetime. This is due to the pick-off processes in which positron from the positronium may annihilate with one of the surroundings electrons. Positronium may be formed in tryplet state as para-positronium (p-Ps), with the average lifetime in a vacuum of $\tau_{p-Ps} = 0.125\ ns$, or in singlet state as ortho-positronium (o-Ps) with the average lifetime in a vacuum of $\tau_{o-Ps} = 142\ ns$. Based on theory by Tao [15] and Eldrup [16] correlation between the lifetime of the ortho-positronium atoms with the free voids size can be determined. These changes of the o-Ps lifetime and intensity of its production are crucial for the biological research. What is important, positronium can also be created in liquids such as water what allows to study living cells. The average lifetime of o-Ps in pure liquid water amounts to about 1.8 ns [17].

Current PALS application in biology

There are few works demonstrating the application of PALS technique to study biological structures. Jean and his group [18], [19] described some applications of positron annihilation techniques in biological systems. They focused on studies of healthy and abnormal skin samples and reported that o-Ps lifetime and therefore free volumes are correlated with the level of skin damage. Both intensity and lifetime of o-Ps were found to be significantly lower in samples with basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) than in normal skin samples. These studies were performed on fixed samples as well as in ambient conditions [3],[4].

Studies conducted by Pietrzak et. al. [20] examined the dependence of PALS parameters in hematocrit and plasma of blood from women with breast cancer before and after the chemotherapy or radiotherapy. It was shown that the mean ortho-positronium lifetime in the blood hematocrits changes from 2.16 ns before the therapy to 2.34 ns after the radiotheraphy. Importantly, it was also found that the o-Ps lifetimes of the patients treated with radiotheraphy and the healthy persons are the same. Results published by Yas et. al. [21] shown dependency of o-Ps lifetime in mause adenocarcinoma tumor over tumor age. It was found that for control (normal tissue) o-Ps lifetime was equal about 2.9

ns, in time, during tumor formation and growth lifetime was significantly decreasing and after 26 days it was equal to around 1.9 ns. Another example of advanced PALS application in studies of cell culture is the paper by Axpe et al. [22], where they employed well defined colorectal cancer cell lines grown in the 3D matrix. Axpe shown how addition of a growth factor $TGF-\beta$ induces changes at the atomic scale in the size of the free volume voids, due to the biological effects. This studies were carried out in 4 C deg. and showed the possibility of using PALS for live cells research.

Some recent PALS studies performed by the J-PET collaboration with simple model micro-organisms unicellular yeasts *Saccharomyces cerevisiae* [23], shows the possibility to observe dynamics of the water sorption by lyophilised (freeze - dry yeast). Lifetime of ortho-positronium was found to change from 2.4 to 2.9 ns (longer-lived component) for lyophilized and aqueous yeasts, respectively. Also some temporal changes of the o-Ps lifetime indicating reorganization of yeast in the molecular scale in the presence of liquid water was observed.

Recently, significant differences in PALS parameters between normal and tumor tissues were also observed by the J-PET group in samples of uterine leiomyomatis and normal myometrium tissues taken from women-patients after surgery [5], [6]. In case of all samples from six patients it was found that mean o-Ps lifetime are of about 2 ns, while for normal tissues this value is of about 50 ps lower. The above discussed results indicate that in all investigations performed to date there is a difference in PALS parameters determined for healthy and cancerous tissues.

o-Ps lifetime as a new diagnostic parameter with J-PET

All studies described in previous section brought important information, showing that PALS technique can be applied in studies of biological structures and human cells and tissues, thus has potential as application in cancer diagnostic. Nevertheless, all shown studies with PAL spectrometer can only be conducted in vitro. With J-PET scanner which is a multi-purpose detector used for investigations with positronium atoms in life-sciences as well as for development of medical diagnostics, such simultaneous PET imaging and PALS measurement is possible, therefore J-PET is capable of imaging properties of positronium produced inside the human body [1], [2].

Currently detector is calibrated and first PALS studies of porous material (XAD4 polymer) were performed, proving J-PET can be successfully used for PALS and gives the same results as a standard spectrometer [27], [28] for studied material. This allows for use of the J-PET scanner for PALS measurement with human tissues. The method of lifetime spectrum reconstruction was prepared and tested. The energy loss is estimated by Time Over Threshold (TOT) technique, which allows to differentiate between deexcitation and annihilation gamma quanta. This method is precisely described in [1], [24], [28].

First such studies were performed on J-PET with Cardiac Myxoma [14] tumor. Studied sample was fixed in formaldehyde in order to avoid influence of tissue decomposing in time of measurement (2 hours). The J-PET data are collected in the triggerless mode by the dedicated electronics [29] and the digital data acquisition system [25]. In Fig.1 (right) obtained with J-PET lifetime spectra with time resolution of about 270 ps (sigma) was acquired. o-Ps lifetime and intensity was calculated by PALS Avalanche program [27] with results $\tau_{o-Ps} = 2.03(01)$ and $I_{o-Ps} = 25.7(1)\%$ respectively. With this measurement we have proven that J-PET can be used not only for PET imaging but also for PALS studies of human tissues. Lifetime value is similar to those measured by Jasińska for uterine leiomyomatis [5].

SUMMARY

Presented idea of applying PALS for biological studies is quite a new concept, so far few studies, including ones conducted by the J-PET collaboration [23], [5],[6] indicate that this technique can be used successfully to differentiate between normal and cancer tissues, therefore can be used in medical diagnostic. Differences between structure and metabolisms of normal and cancer cells, were discussed in this article, but till now there is no explanation what is the reason behind differences observed in PALS. Results for first measurement of human tissue on J-PET detector were presented and are in agreement with results measured on standard PAL spectrometer. Nevertheless detailed studies with high statistic will need to be preformed, main difficulty lies in the fact that myxomas are very rare tumors, and therefore such study will be prolong in time. Also measurement with different types of tumors and comparison with normal tissue would allow for broader analysis. Such information will be crucial for biomedical application. Planned measurements with well defined cell lines possible with use of J-PET scanner will allow to study influence of different factors, like free radicals, reactive oxygen species and glucose metabolism, and thus can lead to some possible model of positronium trapping in living matter and perhaps some new information on carcinogenesis.

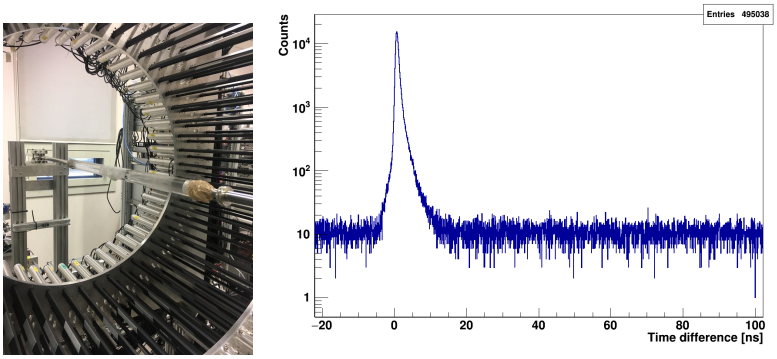

FIGURE 1. (left) Photo of J-PET scanner with Cardiac Myxoma inside the holder during measurement. (right) Positronium lifetime spectrum obtained with the J-PET for cardiac myxoma.

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