The development of a method for determining ortho-Positronium lifetime in extracellular vesicles using Positron Annihilation Lifetime Spectroscopy

J. Niziol[1,2], E. Kubicz[1,2], E. Stępień[1,2], P. Moskal[1,2]

[1] Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Kraków, Poland
[2] Total-Body Jagiellonian-PET Laboratory, Jagiellonian University, Poland

Motivation

Extracellular vesicles (Evs) are regarded as novel diagnostic and prognostic biomarkers for many diseases. Various studies also suggest that EVs have several advantages over conventional synthetic carriers and highlight their potential as biological medicines or drug delivery systems [1]. Use of **Positron Annihilation Lifetime Spectroscopy (PALS)** in this area of research has not been described so far, though it is a promising tool for a direct measurement of EVs, able to provide information at the molecular level [2].

Introduction

EVs are a heterogeneous group of spherical nano-structures assembled from a complex mixture of various lipids and proteins (fig.1). They are released into the extracellular space by different types of cells and can be found in several body fluids.

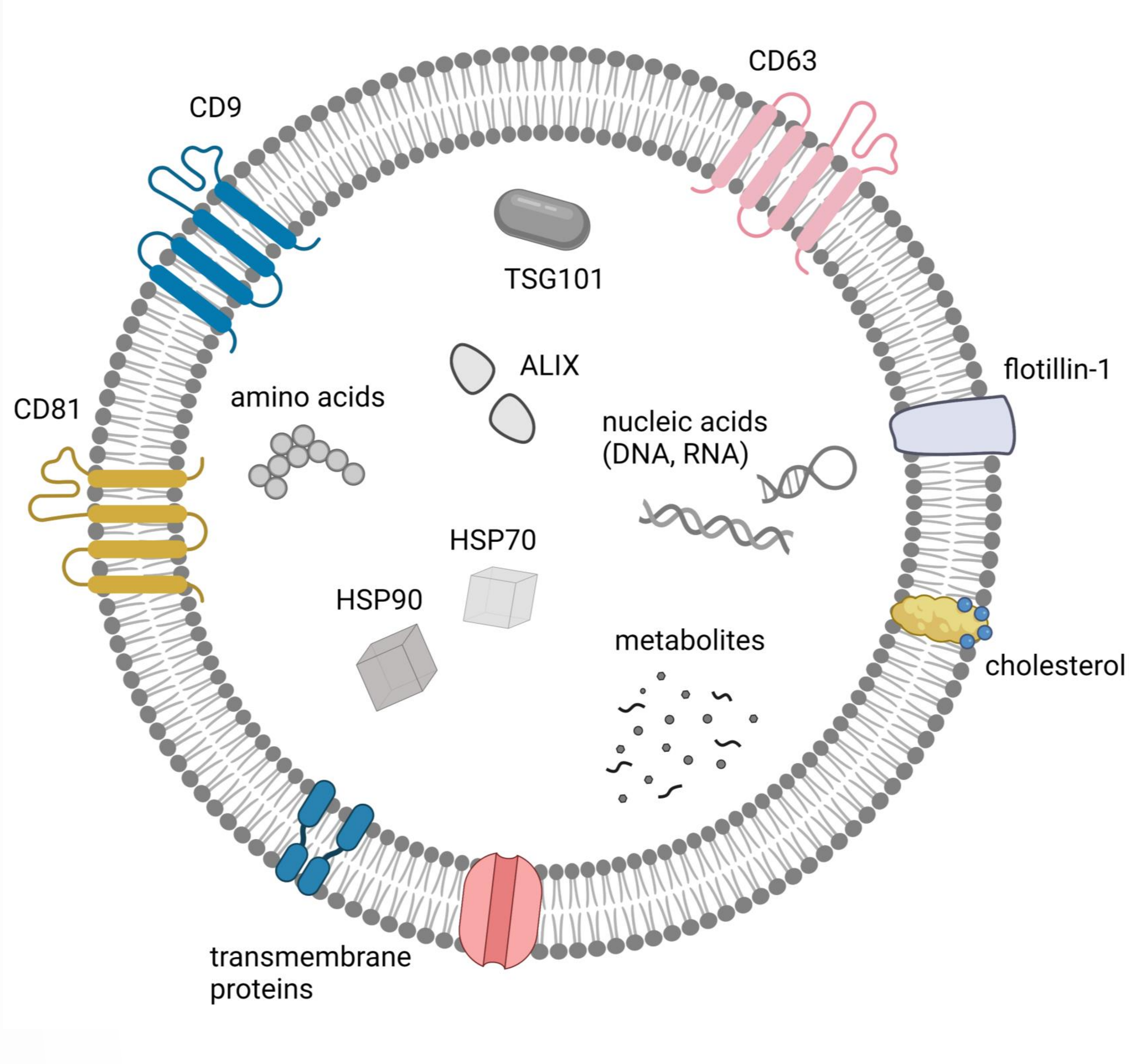

Fig. 1. Scheme of the exemplary exosome structure. [Created with BioRender.com.]

In PALS, the principle of operation is based on thermalized positrons that can form a quasi-bound state with electrons (fig.2), known as **positronium (Ps).** In triplet state (ortho), it localizes within void-content of investigated material and eventually picks-off an electron from neighboring atoms to annihilate within few ns which can then be correlated with the free volume nanohole radius [4].

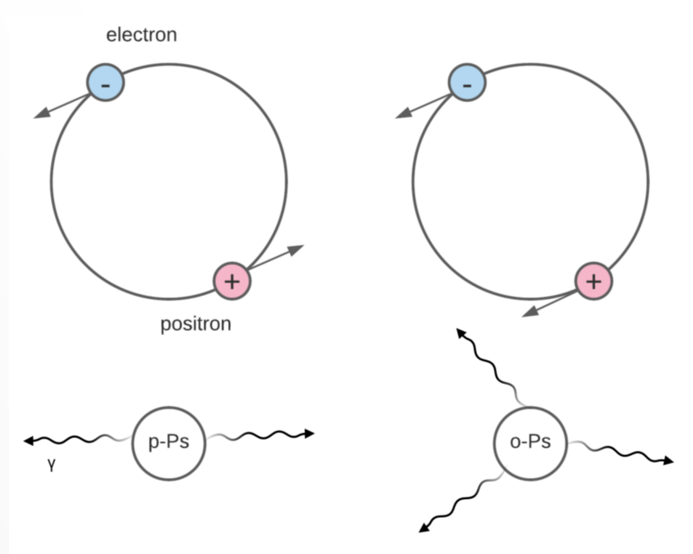

Fig. 2. Positronium atom in two possible quantum states: para (left) and ortho (right), with corresponding decay patterns.

Materials and Methods

The medium collected from **normal pancreatic beta-cell cultures** was subjected to multiple centrifugations and low-vacuum filtration in order to isolate EVs.

EV concentrations in the samples were determined using **qNano technique**.

The positronium lifetime was measured with a pair of scintillation detectors, one detecting the creation of a positron (START) and the other its annihilation (STOP) as shown in fig. 4. The time difference between START and STOP signals is a measure of the positronium lifetime in the material.

To enable studies of liquid samples in **temperature-controlled conditions**, the system was additionally equipped with Lauda LOOP L100 thermostat.

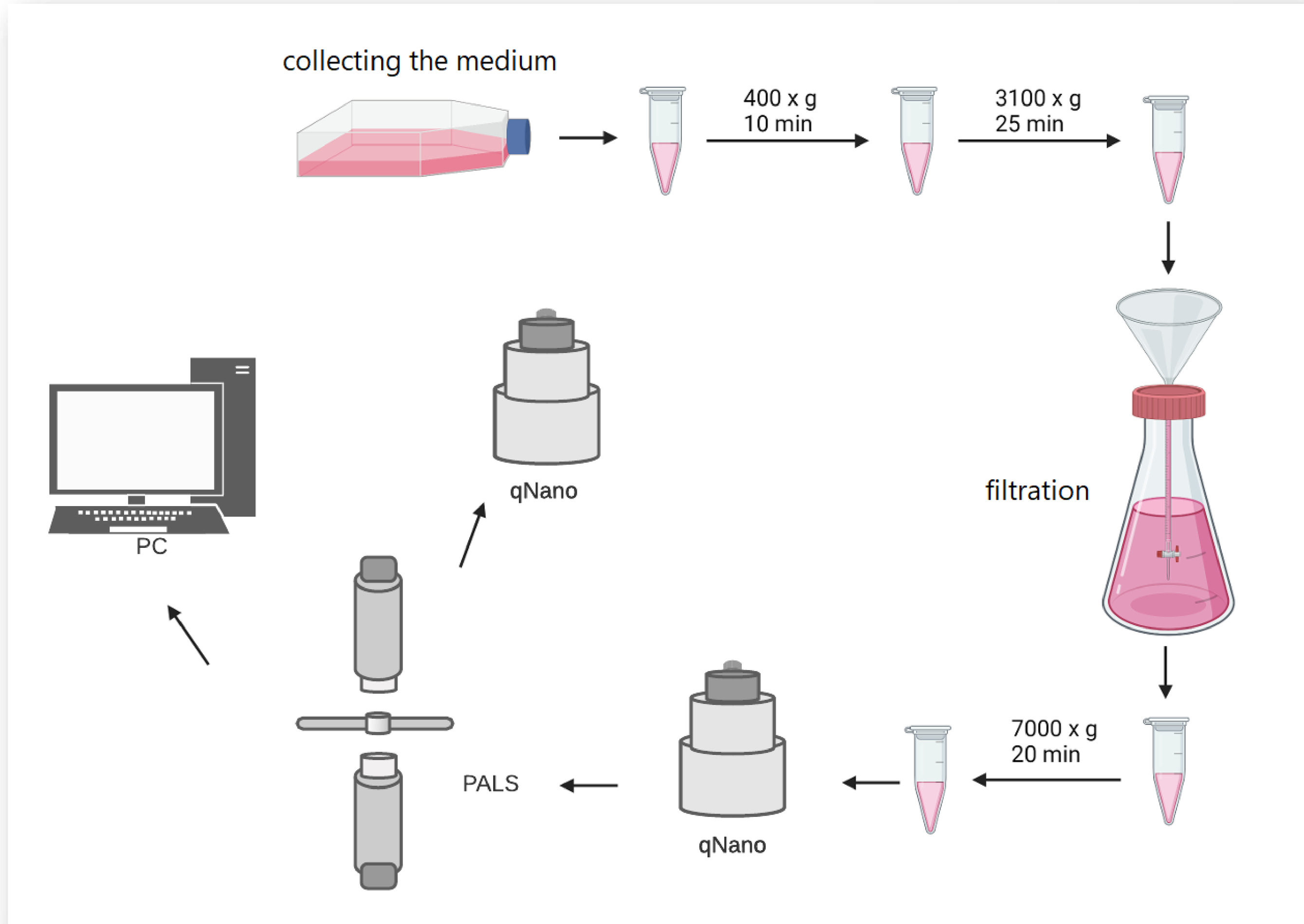

Fig. 3. Scheme describing individual steps of preparation and analysis of EV samples.

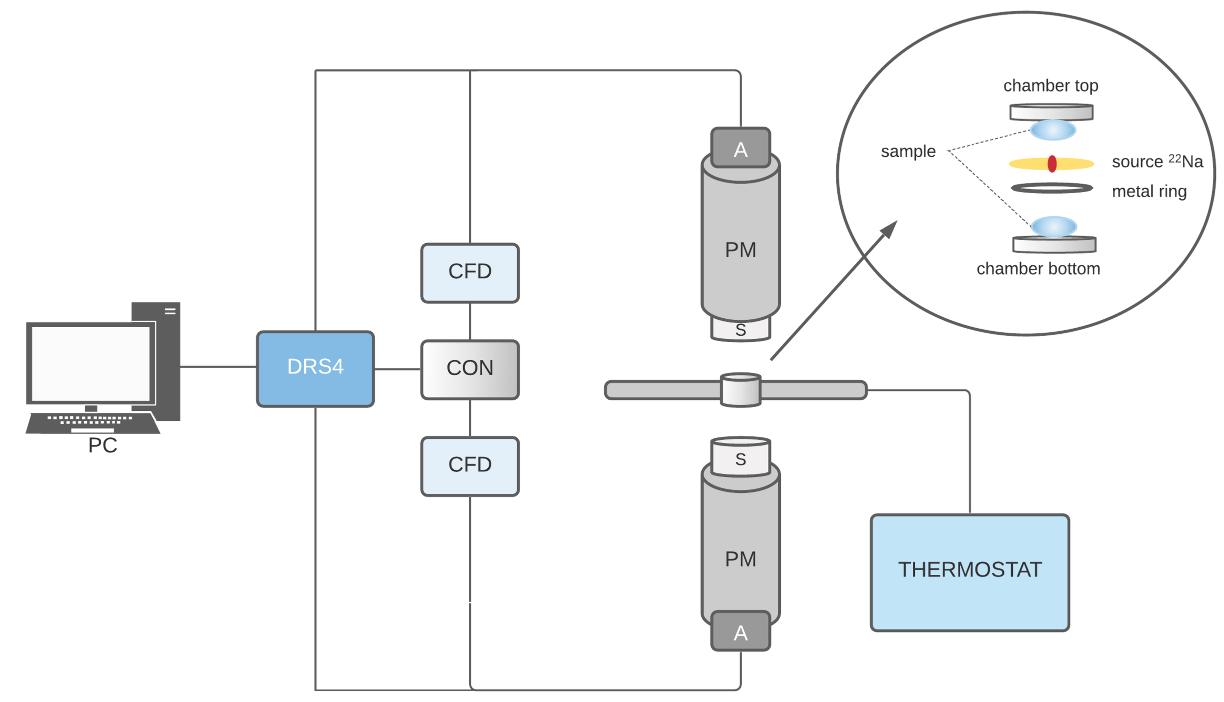

Fig. 4. Scheme of the detection system and chamber construction: DRS4 - evaluation board, CON - coincidence module, CFD - constant fraction discriminator, A - attenuation, PM - photomultiplier, S - scintillator.

Results

Temperature stability of the system was determined. Fig. 5 presents obtained calibration curve, based on which it is possible to accurately determine the temperature of the sample during the measurement.

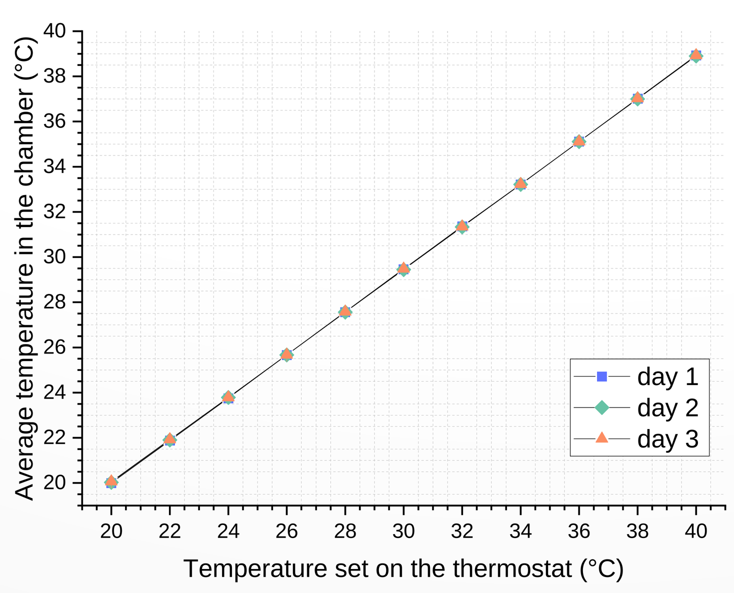

Fig. 5 Calibration curve plotted on the basis of repeatable measurement describing dependence of the temperature reached inside the measuring chamber on the value set on the thermostat.

Two samples of EVs suspended in PBS were used for analysis: from culture carried out under **(1) normoglycemic** and **(2) hyperglycemic** conditions.

EV concentrations in these samples, determined before PALS measurement, were respectively: **(1) 9×10^{10}** and **(2) $6{,}9 \times 10^{10}$ particles/mL.**

The positronium lifetime measured in EV samples was (1) 1.80 ns and (2) 1.77 ns respectively.

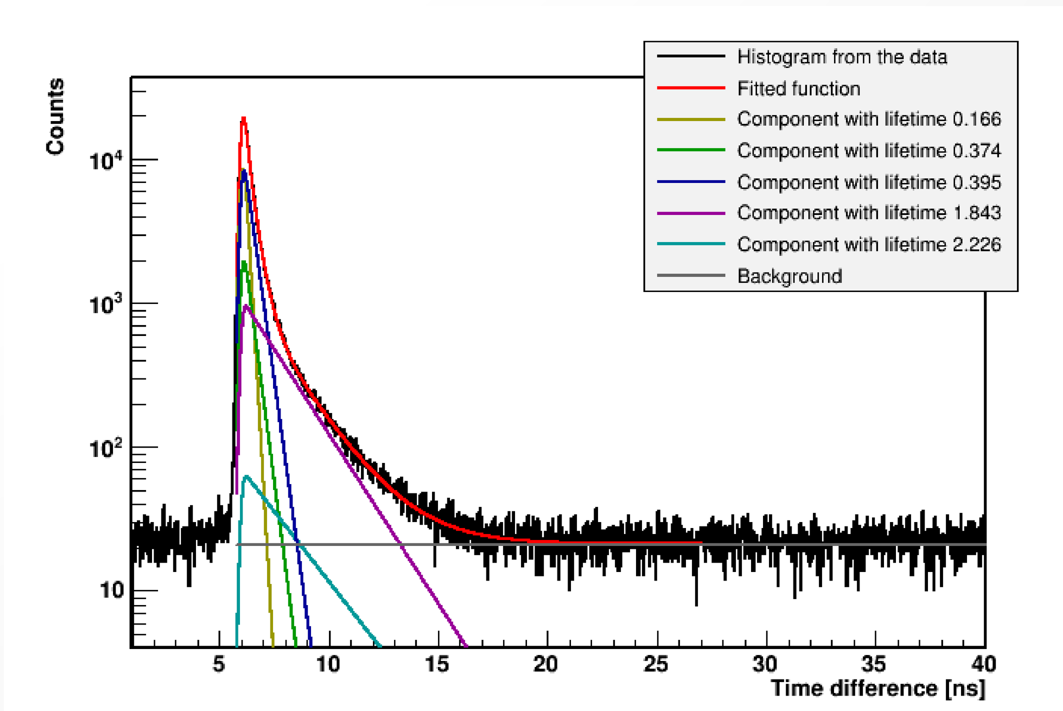

Fig. 6 Exemplary positronium lifetime spectrum (obtained for a sample of EVs isolated from a cell culture under normoglycemic conditions) with superimposed lines representing the distributions of individual components: yellow – pPs, green – annihilation in the source material, turquoise – annihilation in parafilm, blue – free positron annihilation, purple – o-Ps.

References

[1] Surman, M. et al., Current pharmaceutical design vol. 25,2: 132-154 (2019).
[2] Sane, P. et al., The journal of physical chemistry. B vol. 113,7 (2009).
[3] Stępień, E. et al., Expert opinion on therapeutic targets vol. 16,7: 677-88 (2012).
[4] Kubicz, E., Doctoral Thesis, Jagiellonian University (2020).
[5] Nizioł, J., Bachelor Thesis, Jagiellonian University (2021), http://koza.if.uj.edu.pl/theses/.

Acknowledgments

This work was supported by the Foundation for Polish Science through the TEAM POIR.04.04.00-00-4204/17 programme and OPUS 17 nr 2019/33/B/NZ3/01004.

European Funds Smart Growth
FNP Foundation for Polish Science
European Union European Regional Development Fund

Conclusion

Preliminary results suggest strong correlation between mean o-Ps lifetime and EV concentration in the sample. Studied concentrations of EVs were too low, therefore it was mainly the PBS solution that was contributing to the resulting o-Ps lifetime value, and not the EVs itself. Obtained result opens perspective for further research, when applying higher EV to PBS ratio.