Influence of antioxidants on positronium lifetime – studies of melanocyte and melanoma cell cultures with Positron Annihilation Lifetime Spectroscopy

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Cybernetic Modeling of Biological Systems (MCSB 2021)

1. Motivation

→ Positronium as a novel biomarker in cancer diagnostic

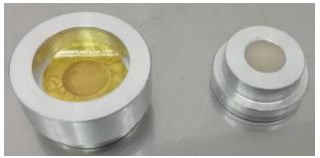
→ Possibility to determine early and advanced stages of carcinogenesis

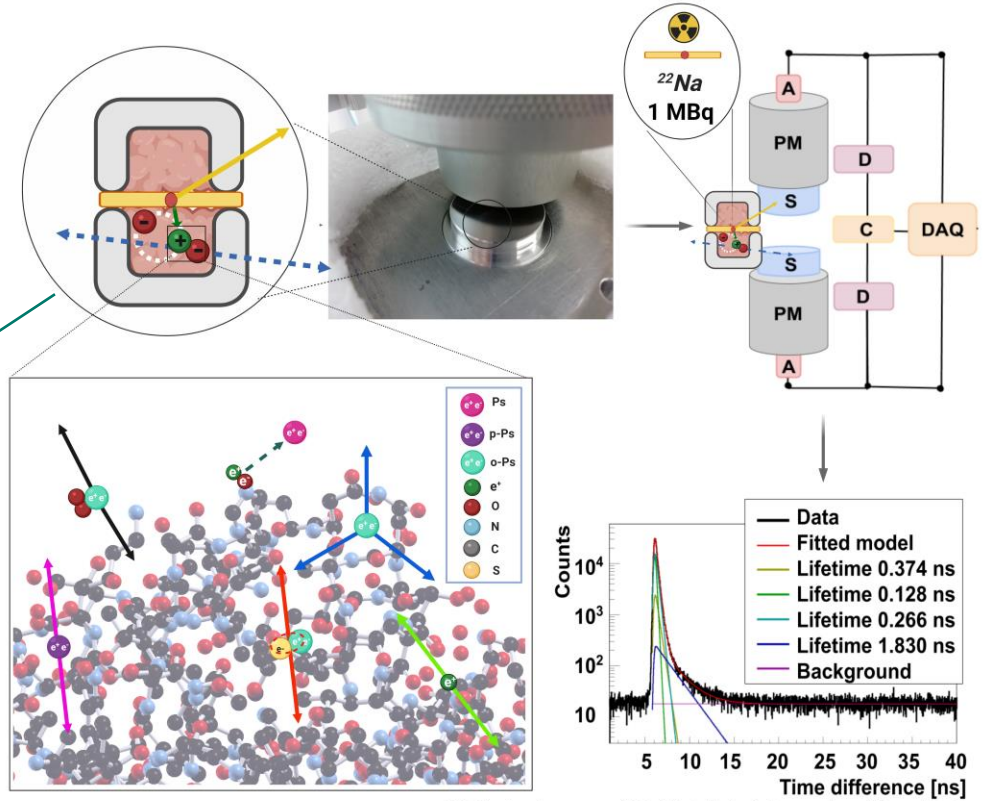
→ Correlation of free radical concentrations with lifetime and intensity of positronium atom producted in human cell cultures of melanoma and melanocytes

2. Positron annihilation lifetime spectorscopy

→ Two BaF_2 detectors with resolution ~250 ps (FWHM)

→ ^{22}Na source in Kapton foil with activity ~ 1 MBq



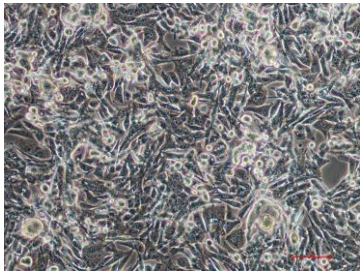


PALS Avalanche program , K. Dulski et al., Analysis procedure of the positronium lifetime spectra for the J-PET detector, Acta Phys. Polon. B48 no. 10, 1611 (2017)

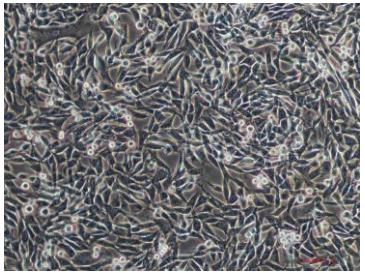
3. PALS studies of cells culture in vitro

Human cell lines:
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC

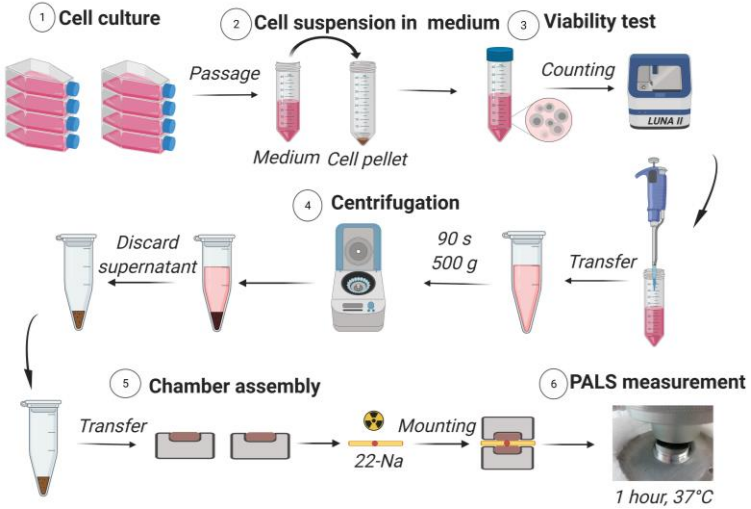
HEMa-LP



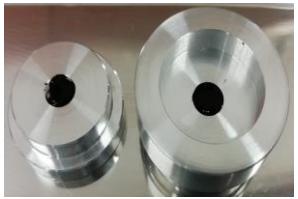
WM115



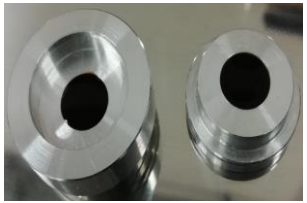
WM266



Each samples contains around >10^8 cells



HEMa-LP

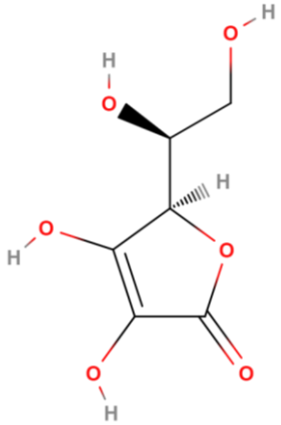


WM 115

WM266-4

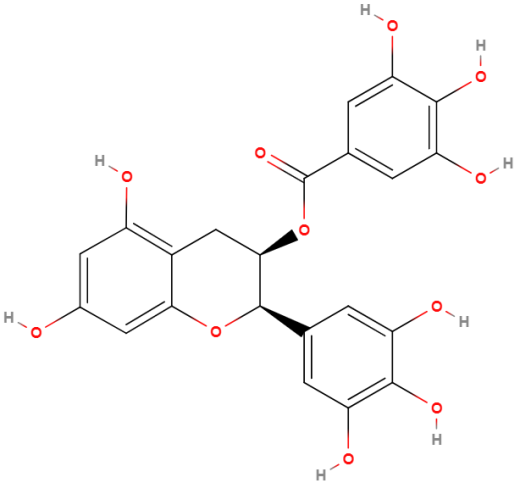
4. PALS - cell culture with Vitamin C and EGCG

FR scavengers → eg. antioxidants, prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition.



Vitamin C (L-ascorbic acid) → found in various foods, functions as an antioxidant

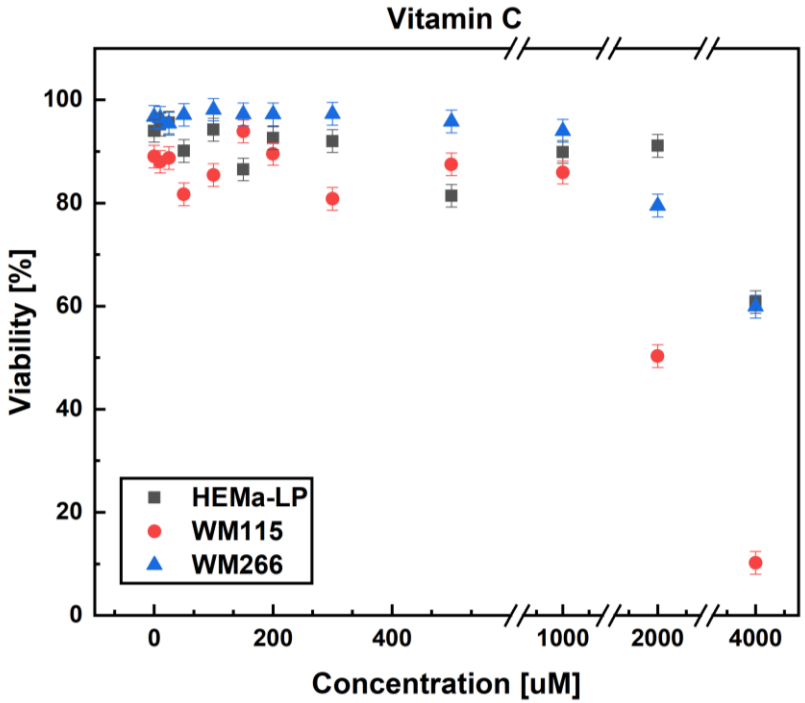
EGCG (Epigallocatechin gallate) → found mostly in green tea, 100x more powerful antioxidant than Vit. C

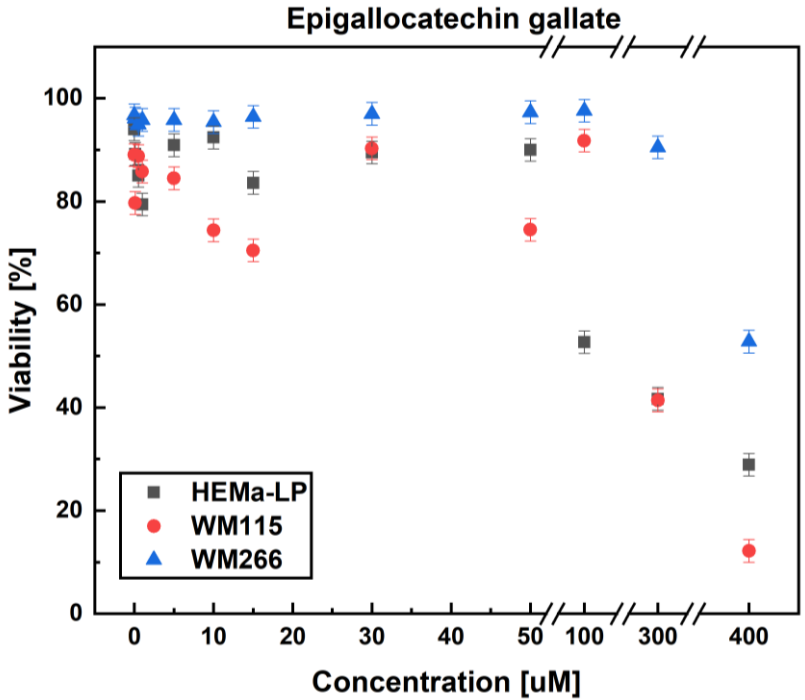


→ Before PALS measurement each flask was incubated for 2 h with media and antioxidant substance in given concentration

5. Antioxidant - viability

Cytotoxicity of EGCG and Vit C. → cell viability was checked after 24 h incubation in given concentration





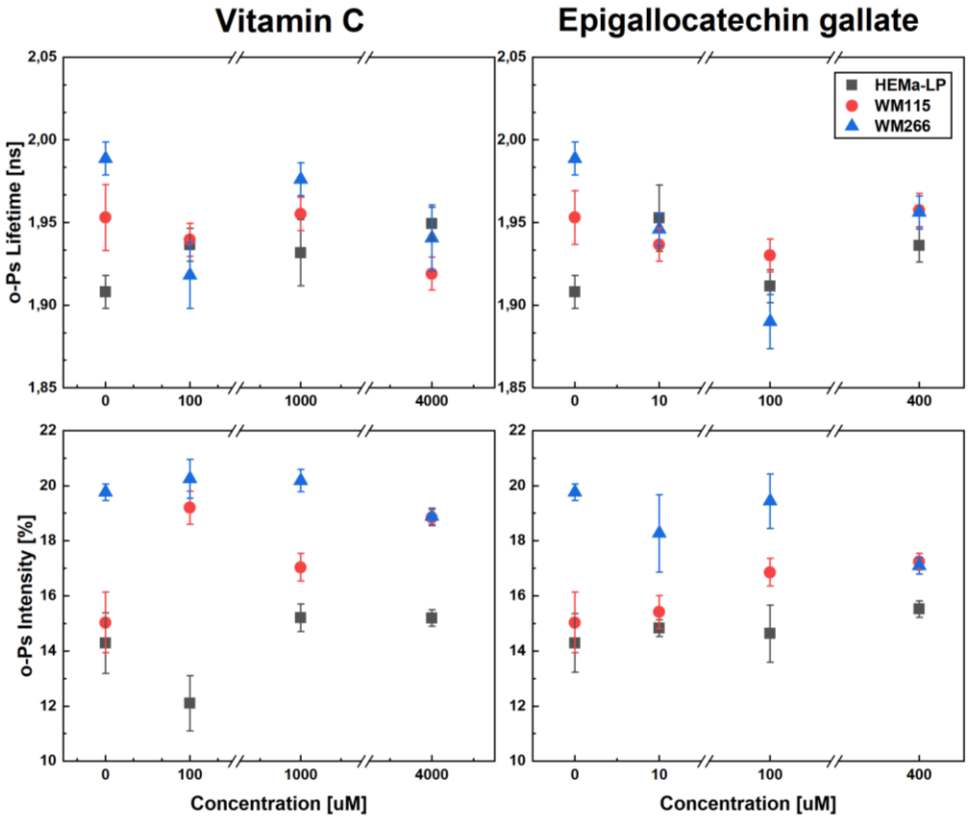
6. Vitamin C, EGCG - PALS results

Rate of change =
$100\% *(V_{before} - V_{after})/ V_{after}$

Vit. C	HEMA	WM115	WM266
Concentration [uM]	Viability RoC [%]	Viability RoC [%]	Viability RoC [%]
0	3,6(1)	6,0(1)	0,2(1)
100	10,4(1)	9,5(1)	0,5(1)
1000	4,7(1)	0,6(1)	0,3(1)
4000	6,7(1)	4,0(1)	1,8(1)

EGCG	HEMA	WM115	WM266
Concentration [uM]	Viability RoC [%]	Viability RoC [%]	Viability RoC [%]
0	3,6(1)	6,0(1)	0,2(1)
10	6,0(1)	1,6(1)	1,4(1)
100	9,5(1)	8,1(1)	0,2(1)
400	4,1(1)	0,1(1)	0,7(1)

7. Vitamin C, EGCG - PALS results



5. Summary and future plans

→ PALS is applicable to study biological structures.

→ Preliminary results shown that PALS parameters differ for normal and cancer cells and tissue.

→ Highest differences in both o-Ps lifetime and intensity can be observed for lowest and highest concentration of vitamin C (100, 4000uM) and 100uM for EGCG.

1. Moskal, P., Jasińska, B., Stępień, E. & Bass, S. D. *Positronium in medicine and biology*. Nature Reviews Physics 1, 527–529 (2019).
2. Moskal, P. & Stępień, E. *Prospects and Clinical Perspectives of Total-Body PET Imaging Using Plastic Scintillators*. PET Clinics 15, 439–452 (2020).
3. Kubicz, E. *Potential for biomedical applications of positron annihilation lifetime spectroscopy (PALS).* in AIP Conference Proceedings 2182, (American Institute of Physics Inc., 2019).

Acknowledgements:
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Thank you for attention