Free radicals influence on the positronium lifetime in melanocytes and melanomas cell cultures

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Objectives

- Positronium, a bound state of positron and electron has been proposed as a novel biomarker for examining cancer cells.
- Our pre-clinical studies have shown significant differences in the lifetime of positronium between normal and neoplastic cells and tissues.
- Concentrations of free radicals, especially reactive oxygen species (ROS) have a significant influence on the properties of positronium, such as its lifetime and production.
- Investigateing the role of antioxidants, such as vitamin C and epigallocatechin gallate (EGCG), on the values of the newly proposed biomarker.
- In vitro cell culture of normal human cell: melanocyte HEMa-LP cell line and two cell lines of melanoma: WM115 (primary melanoma) and WM266-4 (metastatic melanoma) - exposed on various concentrations of vitamin C (100, 1000, 4000 μM) and EGCG (10, 100, 400 μM).

Positron Annihilation Lifetime Spectroscopy

Positronium is an atom consisting of an electron e- and its anti–particle positron e+ its diameter is about 0.2 nm.
It is possible for positronium to be trapped in free spaces between molecules, like for example in cells and tissues (Fig. 1).

Positron can annihilate with an electron not only from Ps, but also with electrons in surrounding matter, mean lifetime value of ortho-positronium trapped in these free volumes can be used to estimate their sizes.
Both lifetime and intensity of o-Ps production gives us an information about structure of given material and can be applied as a novel biomarker for cancer.

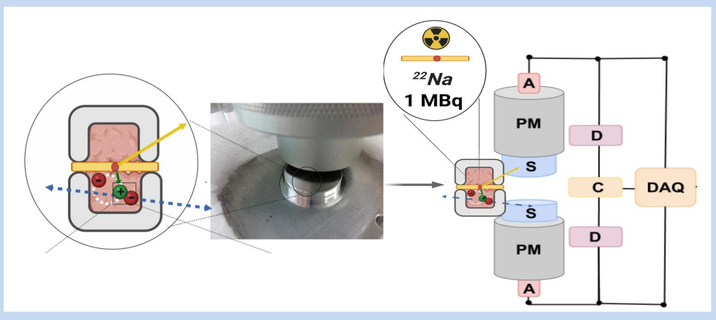

Fig. 1 Exemplary scheme presenting positronium trapping in molecule, PALS setup and positronium lifetime spectra.

Antioxidants and free radicals

Free Radicals 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

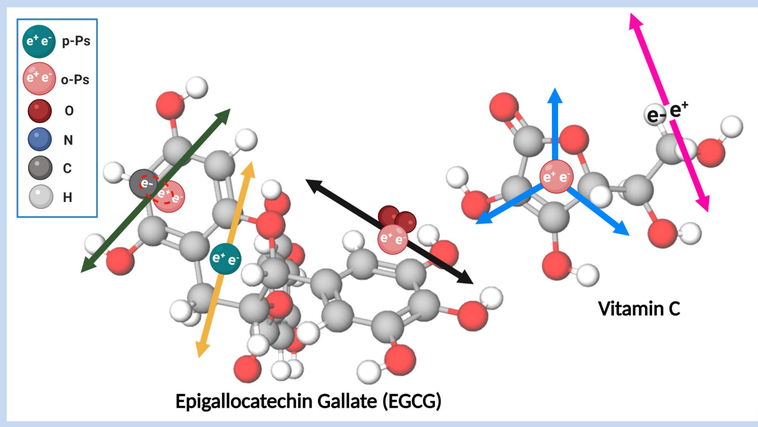

Fig. 2 Schematic view of positronium decay inside (left) EGCG and (right) vitamin C molecules. Around 60% of positrons inside the cells are annihilating directly with an electron (pink arrows). In the rest of the cases, positronium is formed. Positronium atom can be created in two forms: (i) short-lived (125 ps) para-Positronium (p-Ps indicated in teal), decaying into two photons (yellow arrows) or (ii) long-lived (142 ns) ortho-Positronium (o-Ps indicated in coral), which decays into three photons (blue arrows). o-Ps annihilation via pick-off proces - an interaction with an electron from the surrounding molecule (dark green arrows) or through the conversion to p-Ps *via* an interaction with oxygen molecules, which subsequently decays into two photons (black arrows).

Materials and methods

Human cell lines:
1) Melanocytes HEMa-LP
2) Melanoma WM115
3) Melanoma WM266-4

Cells were exposed to:
- Vit. C in concentration from 10 to 4000 μM
- EGCG in concentration from 1 to 400 μM

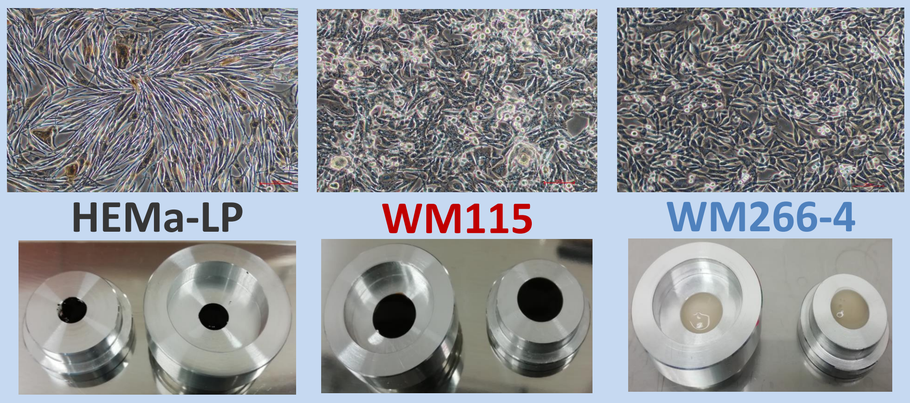

Fig. 3 (top) Micrograph showing cultures and (bottom) cell pellets inside the measurement chamber for (left) melanocytes, (middle) primary melanoma, (right) metastatic melanoma.

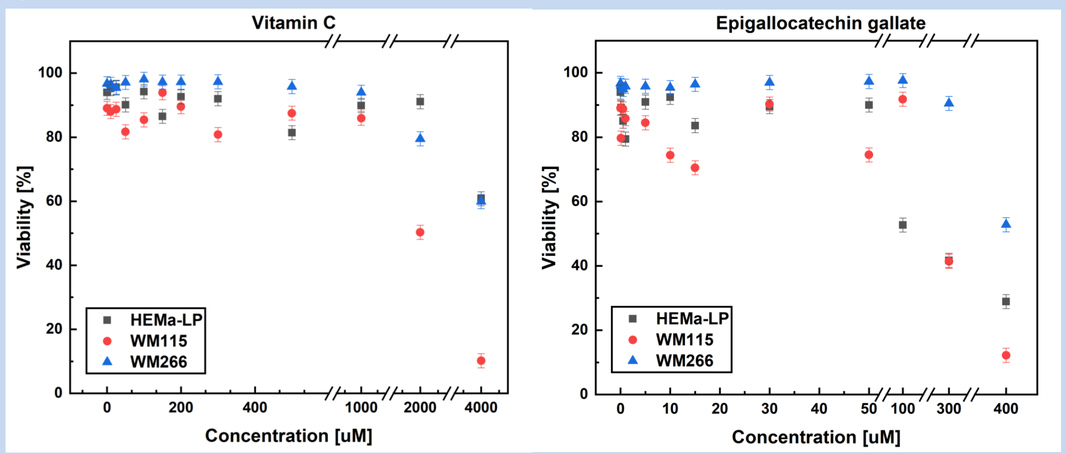

Fig. 4 Viability studies for cells exposed to various concentration of vitamin C and EGCG.

Results

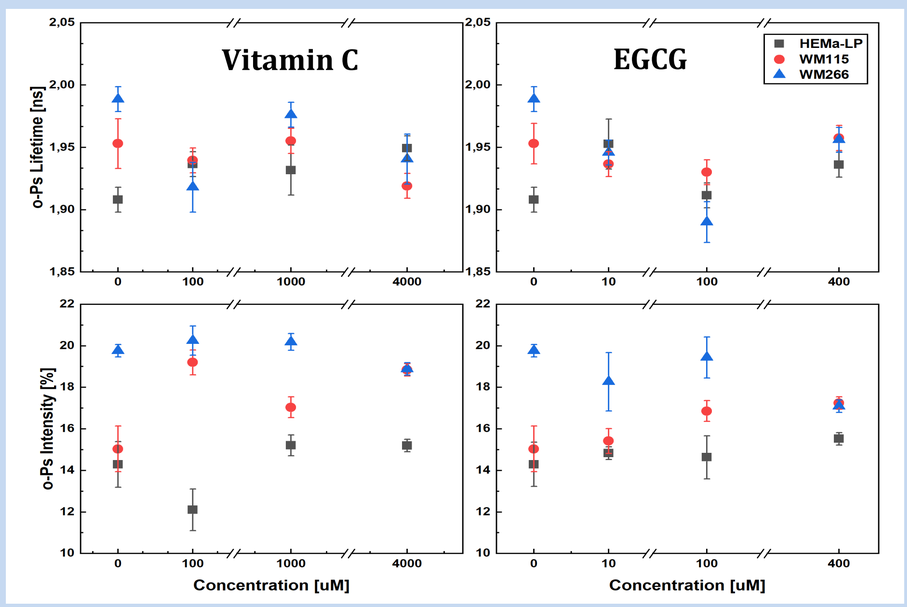

Fig. 5 Mean o-Ps litetime (top) and intensity (bottom) for melanocytes and both cell lines of melanoma exposed to vitamin C (left) and EGCG (right).

Resulting o-Ps lifetime in HEMa-LP, WM115 and WM266-4 cells was equal to 1.91(02)ns, 1.95(03)ns, 1.99(01)ns, respectively in control; 1.93(02)ns, 1.96(01)ns, 1.98(01)ns in 1000 μM concentration of vitamin C and 1.91(02)ns, 1.93(01)ns, 1.89(02)ns in 100μM concentration of EGCG.

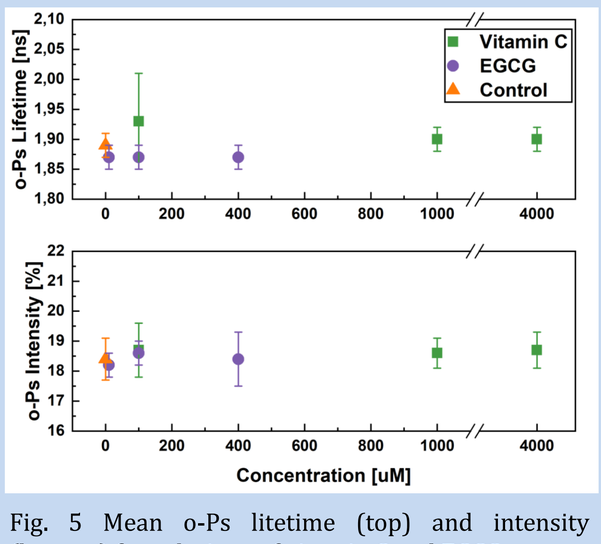

Fig. 5 Mean o-Ps litetime (top) and intensity (bottom) for solutions of vitamin C and EGCG.

No significant differences were observed in measured solutions or culture media without the cells, resulting in o-Ps lifetime equal to 1.91(02)ns, 1.88(01)ns in vitamin C and EGCG solution, respectively.

Concentration [uM]	HEMa-LP	WM115	WM266-4
0	3.6(1)	6.0(1)	0.2(1)
Vitamin C			
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			
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)

Tab. 1 Rate of change of cell viability before and after the measurement, calculated as: $100\%*(V_{before} - V_{after})/ V_{before}$

No significant differences in cells viability before and after the measurement were observed, therefore appropriate conditions for cell measurement were maintained.

Conclusions

- Obtained results showed differences in positronium lifetime, between normal and cancer cell in relation with their malignancy.
- No significant differences were observed in measured solutions or culture media without the cells.
- Outcome of our experiment confirmed the validity of employing positronium as an indicator, which may have a direct impact on better and more accurate diagnostics.

Acknowledgment

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