Biomedical application of Positron Annihilation Lifetime Spectroscopy - in vitro studies of alive normal and cancer cell lines and tissues

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Outline

1. Motivation

2. Cancer vs. normal cells

3. PALS studies of cells cultures in vitro

4. PALS studies of tumor and normal tissues in vitro

5. PALS on J-PET

6. Summary and future plans

1. Motivation

→ Positronium lifetime and intensity are related with temporal dynamics of nanostructures in cells and tissues

→ Possibility to determine early and advanced stages of carcinogenesis by observing changes in biomechanical parameters between normal and cancer cells

→ Combining J-PET scanner with PALS technique – new biomarker in cancer diagnosis

2. Cancer vs. normal cells

4

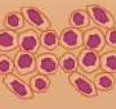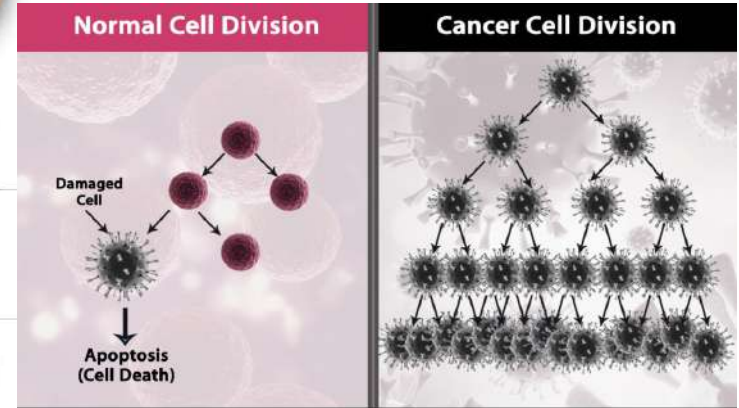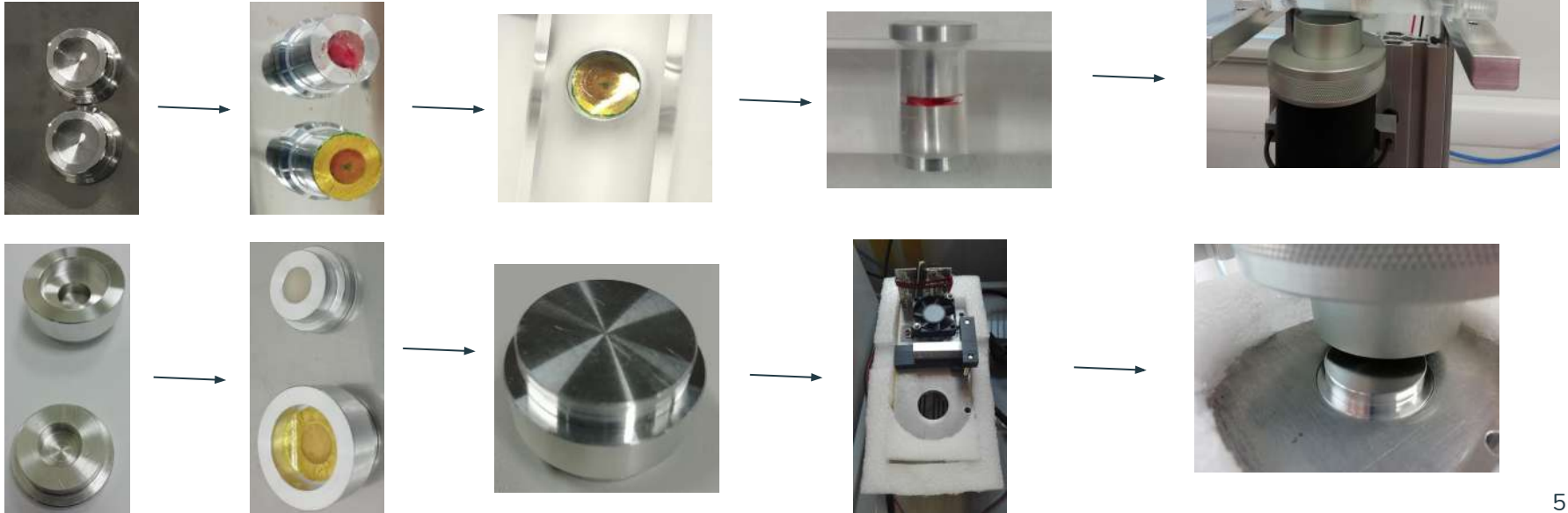# 3. PALS setup

→ Two BaF$_2$ detectors with resolution ~250 ps

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

→ PALS spectra analysis with PALS_Avalanche program developed by K. Dulski – J-PET collaboration

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

→ Cultured in M254/RPMI 1640 medium supplemented with HGMS-2/10% Fetal Bovine Serum, Penicillin 100U/ml and Streptomycin 100 ug/ml
→ Culture was incubated at 37°C in 5% CO_2 humidified atmosphere rinse

HEMa-LP   WM115   WM266

Each samples contains cells from 8 x 75cm^2 flasks, harvest upon 100% confluation (>10^8 cells).

Freeze - dried (liophylized)

Alive Cells in 37 C deg.

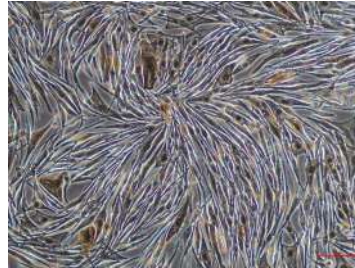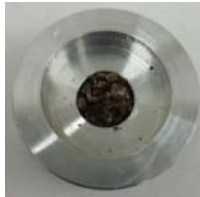HEMa-LP   WM115   WM266   HEMa-LP   WM115   WM266

6

3. PALS - cells culture - freeze dried

Human cell lines:
1) Melanocytes HEMa-LP from ThermoFisher
2) Melanoma WM115 from ATCC
3) Melanoma WM266 from ATCC
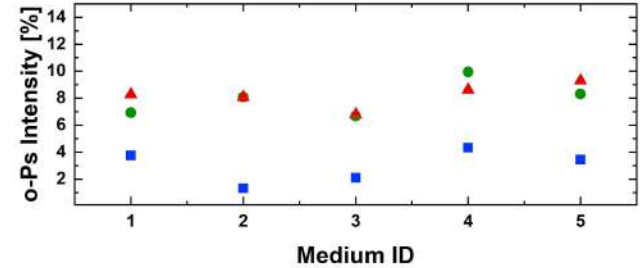
Cells were freeze - dried in -80 C deg., 0.0375 mbar for 24 h

Freeze Mediums:
1) M254/RPMI 1640+ P/S+ 20% FBS + 10% DMSO
2) 10% DMSO + PBS w/o $^{2+}$Ca, $^{2+}$Mg
3) PBS w/o $^{2+}$Ca, $^{2+}$Mg
4) 1.5 M PROH(propylene glycol) + 0.5 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg
5) 0.25 M D-trehalose in PBS w/o $^{2+}$Ca, $^{2+}$Mg

3. PALS - cells culture -alive
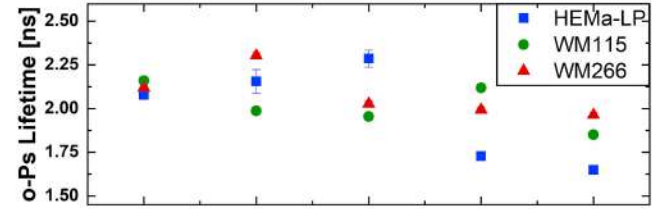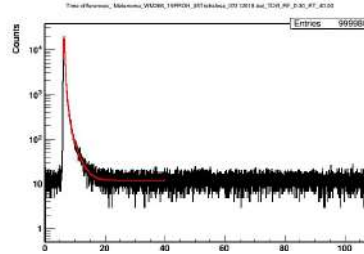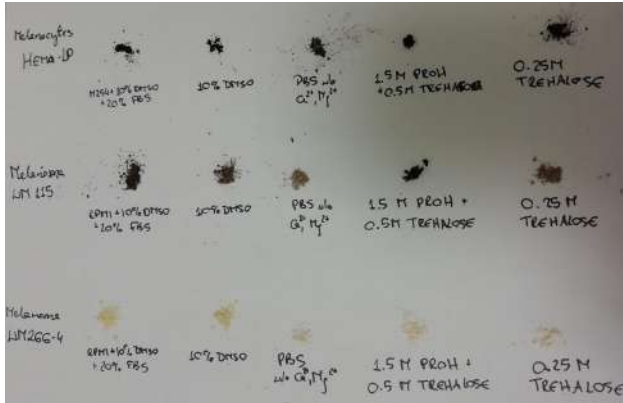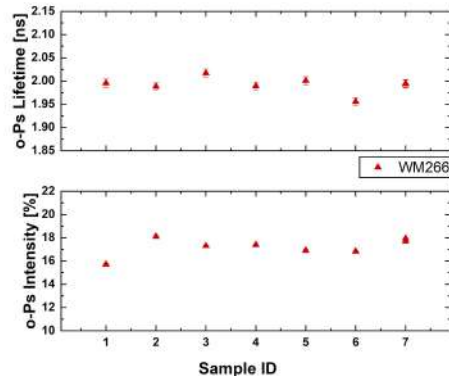
Human cell lines:

1) Melanocytes HEMa-LP from ThermoFisher

2) Melanoma WM115 from ATCC

3) Melanoma WM266 from ATCC

→ Cells were measured in 37 C deg. for 1 h

Cell Line	Viability before [%]	Viability after [%]
HEMa-LP	89.1	87.3
WM115	94.8	90.1
WM266	97.4	96.4

→ For WM266 cell line 7 repetitive measurements were done

→ Results from all are the in the line with each other within 2 sigma uncertainty
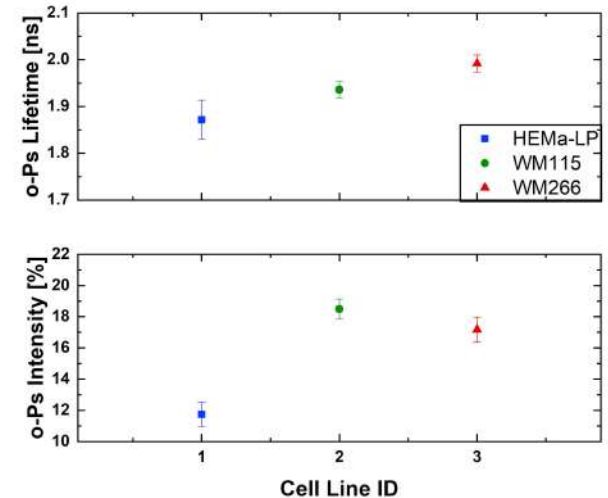
→ At least 2 repetition from each cell line were measured

→ Given results are calculated as an average value from all repetitive measurements

8

4. PALS studies of tumor and normal tissues in vitro

Cardiac Myxoma

→ primitive connective tissue tumor (benign), very rare in comparison to metastatic tumors
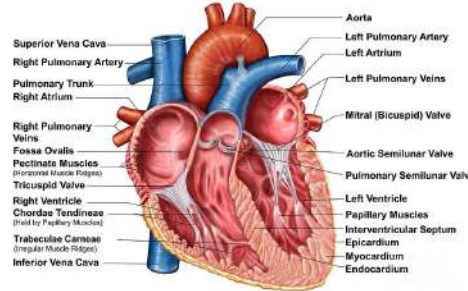→ 75 % of them are located in the left atrium
→ occur mainly in people over the age of 50

Not fixed - tumor sample measured with mediastinal adipose tissue for comparison

Fixed in formalin, tumor cut into ~2 mm thick samples

Patient ID	Sex	Age	Sample ID
1	woman	72	1-6
2	man	61	7
3	man	59	8-10
4	woman	54	11-13

Patient ID	Sex	Age
1	man	70
2	man	58
3	woman	59
4	woman	85
5	woman	60
6	man	64

https://healthjade.com/human-heart-health/

9

4. PALS - tissues in vitro - fixed

Cardiac Myxoma - fixed

Fixed in formalin, samples ~2 mm thick
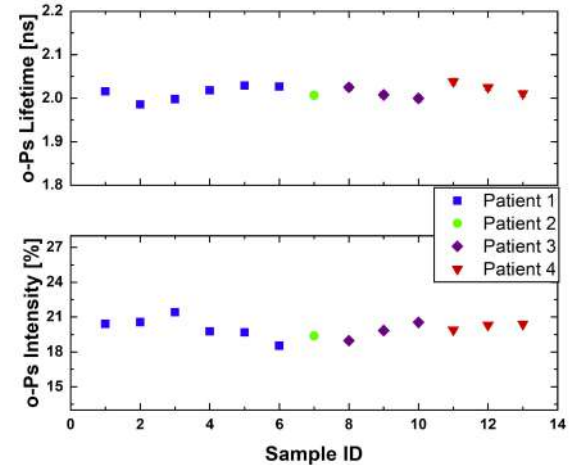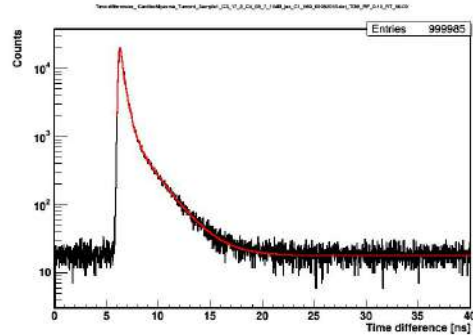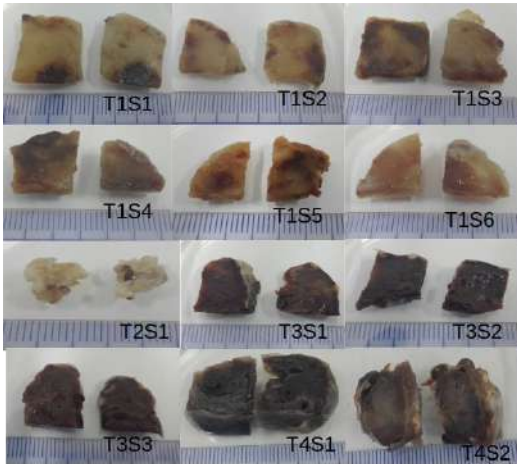
Patient ID	Sex	Age	Sample ID
1	woman	72	1-6
2	man	61	7
3	man	59	8-10
4	woman	54	11-13

10

4. PALS - tissues in vitro - not fixed

Cardiac Myxoma - not fixed

Not fixed - tumor sample measured with mediastinal adipose tissue for comparison

Patient ID	Sex	Age
1	man	70
2	man	58
3	woman	59
4	woman	85
5	woman	60
6	man	64
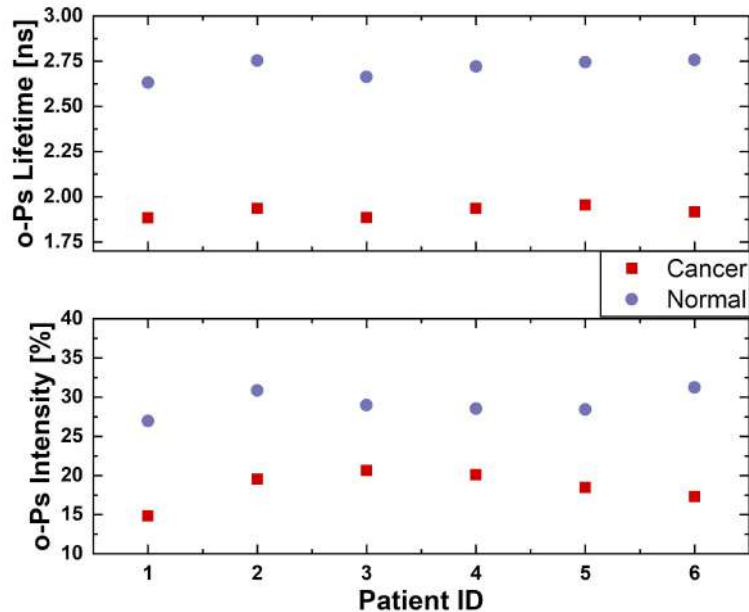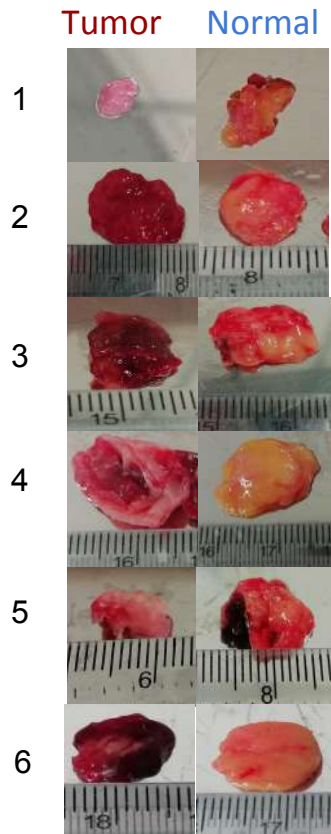
4. PALS - cell vs tissues in vitro - not fixed

Cardiac Myxoma

→ Tumor sample placed in Collagenase II 200U/ml soluble in DMEM+10% FBS + P/S for 48 h
→ Squeezed through 70 um nylon mesh to isolate cells from extracellular matrix
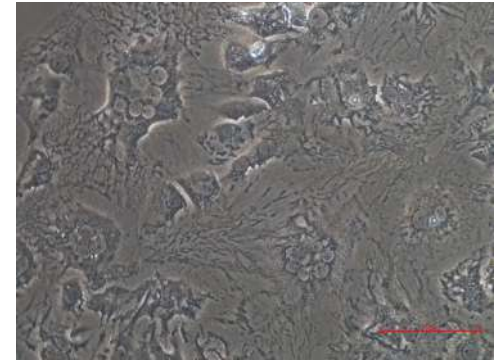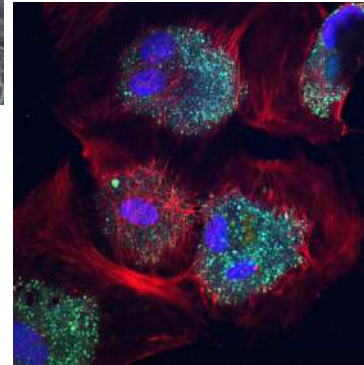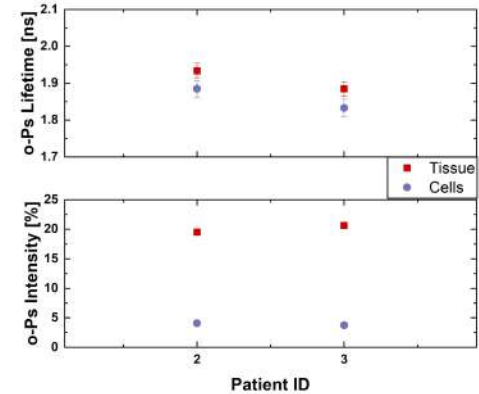→ Cells were seeded on flasks and culture in DMEM + 10% FBS + P/S
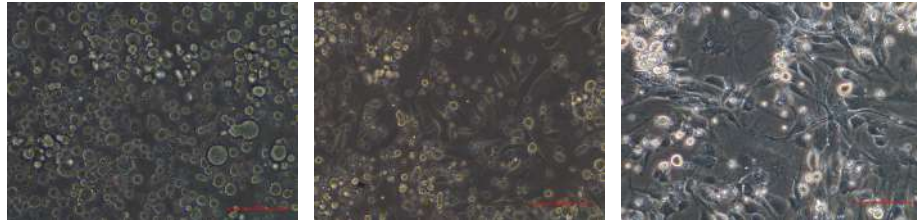
0 h   24 h   72 h

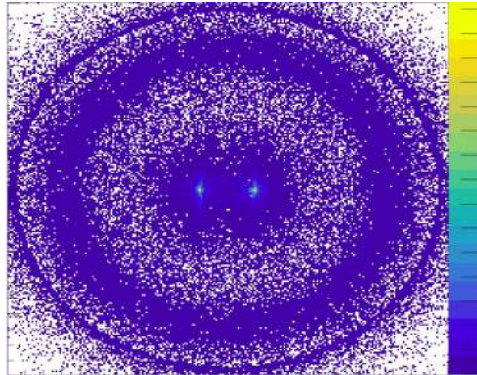| Patient ID	Sex	Age
2	man	58
3	woman	59

5. PALS on J-PET

→ Preliminary research proved PALS technique can be applied on J-PET
→ Studies with cardiac myxoma and colon cancer increasing statistic of the study
→ Cancer and normal tissue sample can be measured at the same time
→ o - Ps lifetime and intensity - new diagnostic biomarker for cancer

13
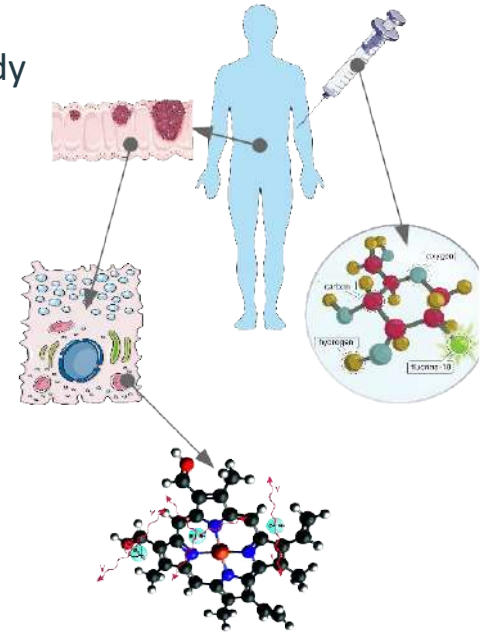
6. Summary and future plans

→ PALS is applicable to study biological structures

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

→ First studies of human tissue on JPET scanner proves that o-Ps lifetime can be used as additional diagnostic parameter

→ Studies with alive cell cultures and tissues – comparing normal vs cancer

→ Checking for possible o-Ps formation model in living cells

Thank you for attention