S9: Molecular and mechanical markers of various pathologies

S9-1 Early stage of essential hypertension monitoring

Kvetoslava Burda [a], Magdalena Kaczmarska [a], Maria Fornal[b], Franz Messerli[c], Jozef Korecki[a], Tomasz Grodzicki [b]

[a] *AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, Poland*
[b] *Collegium Medicum, Department of Internal Medicine and Gerontology, Jagiellonian University, Poland*
[c] *Division of Cardiology, Columbia University College of Physicians and Surgeons, St. Luke's-Roosevelt Hospital, USA*

We searched for differences in the RBC membrane skeleton structure and O_2 membrane permeability between RBCs from patients with both essential arterial hypertension and hypercholesterolemia, from patients having only hypercholesterolemia and from healthy donors. The topography of RBCs and the content of various hemoglobin forms (Hb) were detected using atomic force microscopy and Moessbauer spectroscopy, respectively. We found that the membrane skeleton of RBCs from healthy donors displays a well-known honeycomb pattern, whereas in patients with essential hypertension and/or hypercholesterolemia, who had never received anti-hypertensive therapy, it displays a corncob pattern. Moessbauer results indicate an impaired oxygen release by Hb in RBCs of patients with hypertension under low oxygen pressure which if present in vivo may cause hypoxemia and, in turn, further increase of blood pressure.

[1] M. Kaczmarska, M. Fornal, F. H. Messerli, J. Korecki, T. Grodzicki, K. Burda Cell Biochem Biophys DOI 10.1007/s12013-013-9613-9

This work was partially supported by the AGH UST statutory tasks No. 11.11.220.01/3 within subsidy of the Ministry of Science and Higher Education.

S9-2 Label-free methods in diagnostics and prognostics of malignant melanoma

Tomasz Kobiela

Warsaw University of Technology, Faculty of Chemistry, Chair of Drug and Cosmetics Biotechnology, Poland

Malignant melanoma is one of the most aggressive type of the skin cancer, which can metastasize to every organ in the human body. Thus, an early and proper melanoma diagnosis influences significantly the therapy efficiency. The melanoma recognition is still difficult, and generally, relies on subjective assessments. The technological development of label-free methods such as atomic force microscopy (AFM) or quartz crystal microbalance (QCM) has opened new perspectives for melanoma and its biomarker detection.

We have compared the recognition of mannose type glycans in melanocytes (HEMa-LP) and melanoma cells originating from the radial growth phase (WM35) and from lung metastasis

(A375-P). The glycosylation level on their surfaces was probed using lectin concanavalin A (Con A). The interactions of Con A with surface glycans were quantified with both AFM and QCM techniques that revealed the presence of various glycan structural groups in a cell-dependent manner. The glycans present on WM35 cell surface are rather short and less ramified while in A375-P cells, Con A binds to long, branched mannose and glucose types of oligosaccharides.

The results might be further used in the development of a biosensor for the sensitive screening of malignant melanoma cells. This methodology may also allow the study of the effects of new substances on the inhibition of epithelial-mesenchymal transition to develop the procedure that allows the characterization of new therapeutic agents for primary and advanced melanoma.

S9-3 Advanced vibrational imaging techniques to aid clinical research

Tomasz P. Wrobel [a], Paulina Koziol [a], Natalia Piergies [a], Ewa Pieta [a], Czeslawa Paluszkiewicz [a], Maria Fornal [b], Tomasz Grodzicki [b], Wojciech Kwiatek [a]

a Institute of Nuclear Physics Polish Academy of Sciences, Poland
b Jagiellonian University, Collegium Medicum, Department of Internal Medicine and Gerontology, Poland

Modern molecular imaging modalities based on chemical contrast provide a unique set of tools available to a clinical researcher. These include infrared and Raman spectroscopy techniques also coupled to Atomic Force Microscopes allowing to probe the samples on scales ranging from a few nanometers to centimeters. A short presentation of IFJ PAN Equipment capabilities and a description of a few relevant examples will be made, starting with a most recent work on erythrocyte oxygenation status in human blood. Here, Raman spectroscopy was applied to obtain unique information about the heme-oxygenation levels in red blood cells in the context of early hypertensive status, also called prehypertension. The next example covers the opportunities of FT-IR imaging in aiding histopathology of pancreatic cancer.
This research was performed using equipment purchased in the frame of the project co-funded by the Malopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project No. MRPO.05.01.00-12-013/15. TPW is supported from the „Pancreatic cancer comprehensive histopathology based on IR chemical imaging" project, which is carried out within the Homing programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. It was also supported by the Collegium Medicum of the Jagiellonian University project K/ZDS/007195.

S9-4 Effect of dietary carotenoids on erythrocytes from diabetic patients: a spectroscopic study

Joanna Fiedor [a], Mateusz Przetocki [a], Grzegorz Gajos [b], Józef Korecki [a], Kvetoslava Burda [a]

[a] *AGH-University of Science and Technology, Faculty of Physics and Applied Computer Science, Department of Medical Physics and Biophysics, Poland*
[b] *Jagiellonian University Medical College, Faculty of Medicine, Department of Coronary Artery Disease and Heart Failure, Poland*

Carotenoids (Crts) are structurally and functionally a very diverse group of natural pigments of isoprenoid type. They are synthesised by all organisms capable of conducting photosynthesis. Up to date ~700 Crts have been described, out of which only ~50 become constituents of the human diet, and ~20 is found in human blood and tissues [1]. Crts are known to be efficient physical quenchers of $^1O_2$ and scavengers of other reactive oxygen species (ROS). They also act as chemical quenchers undergoing irreversible modifications [2]. Their antioxidant activity is of special significance to human health [3]. Uncontrolled overproduction of ROS may lead to losing antioxidant-ROS balance resulting in "oxidative stress", a critical factor of the number of pathogenic processes of chronic diseases. Diabetes is recognized as the world's fastest growing chronic condition related to elevated blood glucose level. It considerably increases the risk of cardiovascular disorders, eye and foot failure, affects urinary tract and mental health. On the cellular level, it was shown to modify properties of erythrocyte's membranes.
Here, we would like to present the results of experiments taken on isolated erythrocytes from healthy and diabetic donors. The cells were subjected to Crt treatment in the presence or absence of exogenous ROS. To monitor cellular response UV-VIS absorption and Mössbauer spectroscopies were applied, delivering informations on i.a. the states of haemoglobin and its ability to bind oxygen.

1. J. Nutr. 1989, 119, 101.

2. Biochim. Biophys. Acta 2005, 1709, 1.

3. Nutrients, 2014, 6, 466.

This work was partially supported by the Faculty of Physics and Applied Computer Science AGH UST statutory tasks No. 11.11.220.01/3 within subsidy of the Ministry of Science and Higher Education.