The role of molecular composition of endothelial and pancreatic beta-cell extracellular vesicle in diabetic endothelial cell dysfunction - impact on the targeted cell membrane properties

This is an interdisciplinary project investigating the role released nano- and microfragments of cell membranes in vascular dysfunction caused by diabetes. **Nano- and microfragments of cell membranes** also called extracellular vesicles (**EVs**) are classified according to their differences in size (diameter) and mechanism of their formation, into subgroups, including **exosomes** (**Ex**) with a diameter of 30 to 100 nm, **ectosomes** also named micvesicles (**MVs**) with a diameter of 100 nm to 1 μm, and apoptotic bodies (AB) with sizes between 1 and 5 μm. They are released by number of cells like blood cells, endothelial cells (cells which are lining a blood vessel), gland or cancer cells and many others. They are present in every body fluids, including blood, urine, saliva, amniotic fluid or even tears. They can transfer a number of biomolecules, like proteins, lipids, short fragments of nucleic acids (RNA mostly). In this project, we want to explore the lipid and protein content of EVs produced by hyperglycemic endothelial and pancreatic cells.

Hyperglycemia is a state when glucose (a simple sugar) concentration exceeds a normal level. In laboratory (in vitro) conditions we can use hyperglycemia when glucose concentration is 25 mM in compare to normoglycemic conditions when glucose concentration is 5 mM. In clinical situation, hyperglycemia is observed in patients having diabetes or glucose intolerance. Diabetes is a very dangerous and insidious disease. In Poland, almost 10% of population suffer from diabetes (about 3 million people), one third of them are unaware of their illness, because they were never examined. A normal blood sugar concentration is around 1 teaspoon in an average man. In patients having glucose intolerance, a blood sugar concentration can exceed the level of 7 mM or even more. A persistent elevation in blood glucose leads to glucose toxicity, which contributes to cell dysfunction also endothelial cell dysfunction (ECD) and the number of pathologies grouped together as diabetic complications.

In stressing, hyperglycemic conditions, endothelial cells can release more EVs and those having different molecular content, including altered lipid composition and protein cargo. By means of contemporary methods including mass spectrometry (Secondary Ions Mass Spectrometry – SIMS, mass spectrometry – MS) and routine biochemical methods, we plan to assess lipid content on a single EV level. Such approach needs to use very sensitive and high resolution instrumentation, and SIMS fits perfectly to do this analysis. Changed lipid content influence membrane lipid organization, which we want also investigate by a very novel approach, Positronium Anihilation Life-time Spectroscopy (PALS) is a technique which allows us to show differences in lipid organization in EVs.

The mechanisms of EV internalization to endothelial cells and their impact on ECD will be explored by a novel high resolution technique called Stimulated Emission Depletion (STED). This technique awarded by the Noble Prize in Chemistry in 2014 will allow us to investigate how EVs can internalize to endothelial cells. The impact of EVs on endothelial cells will be explored as changes in cell membrane fluidity, causing their dysfunction by a possible lipid-protein interaction and channel in ion channel distribution or reactivity. This parts or our research will involve electrophysiology methods on a cellular level.

We are an interdisciplinary group of professionals headed by Prof Ewa Łucja Stępień from the Department of Medical Physics of the Marian Smoluchowski Institute of Physics at the Jagiellonian University in Cracow and we are very dedicated to do such research.

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