

# Spectrometric study of biomolecular differences of $\beta$ -cell EVs subpopulations from hyperglycemic conditions

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Extracellular vesicles (EVs) are a spherical shape structures surrounded by a protein-lipid membrane. EVs are important in the detection of various diseases, such as cardiovascular disease or cancer, and may have a high therapeutic potential [1]. The molecular composition and size of EVs membranes vary depending on the source cells, the current stage of development and environmental conditions. Their basic classification distinguishes three subpopulations: exosomes derived from endosomes (50–150 nm), microbubbles budding in the cell membrane (100–1000 nm) and apoptotic bodies (100–5000 nm). Specific features of these EVs subgroups have been proposed, but there are still no standardized markers to distinguish these populations [2].

In the work, we propose the use of secondary ion mass spectrometry with a time-of-flight analyzer (ToF-SIMS) to assess the differences in the molecular composition of EV subpopulations: exosomes, ectosomes and a mixture of both populations. EVs, derived from pancreatic  $\beta$ -cells grown under hyperglycemia conditions (HC), were purified by Low-Vacuum Filtration and concentrated by ultracentrifugation. ToF-SIMS, as a highly sensitive qualitative technique, made it possible to perform a comparative analysis of the tested samples. During the analysis, significant differences in the intensities of the characteristic peaks of amino acids and individual lipid groups were revealed. The demonstrated changes concern EV subpopulations and their mixture obtained from glycerin conditions. It can be assumed that various EV subpopulations derived from pancreatic  $\beta$ -cell cultures are characterized by a changed molecular composition related to biogenesis of the discussed structures. The external environment has a significant impact on the protein-lipid EV membrane composition.

Keywords: extracellular vesicles;  $\beta$ -cell; ToF-SIMS; hyperglycemia; lipidomic;

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**Primary authors:** MARZEC, Magdalena (Jagiellonian University); RZAÇA, Carina (Zakład fizyki medycznej WFAIS UJ); KAMIŃSKA, Agnieszka (Uniwersytet Jagielloński); STĘPIEŃ, Ewa (Jagiellonian University)

**Presenter:** MARZEC, Magdalena (Jagiellonian University)

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