ToF-SIMS testing of the molecular composition of urinary extracellular vesicles to determine symptoms of diabetic kidney disease

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We analyzed extracellular vesicles (EVs) isolated from the morning urine of patients with type 1 diabetes mellitus (T1DM). EVs are spherical cellular structures surrounded by a lipid bilayer with sizes from 30 to 1000 nm. Due to the constantly growing importance of EVs in the diagnosis and treatment of diseases, more attempts are made to effectively isolate, detect and analyze of EVs [1]. Chronic kidney disease (CKD) is currently an increasing clinical problem. Hence, there is a need to identify the early biomarkers of CKD. EVs can serve as non-invasive indicators to detect changes in a kidney and implement effective treatment before irreversible structural changes in the kidney occur. However, the preparation of EVs for nanoscale research is difficult for several reasons. First, the isolation of EVs from the conditioned medium where the concentration of EVs is low. Here, EVs were purified by a Low-Vacuum Filtration (L-VF) technique and concentrated by the ultracentrifugation process [2]. Second, it is the isolation of individual molecular components, like lipids, which are sensitive to each stage of preparation. Therefore, the possibility of testing them in their native state, without the need for extraction, is extremely promising. In the case of Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS), there is no need to perform such preparations or labelling, which allows the structure to be examined in a semi-native state [3].

The aim of the study is to develop a method of early detection of the first microvascular symptoms including CKD. The detection procedure will be based on the use of two physical methods, Raman Spectroscopy (SR) and ToF-SIMS to precisely determine the molecular compounds whose EVs composition changes during early kidney damage. The study recruited 36 patients with T1DM at the University Hospital in Krakow (*no*. 1072.6120.109.2019). Here we present the preliminary results of ToF-SIMS analysis of urinary EVs, showing the diversity in individual amino acids and the lipid content.

**References:**

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