

In order to function properly, all living organisms require a coordinated functioning of many different organs. Homeostasis of an organism is only possible with long-distance communication between cells in different parts of the body, which is mediated by the microvesicles.

Microvesicles are natural, round particles formed from lipid bilayer, differing significantly in size, composition and origin. The diameter of the smallest microvesicles is in the range of 30-100nm. They are synthesized inside living cells, in multivesicular bodies and released into the extracellular environment. The cargo loaded inside the microvesicles carries information to other cells, in the form of proteins, lipids and nucleic acids. One of the most important type of cargo carried by microvesicles are miRNA particles. These are short (17-20 nucleotides) RNA fragments, which regulate gene expression. In the research project we plan to synthesize artificial exosomes for targeted delivery of therapeutic miRNA particles on the endothelial model of diabetes.

Microvascular complications, damaging the small blood vessels are one of the most severe consequences of diabetes. The changes affect strongly the endothelial cells, in which we observe a wide range of functional, biochemical and structural changes, resulting in the lowered proliferation and cell migration, as well as impaired wound healing. Studies have shown, that these changes are accompanied by the alterations in the level of various miRNA particles, in particular, a heightened level of hsa-miR-221-3p miRNA. It has been shown that blocking this molecule results in the increased migration of cells, cultured in hyperglycemic conditions. In our project, the particle was chosen as a cargo delivered by our drug delivery system (artificial exosomes) to the cells.

Our artificial exosomes will be coated with Del-1 (Developmental endothelial locus-1) protein, which is excreted by the endothelial cells and has been shown to mediate the connection between natural microvesicles and cells and, in turn, facilitate the delivery of their cargo. We will synthesize a series of synthetic exosomes with varying lipid composition (mimicking the natural ones). The vesicles coated with the Del-1 protein will be investigated in terms of their structural and physical (size, shape, size distribution, surface charge, stability), as well as functional properties (cytotoxicity and interactions with natural plasma proteins).

A chosen class of synthesized exosomes, exhibiting the most promising properties, will be subjected to the final tests. The exosomes will be loaded with hsa-miR-221-3p miRNA molecules and we will investigate their uptake by the endothelial cells, as well as the impact of the delivered miRNA molecule on their migration and the level of extracellular enzymes – metalloproteinases (elevated levels of these enzymes have been observed in diabetes). The same set of tests will be performed using natural exosomes, isolated from the culture medium of the endothelial cells. These tests will allow us to observe the differences, in terms of the cargo delivery efficiency, between natural and artificial exosomes (and will serve as the final confirmation of the validity of the research hypothesis).

A thorough investigation of the influence of various lipid composition of the artificial exosomes on the efficiency of cargo delivery will allow us to choose the most promising combination for further studies. Our studies will be also useful for the characterization of the biological activity of artificial exosomes.