Extracellular vesicles modifications for future drug delivery systems

The project goal: The project aims to develop ***engineered extracellular vesicles*** *(**eEVs**),* which will be entirely produced by ***mesenchymal stem cells*** (***MSCs***), and then modified by ***glycoengineering*** methods. The main goal is to design ***eEVs*** (namely ***exosomes***) with biomimetic properties for drug delivery systems (***DDS***) to better recognize a target cell**.**

My goal is to move biomedical engineering and material sciences towards nanomedicine and theranostics.

Reasons for attempting a proposed research topic: To deliver drugs, genes, and vaccines in therapeutics, for the treatment of cancer, a variety of ***DDS*** is proposed, most used are ***liposomes***. However, liposomes have number of limitations, including the main as non-selective targeting to a desired cell. Several strategies have been proposed to develop new biomimetic materials for ***DDS***, most of them are based on construction of liposome-like structures, which mimic natural biomembranes, decorated with plasma proteins to provide better stability. On the opposite site, there are very resistant nanocarriers, like dendrimers or nanotubes, which accumulate inside a cell triggering cytotoxic effect. To avoid the shortcomings resulting from liposomes instability and cytotoxicity of nanomaterial, I propose naturally occurring exosomes as ***DDS***. In my research, I have pioneered in Poland investigation on ***EVs*** as ***biomarkers*** of different pathologies, and I contributed significantly to improve the knowledge about ***EVs*** structure, properties, and activity in *diabetes* , *cardiovascular diseases*, and cancer, mostly *melanoma*. Together with my collaborators, I found that melanoma-derived ***EVs*** have different ***glycosylation*** profile. ***Glycosylation*** is a process in which sugars are added to proteins or lipids under the control of enzymes, which occurs in the cell and plays a crucial role in functioning of glycoproteins and controlling biological processes, such as cell-cell recognition. The importance of protein ***glycosylation*** for the biotech industry is highlighted by the fact that approximately 70% of therapeutic proteins, approved or in (pre-)clinical studies, are ***glycoproteins***. As the first in Poland, I was also involved in the use of a fusion protein - ***lactadherin*** for the recognition of EVs in order to develop systems for the capture of ***EVs***.

Description of research: My research hypothesis assumes that enzymatic and biochemical modifications of ***EVs*** (***exosomes***) will facilitate targeted delivery of encapsulated bioactive components to diseased cells, namely to the two tapes of cells, which are contributing to tumour growth: ***cancer cells*** and ***endothelial cells***. The research will be carried out at the Jagiellonian University (JU) in the new establish Theranostics Center. I will use ***exosomes*** with a diameter of 50 to 100 nm, which are nano-sized double-layered membrane entities produced by most cell types and released into biological fluids to transfer bioactive molecules between cells. ***Exosomes*** will be produced by mesenchymal stem cells and then genetically engineered to obtain exosomes containing the ***lactadherin*** protein followed by enzymatic digestion to remove glycans. This process is called ***glycoengineering.*** Such glycoengineered ***eEVs***, will be selectively isolated and characterized to define their size, find a specific ***glycosylation*** pattern, charge and molecular density. Then biological *in vitro* tests will be performed, to investigate the cell uptake and fate. These tests will be done with primary and metastatic carcinoma cell lines (melanoma cells) and microvascular endothelial cells.

Expected results: In my project, I expect to show the role of ***lactadherin*** in exosome uptake and reveal surface ***glycan pattern*** as a potential novel indicators of ***EV*** heterogeneity. I want to establish ***glycoengineering*** as a useful attempt to research cell–extracellular vesicle interactions for biomedical applications, especially to design new drug delivery system (***DDS***). I expect to obtain the up-scaled production of ***engineered EVs*** for further research on biomimetic materials based on ***glycan*** modifications.