A comparative analysis of the ability to secrete exosomes by different types of human mesenchymal stem/stromal cells (MSCs) depending on the source and environmental factors

The aim of the project is to perform a comparative analysis of the ability to secrete extracellular vesicles (particularly exosomes) by different types of human mesenchymal stem/stromal cells (MSCs) depending on the source and environmental factors.

MSCs have regenerative properties - through secretion of different immunomodulatory factors they can modulate the process of inflammation and the reparation processes in situ. These factors are released into the environment encapsulated within exosomes, which are small structures, spherical in shape and surrounded by two-layer lipid film. They are the smallest of all extracellular vesicles (EV) and play a key role in cell to cell communication. Exosomes originate from endosomal compartments, known as multi-vesicular bodies (MVBs) and are involved in intercellular communication via the transfer of numerous membrane receptors, proteins, lipids, RNA and miRNA between cells. As exosomes contain many different factors, they can have a great therapeutic potential as MSCs themselves. There are several potential advantages of exosomes when compared to MSCs alone, which are: 1) inability to self-replicate suggesting no risk of uncontrolled growth; 2) limited potential to trigger the immune system and 3) easier transport and storage, which makes cellular-free treatment more promising when compared to standard MSC-based therapies. While MSCs obtained from different sources usually differ when it comes to clonogenic properties, proliferation potential, secretory profile and senescence rate, we hypothesize that different types of MSCs secrete different levels of exosomes and therefore differ considering their therapeutic potential.

In this project, MSCs will be derived from Wharton's jelly of human umbilical cord (WJ-MSCs - *Wharton's jelly-derived MSCs*) and human adipose tissue (ASCs - *adipose tissue-derived MSCs* and DFAT - *dedifferentiated fat cells*). The umbilical cord and adipose tissue are the most frequently chosen sources of MSCs, as they are easily accessible. Cells derived from those tissues differ when it comes to neuroprotective and angiogenic properties. Moreover, ASCs and DFAT, being two subpopulations of cells originating from the same tissue, differ when it comes to pluripotent gene expression level and secretory profile.

Despite many ongoing experiments regarding MSCs-derived EVs, no comparative analysis considering WJ-MSCs, ASCs and DFAT-derived exosomes is available. In this project, we aim to investigate the number of exosomes isolated from different types of MSCs. We hypothesize that the regenerative and neuroprotective properties of MSCs and their ability to modulate the inflammation process strictly correlates with the number of secreted exosomes. We also intend to analyze the impact of MSCs co-culture with injured tissue on the quantity of released exosomes, as we suspect that the injured tissue stimulates MSCs to secrete higher levels of exosomes and therefore increases their regenerative and neuroprotective properties. Moreover we would like to compare the neuroprotective effect of MSCs to the effect of MSCs-derived exosomes on the injured tissue. We also aim to investigate the impact that selected factors (BDNF, IL-2, IL-1-β) have on the number of released exosomes.

The results of the study will provide basic knowledge about paracrine activity of different types of MSCs. Considering the therapeutic properties of MSCs which might correlate with the number and content of the secreted exosomes, the results of this study will provide information about the most efficient source for obtaining exosomes that could be used in future cellular-free therapies.