OPTIMIZATION OF IN VITRO CELL CULTURE CONDITIONS FOR HUMAN MESENCHYMAL STEM CELLS OF DIFFERENT ORIGIN FOR APPLICATIONS IN TISSUE ENGINEERING

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Introduction
Mesenchymal stem cells (MSCs) are multipotent cells derived from different body tissues. They possess several features, such as robust differentiation capacity and immunosuppressive properties, which make them one of the most promising cell types for potential applicability in tissue regeneration. Their derivatives such as extracellular vesicles (EVs) also very promising due to the fact that they carry bioactive proteins and small nucleic acids such as mRNA and miRNA, which may have positive impact on different functions of target cells. Unfortunately, MSCs are routinely cultured in conditions containing fetal bovine serum that is a rich source of proteins and EVs of animal origin, which may be harmful for patients. If we want to transfer MSCs and MSCs EVs technology to the clinic we need to look for better culture conditions that support robust cell growth but are safe for humans.
Thus, the aim of this study was to select the most optimal serum-free, xeno-free culture medium for harvesting safe MSCs and MSC-EVs for the purpose of regeneration.

Materials and Methods
Human umbilical cord-derived MSCs (UC-MSCs) and adipose tissue-derived MSCs (AT-MSCs) were cultured in several different media. Cell proliferation, viability, metabolic activity (ATP concentration), multiantigenic phenotype (flow cytometry), and differentiation potential as well as senescence rate were measured after specified number of passages. mRNA levels for genes connected with differentiation, and cells secretome were analysed. MSC-EVs were isolated from conditioned media by ultracentrifugation at 100000g and characterised according to International Society for Extracellular Vesicles guidelines. Phenotype and transcriptome analysis of EVs, was performed as well as functional studies.

Results and Discussion
Our results indicated differences between MSCs cultured in the tested media including differences in proliferation rate, metabolic activity, longevity and gene expression levels that allowed for selection of most optimal one. We also observed that MSC-EVs were enriched in transcripts crucial for stimulation of cell differentiation.

Conclusions
Our data allowed us to choose most optimal media for MSCs culture and MSC-EVs harvest for purposes of tissue regeneration. However, further experiments are required for transfer of these results into clinic.

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