OPTIMIZED MULTIPARAMETER CHARACTERIZATION OF STEM CELL-DERIVED EXTRACELLULAR VESICLES USING CLASSICAL AND IMAGING FLOW CYTOMETRY PLATFORMS

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Introduction
The utilization of stem cells (SCs) combined with biopolymer scaffolds brings new hopes in a field of tissue regeneration. The unique properties of SCs involve not only their ability to directly differentiate in the site of injury, but also to release several paracrine factors, including extracellular vesicles (EVs), that are a heterogeneous group of small vesicular structures shed from the cell surface. Importantly, several data have demonstrated that EVs secreted by SCs populations cultured on biopolymer scaffolds may enclose bioactive content in the form of proteins and nucleic acids and transfer their cargo to the target cells. Thus, growing interest is placed on the utilization of those EVs in the field of biomedical research. However, there is still lack of standardized methods of EVs characterization which limits their applicability and affects data reproducibility. As an example, typical flow cytometry-based protocols, commonly used for cells phenotyping, may be inadequate for the characterization of EVs as particles with size close to the detection limit of conventional cytometers. Thus, the aim of this study was to optimize the use of two flow cytometry platforms for the multiparameter analysis of EVs isolated from different types of SCs populations.

Materials and Methods
EV samples were obtained from human iPS- and mesenchymal SCs (MSCs)- conditioned media by ultracentrifugation method. Next, high resolution flow cytometer Apogee A60 Micro-Plus dedicated to small particle applications was utilized to examine EVs phenotype, including expression of tetraspanins and surface markers. Furthermore, RNA Select dye was used to evaluate the content of RNA and the integrity of analyzed vesicles. Additionally, imaging flow cytometry platform (Image Stream Mk II) was also employed to visualize EVs on the single particle level.

Results and Discussion
Our results have revealed that two tested flow cytometry systems may be utilized for the phenotypic characterization of EVs secreted by human SCs populations. However, the conventional immunofluorescence staining and gating strategy protocols have to be thoroughly optimized. We have performed several controls and demonstrated the importance of additional preparation of reagent solutions prior to EVs staining. Additionally, the analysis of calibrating beads allowed to confirm the relative size of EVs and detection limits of tested cytometers. Importantly, utilization of Apogee and Image Stream systems enabled us to demonstrate the difference in the RNA content between iPS- and MSCs-derived EVs. Finally, we have also confirmed the presence of EVs subpopulations containing differential expression of exosomal markers, as well as surface markers characteristic for their parental cells.

Conclusions
In conclusion, we have demonstrated that the utilization of high-resolution flow cytometry platforms is a convenient method for the multiparameter characterization of EVs produced by different types of SCs populations, including those cultured on selected biocompatible polymer scaffolds.

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