



ISEV2020 Abstract Book

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Mission Statement

Advancing extracellular vesicle research globally.

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Our vision is to be the leading advocate and guide of extracellular vesicle research and to advance the understanding of extracellular vesicle biology.

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PS13: Advances in Characterization of EV-Associated Molecules

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PS13.01

Evaluating the impact of culture conditions on human mesenchymal stromal cell-derived extracellular vesicles molecular fingerprint through FTIR spectroscopy

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Introduction: Increasing evidence has proposed extracellular vesicles (EVs) as mediators of many of the therapeutic features of mesenchymal stromal cells (MSC) that have been widely studied in clinical trials over the last years. These EVs have been recognized as nanocarriers of important biological information, which play a central role in cell-to-cell communication. In this context, EVs can be used as an alternative to a cell-based therapy, with reduced risks. The present work aimed to evaluate the impact of different culture conditions on the MSC-derived EVs molecular composition through Fourier-Transform InfraRed (FTIR) spectroscopy.

Methods: EVs derived from MSC from different sources, expanded in two different culture media ((xenogeneic -free (XF) vs serum-containing medium (FBS)) were characterized by FTIR spectroscopy, a highly sensitive, fast and high throughput technique. Moreover, principal component analysis (PCA) of pre-processed FTIR spectra of purified EVs was conducted, enabling the evaluation of the replica variance of the EVs chemical fingerprint in a reduced dimensionality space. For that, different pre-processing methods were studied as baseline correction, standard normal variation and first and second derivative.

Results: EVs secreted by MSCs cultured with serum-containing medium presented a more homogenous chemical fingerprint than EVs obtained with XF medium. The regression vector of the PCA enabled to identify relevant spectral bands that enabled the separation of samples in the score-plot of the previous analysis. Ratios between these spectral bands were

determined, since these attenuate artefacts due to cell quantity and baseline distortions underneath each band. Statistically inference analysis of the ratios of spectral bands were conducted, by comparing the equality of the means of the populations using appropriate hypothesis tests and considering the significance level of 5%. It was possible to define ratios of spectral bands, that can be used as biomarkers, enabling the discrimination of EVs chemical fingerprint in function of the culture medium used for MSC expansion and the MSC donor.

Summary/Conclusion: This work is a step forward into understanding how different culture conditions affect MSC-derived EVs characteristics.

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PS13.02

Performance qualification for MicroFlow Cytometers: understanding technical limitations to improve your research

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Introduction: As microflow cytometry and other techniques mature as validated modalities for analysing extracellular vesicles (EV), there has been a concerted effort to improve reproducibility. In order for this reproducibility to occur there has to be a critical understanding of advantages and limitations for each technology. For microflow cytometry, several instruments are available to analyse EVs. Each platform has different limitations as well as advantages over other platforms. To provide the optimal data for your specific research, it is critical to understand the limitations of your platform. To accurately define these limitations, a performance qualification (PQ) of your instrument should be undertaken.

Methods: An Apogee A60 platform was used in these experiments. Experiments were designed with expected ranges and cut-offs for acceptance criteria. Initial tests included autosampling of a 96 well plate with either single or double aspiration, single sample reproducibility and