EVALUATING THE IMPACT OF CULTURE CONDITIONS ON HUMAN MESENCHYMAL STEM/STROMAL CELL-DERIVED EXOSOMES THROUGH FTIR SPECTROSCOPY

Ana Fernandes-Platzgummer, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal; The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
ana.fernandes@tecnico.ulisboa.pt
Maria João Canha Pereira, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Luís Ramalhete, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal
Sandra Aleixo, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal; Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Portugal
Cláudia Lobato da Silva, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
Joaquim M. S. Cabral, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
Cecília R.C. Calado, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal

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In the last decade, the therapeutic effects of mesenchymal stem/stromal cells (MSCs) have been attributed to a paracrine activity exerted by extracellular vesicles secreted by MSCs, as exosomes. Their properties as intercellular communication vehicles have led to an increase interest in their use for cell-free therapeutic applications. The present work aimed to evaluate how different culture conditions, as culture medium (xenogeneic -free (XF) vs serum-containing medium), conditioning time (1, 2 and 3 days) and different MSC donors (n=6), affect the chemical characteristics of exosomes. For that, purified MSC-derived exosomes were characterized by Fourier-Transform InfraRed (FTIR) spectroscopy, a highly sensitive, fast and high throughput technique. The principal component analysis (PCA) of pre-processed FTIR spectra of purified exosomes was conducted, enabling the evaluation of the replica variance of the exosomes 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. It was observed that the chemical fingerprint of exosomes is more dependent of the medium used for MSCs cultivation than the MSC donor and conditioning days. Exosomes secreted by MSCs cultured with serum-containing medium presented a more homogenous chemical fingerprint than exosomes obtained with XF medium. Moreover, for a given medium (XF or serum-containing medium), the exosomes chemical fingerprint depends more of the MSC donor than of the conditioning days. The regression vector of the PCA enabled to identified 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 artifacts 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 exosomes chemical fingerprint in function of the medium used for MSC grown and the MSC donor. This work is therefore a step forward into understanding how different culture conditions and MSC donors affect MSC exosomes characteristics.