BIOPROCESS INTEGRATION FOR HUMAN MESENCHYMAL STEM CELLS: FROM UP TO DOWNSTREAM PROCESSING SCALE-UP TO CELL PROTEOME CHARACTERIZATION

Margarida Serra, iBET/ITQB-NOVA, Portugal
mserra@ibet.pt
Bárbara Cunha, iBET/ITQB-NOVA
Tiago Aguiar, iBET/ITQB-NOVA
Sofia B. Carvalho, iBET/ITQB-NOVA
Marta M. Silva, iBET/ITQB-NOVA
Ricardo A. Gomes, iBET/ITQB-NOVA
Manuel J. T. Carrondo, iBET/ITQB-NOVA
Patrícia Gomes-Alves, iBET/ITQB-NOVA
Cristina Peixoto, iBET/ITQB-NOVA
Paula M. Alves, iBET/ITQB-NOVA

Key Words: cell therapy, product characterization, mass spectrometry, human mesenchymal stem cells, scale-up

Human mesenchymal stem cells (hMSC) are relevant cell-based products for autologous and allogeneic therapies. To deliver the required cell numbers and doses to therapy, scaling up production and purification processes (at least to the liter-scale) while ensuring high purity, viability and maintaining cells' critical quality attributes (CQA) and functionality is essential [1].

Therefore, the aim of this work was to prove scalability of an integrated streamlined bioprocess compatible with current good manufacturing practices (cGMP) comprised by cell expansion, harvesting and volume reduction unit operations using human mesenchymal stem cells (hMSC) isolated from bone marrow (BM-MSC) and adipose tissues (AT-MSC).

BM-MSC and AT-MSC expansion and harvesting steps were scaled-up from spinner flasks to 2 L scale stirred tank single-use bioreactor using synthetic microcarriers and xeno-free medium, ensuring high cellular volumetric productivities (50 x 10^6 cell.L^{-1}.day^{-1}), expansion factors (14 - 16 fold) and cell recovery yields (80%). For the concentration step, flat sheet cassettes (FSC) and hollow fiber cartridges (HF) were compared showing a fairly linear scale-up, with a need to slightly decrease the permeate flux (30 - 50 LMH, respectively) to maximize cell recovery yield. Nonetheless, FSC allowed to recover 18% more cells after a volume reduction factor of 50. Overall, at the end of the entire bioprocess more than 65% of viable (> 95%) hMSC could be recovered without compromising cell's CQA of viability, identity and differentiation potential.

"Omic" tools in combination with standard analytical assays allow for a better cell characterization, increasing product and process understanding [2] and are thus fundamental for process development. Thus, alongside the standard quality assays for evaluating hMSC's CQA, a proteomics workflow based on mass spectrometry tools was established to characterize the impact of processing on hMSC' CQA. Overall, through sensitivity, robustness and throughput, this type of workflow provided the identification of specific signatures of the final product. Therefore, it proves to be essential to understand the cells' final quality as well as to evaluate the impact of manufacturing at different stages of processing.

References: [1] Pattasseril J et al, BioProcess Int. 2013, 3, 38–46. [2] Campbell A et al, Stem Cells Transl. Med. 2015, 4, 1155–1163.

The authors acknowledge UniMS – Mass Spectrometry Unit team (ITQB-NOVA/iBET, Oeiras, Portugal), iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344), and Fundação para a Ciência e Tecnologia (FCT, Portugal) for funding the project CARDIOSTEM (MITP-TB/ECE/0013/2013), and the grants SFRH/BD/51940/2012 (MIT-Portugal), SFRH/BD/52302/2013, SFRH/BD/52481/2014, SFRH/BPD/86513/2012