ENGINEERING SCALABLE MANUFACTURING OF HIGH-QUALITY HUMAN MSC FOR CELL THERAPY: FROM UP TO DOWNSTREAM PROCESSING INTEGRATION TO CELL PROTEOME CHARACTERIZATION

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Human mesenchymal stem cells (hMSC) are relevant cell therapy 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.

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, volume reduction and washing unit operations using human mesenchymal stem cells (hMSC) isolated from bone marrow (BM-MSC) and adipose tissues (AT-MSC). Single-use technologies were adopted at different steps of the manufacturing workflow to support process integration and scale-up.

BM-MSC and AT-MSC expansion and harvesting steps were scaled-up from spinner flasks to 2 L single-use stirred tank 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 volume reduction and washing steps, 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 performed better allowing recovering 18% more cells after a volume reduction factor of 50 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 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.

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