EXPANSION STRATEGIES FOR HUMAN MESENCHYMAL STROMAL CELLS CULTURED UNDER XENO-FREE CONDITIONS

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Key Words: Human AB serum, Mesenchymal stromal cells, Microcarriers, Multilayer flasks, Xeno-free conditions.

Choosing the culture system and culture medium used to produce cells are key steps towards a safe, scalable and cost-effective expansion bioprocess for cell therapy purposes. Traditionally, mesenchymal stromal cells (MSC) have been cultured in a defined basal medium (DMEM or alpha-MEM) supplemented with fetal bovine serum (FBS). Although effective, the use of FBS or other animal-derived components in manufacturing processes is highly discouraged by regulatory agencies due to the risk of transmitting xenogeneic infectious agents and immunization. The use of AB human serum (AB HS) as an alternative xeno-free medium supplement for MSC has increasingly gained relevance due to safety and efficiency aspects. Here we have evaluated different scalable culture systems to produce a meaningful number of umbilical cord matrix-derived MSC (UCM MSC) using AB HS for culture medium supplementation during expansion and cryopreservation to enable a xeno-free bioprocess. UCM MSC were cultured in a scalable planar (compact 10-layer flasks and roller bottles) and 3D microcarrier-based culture systems (spinner flasks and stirred tank bioreactor). 10 layer-flasks and roller bottles enabled the production of $2.6\pm0.6x10^4$ and $1.4\pm0.3x10^4$ cells/cm^2 ($4.5\pm1.1x10^7$ and $3.0\pm0.7x10^7$ cells) representing a fold increase of 13.0 and 7.11, respectively. UCM MSC-based microcarrier expansion in the stirred conditions has enabled the production of higher cell densities ($5.0\text{-}23.0x10^4$ cells/cm^2) when compared to planar systems. Nevertheless, due to the moderate harvest efficiency attained (80% for spinner flasks and 53.9% for bioreactor) the total cell number recovered, $3.3\text{-}3.7x10^7$ cells, was lower than expected. After the expansion in the culture systems evaluated, the cells maintained the functional properties: the cells were able to differentiate into adipocytes, chondrocytes and osteocytes and presented a typical immunophenotype profile. The cryopreservation of cells (also using AB HS) was also successfully carried out in plastic bags to facilitate post-thaw manipulation and enable clinical use. No significant difference was observed when comparing pre-cryopreservation and post-thaw viabilities of cells expanded in the planar (T-flasks) and 3D culture system (spinner flask with microcarriers) indicating that cells which expanded on a larger scale in microcarrier-based suspension cultures could be successfully cryopreserved in plastic bags, with a higher cell number (up to $4x10^8$ cells) and under xeno-free conditions. Overall, our results indicated that among the planar culture systems evaluated, compact multilayer vessels could be used to easily produce enough cells to treat a small number of patients, and are therefore recommended for autologous therapies. However, for a fully-controlled and larger-scale cell production, in an autologous and/or allogeneic scenario, stirred tank bioreactors combined with microcarrier technology and xeno-free media can be successfully used, allowing for GMP-compliant expansion.