Spring 5-12-2016

Scalable production of mesenchymal stem/stromal cells from different human sources in microcarrier-based stirred culture systems

Ana Fernandez
Universidade de Lisboa, ana.fernandes@tecnico.ulisboa.pt

Scalable production of mesenchymal stem/stromal cells from different human sources in microcarrier-based stirred culture systems

Ana Fernandes-Platzgummer, Department of Bioengineering and IBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa
Av. Rovisco Pais, 1049-001 Lisboa, Portugal
T: +351210407051, ana.fernandes@tecnico.ulisboa.pt

Cláudia L. da Silva, Department of Bioengineering and IBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
J.M.S. Cabral, Department of Bioengineering and IBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Human mesenchymal stem/stromal cells (MSC) are promising candidates for cell based therapies and the development of microcarrier-based MSC cultures in scalable stirred bioreactors combined with well-defined serum-/xenogeneic (xeno)-free medium formulations represent important milestones for the clinical-scale production of human MSC. In this work, we optimized our previously established xeno-free microcarrier-based culture system, towards the large-scale production of human bone marrow (BM)-derived MSC and adipose tissue-derived stem/stromal cells (ASC) and we have established a stirred culture system combining gelatin-based microcarriers and xeno-free culture medium for the expansion of umbilical cord matrix (UCM)-derived MSC. In addition, we tested the ability of using a human platelet lysate-based culture supplement to effectively isolate MSC from UCM, as well as to expand these cells under stirred conditions using plastic microcarriers.

By rationally changing the seeding protocol in BM MSC and ASC stirred cultures, initial cell adhesion was successfully increased from 30-40% to approximately 95%. The maximization of initial cell adhesion combined with an optimized feeding regimen in the first days of culture led to higher final cell densities in a shorter culture period (7 *versus* 14 days) compared to our previous report. To enhance the scalability of this xeno-free system, ready-to-use microcarriers Synthemax® II and Enhanced Attachment® were evaluated in comparison to the previously tested pre-coated plastic microcarriers. BM MSC cultures using these beads attained final cell densities identical to cultures with pre-coated plastic microcarriers, demonstrating that ready-to-use microcarriers can be advantageous alternatives for this xeno-free microcarrier-based culture system. When using UCM MSC, cell expansion in the spinner flaks enabled the production of 240,000 cells per mL after 5 days of culture (5 - fold) with xeno-free culture medium. The optimized protocol was then successfully scaled-up to a stirred-tank bioreactor yielding 120 million cells.

We also demonstrate that a human platelet lysate-based product can be an effective supplement for the successful isolation and scalable expansion of UCM MSC under stirred conditions. Upon an initial 54% cell adhesion to plastic beads, UCM MSC were able to expand by > 13 fold after 5-6 days in culture.

Importantly, all expanded cells maintained their characteristic immunophenotype, multilineage differentiation potential and hematopoietic stem/progenitor cell supportive capacity.

We anticipate that this microcarrier-based culture platforms will enable a cost effective, reproducible protocol for the efficient manufacturing of MSC from different human sources, for potential applications in Regenerative Medicine and Cell Therapy settings.