

## A MICROCARRIER-BASED STIRRED CULTURE SYSTEM FOR THE EXPANSION OF HUMAN MESENCHYMAL STEM CELLS

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The growing knowledge of the intrinsic immunologic properties and differentiative potential of human mesenchymal stem cells (MSC) intensified the research on their clinical applications. Due to the very low percentage of MSC in their niches, a rapid *ex-vivo* expansion method is needed to meet the highly demanding dose of MSC for clinical settings.

MSC cultures have typically been performed in traditional culture flasks under static conditions, which are limited in terms of cell number generated, their non-homogeneous nature and difficulty of monitoring. In this context, bioreactor systems have been developed as alternatives to standard flask cultures for *in vitro* culture. However, reports of MSC expansion in bioreactor systems are scarce and cell numbers generated are still insufficient.

In this work we investigated the *in vitro* three-dimensional expansion of bone marrow (BM) derived MSC in spinner flasks. Gelatin microcarriers (*Cultispher S*) were used as matrix to sustain cell adhesion and proliferation under stirred conditions. Expansion of MSC was performed using MesenPRO™, a commercially available medium supplemented with 2% fetal bovine serum (FBS).

In a first preliminary experiment, we observed that: (i) only half of the inoculated cells adhered properly to the beads, (ii) cell culture displayed a long lag phase (approximately 10 days), and (iii) glucose starvation occurred upon day 13. Nevertheless, the cells that successfully adhered to the microcarriers were expanded reaching a fold increase of 5-6 by day 21.

In order to improve cell seeding efficiency, beads were incubated overnight with FBS to mediate cell adhesion to gelatine microcarriers. Regarding the feeding regime, a more frequent medium change was performed (25% each day rather than each two days) in order to prevent nutrient starvation and/or metabolic by-product accumulation, as well as to avoid the over-dilution of important autocrine factors affecting cell growth. Under these optimized conditions, a 100% seeding efficiency was achieved and no lag phase was observed. The maximum cell density was obtained at day 8,  $3.9 \cdot 10^5$  cell/mL, corresponding to a fold increase of  $8 \pm 2$ . The specific growth rate was  $0.46 \text{ d}^{-1}$ , which was twice higher than that obtained in the preliminary experiment. After the initiation of the exponential growth rate, the specific consumption and production rates of metabolites maintained roughly constant, being  $q_{\text{glucose}} = 5.4 \cdot 10^{-9}$ ,  $q_{\text{lactate}} = 1 \cdot 10^{-8}$ ,  $q_{\text{glutamine}} = 9.0 \cdot 10^{-10}$  and  $q_{\text{ammonia}} = 1.0 \cdot 10^{-9} \text{ mmol} \cdot \text{cell}^{-1} \cdot \text{d}^{-1}$ . During the time expansion course lactate and ammonium concentration values were below 12 mM and 3 mM, respectively.

After the expansion, MSC were able to maintain their differentiation potential into adipogenic and osteogenic lineages, as well as their clonogenic ability as assessed

by the CFU-F assay. Harvested cells expressed >90% of CD73, CD90 and CD105, as measured by flow cytometry.

These results demonstrated that a low serum containing medium is adequate for MSC expansion in a stirred system, which is potentially advantageous to the conventionally used media supplemented with FBS at 10-20% content. Ongoing studies focus on the addition of vacant microcarriers at a specific time point of the culture to increase the available growth surface, potentially extending the proliferation stage of MSC. This would turn possible cell subcultivation without a harvesting step, improving the scalability of the expansion process.