Growth Kinetics of Human Mesenchymal Stem Cells in the Mobius® CellReady Single-use Bioreactor

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Abstract

The increased knowledge of the differentiation capability as well as of the immunologic properties of human mesenchymal stem cells (hMSCs) has stimulated the interest in their use as therapeutic agents. To date, several clinical trials using hMSCs are underway in a variety of indications. However, a key hurdle in the clinical application of hMSCs is the high cost of manufacturing. In addition, current practices using multilayer flatbed cultures do not allow a constant monitoring of cells during the manufacturing process and introduce a high degree of variability. Thus, flatbed cultures can be regarded as sub-optimal for the manufacturing of clinical grade hMSCs.

To overcome these deficiencies the use of stirred tank bioreactors provides an attractive alternative. In these cultures, hMSCs can be grown on microcarriers and samples of cells and medium can be analyzed throughout the process. Here we show the utility of EMD Millipore's Mobius® CellReady 3L single use bioreactor for the expansion of hMSCs. Cells can be seeded directly into the bioreactor without prior need to attach the cells to microcarriers. After 2 weeks of culture, cells reach densities greater than 2×10^5 cells/mL while maintaining their identity as shown by the surface expression of CD105, CD90 and CD73 and the absence of CD14, CD34 and CD45. In addition the ability to differentiate to the adipogenic, chondrogenic, and osteogenic lineages is retained after culture. Furthermore, the capacity to monitor the metabolism of cells facilitates a feeding strategy to maximize cell number.

Taken together, these results show the feasibility of expanding hMSCs in the Mobius® CellReady 3L Bioreactor. This system provides a cost-effective approach for the production of clinical grade hMSCs and allows for a close monitoring of the process.

Materials

Mobius® CellReady 3L Bioreactor (EMD Millipore, CR0003L200); in-house derived bone marrow (Lonza 1M-125) hMSCs; Collagen Microcarriers (Solohill, C102-1521); Corning ® CellSTACK ® Cell Culture Chambers (Cat. #: 3270)

Method

hMSCs were cultured with 8 ng/mL bFGF and 10% MSC-screened FBS media in 5% CO_2 at 37°C incubator or bioreactors

Results

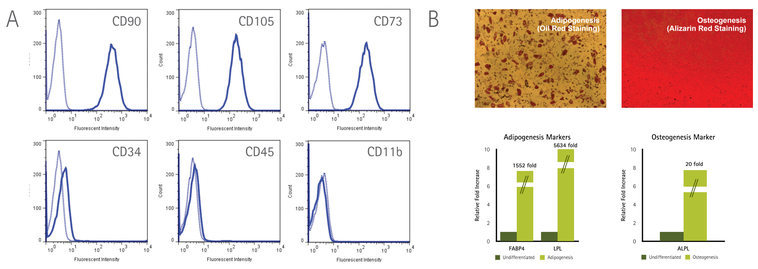

FIGURE 1. Characterization of in-house derived human mesenchymal stem cells. A) After establishing the cells in the lab, hMSCs were characterized by flow cytometry. CD90$^+$, CD105$^+$, CD73$^+$, CD34$^-$, CD45$^-$, CD11b$^-$ cell surface antigen profiles were expressed as expected (guidance from Dominici, et al., 2006); **B)** Both immunostaining and qPCR results showed that hMSCs retained differentiation ability toward adipogenic, osteogenic and chondrogenic lineages.

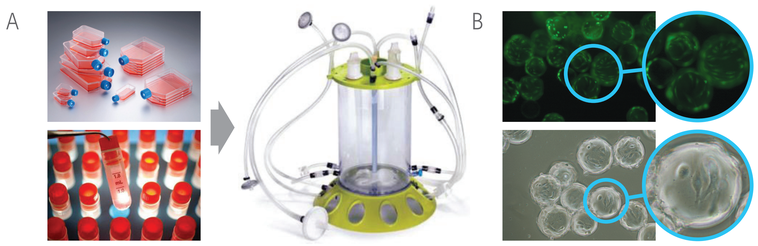

FIGURE 2. hMSCs expansion in Mobius® CellReady 3L bioreactor on microcarriers. A) hMSCs can be either expanded first on T-flasks or thawed and directly seeded into bioreactor with microcarriers; **B)** Morphology of hMSCs on microcarriers after expansion in the bioreactor (Upper: CellTracker Green; Lower: Bright Field).

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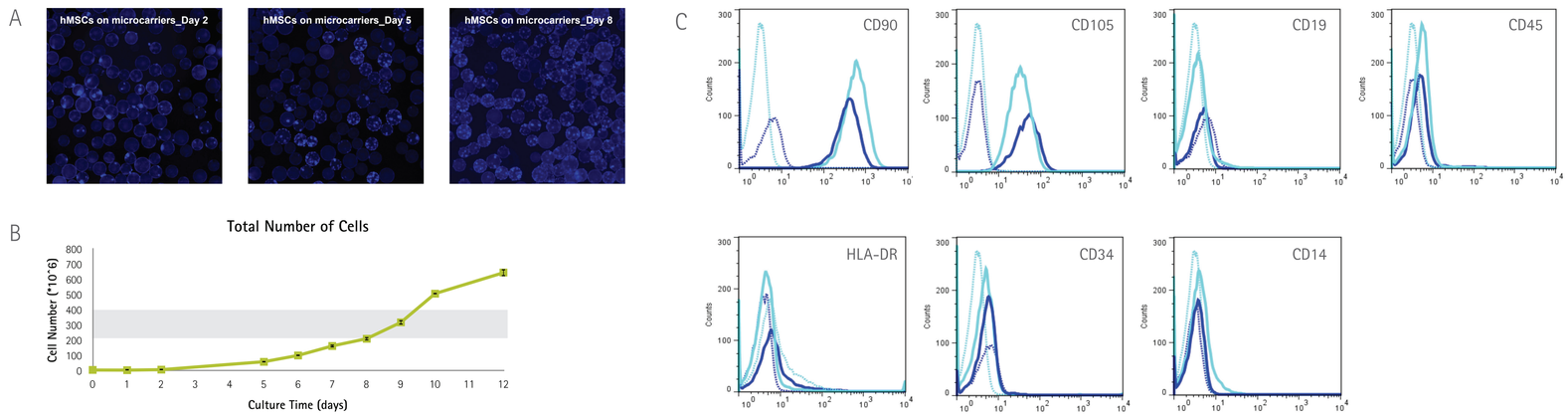

FIGURE 3. Comparison of hMSCs growth in Mobius® CellReady 3L bioreactor with 10-Stack CellSTACK® multilayered flask. A) Time-course staining of hMSCs on microcarriers in Mobius® CellReady 3L bioreactor. After expansion, hMSCs not only proliferated on initial seeded microcarriers but also colonized empty microcarriers. **B)** After 1-3 days lag phase, hMSCs expanded quickly in bioreactor and reached maximum cell number (~600 million cells) in 12 days. The grey bar represents the expected cell number from a 10-Stack CellSTACK® in 14 days; **C)** Flow cytometry data illustrated no difference in cell surface antigen expression between hMSCs expanded in CellSTACK® and bioreactor with microcarriers (dotted line: isotype control; **dark blue line: bioreactor**; light blue line: CellSTACK®) .

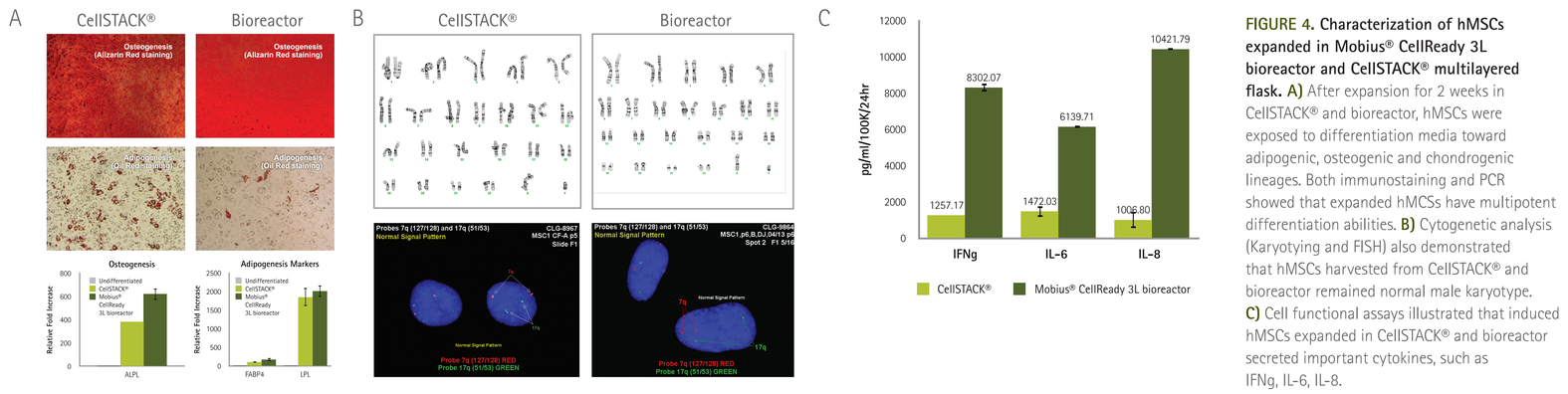

FIGURE 4. Characterization of hMSCs expanded in Mobius® CellReady 3L bioreactor and CellSTACK® multilayered flask. A) After expansion for 2 weeks in CellSTACK® and bioreactor, hMSCs were exposed to differentiation media toward adipogenic, osteogenic and chondrogenic lineages. Both immunostaining and PCR showed that expanded hMCSs have multipotent differentiation abilities. **B)** Cytogenetic analysis (Karyotying and FISH) also demonstrated that hMSCs harvested from CellSTACK® and bioreactor remained normal male karyotype. **C)** Cell functional assays illustrated that induced hMSCs expanded in CellSTACK® and bioreactor secreted important cytokines, such as IFNg, IL-6, IL-8.

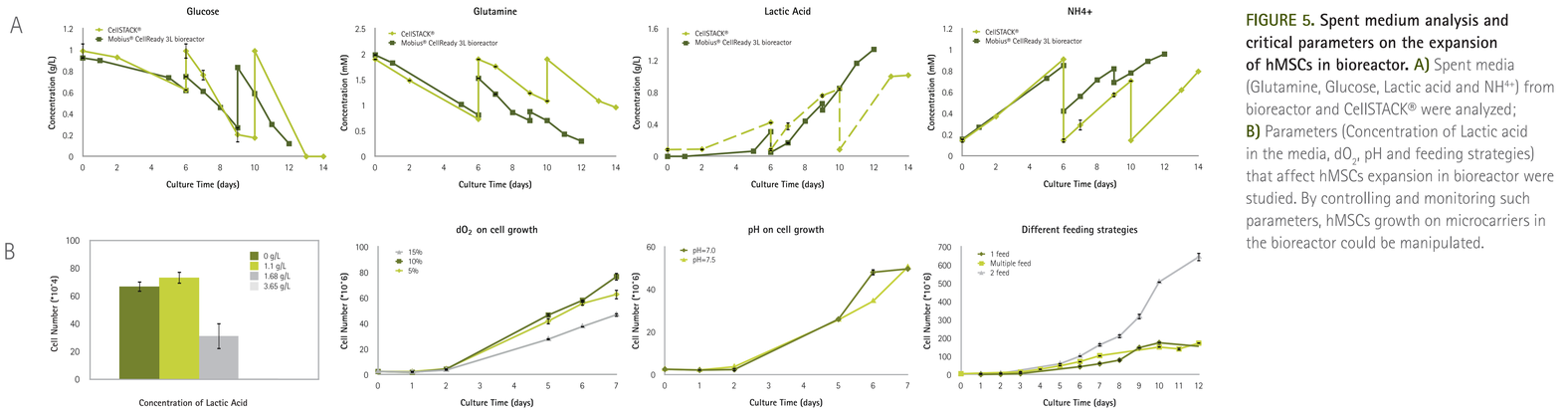

FIGURE 5. Spent medium analysis and critical parameters on the expansion of hMSCs in bioreactor. A) Spent media (Glutamine, Glucose, Lactic acid and NH^{4+}) from bioreactor and CellSTACK® were analyzed; **B)** Parameters (Concentration of Lactic acid in the media, dO_2, pH and feeding strategies) that affect hMSCs expansion in bioreactor were studied. By controlling and monitoring such parameters, hMSCs growth on microcarriers in the bioreactor could be manipulated.

Conclusions and Summary

- hMSCs can be successfully expanded in 3L bioreactor with microcarriers.
- hMSCs expanded in bioreactor showed similarities in flow cytometry profiles, differentiation abilities, functional assays as cells produced in 2D CellSTACK®.
- The Mobius® CellReady 3L bioreactor process can be controlled and monitored.
- Future plans include scaling up the process in Mobius® 50 L bioreactor with microcarriers.

	Mobius® CellReady (3L)	CellSTACK® (10-stack)
Medium	2.4 L	6 L
PBS	2 ~ 4 L	2 ~ 4 L
Trypsin	0.4 ~ 0.6 L	0.4 ~ 0.6 L