Growth Kinetics of Human Mesenchymal Stem Cells in a 3L Single-Use, Stirred Tank Bioreactor

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Human mesenchymal stem cells hold great promise as therapeutic agents because of their differentiation ability (thus their potential to replace damaged tissue) and for their immunomodulatory properties. A large number of clinical trials are underway that are using hMSCs in a variety of indications, including bone/cartilage disease, cancer, heart disease, gastrointestinal disease, diabetes, autoimmunity and neurodegenerative diseases;[1] hMSCs are also being used in drug discovery as a replacement for primary cells and animal models for initial toxicity and effector function screening of new compounds.[2] However, a key challenge remains for both drug discovery and clinical applications: obtaining a sufficient number of cells at reasonable cost.[3]

The large-scale, industrialized production of hMSCs is necessary to advance these cells into clinical trials and to deliver the large quantities needed for drug discovery screening and lead optimization. Bridging the gap between basic research and large scale manufacturing of stem cells for clinical trials requires the expansion of well-characterized cells produced under tightly controlled, consistent, reproducible culture conditions that adhere to current Good Manufacturing Practice (cGMP) standards. cGMP stem cell culture systems require well-defined, optimized processes that support stem cell expansion and differentiation to ensure consistent cell populations with uniform properties and predictable behaviors. Additionally, vessels used for expansion must allow rapid analysis of small volumes containing the actual cells to confirm that the expansion and harvest methods are yielding the expected cell populations. As stem cells are the product, the sample size must be small enough to ensure that valuable product is not wasted.

Human mesenchymal stem cells have historically been isolated based on the ability of these cells to form adherent cell layers in culture and the concomitant lack of adherence of other cells in the bone marrow stroma such as hematopoietic stem cells, adipocytes, and macrophages.[1] However, the multilayer flatbed culture (2D) methods currently used for stem cell expansion are cumbersome, time-consuming, do not allow for constant monitoring of cell characteristics throughout the expansion process, and they introduce a high degree of variability. These limitations make this method sub-optimal for the manufacturing of clinical grade hMSCs. Furthermore, the culture protocols for multilayer vessels require high labor cost, which in turn results in high cost of goods overall. Thus, the development of culture conditions that can be monitored and derive high number of stem cells at low cost is warranted.

One possible solution for overcoming the limitations of 2D multilayer flatbed culture methods is the use of stirred tank bioreactors in which the stem cells are grown on a microcarrier scaffold for suspension (3D). In these 3D cultures, cell samples and medium can be analyzed throughout the expansion process and the growth process can be tightly controlled (e.g. oxygen, pH, glucose, lactate, and ammonia). EMD Millipore tested the utility of its 3L single use bench-scale bioreactor (Mobius® CellReady, EMD Millipore, Billerica, MA) for the expansion of hMSCs.

Results and Discussion

For 3D hMSC expansion, cells can either be expanded first on T-flasks or thawed and directly seeded in the Mobius® CellReady 3L Bioreactor on microcarriers (Figure 2). To determine the best parameters for 3D expansion, various cell and microcarrier seeding densities were investigated (Figure 3). Optimal cell seeding density was demonstrated at 5 million cells/L and optimal microcarrier seeding density was demonstrated at a concentration of 15 g/L.

To study the effects of aeration on the growth of hMSCs in the bioreactor, 5 million cells/L were seeded per bioreactor, allowed to grow for six days and then various sparge strategies were executed, including aeration through minisparge and open-tube at both high and low air flow rates (Figure 4). By day 12, cell growth reached a maximum in the control bioreactor, which could either be due to shear force by air bubbles or a preferred lower oxygen culture.

The impact of lactate and glucose concentration and pH on the expansion of hMSC on microcarriers in suspension culture was tested (data not shown). hMSCs were able to grow under conditions of no glucose by consuming other nutrients in the medium; however, the growth rate was reduced. hMSCs were able to expand under conditions of high glucose concentration (4.5g/L) without significantly reducing growth rate. High concentrations of lactate (>1/66g/L) inhibit hMSC growth on microcarriers, which is consistent with 2D culture results. Finally, optimal growth of hMSCs occurs at pH=7.6; cell growth was significantly reduced at low pH (<6.8).

The growth rate of hMSCs was then compared in 2D and 3D cultures (Figure 5). hMSCs not only proliferated on microcarriers in the bioreactor, but also colonized empty microcarriers. After a 1-3 day lag phase, hMSCs expanded quickly in the bioreactor and reached maximum cell number (~600 million cells) in 12 days, which is roughly 50 percent greater than expected from growth in 2D culture after 14 days. Flow cytometry data illustrated no difference in cell surface antigen expression between hMSCs expanded via 2D or 3D culture.

hMSCs were characterized following expansion for two weeks in 2D and 3D cultures (Figure 6). Expanded cells were exposed to differentiation media toward adipogenic, osteogenic and chondrogenic lineages, cytogenetic analysis and cell functional analysis. Regardless of method of expansion, expanded hMSCs demonstrated multipotent differentiation abilities, normal male karyotype and the ability to secrete important cytokines, including interferon-gamma, interleukin-6 and interleukin-8.

Spent media (glutamine, glucose, lactic acid and NH^{4+}) from 2D and 3D culture were analyzed and compared (Figure 7). Additionally, multiple parameters that potentially affect hMSC expansion in 3D cultured were examined, including the concentration of lactic acid in the media, dO_2, pH and feeding strategies (Figure 7). The capacity to monitor and control the metabolism of cells in a bioreactor facilitates a feeding strategy to maximize cell number.

Conclusion

Taken together, these data demonstrate that expansion of hMSCs in 3D culture demonstrate similar flow cytometry profiles, differentiation ability and functional status as hMSCs expanded in 2D culture. After two weeks, 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.

The production of well-characterized hMSCs in stirred tank bioreactors using microcarriers is the first step towards establishing a scalable, single use production process for the large scale, industrialization

of stem cells. The Mobius® CellReady 3L bioreactor presents several advantages compared to multilayer flatbed cultures, including lower overall cost, ease of use and the ability to closely monitor process parameters throughout expansion and make adjustments while cells are still viable, if needed.

Figure Legend

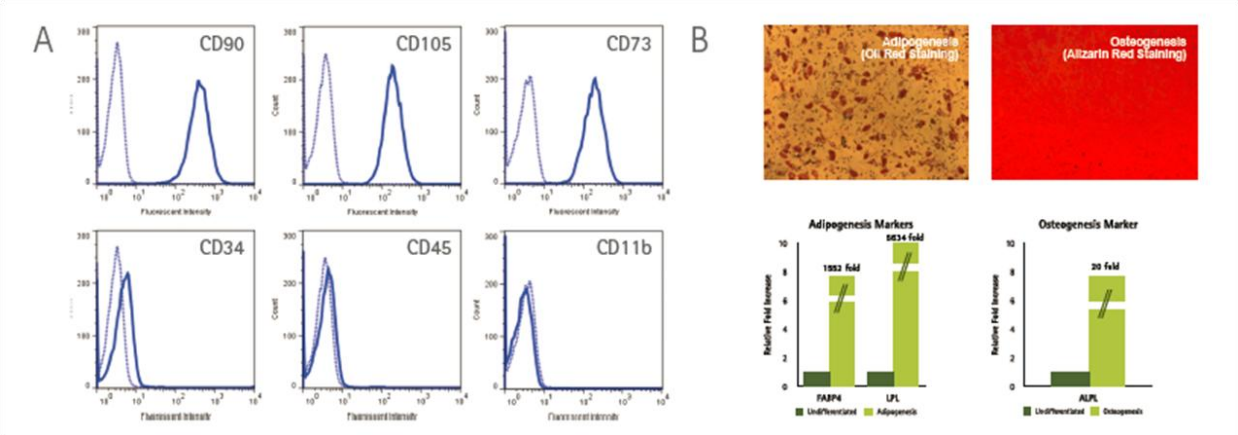


FIGURE 1: (A) In-house derived hMSCs express CD90^{+}, CD105^{+}, CD73^{+}, CD34^{-}, CD45^{-}, CD11b^{-} cell surface antigen profiles; (B) In-house derived hMSCs retain differentiation ability toward adipogenic, osteogenic and chondrogenic lineages.

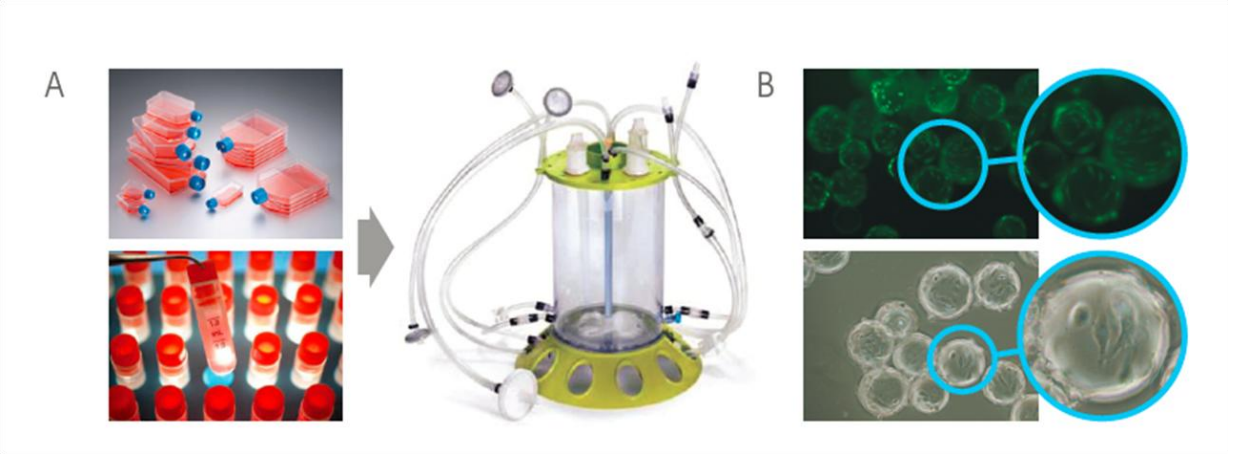
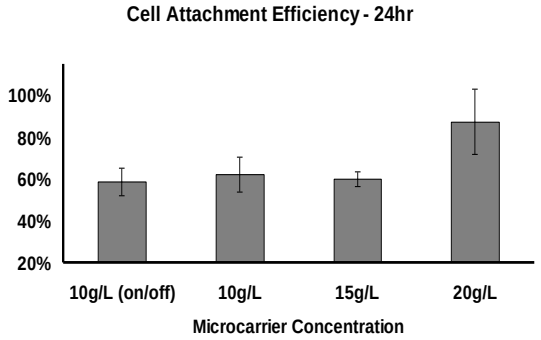
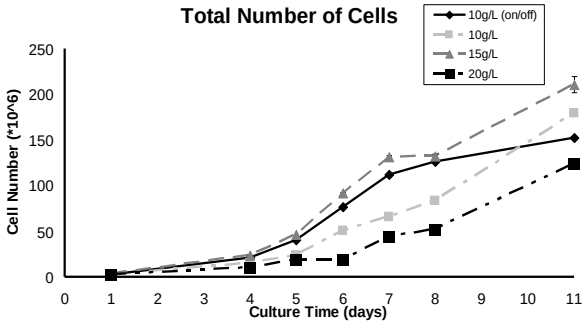


FIGURE 2: (A) hMSCs can be either expanded first on T-flasks or thawed and directly seeded into Mobius® CellReady 3L bioreactor with microcarriers; (B) Morphology of hMSCs on microcarriers after expansion in the bioreactor (Upper: CellTracker Green; Lower: Bright Field).



A

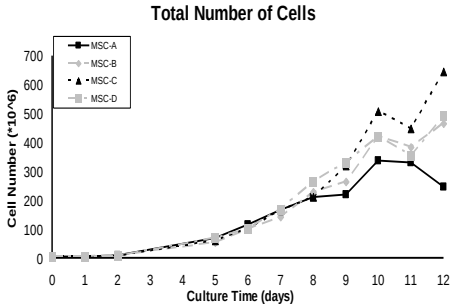


B

Figure 3: (A) hMSC attachment affinity 24 hours; (B) Microcarrier concentration of 15g/L demonstrates maximum hMSC expansion after 11 days.

A

MSC1	dO2	Sparge Strategy
Bioreactor A	20% dO2	Open tube, high air flow rate
Bioreactor B	20% dO2	Mini-sparge, high air flow rate
Bioreactor C	Control	
Bioreactor D	20% dO2	Open tube, low air flow rate



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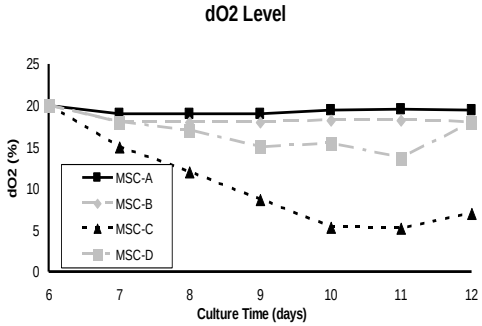

C

Figure 4: (A) Sparge strategies; (B) Cell number reached a maximum in the control bioreactor; (C) dO2 level remained fairly constant except in the control bioreactor.

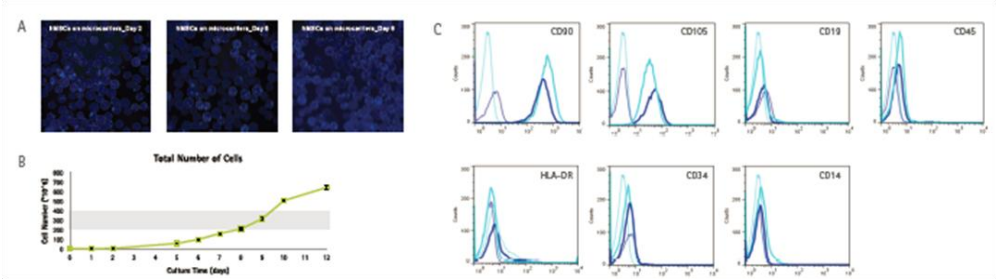

FIGURE 5: (A) hMSCs not only proliferated on initial seeded microcarriers but also colonized empty microcarriers; (B) hMSCs expanded quickly in the bioreactor and reached maximum cell number (~600 million cells) in 12 days. The grey bar represents the expected cell number from a 10-Stack CellSTACK® in 14 days; (C) No difference in cell surface antigen expression between hMSCs expanded in CellSTACK® and a bioreactor with microcarriers (dotted line: isotype control; dark blue line: bioreactor; light blue line: CellSTACK®).

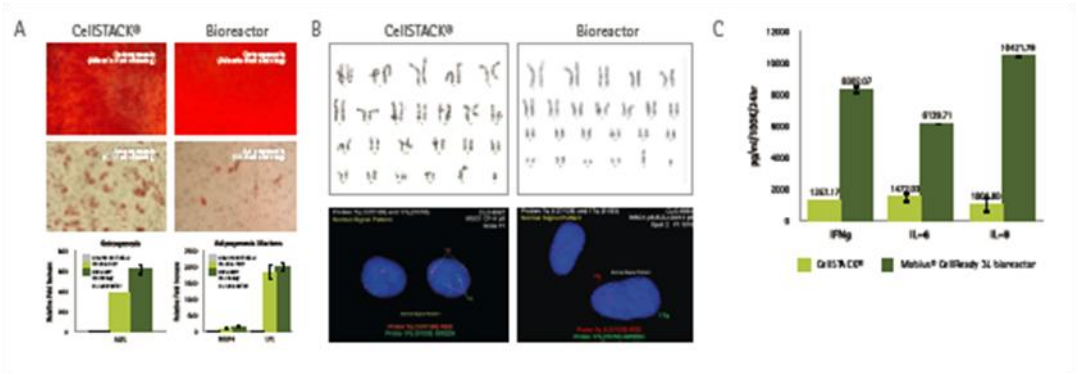

FIGURE 6: (A) After expansion for 2 weeks in CellSTACK® and Mobius® CellReady 3L bioreactor, hMSCs demonstrate multipotent differentiation abilities; (B) Expanded hMSCs retain normal male karyotype; (C) Induced expanded hMSCs secrete important cytokines, including IFN-γ, IL-6 and IL-8.

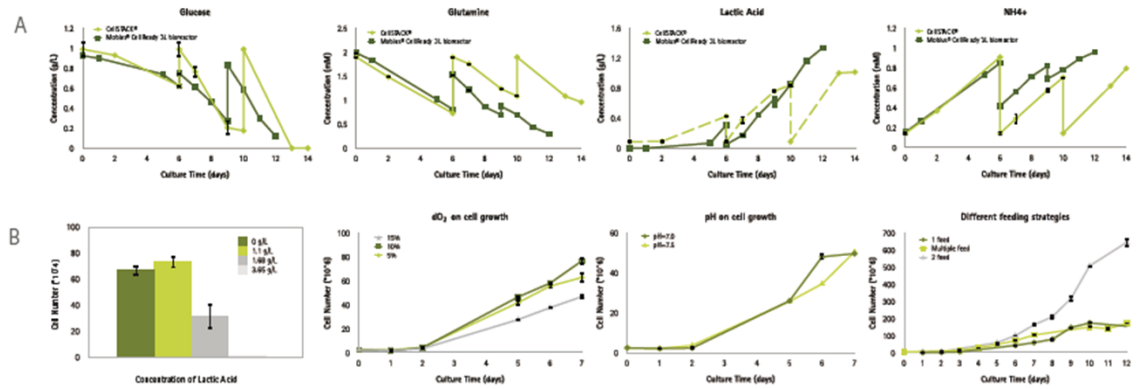

FIGURE 7: (A) Spent media (Glutamine, Glucose, Lactic acid and NH4+) from bioreactor and CellSTACK® were analyzed; (B) Parameters (Concentration of Lactic acid in the media, dO_2, pH and feeding strategies) that affect hMSCs expansion in bioreactor were studied.

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[1] Jing, D., Parikh, A, Canty, J, and Tzanakakis, E. *Tissue Eng Part B Rev*. 2008 Dec; 14(4): 393–406.

[2] Kitambi SS and Chandrasekar. Stem cells: a model for screening, discovery and development of drugs. *Stem Cells and Cloning: Advances and Applications.* 2011 Sept; 4: 51–59.

[3] Rowley, J, Abraham E, Campbell A, Brandwein H, and Oh S. Meeting Lot-Size Challenges of Manufacturing Adherent Cells for Therapy. *BioProcess International*. 2012 Mar; 10(S3): 16–22.