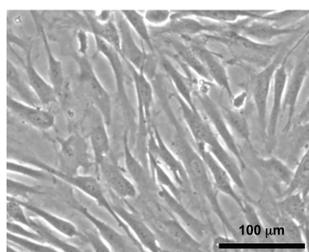


# Identity and Purity Characterization of Human MSCs Expanded in a 3L Stirred Tank Bioreactor

Julie Murrell, Sandhya Punreddy, Manjula Aysola, Kimberly Mann, Anjali Verma, Ellen Binder\*, Donghui Jing, Daniel Kehoe, Neethu Sunil, Knut Niss, Martha Rook  
EMD Millipore Corporation, 80 Ashby Rd, Bedford, MA 01730 USA; \* Merck Millipore, Frankfurter Str. 250, Darmstadt, Germany

## Objective

Here we describe implementation of a panel of assays that characterizes the cell state and the identity and purity of human mesenchymal stem cells (hMSCs). Cell state assays include viability, apoptosis and cell cycle. Identity and purity were assessed using a panel of positive and negative markers based on Dominici, et al., (2006) as well as markers that measure the activation state of the cells. The combination of cell state and marker panels for identity and purity can be used as a process monitoring tool for expansion of hMSCs in addition to a quality control assessment following expansion.



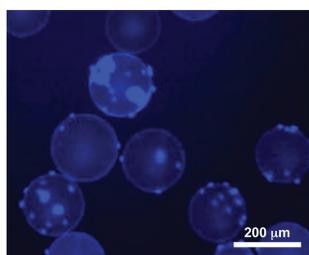
hMSCs on tissue culture flask

## Background

hMSCs are a population of well characterized adult stem cells that can be derived from many tissues including bone marrow and adipose tissue. Due to their potential ability to replace damaged tissue and to provide extracellular factors, they are an attractive target for clinical studies as therapeutic agents and many clinical trials are currently under way. Current multilayer flatbed culture expansion paradigms are cumbersome, time consuming and typically limited in the ability to monitor cell characteristics during the expansion process. We have described a new expansion paradigm that uses a three liter, single use, stirred tank bioreactor with a microcarrier scaffold for suspension expansion of hMSCs (see Jing, et al., poster no. 79). The bioreactor enables sampling throughout the run enabling monitoring of the cells to give an indication of the quality of the expanded cells and to ensure the hMSCs are in the desired undifferentiated state. Here we describe the use of a panel of flow cytometry assays to monitor the hMSC expansion process.



Mobius® CellReady 3L Bioreactor



DAPI staining of hMSC nuclei on microcarriers

## Methods

hMSCs were grown on collagen-coated T150 flasks for use as controls. hMSCs were also seeded onto collagen-coated microcarriers (Solohill) and used in Mobius® CellReady 3L bioreactors for suspension culture (see Jing, et al., poster no.79 for details). Reactors were run for up to 14 days, while flasks were typically cultured for 4 days. Cells were harvested by incubation in 0.05% trypsin. A panel of 14 antibodies against cell surface proteins and 5 isotype matched control antibodies were used for routine analysis with a Guava easyCyte™ 8HT 2 laser, 8 channel flow cytometer. Additionally, ViaCount®, Guava Nexin® and Cell Cycle® kits were used to assess the health of the bioreactor cultures by measuring cell viability, percent of early and late apoptotic cells and the cell cycle population distribution. The Guava® platform requires fewer cells for analysis compared to a traditional flow cytometer, making it more amenable for use in monitoring assays when the number of cells is limiting, e.g., early in the run.



Guava® easyCyte™ 8HT

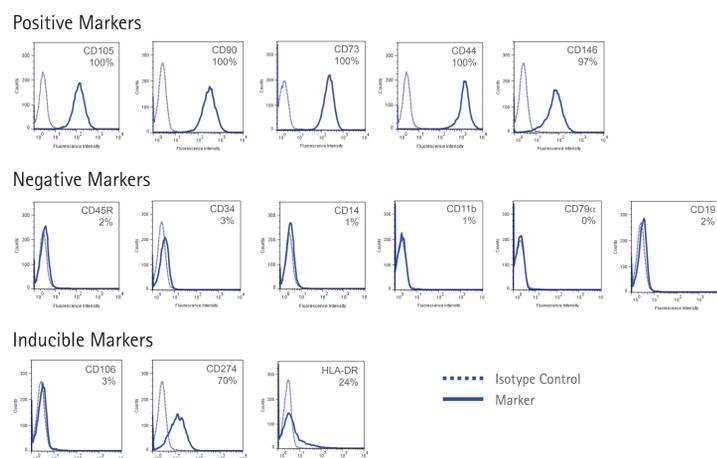
Corresponding Author: julie.murrell@merckgroup.com

Millipore, Mobius, Viacount, Guava Nexin, Cell Cycle and Guava are registered trademarks of Merck KGaA, Darmstadt, Germany. Merck Millipore, the M mark, and easyCyte are trademarks of Merck KGaA, Darmstadt, Germany. CellSTACK is a registered trademark of Corning, Inc. Lit. No. PS10020000 © 2012 EMD Millipore Corporation, Billerica, MA, USA. All rights reserved.

Merck Millipore is a Division of MERCK

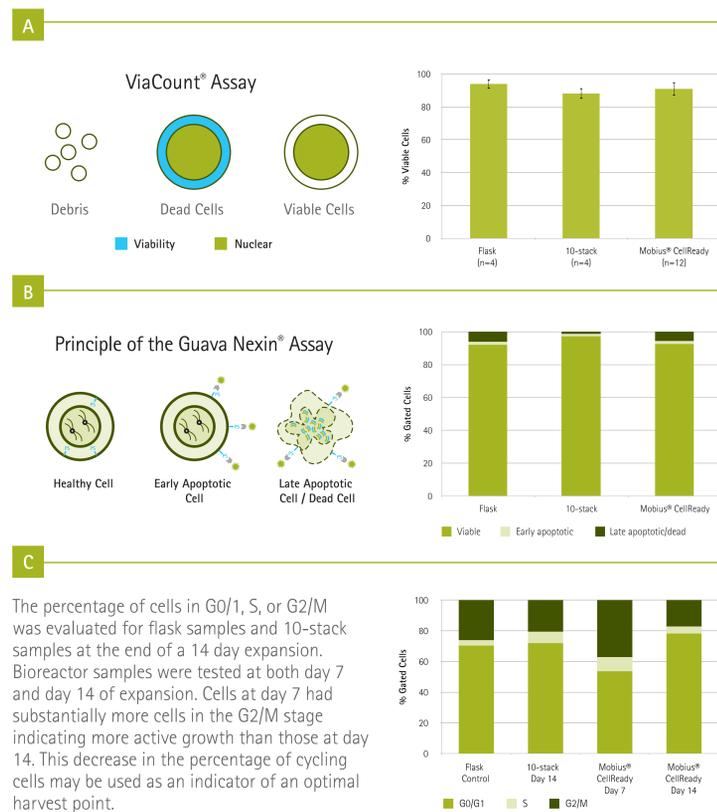
## Figure 1: Surface Antigen Expression of Expanded hMSCs

hMSCs that were expanded in the Mobius® CellReady 3L bioreactor were characterized by flow cytometry. The cells expressed markers CD105, CD90, CD73, CD44, and CD146 which are associated with hMSCs. The cells did not express the hematopoietic markers CD45, CD34, CD14, CD11b, CD79α, or CD19. The activation state of the cells was evaluated by measuring inducible markers CD106, CD274 and HLA-DR; cells in their baseline, uninduced state are shown below.



## Figure 2: Cell Health Monitoring

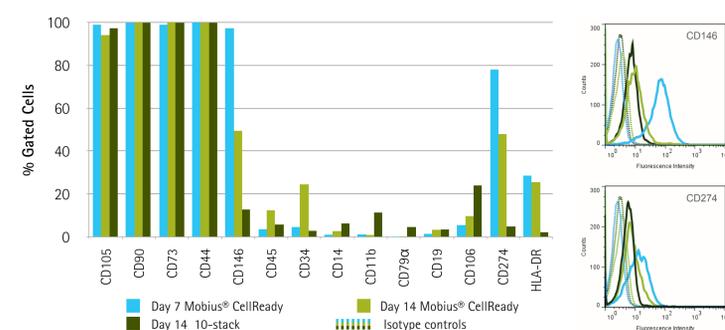
Assays for Viable Cell Number, Apoptosis and Cell Cycle Phase were run on flask controls and hMSCs expanded in 10-stacks or Mobius® CellReady 3L bioreactors with microcarriers. **A)** No difference in viability was observed between cells grown in flasks, 10-stacks and Mobius® CellReady 3L bioreactors with microcarriers. **B)** No difference observed in the population of early or late apoptotic cells when hMSCs were grown on microcarriers in bioreactors compared to flask-based growth.



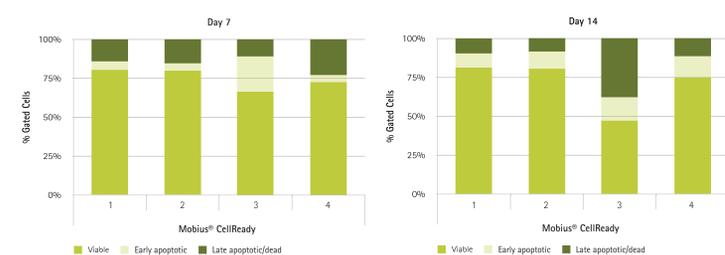
The percentage of cells in G0/1, S, or G2/M was evaluated for flask samples and 10-stack samples at the end of a 14 day expansion. Bioreactor samples were tested at both day 7 and day 14 of expansion. Cells at day 7 had substantially more cells in the G2/M stage indicating more active growth than those at day 14. This decrease in the percentage of cycling cells may be used as an indicator of an optimal harvest point.

## Figure 3: Cell Characterization as a Monitoring Tool During Expansion

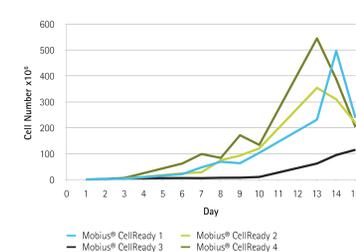
Surface antigen expression of hMSCs grown in Mobius® CellReady 3L bioreactors and in 10-stack CellSTACK® were compared. Cells from bioreactors were analyzed at day 7 and day 14, while cells from the 10-stack could only be analyzed at day 14. While most markers were consistent between both bioreactor and 10-stack throughout expansion, CD146 and CD274 were highly expressed in bioreactor day 7 cells and had decreased expression in both bioreactors and 10-stacks at day 14.



## Figure 4: Apoptosis Analysis as a Process Quality Indicator



During a bioreactor process optimization experiment where each bioreactor was a different condition, day 7 analysis showed an increased population of early apoptotic cells in bioreactor 3. By day 14, viability in bioreactor 3 had decreased to 42% and the late apoptotic/dead cell population increased to 34%. This increased apoptotic population was correlated with poor cell growth characteristics for bioreactor 3.



## Conclusions and Summary

Expansion of hMSCs in stirred tank bioreactors using microcarriers generates cells with equivalent cell health and antigen marker expression as traditional flask based expansion.

The ability to sample and monitor cells during expansion in a bioreactor allows changes in the cell health or identity to be correlated to a need for adjustments to operating parameters or optimal harvest time points.

The combination of cell state and marker panels for identity and purity can be considered a process monitoring tool for expansion of hMSCs in addition to a quality control assessment following expansion.