

## Optimization of a Scalable Single-Use Manufacturing Platform for Expansion of High Quality Human Mesenchymal Stem Cells

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### Abstract

- Human mesenchymal stem cells (hMSC) isolated from various tissues represent the most highly utilized cell type for cell therapy and regenerative medicine applications.
- Given that large numbers of high quality cells are required for many applications and that a large surface area requirement for cell expansion is necessary, a search for a practical manufacturing platform for cell expansion has been ongoing over the last decade.
- Feasibility studies have demonstrated the utility of using microcarriers in dynamic cultures for expansion of hMSC and the results have positioned this technology as the platform of choice for the cost-effective generation of cells.
- Microcarrier cultures performed in fully closed single-use systems provide a platform for cell expansion and are a logical extension of traditional manufacturing technologies.
- The use of high quality cell banks and bioprocess medium in these platforms may significantly impact overall performance.

### Materials

Allegro™ Unison hBM MSC and Medium



- Allegro Unison hMSC Basal Medium:** Lays foundation for successful culture.
- Allegro Unison hMSC Media Supplement A:** Potent supplement allowing for rapid expansion.
- Allegro Unison hMSC Media Supplement R:** Bioreactor feed for effective, simple, scalable and economic microcarrier expansion.
- Allegro Unison hBM MSC:** Bone marrow-derived hMSC designed for scale-up process development. Each vial of cells is guaranteed to expand 10-fold within 1 week when paired with our media systems.

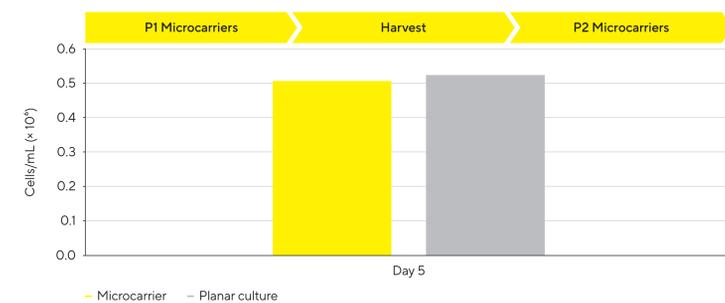
Allegro Microcarrier Delivery System (AMDS)



- SoloHill® Microcarriers:** Delivered ready-to-use in a single-use format.
- Sterilized by gamma irradiation.
- Allows one to directly seed bioreactors – no additional preparation needed.

### Performance

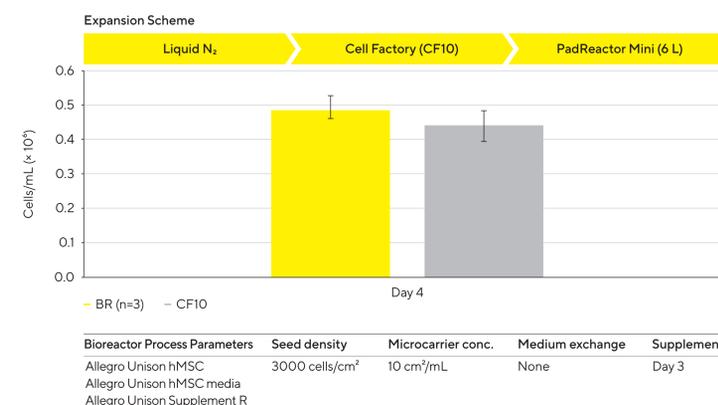
**Figure 1**  
Serial Passage Studies



Serial passage of Allegro Unison hBM MSC in Allegro Unison medium with Pall SoloHill® collagen microcarriers. Cell densities reached 0.5 million cells per mL and over 16-fold expansion after 5 days in spinner cultures.

### Performance (Continued)

**Figure 2**  
Reproducible and Robust Cell Expansion in 6 Liter PadReactor® Mini Bioreactor



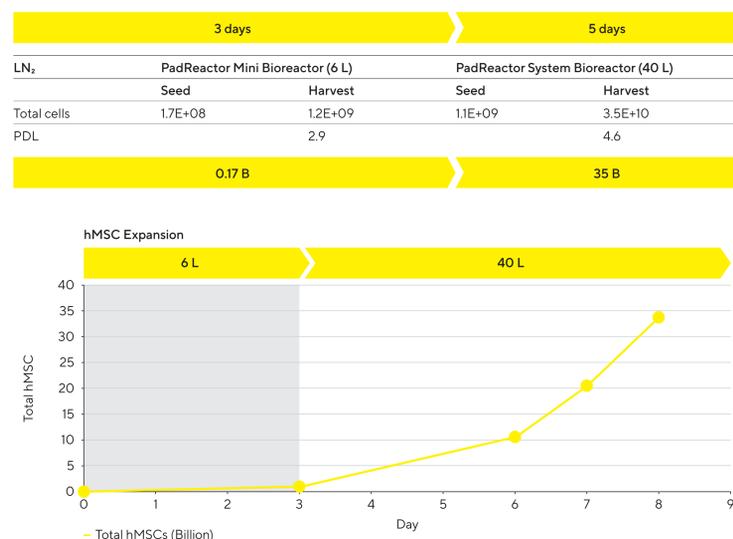
Reproducible expansion of hMSC on SoloHill® microcarriers in PadReactor bioreactors. Cell densities reached approximately 0.5 million cells per mL and over twenty-fold expansion was reproducibly achieved in fed-batch microcarrier cultures in the PadReactor Mini bioreactor at 6 L scale in 4 days (BR n = 3).

**Figure 3**  
Expansion using PadReactor System and Pall SoloHill® Microcarriers



Expansion scheme for Allegro Unison hBM MSC and medium on Pall SoloHill® microcarriers in the Allegro Microcarrier Delivery System (AMDS) using Pall's PadReactor System. Cells thawed from liquid nitrogen were seeded directly onto collagen microcarriers in the PadReactor Mini bioreactor at 6 L and expanded for 3 days. Cells were harvested and seeded onto collagen microcarriers (AMDS) in the PadReactor 50 system at 40 L and cultured for 5 days.

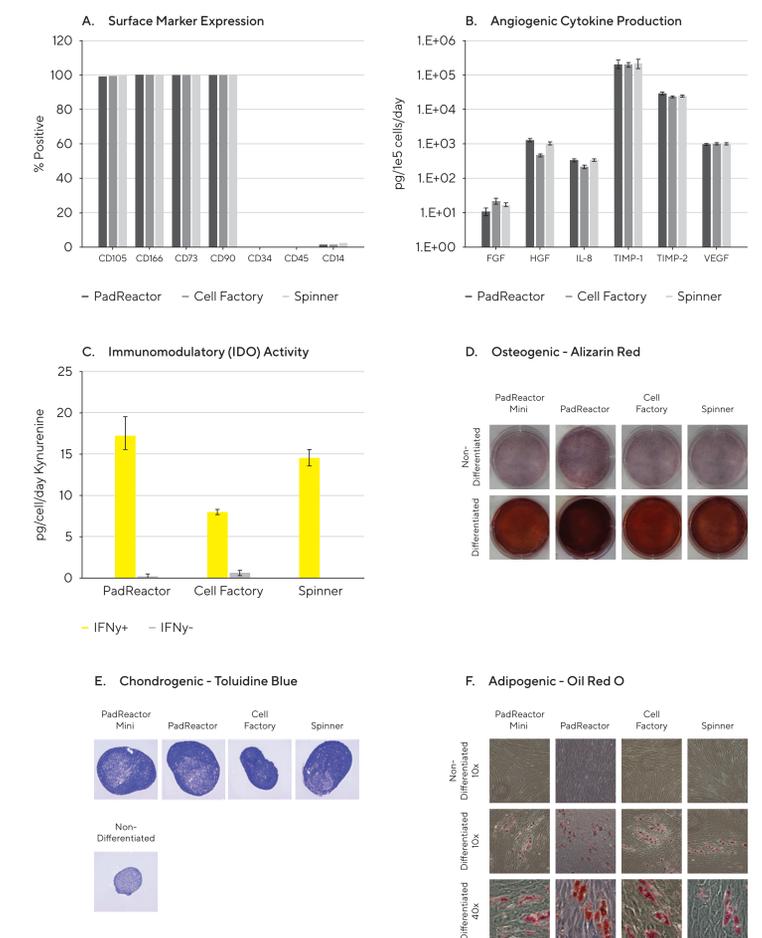
**Figure 4**  
PadReactor System Batch-Fed Process Generates Billions of hMSC in 8 Days



Allegro Unison hBM MSCs seeded onto Pall SoloHill® Collagen microcarriers (AMDS) in the PadReactor Mini bioreactor at 6 L were cultured for 3 days. These cells were used to seed a PadReactor 50 system at 40 L containing collagen microcarriers (AMDS) and were cultured for an additional 5 days. Overall, this batch-fed process resulted in greater than a 200-fold cell expansion.

### Performance (Continued)

**Figure 5**  
PadReactor-Expanded hMSC Retain Critical Quality Attributes



Relevant hMSC characteristics are retained when Allegro Unison hMSC are expanded in a Cell Factory or SoloHill® collagen coated microcarriers in a PadReactor system or spinner flasks. (A) Immunophenotype analysis of hMSC show comparable surface marker expression. (B) Angiogenic cytokine secretion is maintained. (C) Immunomodulatory (IDO) data within historical norms for all cells. The cells maintain multilineage differentiative potential. (D) Osteogenic differentiation capacity. (E) Chondrogenic differentiation capacity. (F) Adipogenic differentiation capacity.

### Conclusions

- Pall's single-use, rapid and robust manufacturing platform enables a 200-fold expansion to nearly 35 billion hMSC from a one passage fed-batch microcarrier culture in a single-use reactor seed train in only 8 days.
- Allegro Unison hMSC media reproducibly supports > 500 M cells/L of media consumed.
- Expanded hMSC maintain critical quality characteristics, including surface marker expression, angiogenic cytokine secretion, multi-lineage differentiation, and inducible immunomodulatory functions.
- This system has the potential to be rapidly implemented at multiple sites enabling product developers to quickly produce tens of billions of high quality hMSC for regenerative medicine product development programs.