Microcarrier-Based Xeno-Free Expansion of Human Mesenchymal Stromal Cells in a Single-Use Stirred-Tank Bioreactor

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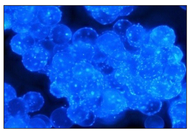
ABSTRACT

- Systems for isolation and expansion of mesenchymal stem/stromal cells (hMSC) from tissues using xeno-free media are under development and some commercially available platforms are already becoming available from various vendors.
- Over the past decade, much data has been generated demonstrating the feasibility of using microcarriers in suspension cultures for expansion of hMSC, however, many systems still use media that contains fetal bovine serum. Here we identified microcarriers that support xeno-free expansion of cells and demonstrate their use in a scale-down model of Pall's single-use Allegro™ STR bioreactor.
- Performance of commercially-available microcarriers and a novel, xeno-free microcarrier type were examined in small scale spinners. Microcarriers that promoted cell attachment and growth were selected and conditions that supported growth were optimized and compared to a high-performing collagen-coated microcarrier.
- The xeno-free microcarriers provided the highest cell numbers (0.46 B cells/L) followed by SoloHill collagen-coated microcarriers (0.32 B cells/L) and SoloHill Star-Plus (0.25 B cells/L).
- The xeno-free microcarrier was implemented in the scale-down model of the Allegro STR bioreactor contains a bottom-mounted impeller. Cell numbers reached 0.42 B cells/L in 6 days. Standard cell characterization assays revealed that cells retained critical quality attributes indicative of cell type.
- Results from this proof of concept study demonstrate the use of a complete single-use technology platform including, microcarriers and a scale-down model of Pall's Allegro STR bioreactor.

MATERIALS

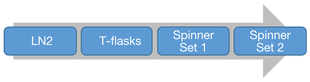
- Commercially-available bone marrow-derived human mesenchymal cells and medium.
- Single-use spinners and cell factories.
  - Disposable spinners used for small-scale feasibility studies.
  - Multi-plate cell culture vessels used in seed train for bioreactor experiments.
- Microcarriers: SoloHill microcarriers, including a novel xeno-free prototype.
  - Plastic, Plastic Plus, Star-Plus, Collagen, Hillex II, xeno-free prototype.
  - Microcarriers allow for direct transfer out of flatware culture and into space-efficient 3D cultures with minimal development time.
  - Excellent attachment and growth across multiple cell types.
  - No hydration needed, simply sterilize and use.
  - Also offered in a pre-sterilized, ready-to-use format: The Allegro Microcarrier Delivery System (AMDS).
  - Solid core design able to withstand mechanical harvest methods.
- CerCell's Pall-configured 3.2 L single-use bioreactor
  - Bottom-mounted impeller design
  - Ideal for use in seed train with Pall's larger-scale Allegro STR bioreactor platforms.



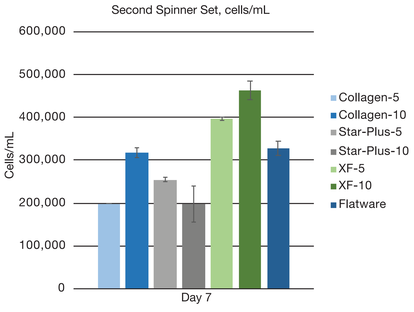
PERFORMANCE

Figure 1

Microcarrier screen performed in 125 mL disposable spinners.



Process Parameters	
Seeding density	3000 cells/cm²
Microcarrier concentration	5 and 10 cm²/mL
Medium exchange	None
Supplement	Day 3



Expansion of hMSC on SoloHill microcarriers serial-passaged in spinners. The xeno-free microcarrier at 10 cm²/mL provided the highest cell numbers (4.6 x 10^5 cells/mL) followed by SoloHill collagen-coated microcarriers at 10 cm²/mL (3.2 x 10^5 cells/mL) and SoloHill Star-Plus at 5 cm²/mL (2.5 x 10^5 cells/mL). Plastic, Plastic Plus and Hillex II did not support adequate growth under the conditions tested (data not shown).

Figure 2

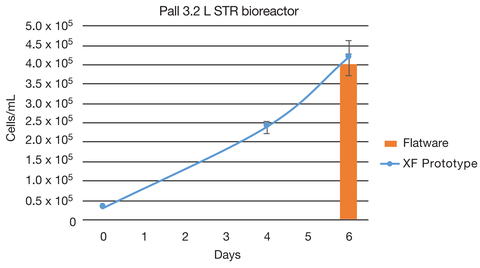
Reproducible expansion of hMSC in 3.2 L scale-down model of Pall's Allegro STR bioreactor

Expansion scheme for hMSC and on SoloHill xeno-free microcarriers using the Pall 3.2 L single-use bioreactor. Cells thawed from liquid nitrogen were seeded onto multi-plate cell culture vessels and expanded for 3 days. Cells were harvested and seeded onto xeno-free microcarriers in the Pall 3.2 L single-use bioreactor at 2.5 L volume and cultured for 6 days.

Figure 3

Pall 3.2 L STR bioreactor batch-fed process

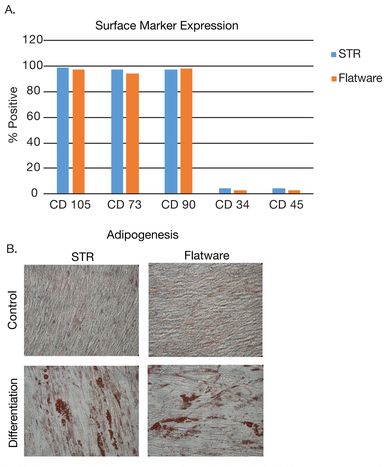
Process Parameters	
Seeding density	3000 cells/cm²
Microcarrier concentration	10 cm²/mL
Medium exchange	None
Supplement	Day 3



hMSC on SoloHill xeno-free microcarriers were cultured in the Pall 3.2 L standard single-use bioreactor. This batch-fed process yielded 0.42 B cells/L in 6 days.

Figure 4

Pall 3.2 L STR bioreactor expanded hMSC retain critical quality attributes



Cell characteristics are retained when hMSCs are expanded in a multi-plate cell culture vessel or on SoloHill microcarriers in a Pall 3.2 L STR bioreactor or spinner flasks. (A) Immunophenotype analysis of hMSCs show comparable surface marker expression. The cells maintain differentiative potential. (B) Adipogenic differentiation capacity.

CONCLUSIONS

- The newly-developed, xeno-free microcarriers support expansion of cells in a scale-down version of Pall's single-use stirred tank reactor, which contains a bottom-mounted impeller.
- This fed-batch, xeno-free microcarrier culture enabled cell numbers to reach 0.42 B cells/L in 6 days.
- Expanded hMSC maintained critical quality attributes, including surface marker expression and multi-lineage differentiation capacity.
- Results from this study demonstrate that hMSC can be successfully propagated on xeno-free microcarriers in a single-use stirred tank reactor.
- These studies provide the basis for efficient and reproducible generation of high quality cells in a xeno-free system.

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