DEVELOPMENT OF SCALABLE MANUFACTURING PROCESSES FOR BONE-MARROW DERIVED MESENCHYMAL STEM CELLS IN A LOW SHEAR, SINGLE USE BIOREACTOR SYSTEM

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Introduction

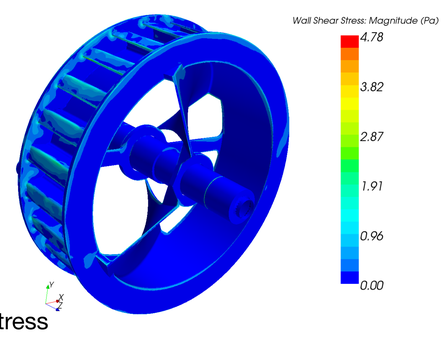
Stem cell therapies are rapidly progressing from early research through clinical trials, and most clinical batches to date have been produced in two dimensional (2D) culture. However, 2D cultures have significant economic and regulatory drawbacks for scale up. One of the scalable approaches for large volume production of anchorage dependent cells in clinical and commercial manufacturing is to grow them on microcarriers (MCs) suspended in a bioreactor as a 3D culture. Culturing cells on MCs allows the entire volume of the bioreactor to be utilized under homogeneous conditions, thereby maximizing the number of cells that may potentially be grown and harvested in a given space. Furthermore, single use bioreactors offer advantages for this application such as ease of use and elimination of expensive and laborious cleaning requirements between batches.

However, the 3D culturing of anchorage dependent cells on MCs has various process challenges in order to achieve high final cell densities including: 1) overcoming shear stress during scale up, 2) achieving efficient cell attachment during seeding, and 3) high yield cell harvest from MCs while maintaining desired cell quality and efficacy.

This study highlights the benefits of PBS Vertical-Wheel™ mixing technology at 3L scale by demonstrating the growth of large numbers of human bone marrow derived mesenchymal stromal cells (hBM-MSC) both in serum-containing and in xeno-free medium. In addition, key operation parameters such as microcarrier types and concentrations, seeding conditions, agitation speeds, feeding regimens, and length of runs were evaluated to optimize the cell culture process.

PBS Vertical-Wheel™ Mixing Technology

1) The vertically-oriented impeller creates tangential fluid motion to suspend cells and MCs effectively
2) The oppositely-oriented internal vanes of the impeller guide the liquid bi-axially, which results in a cutting and folding fluid motion that achieves fast mixing with low agitation speed
3) The large radius of the impeller helps dissipate its rotational energy to the liquid over a large contact area, minimizing shear stress

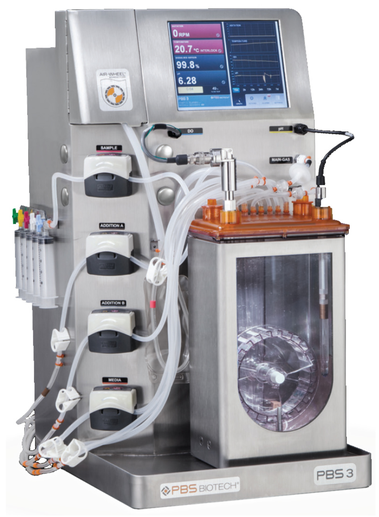


These 3 key design elements combine to offer gentle and uniform fluid mixing and efficient particle suspension inside the vessel, using low power input and agitation speeds. The low shear stress improves cell attachment to the MCs, while the homogeneous mixing promotes uniform attachment for higher percentage colonization of MCs.

PBS3 MagDrive Bioreactor

The newly released PBS 3 MagDrive model (PBS 3 *MAG*) uses the same Vertical-Wheel™ technology as the previous PBS 3 AirDrive model (PBS 3 *AIR*), but gathers its rotational power from magnetic coupling instead of buoyancy of sparged gas bubbles. PBS MagDrive bioreactors are designed especially for shear sensitive cell therapy applications without foaming problems. Benefits of these bioreactors include:

- No need for anti-foaming agents or shear protectants in culture media
- Lower working volumes for *in situ* cell harvesting
- Optional secondary heating system for static seeding without temperature drop
- Optimal for culturing shear sensitive cell aggregates or anchorage dependent cells on MCs



PBS 3 *MAG*

Methods and Materials

MSC Culture: A cell bank of hBM-MSCs (cat.#70022, Stem Cell Technologies) was prepared by expanding cells on TC flasks using the Mesencult-SF Culture Kit and the Mesencult-ACF Dissociation Kit (Stem Cell Technologies) as described by the manufacturer. These cell banks were used as starting material and seed stock for process development and bioreactor experiments. Different culture media and MCs were screened in 2D and spinner cultures. Conditions that appeared promising were then scaled up into the PBS 3 MagDrive bioreactor. Half-volume feeding was performed every 2-4 days, depending on metabolite levels and cell density.

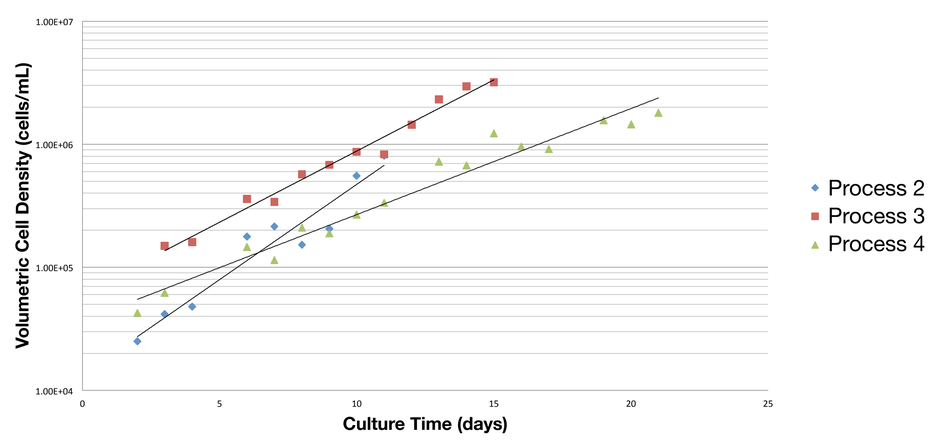
Cell Growth and Proliferation: Culture growth was monitored by withdrawing samples from the culture vessel, removing cells from MCs, staining with trypan blue, and manually counting the number of cells using a hemocytometer. MC colonization was monitored microscopically.

Inoculum Generation: For all runs but one, the inoculum was prepared by thawing a vial from the cell bank and expanding it for one passage in T-flasks. For the run seeded from 3D culture, a frozen vial containing 2 million cells was thawed and seeded directly onto a 100 mL culture media containing MCs in a spinner flask. These cells were then passaged and expanded further in a 500 mL culture with MCs in a spinner flask, prior to being passaged to inoculate the PBS 3 MagDrive bioreactor with 2L working volume.

Results and Discussion

Maximum Cell Density: Previous report showed that hMSC's could be expanded 10 folds to a maximum cell concentration of $3x10^5$ cells/mL in xeno-free conditions in PBS 3 *AIR* and 45 folds to a maximum density of $9x10^5$ cells/mL in serum containing medium in PBS 3 *MAG*. Through process development, a record high maximum cell density of $3x10^6$ cells/mL was achieved with a total cell number of 6 billion cells in 2L working volumes. The growth curve of the batches with different seeding densities and MC concentrations indicates that the specific growth rates of the batches were not significantly affected.

Growth Curves of hMSC's in PBS 3 MagDrive Bioreactor



Seed Train in 3D Culture: Methods of growing inoculum cells on MCs were developed in parallel with this process optimization by seeding a PBS 3 MagDrive bioreactor with cells grown in 3D. The bioreactor of Process 4 was seeded from cells grown in spinners, whereas those of Process 1-3 were inoculated from cells grown in 2D cultures. The thawed cells from a cell bank vial were directly seeded onto MCs in a 100 mL spinner and passaged to a 500 mL culture prior to being passaged in a PBS 3 MagDrive bioreactor. The slower growth rate in Process 4 might be due to the difference in the seed culture preparation before inoculating the bioreactor, including the extra passaging and cultivation time. Nevertheless, the maximum cell density of 1.9 $x10^6$ cells/mL in Process 4 demonstrates a proof of concept for 3D seed train to larger bioreactors.

Summary of Process Improvement

Process	Seeding Density (Cells/mL)	Maximum Cell Density (Cells/mL)	Expansion Ratio	Medium Type	MC Type	Seed Culture	Doubling Time (Days)
1	2×10^4	1.4×10^5	7	Xeno-free	A (1x)	2D	2.8
2	2.4×10^4	8×10^5	33	With serum	B (1x)	2D	1.9
3	3.6×10^4	3×10^6	83	With serum	B (2x)	2D	2.6
4	4.6×10^4	1.9×10^6	41	With serum	B (2x)	3D	3.2

Conclusions and Future Goals

hBM-MSCs can be grown in 3D culture to commercially relevant cell densities with the proper choices of process conditions and equipment, with 6 billion cells produced in a 2L working volume culture. Direct passaging of the inoculum grown in 3D to a bioreactor makes the seed transfer simpler and scalable, minimizing if not eliminating the need for larger number of 2D cultures. The PBS MagDrive product line is uniquely suited for scale up of shear sensitive cultures, from the 0.1L and 0.5L PBS-mini units, to the 3L, 15L, and 80L production bioreactors. Effects of the 3D passaging method and the 3D culturing conditions on quality of harvested cells will be assessed by flow cytometry, trilineage differentiation, and colony forming unit assays.

Furthermore, our aim is to provide a complete, scalable cell therapy product manufacturing process, from a frozen cell bank to a production bioreactor up to 80L scale, that can produce 150 billion cells per batch using single use bioreactors.