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Growth of Human Mesenchymal Stem/Stromal Cells on Sartorius Collagen Microcarriers in 50 L Bioreactor

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Introduction

There is a significant need for efficient single-use systems that can be incorporated into completely closed systems and used to generate cells for process development studies, pre-clinical testing, seed train applications, or for large scale manufacturing. Although various platforms for expansion of cells exist, these systems are often not comprehensive enough to allow for rapid implementation by researchers. These systems may be cumbersome and inconsistent in supplying high quality cells for the intended purposes. A complete system for the generation of sufficient cell numbers that utilizes a bioreactor platform which is robust and easy-to-use is therefore highly desirable.

We have previously demonstrated efficient expansion of human mesenchymal stem/stromal (hMSC) in the PadReactor® system single-use bioreactor to the 40 L scale. This system utilized commercially-available, well-characterized cells, specially-formulated growth media and supplements, microcarriers, a single-use bioreactor and a harvest method that could be used to generate tens of billions of cells in 7 days of culture. Here we extend these finding by employing these components in a new bioreactor type which contains a bottom-mounted impellor. The Allegro™ STR bioreactor is a new stirred tank bioreactor platform which is scalable, compact, ergonomic and designed to maximize usability and process assurance.

hMSC and medium were cultured at 30 L scale in microcarrier-based, fed-batch cultures in the Allegro STR 50 L bioreactor for 6 days. Cells harvested from microcarriers retained critical quality attributes when examined in standard cell characterization assays. The results obtained in this study lay the groundwork for a complete system for efficient generation of high quality cells for process development studies or large scale production. Together, this system provides a practical manufacturing platform for the expansion of cells.

Materials and Methods

Seeding Allegro STR 50 Bioreactor and Culture Parameters

Commercially-available cells and medium were used in these studies. Bone marrow-derived human mesenchymal stem cells were thawed from liquid nitrogen storage and seeded at 3000 cells/cm² onto 5 × 10-stack cell factories and cultured for 3 days. The cells were harvested from the cell factories and the Allegro STR 50 L single-use bioreactor (Pall, cat# 6412-0631R) was seeded at 3000 cells/cm² onto irradiated collagen microcarriers via the Allegro Microcarrier Delivery System (AMDS), (Pall, cat# AMDS05CS100). Cells from the harvested cell factories were also used to seed a 200 mL disposable spinner flask (Corning®) containing irradiated collagen microcarriers. This would serve as a cell control and is referred to as SP1. T25 T-Flasks were also seeded for use as flatware controls. The operating parameters for the Allegro STR 50 L bioreactor culture were set up as shown in Table 1. Following an attachment period of 1 – 2 hours, 200 mL of culture was removed from the Allegro STR 50 bioreactor and transferred to another 200 mL disposable spinner flask to serve as a bioreactor control (SP2). The culture duration was 6 days, with a bioreactor feed being added at day 3. Samples were collected from the bioreactor and controls for observation, images, attachment and distribution measurements, metabolic analysis, cell counts and characterization.

Table 1

Operating parameters for Allegro STR 50 bioreactor run

Parameter	Value
Microcarrier	Collagen, irradiated, AMDS
Microcarrier Density	10 cm²/mL
Cell Type	hMSC
Seeding Density	3000/cm²
Stir Speed	30 rpm
Temperature	37.0 °C
pH	7.35
Dissolved oxygen (DO)	50%
Primary Control	Overlay
Secondary Control	Base addition
Maintenance	Fed batch supplement added at day 3
Culture Duration	6 days
Enzyme	TrypLE®

Cell Attachment to Microcarriers

Samples were collected at approximately 24 hours post inoculation, fixed in 4% formalin and stained with the nuclear stain 4', 6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI, VWR, cat# 422801-BL). This staining allowed for the visualization of the nuclei of the cells attached to the microcarriers via fluorescence microscopy (Nikon* TiE, Nis Elements software) and thus the calculation of percent cell attachment on the microcarrier population.

Cell Growth Assessment

Cell counts were performed on representative samples collected from spinners or the bioreactor. To harvest the cells for counting, the samples were transferred to conical-bottom tubes and the cell-laden microcarriers were allowed to settle. The supernatant was aspirated and the microcarriers were washed twice with 0.13 mL/cm² calcium and magnesium-free phosphate-buffered saline (PBS, VWR). The PBS was aspirated and 0.02 mL/cm² of the recombinant enzyme TrypLE® (ThermoFisher) was added at 37 °C to dissociate the cells from the microcarriers. After cells were rounded and on the verge of dislodging (5 – 10 minutes), the microcarrier-cell suspension was gently triturated up and down with a pipette to dislodge the cells into a single cell suspension.

In the case of small volume samples (5 – 10 mL), the cells and microcarriers were then passed over a 70 micron cell strainer (VWR) fitted onto a sterile 50 mL tube (VWR), allowing the cells to pass through the strainer and into the tube while retaining the beads on the strainer. For larger volume samples (10 – 100 mL), the cells and microcarriers were passed over a 70 micron stainless steel sieve (Bellco*) fitted onto a sterile 250 mL beaker (Nalgene), allowing the cells to pass through while retaining the beads on the sieve. To quench the enzyme reaction and collect any remaining cells and beads, a volume of complete medium (CM) equal to that added for TrypLE® was added to the tube originally containing the cells and beads and then was passed over the strainer or sieve, rinsing the microcarriers and allowing the remaining cells to pass through the strainer and into the container. This step was repeated twice. Representative samples were collected from the cell suspension and cell counts were performed using a Nucleocounter NC-200 (ChemoMetec*). The retained microcarriers were dried and weighed to determine surface area and thus the calculation of cells per cm² for each sample.

Bioreactor Harvest, Allegro STR 50 Bioreactor

All controllers were stopped and the cell-laden microcarriers were allowed to settle for 6 minutes in the bioreactor. The medium was pumped off and 50 L PBS was added to the bioreactor at room temperature with stirring at 30 rpm. Agitation was stopped and the microcarriers were allowed to settle. The PBS was pumped off and 6 Lof 37 °C pre-warmed TrypLE® was added to the bioreactor. The slurry was agitated briefly (5 seconds) to mix and incubated for 10 minutes. An additional brief agitation was introduced at the 5 minute mark to redistribute the slurry. Following the 10 minute incubation, agitation was started again at 35 rpm and a small sample was collected to verify that the cells were detaching from the microcarriers. Upon verification of cell detachment, 6 L of complete medium (CM) was added to the bioreactor. The slurry was pumped from the bioreactor and ultimately through a 70 micron screen and into a cell collection bag. Six and one-half liters of PBS was then added to the bioreactor to collect any remaining cells and microcarriers. This wash was pumped from the bioreactor, through the screen and into the cell collection bag. A second wash was performed in a similar manner. Representative samples were collected from the cell collection bag for cell counts, viability and characterization studies.

Cryopreservation

Harvested cells were centrifuged at 200 × g for 10 minutes at 4 °C, cell pellets were resuspended at 5 × 106 viable cells/mL in Cryostor CS5 cryopreservation medium (BioLife Solutions) and aliquoted into freezing vials at 1 mL/vial. Vials were transferred to a freezing container (Corning®) and placed at -80 °C overnight followed by storage in the vapor phase of liquid nitrogen.

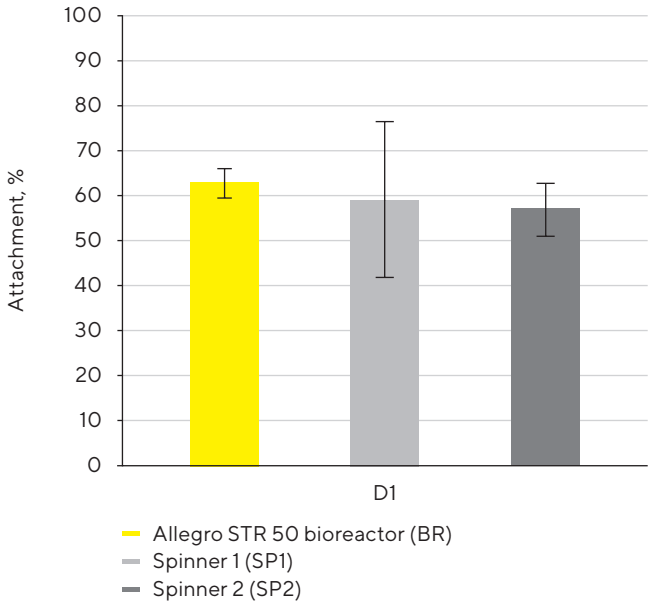
Results and Discussion

Seeding the Allegro STR 50 L Bioreactor and Attachment to Microcarriers

Cells harvested from the 10-stack cell factories after 3 days resulted in a harvest density of 54,000 cells/cm². These cells were used to inoculate the Allegro STR 50 L bioreactor and controls at 3000 cells/cm². The attachment percentages of samples collected from the Allegro STR 50 bioreactor and spinner controls one day post inoculation, showed a ranged from 57 – 63%, as shown in Figure 1. The Allegro STR 50 bioreactor microcarriers demonstrated the highest cell attachment with 63% of the microcarriers having at least one cell attached. SP1 and SP2 followed with 59% and 57%, respectively.

Figure 1

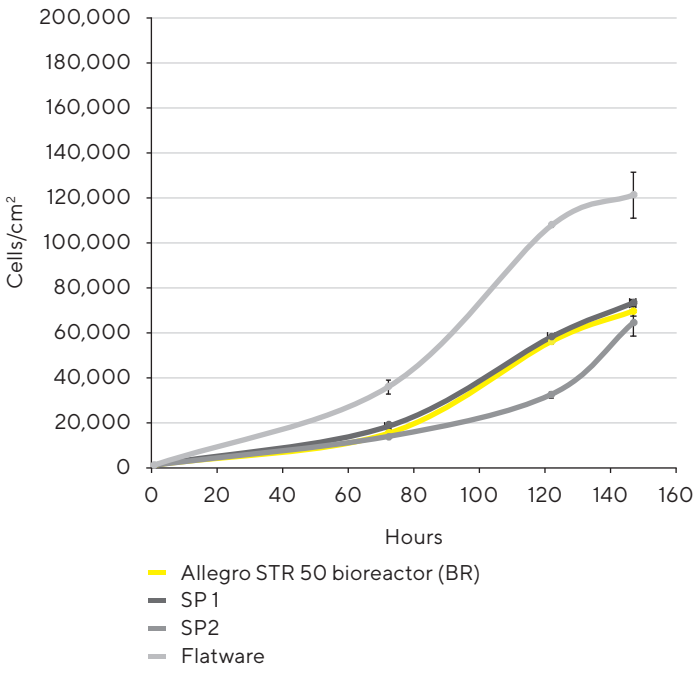
Attachment results for the Allegro STR 50 L bioreactor and spinner controls



Cell Growth and Harvest

A growth curve generated from the small scale cell count samples (5 – 100 mL) harvested from the Allegro STR 50 bioreactor and controls is shown in Figure 2. Samples were collected at days 3, 5 and 6. The t-flask cell counts were highest on a cells/cm² basis, reaching 120,000 cells/cm² by day 6. The Allegro STR 50 bioreactor, SP1 and SP2 conditions reached 71,000, 74,000 and 65,000 cells/cm² by day 6, respectively. This result is consistent with what has been seen historically, where the flatware condition outpaces the microcarrier conditions on a cells/cm² basis. Viability was greater than 90% for all conditions.

Figure 2
Growth curve from samples collected at days 3, 5 and 6 from the Allegro STR 50 bioreactor and controls



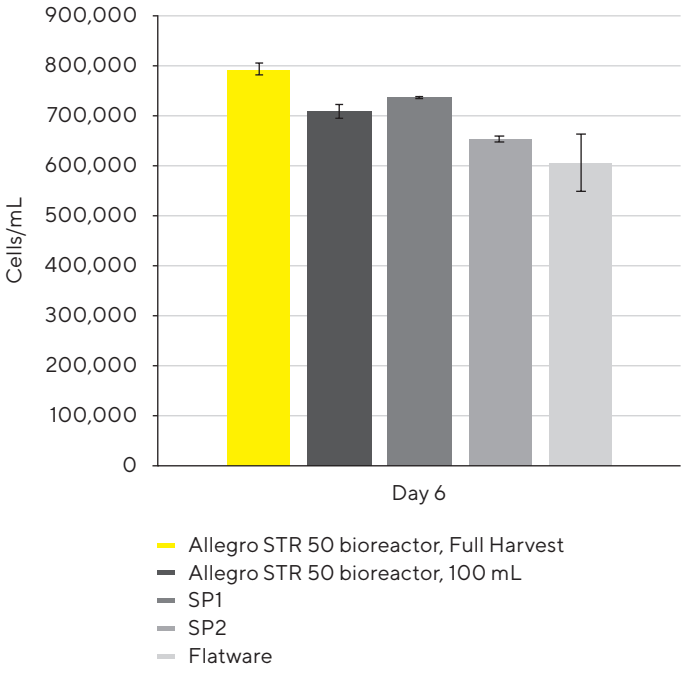
Cell counts in cells/mL from the day 6 cultures are shown in Figure 3 to demonstrate one of the advantages microcarrier cultures have over flatware. Due to the ability to easily increase the surface area by the introduction of additional of microcarriers, one can push the system, allowing for the potential to reach significantly higher cell numbers than in flatware in the same volume of medium. At a microcarrier density of 10 cm^2/mL, the Allegro STR 50 bioreactor (100 mL sample), SP1 and SP2 samples reached day 6 cell densities of 750,000, 740,000 and 650,000 cells/mL, respectively. This is compared to 610,000 cells/mL for the flatware control, which is typically limited to a 5 cm^2/mL surface area-to-volume ratio due to gas exchange requirements. So the ability to increase microcarrier density of the microcarrier cultures allowed for a greater overall number of cells/mL than the flatware culture.

Viability was shown to be greater than 90% for all conditions. Although the initial attachment for the Allegro STR 50 bioreactor was somewhat low at 63%, the percent attachment increased to greater than 95% during the course of the culture as the microcarriers began forming bead-bead bridges, allowing cells to populate virtually the entire population of microcarriers, with aggregate sizes ranging from 2 – 50+ microcarriers.

Harvest Efficiency

Results from the full harvest are included in Figure 3. A 100 mL representative sample was collected from the Allegro STR 50 bioreactor (designated as Allegro STR 50 bioreactor, 100 mL) prior to performing the full scale harvest of the bioreactor. The cell count results from this sample serve as a baseline number in determining the harvest efficiency of the full-scale harvest. The target metric for harvest efficiency for the full scale harvest is to achieve harvested cell numbers ≥ 85% of those achieved from the 100 mL sample. Samples from the Allegro STR 50 bioreactor full harvest and the Allegro STR 50 bioreactor 100 mL sample reached 790,000 and 710,000 cells/mL, respectively, demonstrating an efficient harvest. Oftentimes, the full harvest numbers are greater than the 100 mL sample. This could be due to the fact that, prior to harvesting cells from the 100 mL microcarrier sample, it must first be collected from the bioreactor using a sampling manifold. The nature of the sample collection process using this sampling manifold introduces additional shear, resulting in the potential loss of cells prior to initiating the enzymatic digestion step.

Figure 3
Day 6 growth results in cells/mL for the Allegro STR 50 L bioreactor study

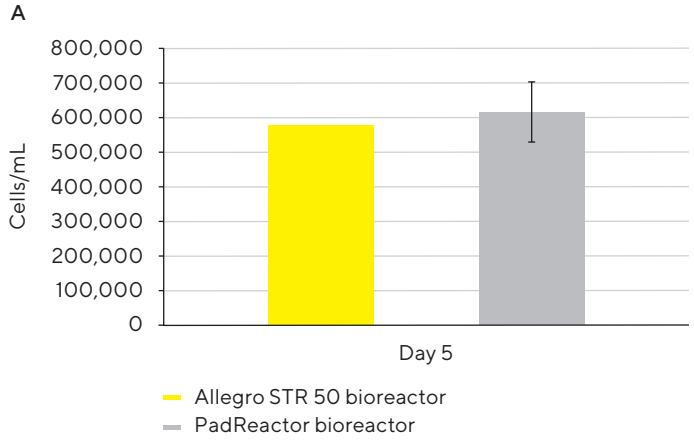


Comparison to PadReactor® Bioreactor Results

The results achieved in the Allegro STR 50 L bioreactor are comparable to those obtained with the PadReactor® bioreactor platform. Previously, it was demonstrated that hMSC could be successfully grown in the PadReactor® bioreactor platform at 40 L on collagen microcarriers. The culture duration for the PadReactor® bioreactor runs were out to 5 days. As shown in Figure 4, the hMSC cell yield from the Allegro STR 50 bioreactor at day 5 is comparable to the previously achieved PadReactor® bioreactor results. Viability of harvested cells from both bioreactors was also comparable. The same donor and medium used in the PadReactor® bioreactor runs were used in the Allegro STR 50 L bioreactor.

Figure 4

(A) hMSC cell yield from the Allegro STR 50 bioreactor at day 5 is comparable to the PadReactor® bioreactor results. (B) Viability of harvested cells from both bioreactors was also comparable. The same donor and medium used in the PadReactor® bioreactor runs were used in the Allegro STR 50 L bioreactor.



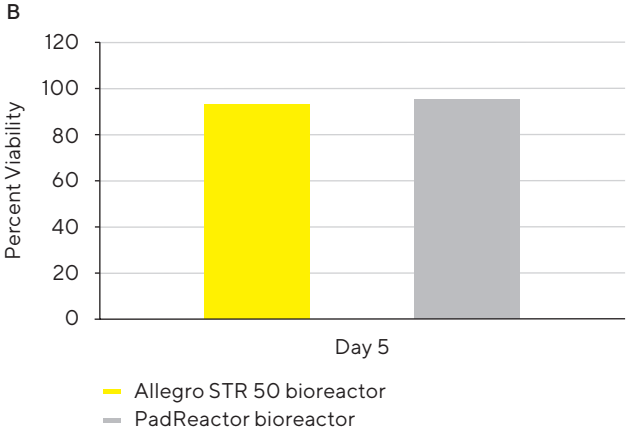


Figure 5 below shows the results from the full expansion process of going from liquid nitrogen to the Allegro STR 50 L bioreactor. The starting number of cells required to seed the cell factories was 95 M. This number was expanded for 3 days, generating more than enough cells (1.7 B cells), to seed the Allegro STR 50 bioreactor at 30 L. Following a 6 day expansion in the Allegro STR 50 bioreactor, a total of 22 B cells were harvested. The average viability of the harvested cells was 97%.

Figure 5

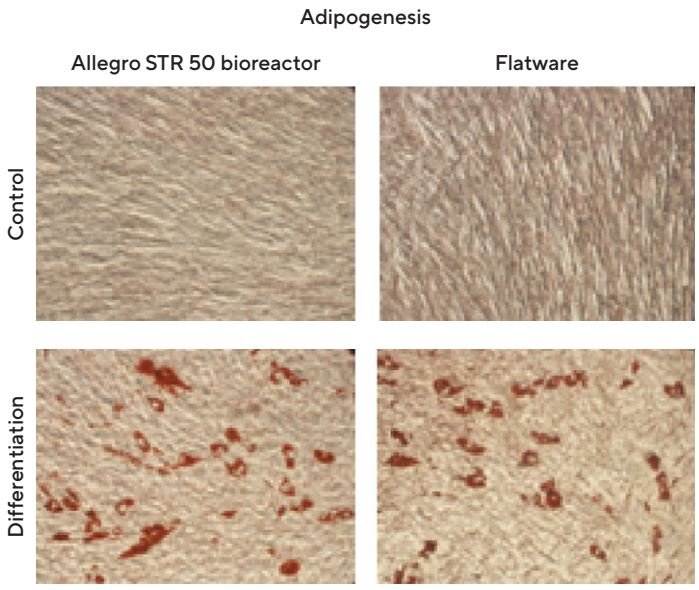
Results from the expansion of cells from liquid nitrogen to the Allegro STR 50 bioreactor

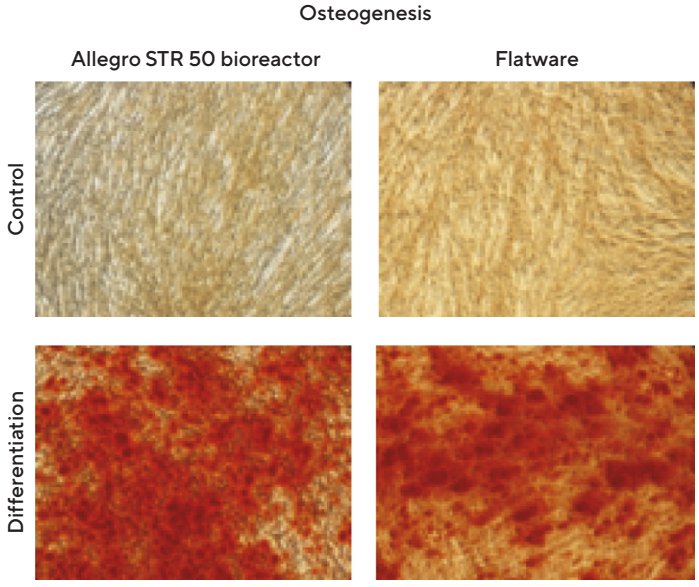
3 days			6 days	
Frozen Vials	**Cell Factories**		**Allegro STR 50 Bioreactor (30 L)**	
	Seed	Harvest	Seed	Harvest
Total Cells	9.5×10^7	1.7×10^9	0.9×10^8	2.2×10^{10}

Cell Characterization

The cells collected from this study were frozen down for characterization. The testing revealed that the cells maintained multilineage differentiative potential, as shown in Figure 6, demonstrating adipogenic and osteogenic differentiation.

Figure 6
Cell characteristics are retained when hMSCs are expanded on SoloHill® microcarriers in the Allegro STR 50 bioreactor. The cells maintained differentiative potential, demonstrating adipogenic and osteogenic differentiation capacity.





Conclusions

The Allegro STR 50 bioreactor supports expansion of human mesencyhmal stromal | stem cells on microcarriers delivered into the bioreactor via the Allegro Microcarrier Delivery System (AMDS). This microcarrier culture enabled cell numbers to reach 0.79 B cells/L in 6 days, with a total of 22 B cells being obtained after harvest. Cell yield and viability obtained in Pall's Allegro STR 50 bioreactor was comparable to Pall's PadReactor® bioreactor results. Expanded hMSC maintained critical quality attributes, including multi-lineage differentiation capacity. The results obtained in this study lay the groundwork for a complete system for efficient generation of high quality cells. Together, Pall's Allegro STR 50 bioreactor and SoloHill® microcarriers provide a practical manufacturing platform for dynamic culture and expansion of adherent cells.

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