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2D to 3D Transition of Mesenchymal Stem Cell Expansion Process using the Sartorius MSC Pilot Solution

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Abstract

Mesenchymal Stem Cells (MSCs) are adult multipotent stem cells that can be isolated from various tissues, including bone marrow, adipose tissue, and umbilical cord (Wharton's Jelly). These cells modulate immune responses *in vivo* and can differentiate into several different cell types. The therapeutic properties of MSCs are under numerous clinical investigations for the treatment of various diseases including Graft-versus-Host Disease (GVHD). Given that the clinical cell number requirement for these therapeutic applications is substantial and requires the ability to manufacture large quantities of MSCs under current good manufacturing practices (cGMP), it is essential to establish robust and scalable expansion processes during process development (PD) to ensure success at the manufacturing stage. While traditional workflows often depend on two-dimensional (2D) static methods for cell expansion, this approach becomes increasingly unsustainable with scale, and bottlenecks in production and supply escalate costs and time to market for these life-changing therapies. This application note describes the rapid and successful transition of an established 2D planar culture process for cultivation of Wharton's Jelly MSCs (WJMSC) to three-dimensional (3D) microcarrier-based suspension culture using the Sartorius MSC Pilot Solution.

Find out more: www.sartorius.com/en/applications/cell-and-gene-therapy/cell-therapy/msc/msc-pd

Introduction

Graft-versus-Host Disease (GVHD) is characterized by an allogeneic cytotoxic reaction of donor lymphocytes against host tissue.[1] The standard treatment for GVHD is immunosuppression with the intent of inducing donor-recipient tolerance without eliminating the effectiveness of the grafted tissue. Use of MSCs to treat GVHD is being actively pursued in clinical trials because of the immunosuppressive properties of this cell type.[2]

A phase I clinical trial using WJMSCs to treat GVHD at the University of Kansas Medical School (KUMed) has been completed resulting in promising dosing and safety data.[3] While the phase I trials used cells generated in 2D planar culture processes, future studies would require significantly higher cell numbers rendering conventional 2D methods unsustainable. Based on a dosing regimen of 4B cells per 100 kg patient, the quantity of cells required to complete phase II (50 patients) and phase III (300 patients) equates to a total cell requirement of ~1.4x10^{12} cells. To accommodate this cell number requirement, hundreds to thousands of individual 2D culture vessels would be needed in an expansion seed train. Furthermore, 2D processes are comprised of many manual operations including open handling steps which increase the risk of contamination, and they are further disadvantaged by the lack of process monitoring and control capabilities. Given the current predictions for cell requirements to treat the patient population and the existing limitations of 2D culture systems, a scale-up strategy using a microcarrier-based suspension process to support the additional later-phase clinical trials and subsequent commercialization was pursued.

The Sartorius MSC Pilot Solution is a scalable, semi-automated solution that simplifies cell expansion using a microcarrier-based 3D suspension system (Figure 1). The 3D suspension system accelerates scale-up by streamlining the seed train and significantly reducing the number of open handling steps and potential for contamination. Furthermore, the simplified process leads to decreased costs by reducing labor requirements, the number of quality control assays required to qualify cells, and use of consumables. This application note describes a series of studies in which a rapid transition of a 2D to 3D expansion process of WJMSCs is achieved using key components of the Sartorius MSC Pilot Solution (Figure 1A-F). Briefly, WJMSCs were first seeded in 2D culture vessels then expanded at the 2 L scale

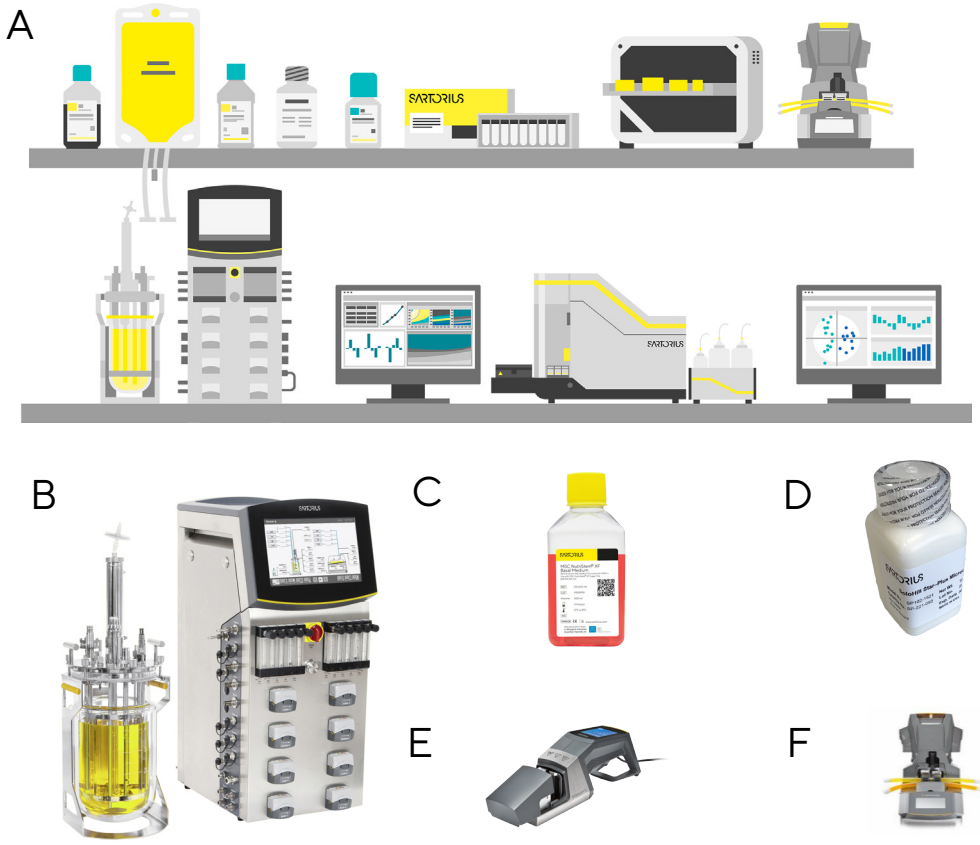


Figure 1. *The Sartorius MSC Pilot Solution.* (A) Schematic of the Sartorius MSC Pilot Solution, Including (B) BioStat® B and Univessel® Glass, (C) MSC NutriStem® XF Medium, (D) Solohill® Collagen Coated Microcarriers in Microcarrier Delivery Systems (MDS), (E) Biosealer® and (F) Biowelder®.

in microcarrier-based, fed-batch cultures in Sartorius reusable Univessel® Glass and single-use Univessel® SU stirred-tank bioreactors with Nutristem® MSC XF medium and SoloHill® microcarriers using processes previously optimized in 200 mL spinner flasks. Manifolds for fluid transfer into and out of the bioreactors were assembled using Sartorius Tuflux® tubing and the Sartorius Biosealer® and Biowelder®. Cells harvested from microcarriers retained critical quality attributes (CQAs) when examined in established cell characterization assays. The results obtained in this study lay the groundwork for efficient generation of high-quality cells for further process development studies, clinical studies, or large-scale production. While additional work is required to scale to larger volumes, this platform provides a robust solution for the expansion of therapeutically active MSCs.

Materials

Key Materials and equipment used:

- Univessel® Glass 2L
- Univessel® SU 2L
- SoloHill® Collagen Coated Microcarriers in MDS
- MSC Nutristem® XF Basal Medium
- MSC Nutristem® XF Supplement Mix
- PLTGold® Human Platelet Lysate
- Biosealer® TC
- Biowelder® TC
- Tuflux® Tubing
- KUMed Human WJMSCs

Methods

Culturing of WJMSCs

Human WJMSC received from KUMed (Passage 2) were expanded in Sartorius Univessel® Glass and Univessel® SU stirred-tank bioreactors and spinners on SoloHill® Collagen Coated Microcarriers (Sartorius, CIR-221-020) or in 2D tissue culture flasks in Nutristem® MSC XF medium (Sartorius, 05-200-1A) supplemented with 5% Human Platelet lysate (Sartorius, PLTGOLD500R). Unless otherwise noted, medium refers to this complete formulation.

Visualization of cell attachment to microcarriers

Samples were collected at approximately 3, 5, and 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 and viability assessment

Cell counts were performed on representative samples collected from planar cell culture vessels, spinner flasks, and bioreactors. To harvest the cells for counting, the samples were transferred to conical-bottom tubes and the cell-laden microcarriers allowed to settle. The supernatant was aspirated and the microcarriers were washed twice with calcium and magnesium-free phosphate-buffered saline (PBS). The PBS was aspirated and 0.04 mL/cm² of the recombinant enzyme TrypLE (0.5X concentrated) 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 for daily cell counts (5 – 10 mL), the cells and microcarriers were then passed over a 70 µm cell strainer fitted onto a sterile 50 mL tube, 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 µm stainless steel sieve fitted onto a sterile 250 mL beaker, 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 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. Representative samples were collected from the cell suspension and cell counts were performed using a Nucleocounter NC-200. The retained microcarriers were dried and weighed to determine surface area and to calculate cells per cm² for each sample. To determine viability, a live/dead staining method was used. Approximately 60 µl of the cell samples were loaded into a Via1-Cassette™ (ChemoMetic, cat no. 941-0012) containing the fluorophores acridine orange (AO, stains both live and dead cells) and 4',6-diamidino-2-phenylindole (DAPI, stains dead cells), and viability was measured by the Nucleocounter NC-200.

Bioreactor seeding and culture conditions

Standard procedures were followed for seeding and culturing cells in Sartorius 2L Univessel® bioreactors. Details describing the seed train used for cell expansion and operating parameters for the bioreactor runs are shown in Table 1. Briefly, for each bioreactor, cells were thawed and seeded onto two T-150 flasks for an initial 4-day cell expansion period. Cells were then passaged to a 2-stack vessel and cultured for an additional 4 days. The cells were harvested from cell stacks and used to seed 2L cultures in Univessel® bioreactors containing irradiated collagen microcarriers (10 cm²/mL) previously

Cell Bank	2 x Tflasks	1 x CellSTACK 2	Univessel® Glass & SU
WJMSCs →	 →	 →	
Microcarrier Type	n/a	n/a	Collagen Coated, Irradiated
Surface area per mL, cm²/mL	7.5	7.5	10
Media type	Nutristem® + 5% PLT	Nutristem® + 5% PLT	Nutristem® + 5% PLT
Media, mL	40	170	2000
Total area, cm²	300	1275	20000
Mass MC, g	n/a	n/a	55.6
Culture duration, days	4	4	6
Harvest enzyme	TrypLE	TrypLE	TrypLE

Table 1. *Seed Train and Operating Parameters for Bioreactor Runs*

transferred to the bioreactors via the Microcarrier Delivery System (MDS). Cells from harvested cell stacks were also used to seed a 200 mL disposable spinner flask containing irradiated collagen microcarriers. Six T-25 flasks were also seeded for use as 2D controls. An intermittent attachment strategy was performed for 5 hours. After this attachment period, initial stir speed was set in both vessels. After Day 2, agitation in the single-use vessel was increased to prevent microcarrier settling. Samples were collected daily from the bioreactors and controls for observation, images, attachment and distribution measurements, metabolic analysis, cell counts, and characterization assays.

Bioreactor Harvest and Cryopreservation

Bioreactor controllers were stopped and the cell-laden microcarriers settled for 1 to 4 minutes in the bioreactor. Approximately 75% of the spent medium (1.5 L) was pumped out of the bioreactor and 2.0 L PBS was added to the bioreactor at room temperature. Agitation was stopped and the microcarriers settled for 2 to 6 minutes. Two additional washes with 2.0 L PBS were sequentially performed. After the final PBS addition, PBS was pumped off and 0.7 L of 37°C pre-warmed 0.5x TrypLE was added to the bioreactor. The slurry was agitated for 15 minutes. Following the 15-minute incubation, a small sample was collected to verify that the cells were detaching from the microcarriers. Upon verification of cell detachment, 0.7 L of medium was added to the bioreactor. The slurry was pumped from the bioreactor and ultimately through a 70 µm screen and into a cell collection bag. After the initial transfer of the microcarrier cell suspension from the bioreactor was complete, an additional 0.7 L of medium was added to the bioreactor to collect remaining cells and microcarriers. This wash was pumped from the bioreactor, through the screen and into the cell collection bag. Representative samples were collected from the cell collection bag for cell counts, viability, and characterization studies. Harvested cells in a final volume of approximately 2.5 to 3.0 L were centrifuged at 400 x g for 10 minutes at 4°C, cell pellets were resuspended at 1x10⁶ viable cells/mL in Cryostor CS10 cryopreservation medium and aliquoted into freezing vials at a 1 mL per vial. Vials were transferred to a freezing container and placed at -80°C overnight followed by storage in the vapor phase of liquid nitrogen.

Plating efficiency

Cells harvested from 3D suspension cultures were concentrated via centrifugation at 400 x g for 10 minutes at 4°C, cell pellets were then resuspended at approximately 1x10⁶ viable cells/mL in cell culture medium and diluted to the appropriate concentration. Cell suspension was then plated onto two T-25 flasks at a cell density of 1.3x10⁶ cells/cm² and vessels were place in a 37°C cell culture incubator overnight. After 24 hours, cells were harvested using TrypLE and cell counts were performed. Plating efficiency (%) was calculated as (observed cell density at 24 hour / seeding density) * 100.

Flow cytometry characterization of human WJMSC

Human WJMSC were characterized for CD markers by flow cytometry using the BD Stemflow Human MSC analysis kit. Briefly, the cells were resuspended at a concentration of 1x10⁶ cells per 100 µL in staining buffer. WJMSC samples were stained with four fluorochromes together including positive and negative staining cocktails. The positive marker cocktail stained for APC CD73, FITC CD90, and Per-

CP-Cy5.5 CD105 (used to positively identify WJMSC) and the negative cocktail stained for PE CD45, PE CD34, PE CD11b, PE CD19, and PE HLA-DR. A PE CD44 antibody conjugate was used as a positive control for compensation set-up and gating of the negative cocktail. For each flow cytometry run, fluorescence minus one control for each fluorochrome and isotype controls for each antibody were used for compensation and nonspecific fluorescence analysis. After singlet cells were gated, MSCs were first identified by their negative stain for all hematopoietic markers and HLA-DR and then identified by their positive stain for CD73 and CD105, and then ultimately by their positive stain for CD90. Samples were washed with 1% FBS solution after staining. LSR II (Becton Dickinson) was used for flow cytometry and analysis was conducted using FACSDiva software.

Potency by Immunosuppression

Immunosuppressive potency of WJMSC was determined by evaluating the ability to inhibit phytohemagglutinin (PHA)-induced proliferation of human peripheral blood mononuclear cells (hPBMC) in co-culture. A known number of WJMSC were seeded in 96-well plates in high glucose DMEM supplemented with 10% FBS. After 24 hours, 10 µg/mL Mitomycin-C was added to inhibit cell proliferation. The treated cells were incubated for an additional 2 hours at 37°C and then washed 5 times with culture media. A known number of hPBMCs were then added to each well, stimulated with PHA to activate T-cell proliferation and co-cultured for 3 days. Inhibition was determined by flow cytometry analysis of T-cell proliferation, compared to a WJMSC-free culture.

Karyotyping

Chromosomal consistency was determined prior to or after cryopreservation by recovering cells that were in growth phase. WJMSCs from the final product were then recovered and fixed in 3:1 Methanol:Acetic Acid. Fixed suspensions were dropped on slides, banded using Giemsa/Trypsin/Leishmans (GTL) and visually evaluated against an in-house standard.

Results

Cell attachment to microcarriers is achieved in 24 hours of 3D suspension-based culture

The first requirement for successful suspension culture of adherent cells on microcarriers is to achieve efficient attachment and even distribution of cells to the microcarrier population. An intermittent attachment strategy was performed, and samples were subsequently retrieved at 2.75, 5.5, and 24 hours to assess attachment and distribution of cells on SoloHill® Collagen Coated Microcarriers. In this study, cell attachment reached greater than 80% by 24 hours of culture in both Univessel® bioreactors (Figure 2A, C). Additionally, the number of cells per microcarrier as measured in cells/bead approached the theoretical number of three cells per bead as calculated from seeding density and microcarrier concentration, indicating that a successful attachment and distribution of cells onto microcarriers had been achieved and conditions were suitable to obtain productive cultures (Figure 2B, D).

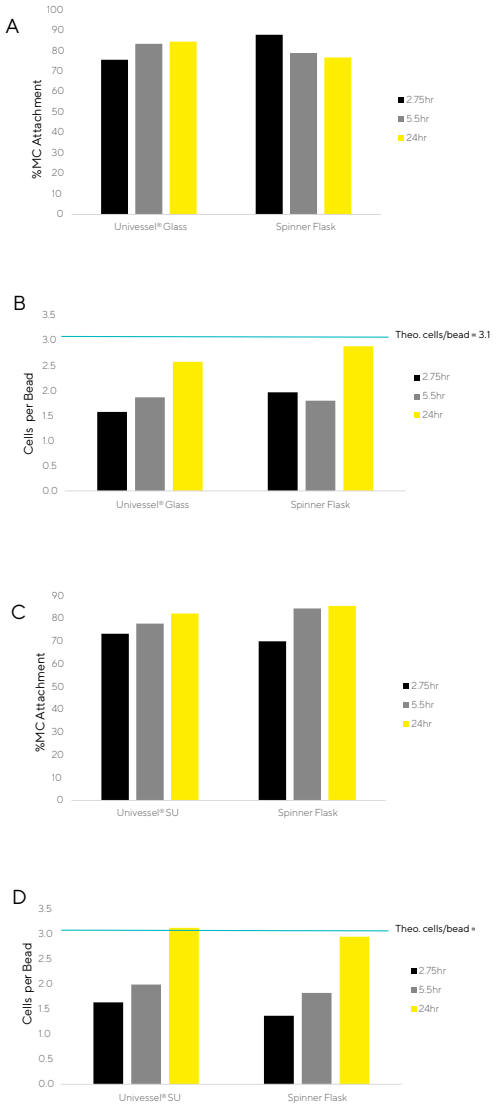


Figure 2. *Cell Attachment to Microcarriers is Achieved in 24 hours of Suspension-Based Culture.* (A, C) Cell attachment is shown as %Microcarrier (MC) Attachment for both Univessel® Glass (A) and SU vessel (C) compared to respective spinner controls. (B, D) Distribution of cells is shown on the right as cells/bead, demonstrating sufficient attachment at 24 hours of culture.

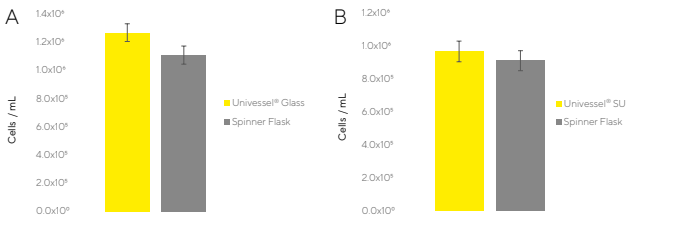
Univessel® stirred-tank bioreactors outperform spinner flasks with respect to cell growth

Following cell attachment onto microcarriers, WJMSCs were expanded in all vessels for six days before samples were harvested to measure cell growth. Cell growth on SoloHill® Collagen Coated Microcarriers in both 2 L Univessel® bioreactors (Glass and SU) was equivalent to or greater than their respective spinner flask controls on Day 6 (Figure 3). Cell density in the Univessel® Glass and SU bioreactors reached 1.3 M and 0.97 M cells/mL, respectively, and total harvested cells from each bioreactor was 1.9 B and 1.6 B. Spinner flask control cultures, on the other hand, reached 1.1 M and 0.85 M cells/mL in each experiment, slightly lower than the bioreactor samples. The benefit of automation and process control enabled by the Univessel® stirred-tank bioreactors likely contributed to the differences observed in cell growth.

To further assess and characterize cell growth, samples from Day 6 cultures were fixed and stained with DAPI to visualize cells under a microscope (Figure 4). Images of cell-laden microcarriers were reflective of the cell numbers obtained from harvest. Tightly clustered aggregates of cells and microcarriers were observed in the samples from all vessels indicating that conditions for cell growth were ideal for propagation in suspension.

Cells harvested from Univessel® stirred-tank bioreactors exhibit higher cell viability than cells harvested from 2D cultures and spinner flasks

In addition to cell growth, viability was measured from cell counts based on a live/dead staining method. According to these calculations, all samples including controls retained a high viability of 95-97% (Figure 3, control data not shown). However, since viability dyes are based on measurement of membrame permeability and do not provide complete information regarding the cell health, a plating efficiency assay was performed on samples from the Univessel® SU bioreactor as well as spinner flask and 2D T-flask control samples to further assess and confirm cell health and viability. In this study, cells harvested from the Univessel® SU bioreactor retained high plating efficiency (PE) of 92% as they approached the initial seeding density of $1.3x10^4$ cells/cm² at 24 hours (Figure 5). On the other hand, the T-flask and spinner flask culture samples displayed significantly lower PE than the bioreactor samples, only reaching approximately $5x10^3$ cells/cm² (PE of 38%) and $6x10^3$ cells/cm² (PE of 47%), respectively. Clearly, the samples grown in spinner flasks and T-flasks, while still viable (as determined by live/dead staining methods) upon harvest, were not sufficiently healthy to attach once plated.



Parameter	Target	Result	
		Glass	SU
Harvest density, cells/mL	≥$6x10^5$	$1.3x10^6$	$9.7x10^5$
Viability, %	≥90	96	97
Harvest efficiency, %	≥90	≥95	≥95
Total cells harvested	≥$1.0x10^9$	$1.9x10^9$	$1.6x10^9$

Figure 3. *Cell Growth in Bioreactor Cultures Exceeds Growth in Spinner-Flask Controls.* Viable cell counts of samples retrieved from 6-day cultures in Univessel® Glass (A) and SU (B) bioreactors and spinner controls. Inset table shows harvest density, percent viability, harvest efficiency, and total cells harvested from each system.

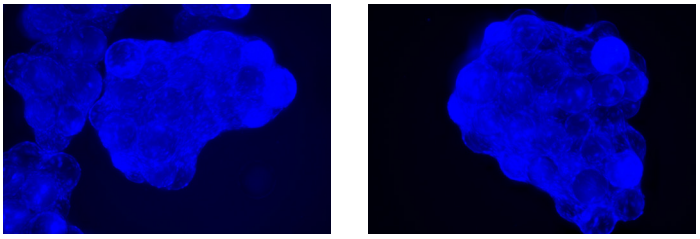


Figure 4. *Aggregates of Cell-Laden Microcarriers in Bioreactors are Comparable to those of Spinner-Flask Controls.* Representative fluorescent images of cell-laden microcarriers. Cells attached to microcarriers are visualized using DAPI staining of microcarrier-cell aggregates retrieved from reactors. Nuclei appear as blue dots when viewed under ultraviolet light. Univessel® Glass bioreactor.

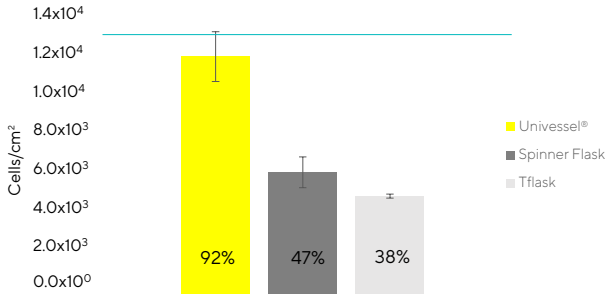
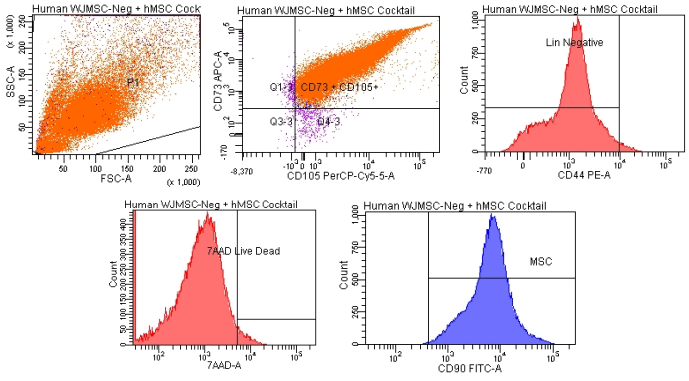


Figure 5. *Cell Viability of MSCs Grown in Univessel® Bioreactors is Greater than those Grown in Spinner Flask Controls.* Viability was measured after growth and harvest from the Univessel® SU after 6-day culture using a plating efficiency assay. The cell density (cells/cm²) compared to the expected cell density indicated by the teal line and plating efficiency% (label within bars) are depicted in the graph.

MSCs maintain phenotypic and functional CQAs after expansion in 3D culture

An important consideration for transitioning from static 2D cultures to suspension 3D cultures is the maintenance of both phenotypic and functional CQAs of the expanded cell population. Here, three assays were performed to verify maintenance of MSC identity and characteristics including surface marker expression, potency of immunosuppression, and karyotype. To determine whether WJMSCs expanded in Univessel® bioreactors retained MSC surface markers recognized by the International Society for Cellular Therapy (ISCT), flow cytometry was performed.[4] These studies revealed that 97% of cells harvested from bioreactors expressed the markers characteristic of MSCs (CD105, CD73, and CD90), while lineage negative markers, CD34, CD45, CD11b, CD19 and HLA-DR were expressed in only 0.7% of the cell population, exceeding established ISCT criteria (Figure 6).

MSCs are being studied for their effectiveness in treating GVHD due to their immunosuppressive properties. Therefore, a functional CQA of particular interest in the current study is the potency of immunosuppression. MSCs harvested from bioreactors retained their ability to suppress PHA-induced hPBMC proliferation when cultured together, inhibiting hPBMC growth by 35% compared to PHA-activated hPBMC samples cultured without WJMSCs (Figure 7). Thus, cells expanded in bioreactors were able to demonstrate appropriate immunosuppressive capacity.



MSC characterization (Specifications)	% Total
Purity (≥ 80%)	94%
MSC characterization (≥ 95% CD73, CD90, CD105)	97.6%
Lin negative (≤ 2% CD34, CD45, CD11b, CD19, HLA-DR)	0.7%
Viability (≥ 80%)	91.6%

Figure 6. *MSC Surface Markers are Maintained after Expansion in 3D Suspension-Based Culture Systems.* Flow cytometric analysis of cell identity using predictive cell surface antigens indicates that cells retained MSC-specific surface markers after expansion in Univessel® bioreactors. 94% of cells exhibited a purity of 80% or greater , and 97.6% expressed positive markers for MSCs (CD73, CD90, CD105), while only 0.7% expressed negative markers (CD34, CD45, CD11b, CD19, HLA-DR). 91.6% of cells met the criteria for viability at 80%.

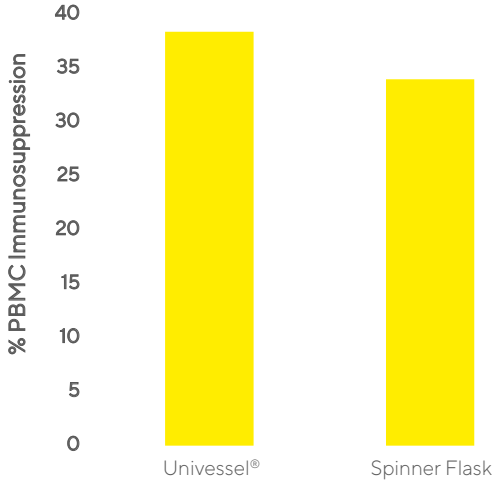


Figure 7. *WJMSCs Expanded in Univessel® Bioreactors Maintain Potency and Immunosuppressive Capacity.* Thirty-eight % inhibition of phytohemagglutinin (PHA)-induced proliferation of human peripheral blood mononuclear cells (hPBMC) in co-culture was observed in samples that had WJMSCs grown in bioreactors added to them, compared to those that did not. Samples of hPBMCs co-cultured with WJMSCs grown in spinner flasks showed slightly less immunosuppression.

Figure 8. *MSCs Retain Normal Karyotype after Expansion in Univessel® Bioreactors.* Representative karyogram from cells expanded in suspension bioreactors indicating retention of chromosomal consistency CQA.

Finally, karyotyping was performed on samples from bioreactor cultures to determine whether chromosomal consistency was maintained after expansion in 3D suspension-based systems. In fact, MSCs expanded in Univessel® bioreactors retained normal karyotypes, as shown in a representative image (Figure 8).

Taken together, the results from these characterization studies confirm that the CQAs of WJMSCs are maintained throughout cell expansion and that transitioning to 3D culture using the Sartorius MSC Pilot Solution is a robust and seamless approach to enable successful scaling and manufacturing.

Discussion

The field of cell therapy and regenerative medicine is rapidly advancing with approved therapies already in commercial manufacturing while new and promising applications are being pursued in research and development, pre-clinical studies, and clinical trials. To date, there are 10 approved MSC therapy products globally available for treatment of various disease conditions and injuries, including thermal burns, receding gums, and complications associated with cell transplantation.[5] However, full-scale industrialization of processes capable of generating the large numbers of MSCs required to treat entire patient populations is still under development.

The studies presented demonstrate the successful transition of MSC cultures from 2D to 3D suspension-based cell expansion systems and scale up to clinically relevant cell numbers using the Sartorius MSC Pilot Solution. A developed 2D process was successfully transferred to both the 2L Univessel® Glass and SU bioreactors utilizing the Biostat® B platform. Results for growth exceeded expected targets, achieving 1.27 B cells/L for the Univessel® Glass and 0.97 B cells/L for the Univessel® SU. Cell viability was also excellent, achieving 96% and 97% for the Univessel® Glass and SU, respectively, harvest efficiency exceeded 95% for each bioreactor run and plating efficiency post-harvest reached 92%.

Speed of process development was demonstrated without sacrificing cell quality, with the present studies completed in only 2 months, providing a rationale for engaging the Sartorius MSC Pilot Solution for process development of cell-based therapies. Additionally, the cell characterization studies described here could be further accelerated with full use and end-to-end integration of the Sartorius systems for cell characterization within the Sartorius MSC Pilot Solution.

Combined with the automated and controlled cell expansion in the Univessel® stirred-tank bioreactors, along with Nutristem® medium, SoloHill® microcarriers, and Sartorius single-use products, these components comprise a complete solution for rapid and cost-effective pilot-stage process development.

The Sartorius MSC Pilot Solution is one in a series of Sartorius scalable solutions that enable seamless process transfer between stages of cell therapy development. The Sartorius MSC Exploration and Characterization Solution as well as the MSC Optimization and Characterization Solution each enable semi-automated, scale-down optimization of suspension-based MSC culture in stirred-tank bioreactors (Ambr®) and can be used to efficiently develop process knowledge. Processes can then be directly transferred to larger volumes using the Sartorius MSC Pilot Solution for 0.6 to 10 L volumes and finally to the Sartorius MSC Manufacturing Solution for 15 to 2000 L clinical-scale production of cGMP stem cell therapies.

Efficiency of clinical-scale production is critical, and with the Sartorius MSC Manufacturing Solution, a simple 4-step seed train achieves the yearly cell number requirement for a phase III clinical trial, which is approximately $1.2x10^{12}$ cells (Figure 9). Following initial expansion, MSCs can be seeded in the 2L Univessel® Bioreactor, then transferred to the Biostat STR® 50 bioreactor at 20 L working volume, followed by expansion in the single-use Biostat STR® 200 bioreactor at 200 L working volume . Using a conservative estimate, the viable harvest yield of the Biostat STR® 200 is $1.0x10^{9}$ cells/L. Thus, at the 200 L scale, each run can generate $2.0x10^{11}$ cells, and implementation of this strategy can provide enough cells to treat the yearly predicted patient population in only 6 manufacturing runs, a significant reduction compared to conventional manufacturing processes. This demonstrates the power of applying Sartorius' scalable

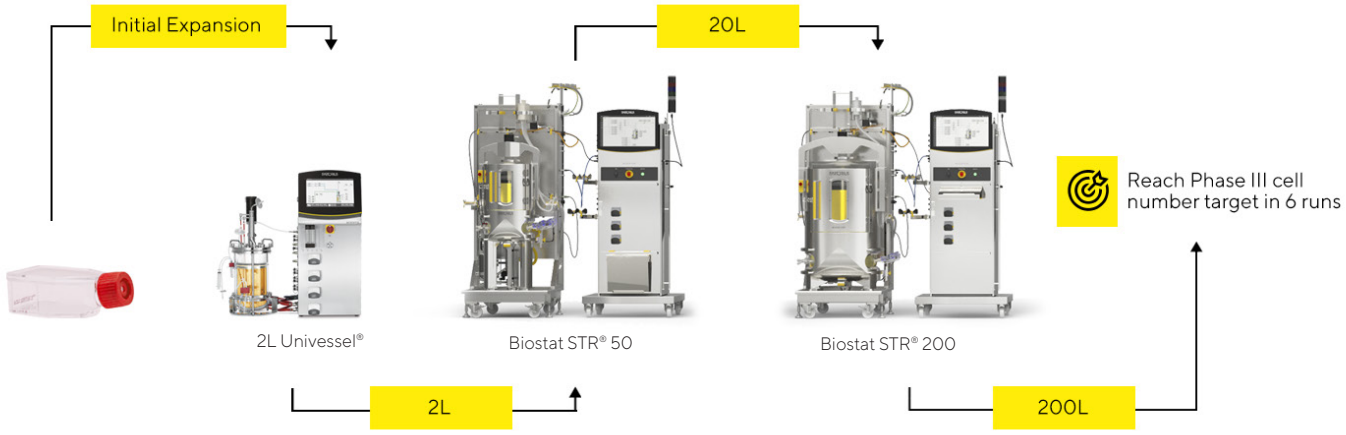


Figure 9. *Scalable Solutions Enable Scale Up to Clinical Cell Number Requirements.* Example of Sartorius industrialized cell therapy manufacturing platform developed to generate predicted patient cell number requirements. The projected scale-up strategy consists of an initial 2D expansion step, then seeding a 2L Univessel® bioreactor, followed by scale up to 20 L in a Biostat STR® 50, and ultimately a 200 L Biostat STR®. Since the viable harvest yield for the Biostat STR® 200 is $1x10^{9}$ cells/L, at the 200 L scale it would only take approximately 6 runs to reach the cell requirement of $1.2x10^{12}$ cells per year for a phase III trial.

solutions to accelerate MSC therapy development and swiftly make MSC treatments available to more patients.

Conclusion

The Sartorius MSC Pilot Solution, along with the subsequent MSC Manufacturing Solution, comprise a scalable offering that provides continuity and enables seamless process transfer for industrialized expansion of adherent cell types. This study provides proof of concept for the successful transition from a 2D static to 3D suspension culture process for cell expansion using the Sartorius MSC Pilot Solution. With these solutions, MSCs can be robustly expanded to large working volumes in a simplified seed train compared to 2D processes, while cells maintain high viability and stem cell relevant CQAs. This, in turn, accelerates time to market and saves on costs of production, ultimately leading to increased accessibility and affordability of these life-saving therapies.

References

[1] Blazar, B., Murphy, W. & Abedi, M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol 12, 443–458 (2012). https://doi.org/10.1038/nri3212

[2] Cheung, T.S., Bertolino, G.M., Giacomini C., Bornhäuser M., Dazzi F., Galleu A. Mesenchymal Stromal Cells for Graft Versus Host Disease: Mechanism-Based Biomarkers. Front. Immunol. 11, 1338 (2020). https://www.frontiersin.org/article/10.3389/fimmu.2020.01338

[3] Soder, R.P., Dawn, B., Weiss, M.L. et al. A Phase I Study to Evaluate Two Doses of Wharton's Jelly-Derived Mesenchymal Stromal Cells for the Treatment of De Novo High-Risk or Steroid-Refractory Acute Graft Versus Host Disease. Stem Cell Rev and Rep 16, 979–991 (2020). https://doi.org/10.1007/s12015-020-10015-8

[4] Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A., Prockop Dj., Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8 (4), 315-7.(2006) doi: 10.1080/14653240600855905. PMID: 16923606

[5] Approved Cellular and Gene Therapy Products. U.S. Food and Drug Administration. Updated June 15, 2021. Accessed September 15, 2021. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

Ordering Information

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Univessel® Glass, 2L	UNIVESSEL-00002
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Biosealer® TC	16391-000
MSC Nutristem® XF Supplement Mix	05-201-1U
MSC Nutristem® XF Basal Medium	05-200-1A
PLTGold® Human Platelet Lysate	PLTGOLD500R
SoloHill® Collagen Coated Microcarriers	CIR-221-020
⅛" Tuflux® Tubing	FSA121869
¼" Tuflux® Tubing	FSA121870

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