

Advancing Microcarrier-Based Stem Cell Culture Processes and Easing Transition to 10 L Single-Use Bioreactors

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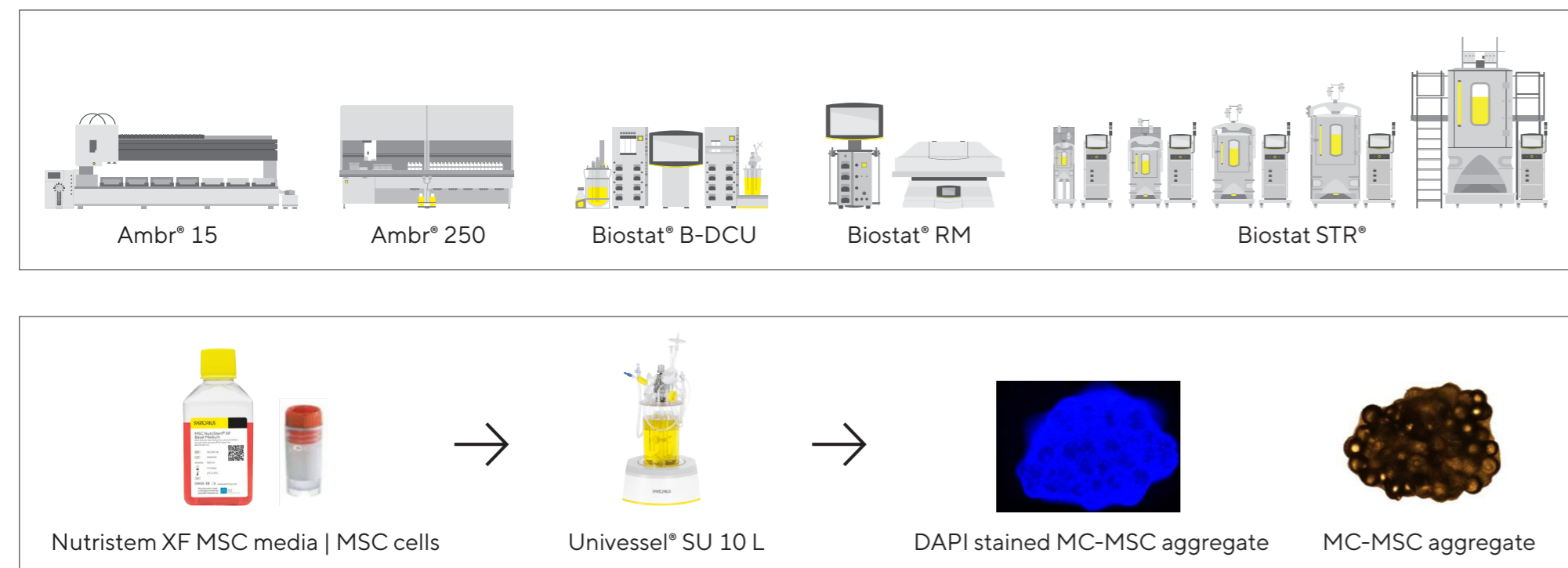
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1. Introduction

To smoothly implement complex cell culture processes at a higher scale, the bioreactor needs to ensure accurate control of process parameters like oxygen supply and pressure. Cell culture processes are important in several fields including regenerative medicine, drug discovery, and biotechnology. Our latest prototype, the 10 L single-use cell therapy vessel, is equipped with a specialized elephant ear impeller. This vessel provides the superior mixing efficiency and gentle agitation needed to maintain cell viability and functionality. Additionally, it features three dip tubes (10%, 20%, and 80% filling volumes) with a diameter of 1/8 inch, enabling precise media addition, exchange, and sampling throughout the culture process.

Using the new 10 L cell therapy single-use vessel, we scaled up bone marrow-derived mesenchymal stem cell culture to manufacturing scale. We used stem cells isolated in the Emerging Therapies Services Lab at Sartorius for 2D and 3D process development. We performed process optimization in a prototype Univessel® Single-Use (SU) 10 L stirred tank bioreactor using MSC NutriStem® XF cell culture media and microcarriers from Sartorius. This study demonstrates the first successful 2D to 3D transition from development to production scale in this 10 L bioreactor, and focuses on controlling these critical parameters.

Figure 1: BMMSC Culture Process in Single-Use Bioreactor Portfolio From PD to CM Scale

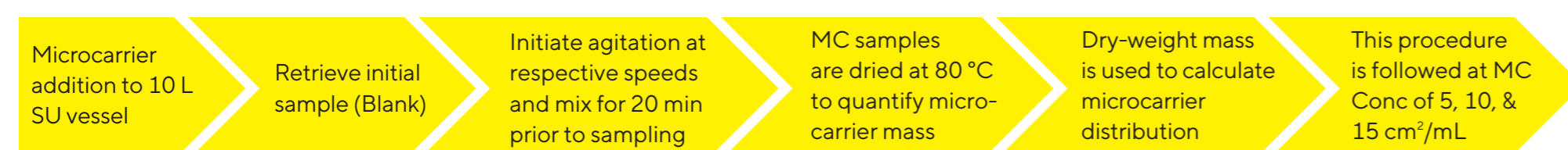


2. Experimental Approach

Bioreactor Mapping Process

We performed microcarrier mapping to evaluate mixing performance and microcarrier integrity in the new 10 L SU bioreactor, and identified optimal stir speeds for mixing and verified maintenance of microcarrier integrity using particulate count analysis. A pressure test was performed on Univessel® SU 10 L to confirm the bioreactor had no tube breakage or leakage. We selected microcarriers with low (1.02) and high (1.1) relative densities to examine the just suspended state (Js) of microcarriers at concentrations of 5, 10, and 15 cm²/mL.

Figure 2: Schematic Process Flow Representing Microcarrier Mapping in 10 L Single-Use Bioreactor

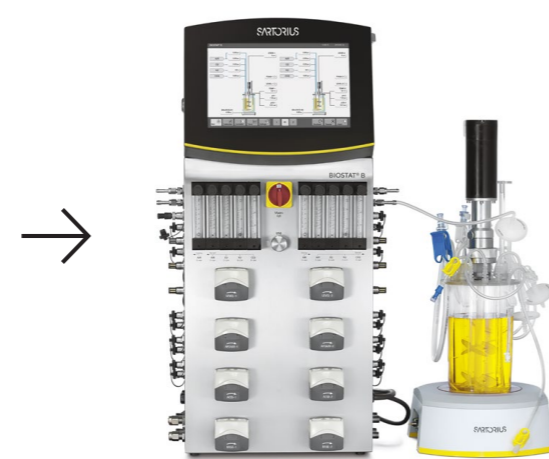


Note. We used a buffered solution of NaCl 6.4 g/L and sodium bicarbonate 3.7 g/L to mimic cell culture medium for the microcarrier mapping studies. To assess mixing, we added microcarriers to the bioreactor which settled at the bottom of the bioreactor. A blank sample was collected, and the run was then initiated by manually setting the impeller speed to the lowest test setting (8 rpm) and 12 mL samples were retrieved. Subsequent samples were taken at different agitation speeds until a maximum speed of 169 rpm was reached. Agitation was maintained at the desired speed for approximately 2 minutes, and samples were then removed from the bottom (1.5 cm from bottom), middle (18 cm from bottom), and top (4 cm from meniscus) of the bioreactor using 10%, 20%, 84% dip tubes.

Mesenchymal Stem Cell Culture Process

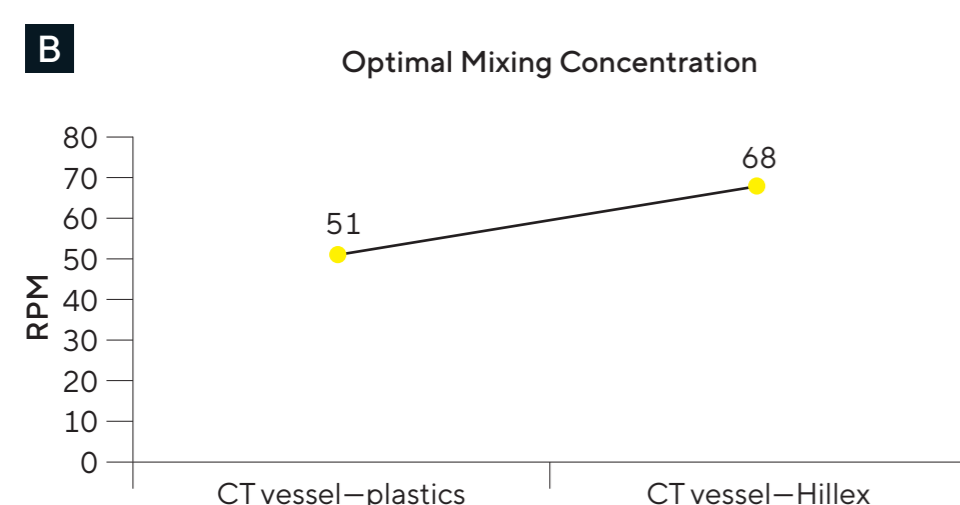
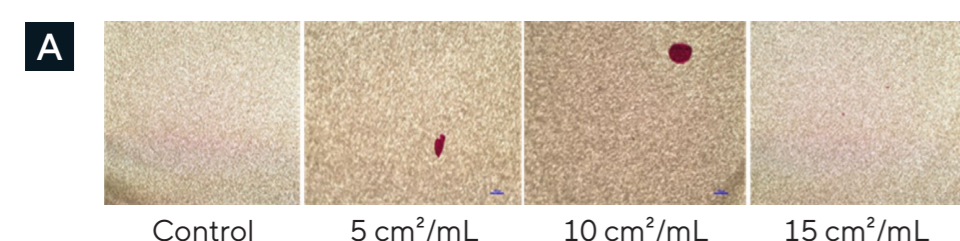
We isolated bone marrow-derived mesenchymal stem cells and performed basic characterization to confirm the stem-like nature of the cells. Cells were expanded in 2D before seeding into the 10 L SU bioreactor. We used a prototype animal component-free microcarrier with a relative density of 1.02 and surface area of 360 cm²/g at a density of 10 cm²/mL.

- MSCs were seeded into a 10 L SU bioreactor at an initial density of 3E3 cells/cm² in defined MSC NutriStem® XF cell culture media supplemented with 5% human platelet lysate and cultured for 6 days
- Control cultures for cells (SP1) and bioreactor (SP2) were maintained in 500 mL spinner flasks for 6 days, and we compared daily cell counts and viability to bioreactor culture
- Sampling was performed at regular intervals to assess cell density, viability, metabolic activity, and product quality through the 20% dip tube

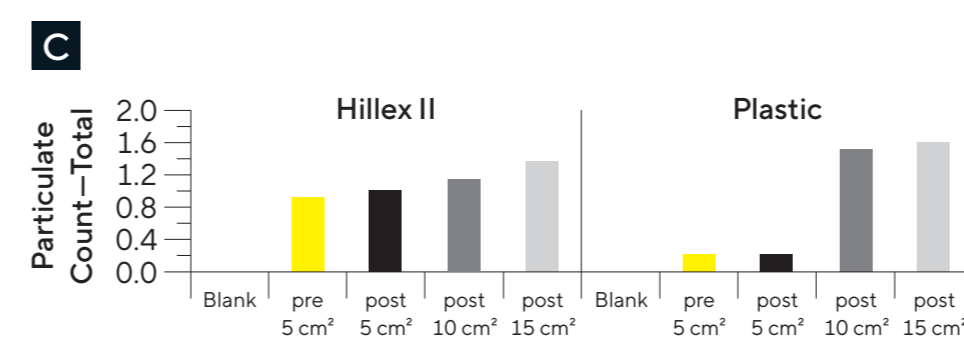


3. Results

Bioreactor Mapping Process



Note. A) Assessing microcarrier breakage under different concentrations at 5, 10, and 15 cm²/mL post culture. Our comprehensive microscopic observations have demonstrated the integrity of microcarriers subjected to different concentrations in various agitation conditions. Through our results, we determined that there is very little microcarrier breakage evident across the tested agitation parameters. B) The optimal mixing concentration (OMC) results highlight the Js for Plastic (at 51 rpm) and Hillex (at 68 rpm) microcarriers in Univessel® SU 10 L. OMC is critical to fine-tuning mixing parameters and enhancing MSC culture performance, ensuring uniform distribution of cells and nutrients while minimizing oxygen and nutrient gradients.

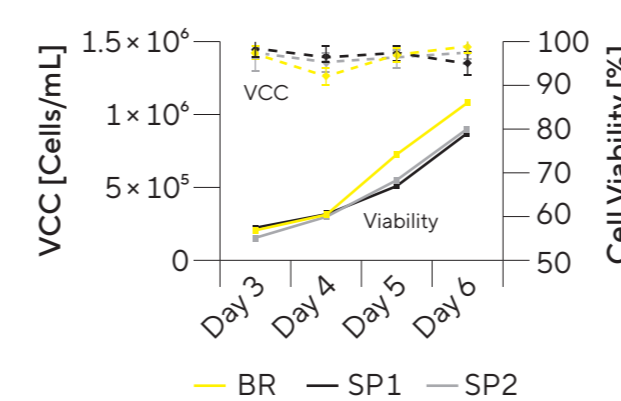


C) Our analysis of particulate count, performed in accordance with USP 788 guidelines, affirms the robustness of our findings. The particulate count consistently remained below the stipulated threshold (not exceeding 12 per mL equal to or greater than 10 µm, and 2 per mL equal to or greater than 25 µm)—indicating the absence of significant microcarrier breakage.

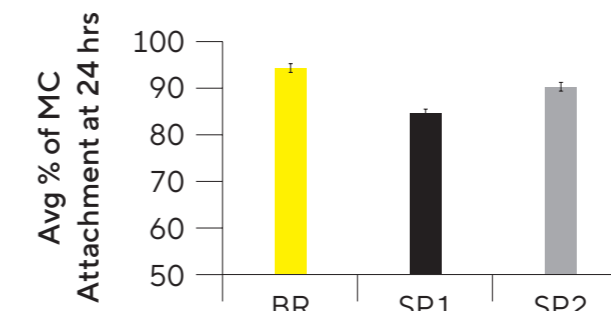
MSC Culture in 10 L SU Bioreactor

MSCs demonstrated robust growth kinetics, reaching a peak density of 1e6 cells/mL at day 6. Viability remained consistently high (> 90%) throughout the culture duration, indicating favorable cell health and proliferation. Growth curve analysis revealed linear growth during the 6-day culture.

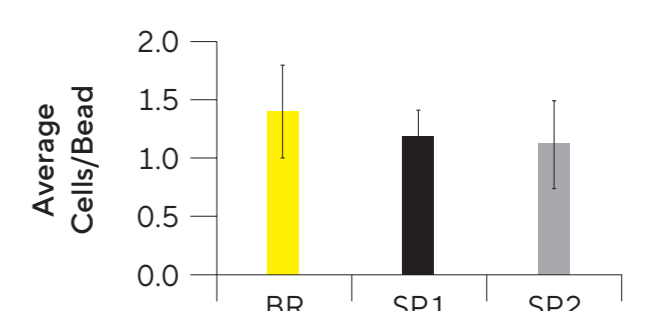
1 Viable Cell Concentration/ Viability



2 Cell Attachment Efficiency



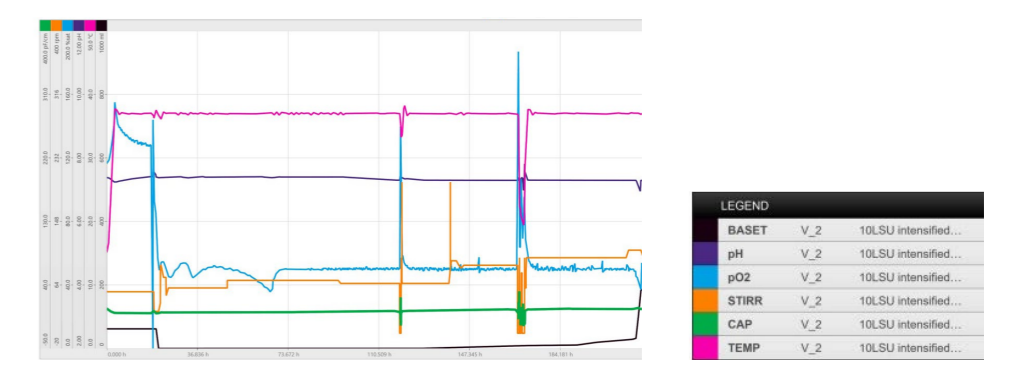
3 Cell/Bead Ratio



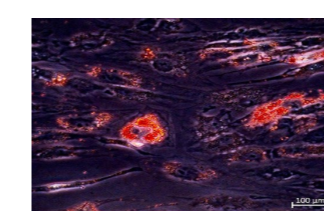
4 Cell Therapy Bioreactor

Microcarrier	Prototype ACF
MC relative density	1.02
MC concentration [cm ² /mL]	10
Cell culture medium	Sartorius MSC NutriStem® XF media
RPM	45–55
Tip speed [m/s]	0.21–0.28

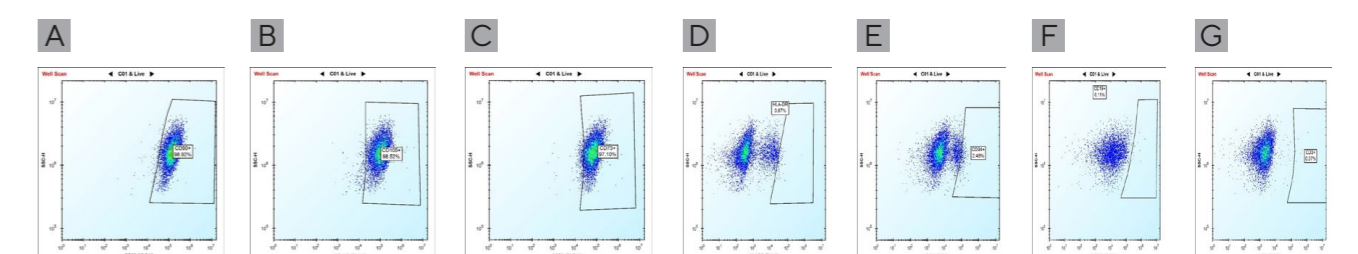
5 Cell Therapy Bioreactor—MFCS Graph



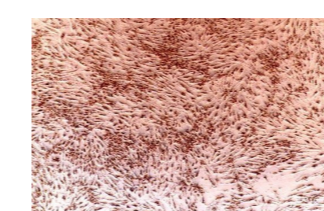
6 Adipogenic Differentiation



8 Adipogenic Differentiation



7 Osteogenic Differentiation



Marker	Expression [%]	Expression
A CD90	98.92	(+ve)
B CD73	97.10	(+ve)
C CD105	98.52	(+ve)
Marker	Expression [%]	Expression
D CD34	2.49	(-ve)
E CD45	0.01	(-ve)
F CD14	0.07	(-ve)
G HLA DR	0.87	(-ve)

Note. 1) Process parameters (VCC; viability) for MSC culture process in 10 L single use bioreactor (SP1—Bioreactor control; SP2—Cell control) 2) Cell attachment was assessed after 24 hours of inoculation. 3) Cells/bead ratio was evaluated after 24 hours of inoculation. 4) After 24 hours of bioreactor inoculation, sample is collected to evaluate cells/bead ratio. 5) Key parameters including dissolved oxygen, pH, and glucose | lactate levels were monitored throughout the culture period. Gas rates were maintained using cascade control. 15 mL/min of air at 100% oxygen was constantly entering the culture through the ring sparger. 6 and 7) Functional assays demonstrated the multilineage differentiation potential of MSCs, confirming their therapeutic relevance. 8) Phenotypic characterization confirmed the retention of MSC-specific surface markers throughout the culture period.

Conclusion

Our bioreactor mapping results demonstrate the reliability and durability of the Univessel® SU 10 L stirred tank bioreactor for microcarrier-based culture. The data not only inform our understanding of agitation-induced stresses on microcarriers, but also bolster our confidence in their suitability for various therapeutic applications. Our study shows the bioreactor's capability to hold microcarrier-based culture in specified agitation conditions, and indicates their potential for scientists performing relevant processes.

Notably, the bioreactor resulted in impressive cell growth and viability, demonstrating the utility of the vessel design as well as the capability to control the critical process parameters that influence cell behavior. These findings highlight the system's suitability for therapeutic applications in cell culture processes.

The new Sartorius Univessel® SU 10 L bioreactor empowers researchers and bioprocess engineers to accelerate the development and production of MSC-based therapies, and drive the field of regenerative medicine forward.

Univessel® SU 10 L

- Single-use stirred tank bioreactor with elephant ear impeller, 10 L maximum working volume
- Presterilized and ready-to-use
- Available in three application-specific vessel designs: essential, perfusion, and cell therapy
- Bioreactor designed for scale-up compatibility with the Sartorius portfolio

Acknowledgement

Thanks to all colleagues involved from ETS team, Bioreactor Technologies, the teams of Application & Testing Labs (Bohemia, USA; Göttingen, GER; Royston, UK) and PD Cell Line, Media & Testing Solution (Ulm, GER).

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