Closed-System Expansion and Downstream Processing of Adherent Cells Using Osilaris™ and Rotea™

Application Note

Highlights

- The Osilaris™ platform integrates seamlessly with the Rotea™ Counterflow Centrifugation System for efficient harvest, wash and concentration.
- MSCs can be cultured on polystyrene microcarriers and be harvested in one single Osilaris™ culture bag with minimal cell loss using a closed workflow
- Harvested cells retain high viability and MSC markers after formulation with the Rotea™

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Introduction

Cell therapy manufacturing requires scalable and controlled expansion platforms that can be integrated into closed, end-to-end workflows. Microcarrier-based expansion is a scalable approach for adherent cell culture, such as mesenchymal stromal cells (MSCs). However, efficient cultivation and downstream processing, such as harvest and concentration of microcarrier cultures can be challenging. In many existing workflows, these stages are not fully integrated, requiring open handling or manual interventions that increase contamination risk, process variability, and operational complexity, limiting translation to GMP-compliant manufacturing.

The Osilaris™ platform addresses these challenges by enabling controlled expansion of adherent cells in microcarrier-based cultures within a closed and scalable system. Its inversion-based rocking mechanism ensures homogeneous culture conditions and control of key parameters like pH and dissolved oxygen (DO). Compatibility with downstream technologies supports closed workflows and reduces manual handling.

In this application note, bone marrow–derived MSCs were expanded on collagen-coated polystyrene microcarriers in the Osilaris™ platform and subsequently harvested using the Thermo Fisher Rotea™ cell processing system (Figure 1). Direct connection of the single-use Osilaris™ adherent culture bag to the Rotea™ system enables a fully closed workflow integrating expansion, harvest, washing, and concentration. The results demonstrate a streamlined process with minimal manual handling, enabling efficient cell harvest. It maintains high viability and MSC marker expression, supporting scalable and GMP-compatible cell therapy manufacturing.

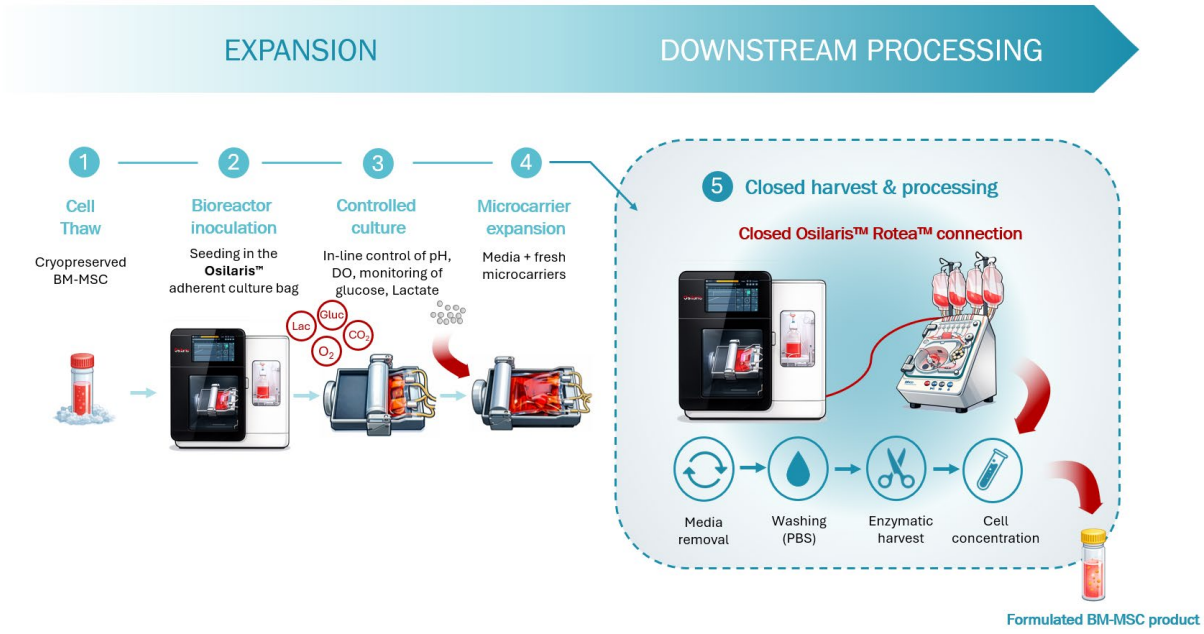

Figure 1 Integrated microcarrier-based MSC expansion and closed harvest workflow. *BM-MSCs are seeded onto collagen-coated microcarriers and expanded in a closed system, with medium and microcarrier addition enabling bead-to-bead transfer. Cells are subsequently detached, harvested, and washed using the Rotea™ system welded in a closed loop with the Osilaris™ platform workflow.*

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Materials and Methods

Cell thaw and bioreactor inoculation

BM-MSCs from two donors at passage 2 were thawed from cryopreservation and directly seeded with 2 million cells into the Osilaris™ adherent culture bag. The culture was initiated at a working volume of 125 mL using αMEM (Capricorn Scientific) supplemented with 10% fetal bovine serum, 1% GlutaMAX™, 1% penicillin/streptomycin, 0.1% βFGF, and 0.1% L-ascorbic acid 2-phosphate. Collagen-coated polystyrene microcarriers (Corning®, catalog no. 3786) provided an initial surface area of 2,625 cm^2.

Controlled bioreactor culture conditions

Parameter	Setpoint
Temperature	37 °C
Rocking speed	90 °/s
Rocking angle	+/- 90 °
Rocking regime	7 hours rocking – 1 hour hold
pH	7.3
DO	75%
Glucose	> 1 mmol/L
Lactate	< 8 mmol/L

Microcarrier expansion

When cells reached a surface density of >2,000 cells/cm^2, expansion was performed by adding fresh medium and microcarriers at 58 g/L. This step was carried out twice, resulting in a final working volume of 1.2 L and a total available surface area of 25,000 cm^2.

Automated closed-system harvest and downstream processing

At a final density of approximately 20,000 cells/cm^2, cells were harvested using the Rotea™ system. The Osilaris™ adherent culture bag was connected to the CTS™ Rotea™ Full Flow Single-Use Kit via sterile welding (Figure 2B), enabling a closed workflow. Using the Rotea™ Protocol Builder a protocol was defined. Harvest was initiated with the Rotea™ GUI System Software by automated removal of spent medium using the Rotea™ fluid management system, followed by three PBS washes. The TrypLE solution (PBS with 10× TrypLE™ and EDTA) was added and incubated under rocking conditions to promote efficient detachment. After approximately 15 minutes, cells were separated from microcarriers using the integrated 100 µm filters of the Osilaris™ adherent bag (Figure 2A) and transferred to an intermediate bag containing 100 mL FBS to stop enzymatic activity (Figure 2B). Cells were then concentrated, washed, and collected in a final cell bag using the Rotea™ system.

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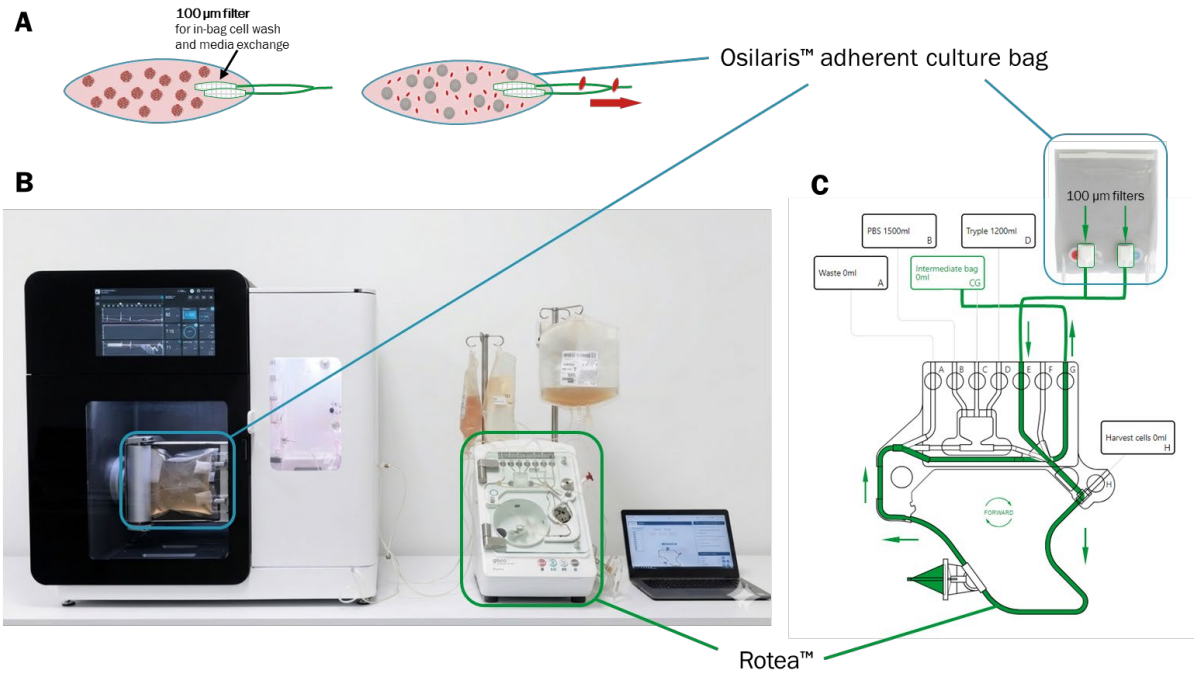

Figure 2 Closed Osilaris™–Rotea™ integration for microcarrier-based MSC harvest. *Cells expanded in the Osilaris™ adherent culture bag, are detached with TrypLE, separated from microcarriers through integrated filters and are harvested directly with the Rotea™ cell processing system for automated washing and concentration.*

Results

Cell visualization

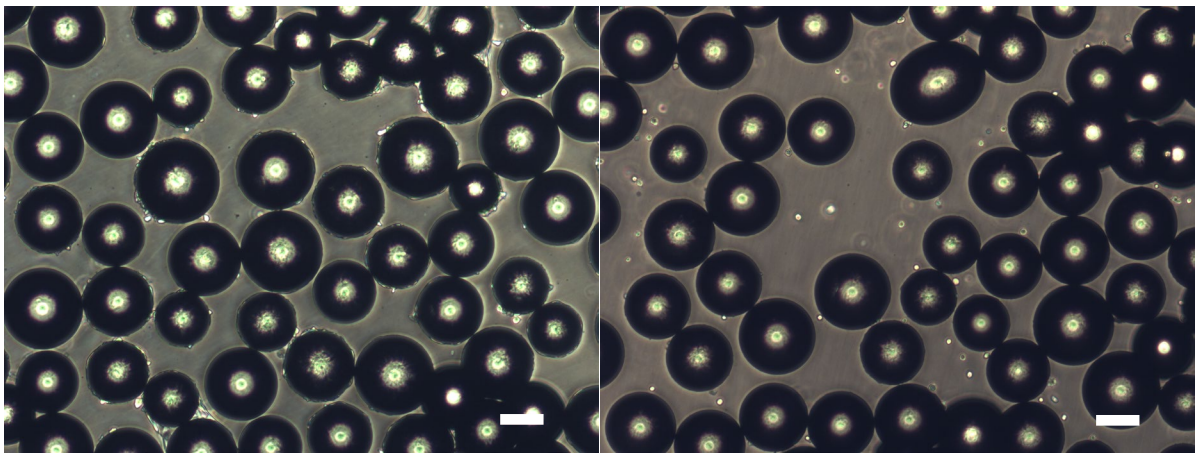

Figure 3 Representative microcarrier cultures at harvest. *Bright-field microscopy images of collagen-coated microcarriers following MSC expansion in the Osilaris platform. Microcarriers from donor 2 (left) prior to harvest show dense and uniform cell coverage. Following incubation with the harvest solution (right), cells are effectively detached from the microcarriers and observed in suspension. Scale bars in white represent 100 µm.*

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Cell viability and yield

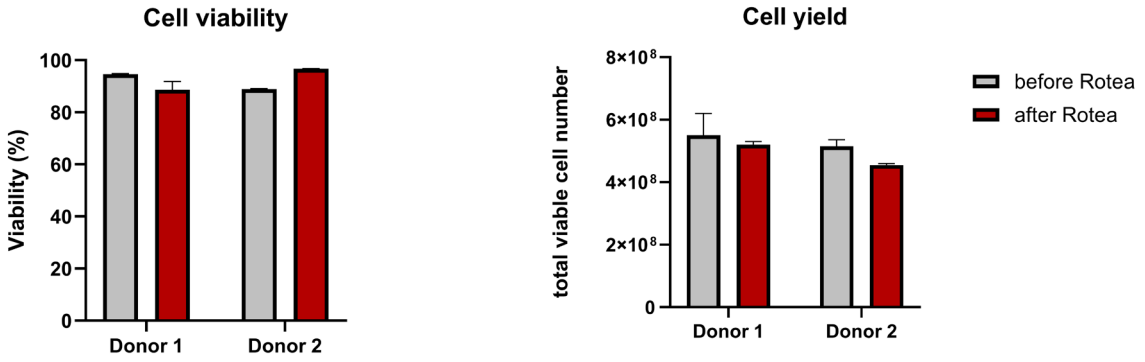

Figure 4 Cell viability and recovery after harvest using the Rotea™ cell processing system. *(Left) Viability of BM-MSCs from two donors before and after Rotea™ processing, showing high cell viability throughout the harvest and processing workflow. (Right) Total cell numbers measured from a sample and after Rotea™ processing. Comparable cell yields were obtained following harvest. These results demonstrate efficient cell recovery during the automated, closed harvest and concentration process, highlighting both the gentleness of the procedure and its robustness across donors.*

Phenotypic markers

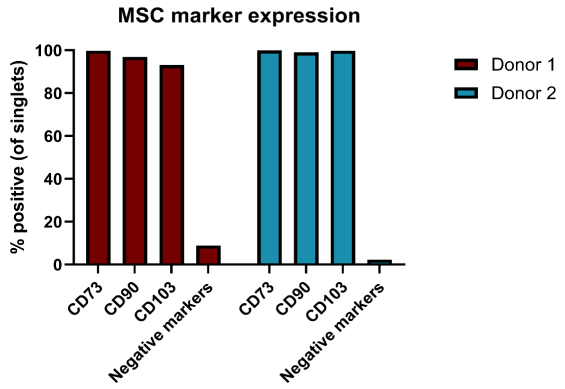

Figure 5 Phenotypic characterization after microcarrier expansion and harvest of MSCs. *Flow cytometry analysis confirms MSC marker expression following expansion and harvest. Cells from both donors show high expression of canonical MSC markers CD73, CD90, and CD105, while remaining negative for lineage markers, consistent with the expected MSC phenotype.*

Discussion

This study demonstrates the successful implementation of a closed and integrated workflow for microcarrier-based MSC expansion and harvest using the Osilaris™ platform in combination with the Rotea™ cell processing system. By directly connecting the Osilaris™ adherent culture bag to the Rotea™ system, expansion, harvest, washing, and concentration steps can be performed within a single automated closed process, minimizing manual handling and reducing contamination risk. This workflow enables efficient recovery of BM-MSCs with high cell viability (>90%) and preservation of key MSC surface markers following processing. These results indicate that integration of upstream and downstream operations can address a key bottleneck in cell therapy manufacturing: maintaining process continuity while preserving cell quality attributes during scale-up and transfer between process steps [1,2].

As the cell therapy field moves toward fully automated and closed manufacturing, integration of upstream and downstream processes is essential to reduce human error, contamination risk, and operational complexity. The Osilaris™ platform enables a streamlined, closed workflow from cryopreservation to clinically relevant cell numbers, incorporating efficient in-bag cell detachment and microcarrier separation via integrated filtration [3]. When combined with the Rotea™ cell

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processing system, this workflow is further strengthened through automated washing and concentration, ensuring high cell viability is preserved through a gentle and controlled harvest process.

Together, these features enable a reproducible and scalable workflow from cell expansion to final product formulation, supporting efficient and GMP-compatible MSC manufacturing.

Furthermore, when combined with downstream fill-and-finish solutions such as the Thermo Fisher Scientific™ Compleo™ system, this integrated workflow provides a comprehensive, closed solution from cell expansion to final product formulation.

References

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2. *Sensebé L, Gadelorge M, Fleury-Cappellesso S. Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review. Stem Cell Res Ther. 2013 Jun 7;4(3):66. doi: 10.1186/scrt217.*
3. *Das, R., Roosloot, R., van Pel, M. et al. Preparing for cell culture scale-out: establishing parity of bioreactor- and flask-expanded mesenchymal stromal cell cultures. J Transl Med 17, 241 (2019). https://doi.org/10.1186/s12967-019-1989-x*

Find out more

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