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Automating hMSC-EV Manufacturing in the Osilaris™ Bioreactor with RoosterBio hMSCs and Media

Application Note

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1) Introduction

Extracellular vesicles (EVs) are lipid-bilayer nanovesicles released by cells that carry lipids, proteins, and nucleic acids. Several classes of EVs exist, including exosomes which are the best studied subtype with a diameter range of 40–120 nm. EVs mediate intercellular communication and can deliver bioactive cargo across membranes. This positions EVs as versatile therapeutics and drug-delivery vehicles. Notably, EVs from mesenchymal stromal/stem cells (MSCs) have demonstrated immunomodulatory and pro-regenerative effects in animal models, potentially with lower immunogenicity and tumorigenicity than whole-cell therapies[1]. Recent studies have demonstrated the safety of local EV administration in clinical use[2]. EVs also capture many benefits of MSCs without the challenges of handling living cells, further motivating their development. Reflecting this promise, MSCs and MSC-derived EVs are being studied across numerous clinical trials, driving demand for robust, economical manufacturing.

2) EV Manufacturing Strategies

Adherent cell-derived EV manufacturing workflows that rely on planar plasticware (2D) scale out by increasing surface area but incur high costs, and aseptic risk, while offering limited environmental control. To address these concerns a variety of bioreactor platforms have been evaluated for MSC and MSC-EV manufacturing including those with built-in matrices for MSC attachment and expansion such as hollow-fiber, fixed bed, and dynamic bed bioreactors. Additionally, microcarrier-based approaches have been used to successfully manufacture MSCs and MSC-EVs, including vertical wheel and stirred-tank bioreactors.

Microcarrier-based culture has several advantages for MSC and MSC-EV bioprocessing. First, with optimized protocols and bioprocessing media, MSCs can achieve >85% attachment in a short period of time. Microcarriers enable xeno-free, fed batch processes to routinely reach >5x10^5 MSCs/ml[3]. Additionally, depending on the chosen microcarrier with optimized protocols, MSC harvest can be straightforward, with enzymatic detachment processes. These high-density cells maintain their tri-lineage potential, canonical surface markers, immunomodulatory IDO (Indoleamine 2,3-dioxygenase) activity, and angiogenic cytokine secretion compared to 2D controls[4]. Finally, the same microcarrier-based workflow provides a scalable foundation for downstream MSC-EV manufacturing.

3) Osilaris is an efficient MSC cultivation platform

Osilaris, developed by Scinus Cell Expansion, is a closed bioreactor system designed for efficient large-fold expansion of cells, consisting of a cabinet and a single-use culture bag. For the controlled expansion of adherent cells, such as MSCs, the system uses microcarriers and the Osilaris culture bag for adherent culture. The culture bag is placed on a rocker platform inside the cabinet (Figure 1A–B). The platform rotates along its longitudinal axis at a predefined speed and angle, ensuring that the microcarriers remain in homogeneous suspension while minimizing shear stress. Additionally, the rocking platform improves nutrient distribution, enabling efficient culture at high microcarrier densities and supporting large-scale cell expansion.

The bag is equipped with in-line pH and dissolved oxygen (DO) sensor patches and a dedicated tubing set that passes through the Carboxygenator, the proprietary gas exchange component of the Osilaris system. This unique element of the bioreactor enables gas exchange by forced diffusion, eliminating the need for an air–liquid interface and antifoaming agents, while allowing precise control of DO and pH throughout the culture, ensuring a homogeneous and reproducible cell culture. Additional access points facilitate convenient medium addition, exchange, and sampling, while maintaining a closed and sterile system. Culture conditions, including pH, DO, and temperature, are automatically regulated by the hardware enclosure, reducing operator intervention and supporting standardized operations under GMP conditions.

A roller mechanism within the platform allows the culture volume to be expanded stepwise to continuously accommodate increasing cell numbers (Figure 1C).

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In combination with bead-to-bead transfer across the microcarriers, this setup enables expansion without the need for enzymatic passaging. Medium compositions are easily exchanged using the flexible tube setup. Taken together, the design of the Osilaris bioreactor establishes a controlled, scalable, and closed environment that supports robust MSC expansion and provides optimal conditions for the generation and harvest of EVs.

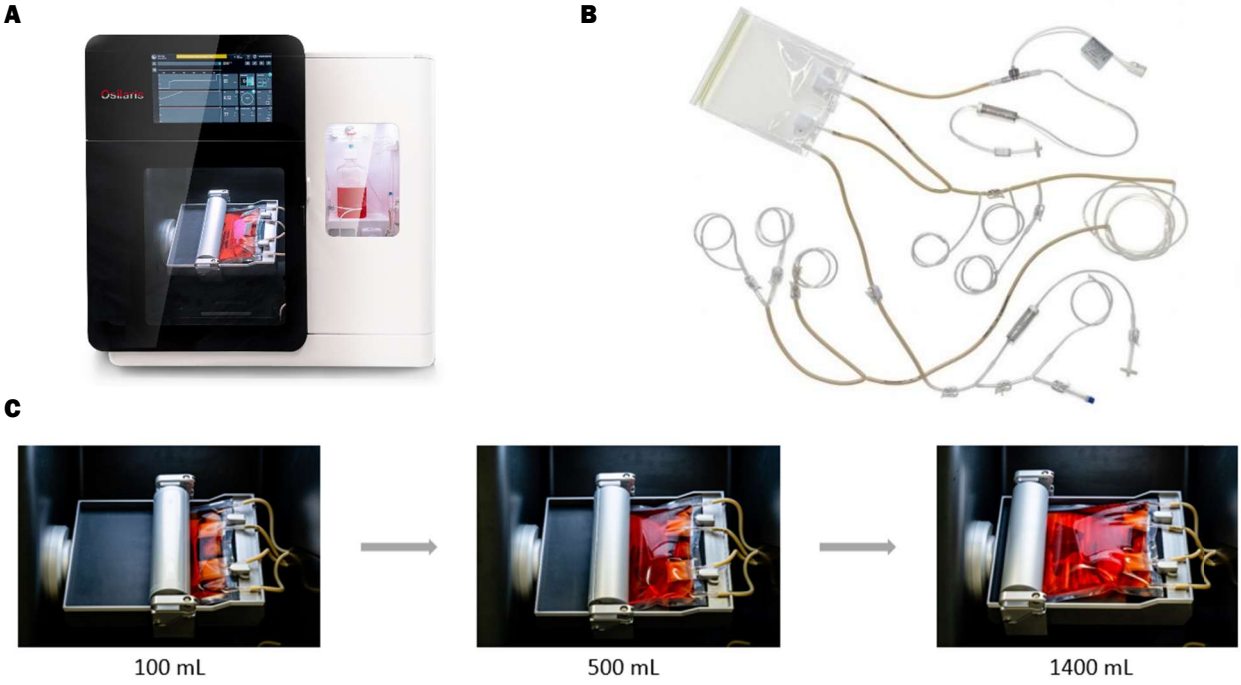

***Figure 1**: The Osilaris bioreactor system. **A.** Osilaris cabinet. **B.** Osilaris adherent culture bag. **C**. Timelapse showcasing the 14-fold expansion of the culture bag volume.*

4) RoosterBio Products Enable High MSC and MSC-EV Yields

RoosterBio's MSC banks and bioprocessing media simplify MSC and MSC-EV manufacturing from early-stage research and development through clinical translation. RoosterVial™ hMSCs and RoosterBio's bioprocessing medias are offered in development grade and cGMP formats, streamlining tech transfer across development stages. The xeno-free RoosterNourish™-MSC-XF expansion medium removes the need for media exchanges in 2D batch and 3D fed-batch culture with RoosterReplenish™-MSC-XF bioreactor feed. In EV collection, chemically-defined, low-particulate RoosterCollect™-EV minimizes background particle counts, increasing confidence that measured particles originate from the cultured cells and reduces downstream processing complexity. RoosterBio's cell and media products have demonstrated enhanced productivity and simplified MSC and MSC-EV manufacturing approaches across production scales. Additionally, RoosterBio supports product developers with development and analytical services, including EV characterization.

5) hMSC growth and EV Production in the Osilaris Bioreactor with RoosterBio Cells and Media

In this study, we explored the potential to generate EVs using a combination of RoosterBio's RoosterVial-MSCs, RoosterNourish-MSC-XF expansion medium, and RoosterCollect-EV collection medium in Scinus' Osilaris™ bioreactor.

RoosterBio's MSCs were cultivated on Synthemax-II-coated dissolvable microcarriers (SM2-DMC; Corning Life Sciences) using the single-use Osilaris culture bags designed for adherent cells. Across three experiments, cultures were established by

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Experimental outline

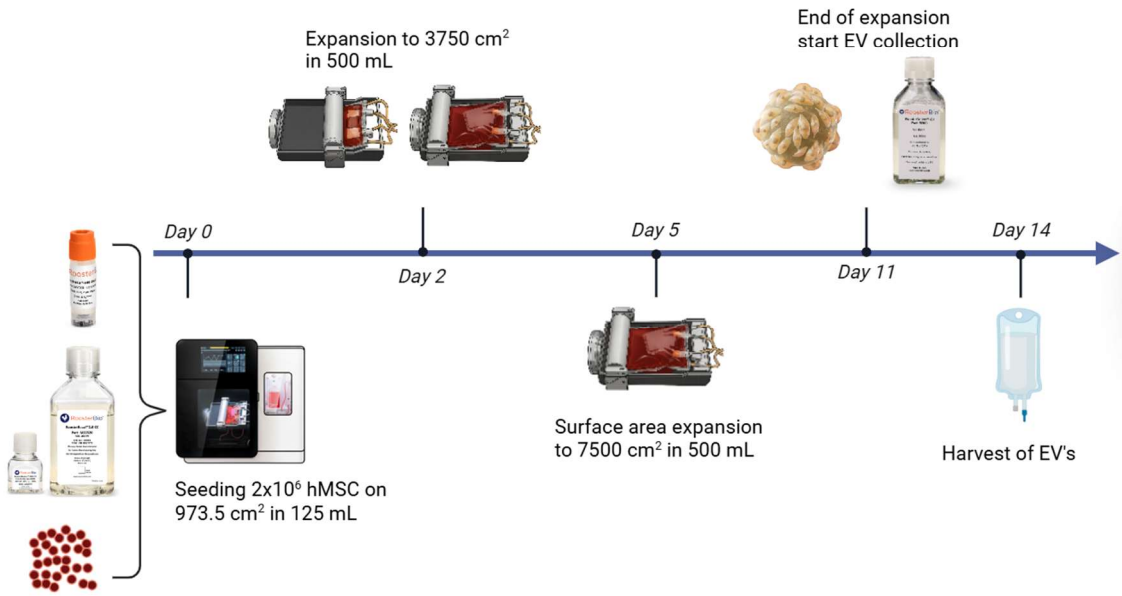

***Figure 2**: Experimental outline of Rooster hMSC expansion and EV collection with Osilaris.*

seeding cells at 2,000 cells/cm² in 125 mL of RoosterNourish-MSC-XF medium (Figure 2), for a total initial surface area of 973.5 cm². To enhance cell attachment and distribution, a mixing regime was implemented, consisting of 15 minutes of rocking followed by a 4-hour horizontal hold. Following an overnight attachment period, the perfusion loop was activated, thereby initiating dissolved oxygen (DO) control at 75% and pH control at 7.3. Subsequently, the mixing regime was adapted to include seven hours of rocking with a one-hour horizontal hold phase designed to enhance bead-to-bead transfer.

In order to promote sustained cell proliferation, the surface area of the culture medium was expanded in a two-step process by introducing fresh microcarriers and expanding the volume of the bag on day 2. During the initial phase of expansion, the Osilaris roller system was employed to expand the bag volume and new microcarriers were added. The resulting media volume was 500 mL with an available surface area of 3750 cm². In the second expansion, a portion of the spent medium was discarded prior to the addition of fresh microcarriers keeping the total volume at 500 mL but increasing the culture surface to 7500 cm². In total, only 1L medium was used during cell expansion in a single run.

After the cultures had reached the desired density, the RoosterNourish-MSC-XF expansion medium was removed and the cell-microcarrier content was washed twice before addition of RoosterCollect-EV to the culture bag. Cells were then maintained for an additional three days, after which EVs were harvested. A monolayer culture was included as the control, which was seeded at the same density as the bioreactor cultures.

Following an 11-day proliferation phase, each condition produced 194 million cells (range 191 – 198 million cells), as shown in Figure 3A. Growth kinetics and final yield were highly consistent across the three replicates, indicating a reproducible expansion protocol. This outcome represents a fold increase of 100-fold from culture initiation. As illustrated in Figure 3B, the glucose concentration was successfully maintained at the desired levels throughout the duration of the cell cultures. As indicated by the data presented in Figure 3C, the accumulation of lactate exhibited comparable trends across all experiments.

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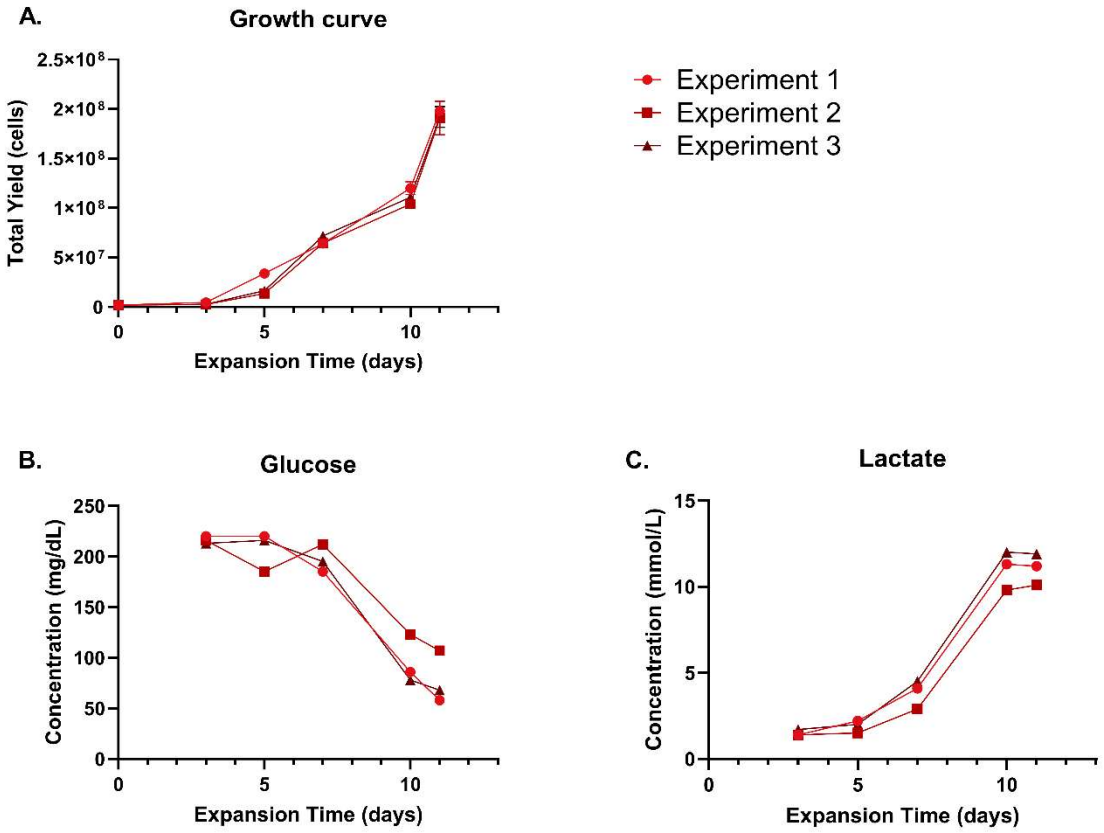

Figure 3: A. *RoosterVial™ hMSCs growth on SM2-coated dissolvable microcarriers in Osilaris using RoosterBio's RoosterNourish-MSC-XF medium.* ***B****. Glucose and* ***C.*** *Lactate concentrations in supernatants from 3 different expansions in the Osilaris bioreactor.*

After the proliferation phase, the RoosterNourish-MSC-XF medium was removed and replaced with the RoosterCollect-EV medium. After 72 hours, EVs were harvested from the bioreactor and were characterized using RoosterBio's analytical services. The Osilaris-based process resulted in higher EV content than monolayer control cultures. EV concentration per mL from bioreactor-collected medium was more than twice as high compared to EV-medium collected from the monolayer control ($2.9x10^9$ EV/mL vs $1.4x10^9$ EV/mL, Figure 4). Nanoparticle tracking analysis (NTA) showed comparable particle sizes across the EV harvest solutions of the different experiments (Figure 5), with all runs and the monolayer control producing particles in the 100 – 400 nm range.

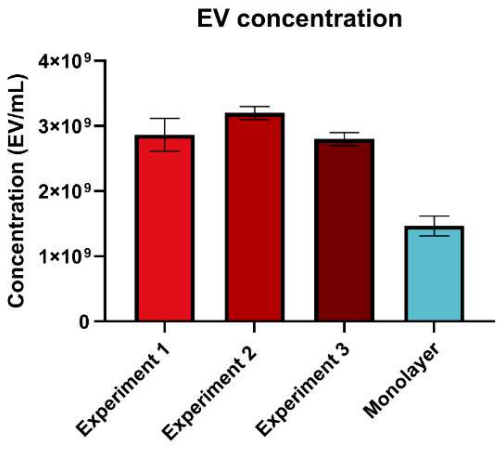

Figure 4: *EV particle concentration in supernatants of three different experimental runs with the Osilaris bioreactor, and one monolayer control. Error bars represent standard deviation of n = 3.*

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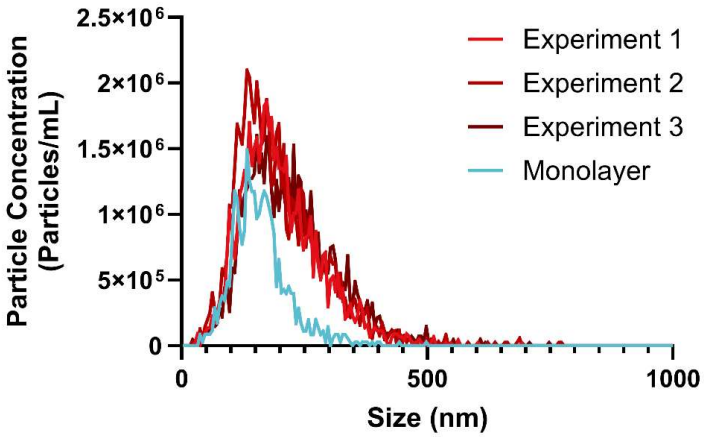

Figure 5: *Size distribution of EV particles collected from three different experimental runs in the Osilaris bioreactor and a reference monolayer flask culture.*

6) Conclusions and recommendations

The Osilaris platform is designed for the efficient scale-up of adherent and suspension cell cultures. In this study, we demonstrated how the system can grow an inoculum of 2 million hMSCs to ~200 million cells for EV production. Although this proof-of-concept study yielded only a fraction of the system's full potential (the Osilaris bioreactor can produce over 1.5 billion hMSCs from the same inoculum in a single closed process) it highlights its ability to support both large-scale cell expansion and EV generation[4].

Suitable for GMP-compliant manufacturing of cell-based products, Osilaris provides an easier, more scalable and cost-effective alternative to flask-based processes. The minimal inoculum, the yield, and densities of more than $5x10^5$ cells/ml in an automated closed-system design, make it also a unique alternative to spinner flasks, fixed beds or wave bag bioreactor designs[3]. In combination with RoosterBio's development grade MSCs and fit-for-purpose bioprocess medium, minimized both operator time and medium use. Additionally, RoosterBio's low-particulate EV-collection medium is designed to deliver robust, high EV-concentration yields.

Results show that this approach is highly reproducible, with cell growth and subsequent EV yield showing high consistency between runs.

This is further corroborated by the metabolic activity observed with concordant and consistent glucose consumption use and lactate production across all production runs. The EV yields of $>2.9x10^9$ EVs/mL was more than double the yield of the monolayer control indicating a highly efficient bioprocess. Although the current protocol describes collection after 3 days, it might be improved through further optimization of the collection duration. While extension of the collection period would be a straightforward approach to increasing yield, other options such as a continuous perfusion collection may also increase production. The flexible setup of the Osilaris single-use bags makes it both easy and safe to validate and implement these process changes.

Taken together, the results in this study demonstrate that the Osilaris bioreactor in combination with RoosterBio's development grade MSCs and bioprocess medium deliver an operator-friendly, robust and more cost-effective EV-production workflow. This approach provides a clear, translatable path from proof-of-concept to clinical manufacturing.

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References:

1. Kou, M., Huang, L., Yang, J., Chiang, Z., Chen, S., Liu, J., Guo, L., Zhang, X., Zhou, X., Xu, X., Yan, X., Wang, Y., Zhang, J., Xu, A., Tse, H. F., & Lian, Q. (2022). Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool?. Cell death & disease, 13(7), 580. https://doi.org/10.1038/s41419-022-05034-x
2. E. Rohde, O. Kaiser, S. Sasse, J. Harre, D. Auer, T. Schally, S. Rund, C. Kals, C. Folie, E. Bayer, M. Gimona, N.K. Prenzler, A. Warnecke, Clinical Phase 1/2 Trial: Initial Results Following First-in-Human Application of Vesicle-Enriched Secretome Fractions in Hearing Loss Patients Receiving Intracochlear Electrode Implantation, Cytotherapy, Volume 27, Issue 5, Supplement, 2025, Page e9, ISSN 1465-3249, https://doi.org/10.1016/j.jcyt.2025.04.018
3. Lembong, J., Kirian, R., Takacs, J. D., Olsen, T. R., Lock, L. T., Rowley, J. A., & Ahsan, T. (2020). Bioreactor Parameters for Microcarrier-Based Human MSC Expansion under Xeno-Free Conditions in a Vertical-Wheel System. Bioengineering, 7(3), 73. https://doi.org/10.3390/bioengineering7030073
4. 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

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