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Leveraging *ExoMX*™ Platform to Industrialize Large-scale Exosome Manufacturing

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Abstract

Background:

- Exosomes, small extracellular vesicles (EVs), derived from mesenchymal stem cells (MSC) retain the therapeutic potentials of parental MSCs in wide-range clinical applications of immunomodulation, regenerative medicine, as well as drug delivery cargos. However, clinical translation of EV production from lab-scale to large-scale is challenging due to their nature characteristics of nanometer particle size, heterogeneity, complex isolation processes with low recovery rate, and labile stability.
- To overcome the common manufacturing hurdles of MSC-EVs products across the path into clinic, we have successfully established the state-of-the-art **ExoMX™** platform by integrating 3D microcarrier-based upstream process and TFF and chromatography based downstream process with unique formulation to maintain EVs stability during the whole complicated process and long-term storage.

Results:

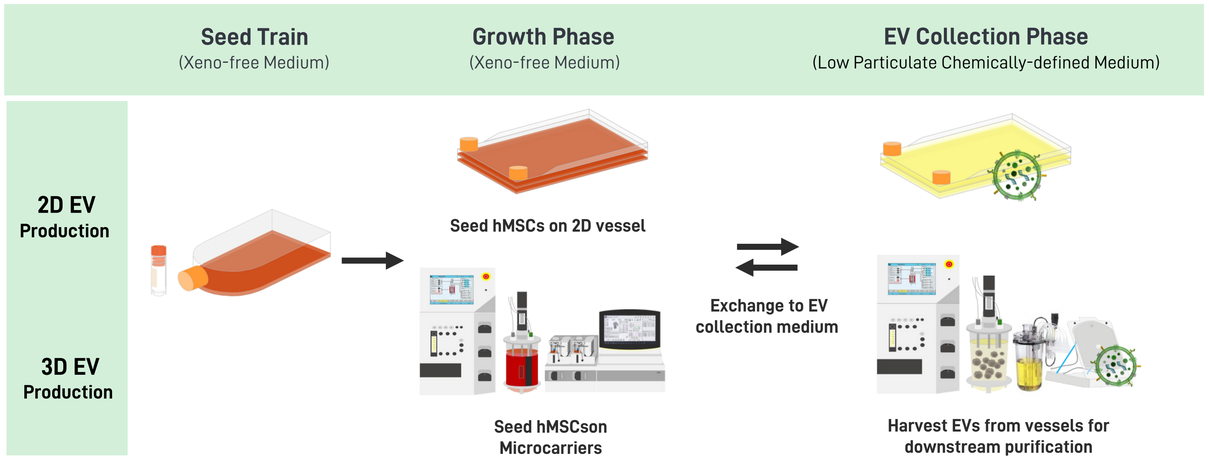
- MSC-EVs production yield was 6-fold boosted to reach around 8x 10^9/mL and identity markers including CD9, CD63, CD81 and Alix were significantly elevated by 3D bioreactor culture process compared to traditional 2D culture.
- Linear scalability was proven from 0.25L to 2L bioreactor culture in our scale-up studies. To obtain large amounts of purified EVs with high purity, total EV recovery rate through 5-step scalable continous purification process was successfully optimized up to 40%.
- Integrity of lipid bilayer structure of final EV products with unique formulation was well-maintained as shown by Cryo-TEM morphology analysis and nanoFCM quantitative analysis.

Revolution of EV Manufacturing Technology

Traditional 2D Culture	ExoMX™ Platform	
Scaling out Small batch size	Scaling up Large batch size	**Scalability** **Mass Production**
Labor intensive	Closed and automated bioreactor process	**Expedited Process** **Reduce COGs**
Poor lot-to-lot Consistency	Process real-time controlled High lot-to-lot consistency	**Automation** **Advanced Process Control**

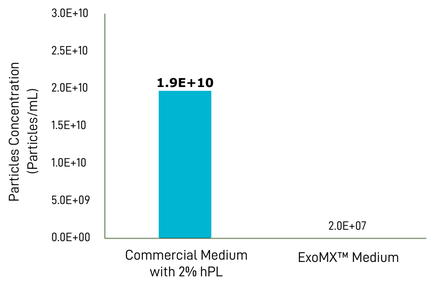
ExoMX™ platform leverages 3D microcarrier-based bioreactor manufacturing technology which can produce sufficient clinical EVs on demands, ranging from 1-100 trillions of EVs, while reducing time, labor, and cost of goods (COGs) for regenerative medicineapplications.

Experimental Design: Comparison of MSC-EV Production Between 2D and 3D Systems

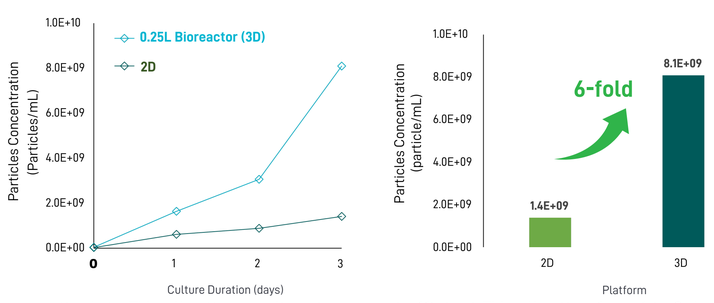


ExoMX™ 3D Bioreactor Process Boosts MSC-EV Production with Low-particulate Chemically-defined Medium

Ultra low noise background



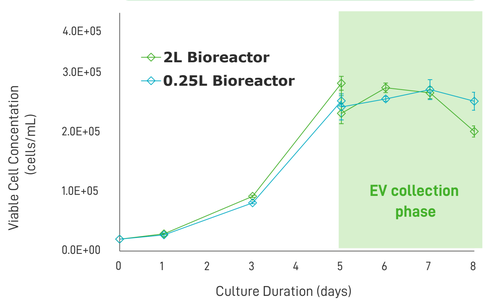
EV production yield is boosted by 3D process



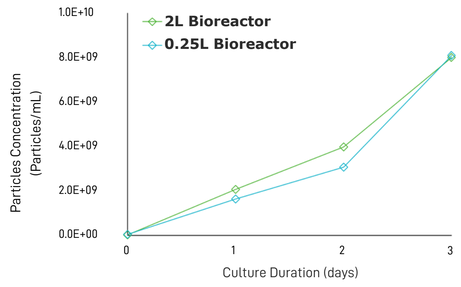
- The ExoMX™ platform uses RoosterCollect-EV, a low-particulate EV collection medium to support efficient EV collection from 1 to 3 days in both 2D planar culture and 3D bioreactor systems.
- Production of hMSC-EVs from 3D bioreactor systems using a microcarrier-based approach increases yield by six-fold compared to the 2D process.

Comparable hMSCExpansion and EV Production Profiles in Scale-up Studies

hMSC Growth Kinetics



EV production profile

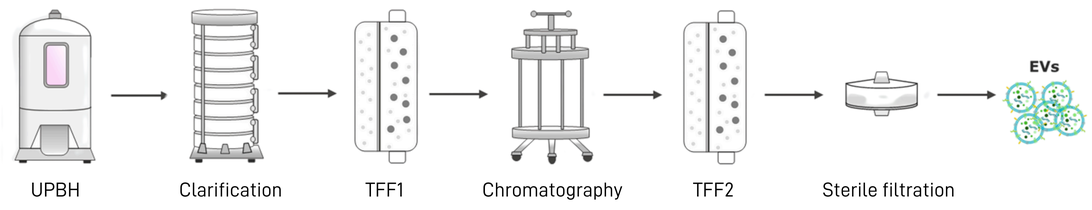


- hMSCwere expanded for 5 days in cell expansion medium and collected for 3 days in 0.25L and 2L bioreactor. Comparable cell density > 250,000 cells/mL by day5. Cell viability were > 90% in both bioreactor scales.
- Particles collected for 1, 2 and 3 days in EV collection medium had similar particles per ml averaging with peaking at ~ 8 billion particles/mL on day 3.

Downstream Purification Process of ExoMX™ Platform

MYCENAX **Downstream Process (DSP)**

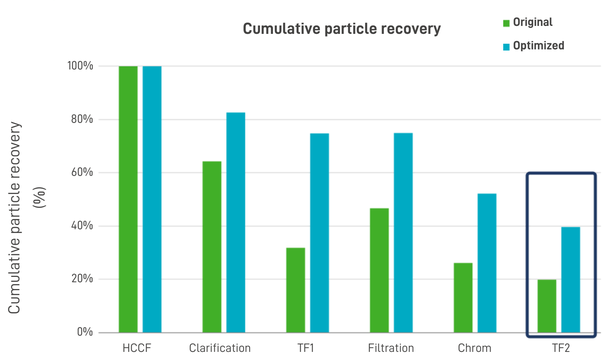
* UPBH, Unprocessed Bulk Harvest

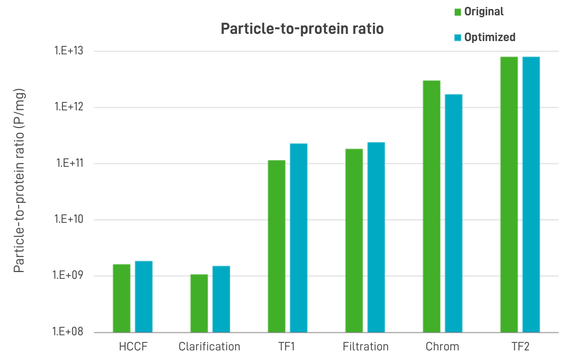


Method	Scale	Process duration	Particle recovery (%)	Purity (particles/mg)
Ultracentrifugation	0.1-0.2 L	4h/run	10-20	8.70E+11
Mycenax Original DSP ★	0.2-50 L ★	4-6 h (for all scale) ★	20-25	1.67E+12 ★

- The recovery and purity of EVs obtained by the 5-step downstream process (DSP) comparable to the centrifugation control.

2-fold improvement of EV recovery rate by optimized DSP

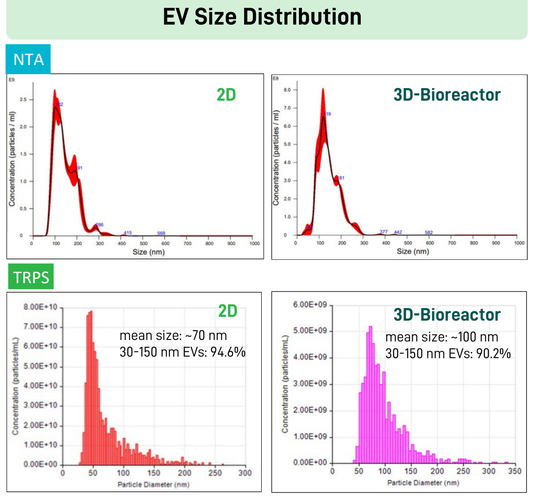




- Addition of reagent-X substantially improves EV recovery rate during the whole 5-step downstream purification process.
- By adding reagent-X within optimized DSP, the total EV recovery rate was increased from 20% to 40%, while the purity (particle/protein ratio) was maintained.

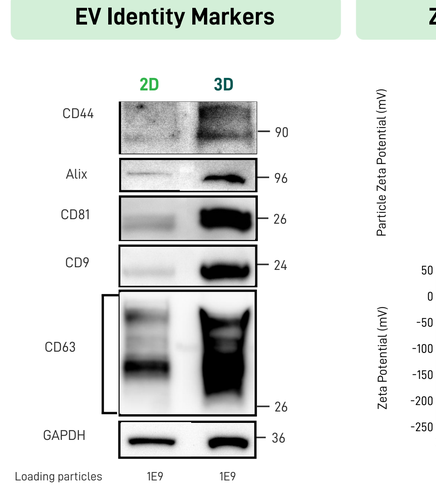
Comparison of CQAs between 2D-EV and 3D-EV

EV Size Distribution



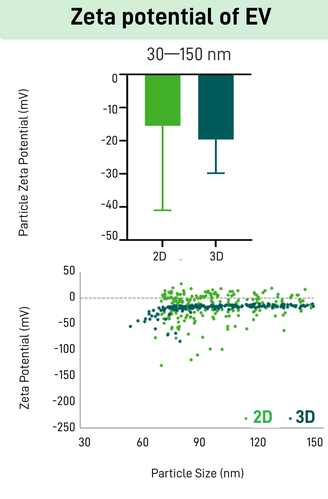
NTA analysis showed a consistent size distribution of purified EVs from both the 2D planar and 3D bioreactor processes, with diameters ranging from ~50-500 nm and a median of ~145 nm. However, TRPS analysis revealed a size range of ~40-350 nm, with the 3D process yielding a larger mean particle size (~100 nm) compared to the 2D process (~70 nm). The percentage of 30-150 nm EVs was 94.6% for 2D and 90.2% for 3D.

EV Identity Markers



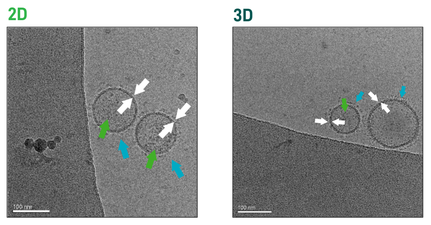
Purified EVs maintained hMSC-specific EV tetraspanin transmembrane markers and cytosolic protein, confirmed by Western blot. When loading the same particle number of EV samples, protein expression level was stronger in 3D-EVs than in 2D-EVs.

Zeta potential of EV



For particles in the 30-150 nm range, the Zeta potential of 2D-EVs was measured at -15.5mV, while the Zeta potential of 3D-EVs was -19.6mV, both measured by TRPS.
For particles in the 30-150 nm range, the zeta potential distribution of 3D-EVs was more condensed than 2D-EVs.

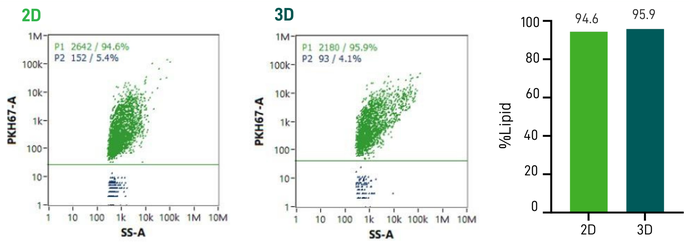
EV Integrity



* The white arrow indicates the lipid bilayer, the green arrow shows the cargo in the EV, and the blue arrow represents the EV corona.

Cryo-TEM analysis was performed to examine the morphology of EVs produced from both the 2D and 3D processes, and it showed characteristic double membrane-surrounded vesicles in both culture systems.

EV Purity



EV samples were labeled with PKH67 dye for the detection of integrated lipid bilayers by using Nano-flow cytometer. The percentage of EV lipid in both 2D- EVs and 3D-EVs was higher than 90%, indicating that the purity of final EV products was highly maintained by **ExoMX™** platform.

Conclusion

- We successfully developed a scalable microcarrier-based stirred tank process for EV production using xenofree hMSCs expansion media and low-particulate chemically-defined EV collection medium.
- The process was developed at the 250mL scale (Ambr® 250) and the linear scalability was successfully demonstrated at the 2L scale in Univessel® SU. EV yield of 2L bioreactor production following 5-step purification process is about 6.4x10^{12}/batch.
- In summary, Mycenax **ExoMX™** platform industralizes EVs large-scale production to meet clinical demand, comprising the advantages of high productivity, high efficiency, scalability, GMP-compliant process, and cost-effectiveness. By leveraging **ExoMX™** platform, the path across early development to commercialization will be accelerated so as to support exosome-based therapeutic fields.
- Further work will be carried out to further understand the biologic characteristics of 3D-EVs produced by **ExoMX™** platform, with future process optimization studies on scaling-up to 50L production.