Exosome production from hMSC using the iCELLis™ fixed-bed bioreactor

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Abstract

We aimed to establish a process for exosome production using human mesenchymal stromal cells (hMSC) in adherent culture, and to evaluate its purification efficiency and impact on functional activity. By comparing the performance of three fixed-bed bioreactors (iCELLis™ Nano, T1, T2), we found that the exosome yield per unit area in the iCELLis Nano bioreactor (2.50×10^{10} particles/cm²) was comparable to that of the T1 bioreactor (2.85×10^{10} particles/cm²), and higher than that of the T2 bioreactor (4.75×10^{9} particles/cm²). We used tangential flow filtration (TFF) for downstream purification, and the particle recovery rate of iCELLis Nano reached 76%, compared to 60% obtained from T1 and 66% from T2. Activity tests showed that the exosomes purified by TFF retained high biological activity (IL6 inhibitory activity 78.91%, IL27 inhibitory activity 91.71%). This study provides an efficient and scalable technological solution for industrial exosome production.

Introduction

Extracellular vesicles (EVs), especially smaller EVs of endosome origin, i.e. exosomes, are increasingly explored for therapeutic applications. To obtain high yields of exosomes, cell culture methods are commonly employed, especially using cell lines such as human embryonic kidney cells (HEK293) and human mesenchymal stromal cells (hMSC). HEK293 cells express exosomes via both adherent and suspension cultures, while hMSC cells express exosomes only in adherent cultures. The adherent scale-up culture methods include cell factories, fixed-bed bioreactors such as iCELLis fixed-bed bioreactor, and microcarrier suspension cultures such as Cytodex™ microcarriers. Suspension scale-up cultures include shake flasks, rocking bioreactors such as the ReadyToProcess WAVE™ 25, and stirred tank bioreactors such as the Xcellerex™ XDR and Xcellerex X-platform bioreactors.

Exosome purification methods include ultrafiltration concentration, density gradient centrifugation, size exclusion chromatography, and multimodal chromatography using Capto™ Core 400/Capto Core 700 resins. TFF technology, due to its high recovery rate and scalability, has gradually replaced traditional ultracentrifugation methods (1).

Our study explores a robust and scalable process for exosome expression from hMSC cells. In the upstream process study, we evaluate the exosome production efficiency of iCELLis Nano fixed-bed bioreactors, while in the downstream process study, we evaluate the TFF purification and characterize the functional activity of the exosomes. The goal is to establish an efficient and stable exosome production platform.

Materials and methods

Materials

Cell line and culture media: Human umbilical cord mesenchymal stromal cells (hUC-MSC); serum-free specialized culture medium.

Bioreactors: iCELLis Nano fixed-bed bioreactor (Cytiva, 0.53 m²), fixed-bed bioreactor 1 (T1, 3 m²), fixed-bed bioreactor 2 (T2, 2 m²).

Purification equipment: Hollow-fiber column, tangential flow filtration systems, ÄKTA flux™ 6 [Cytiva]).

Methods

Cell culture

Parameters	iCELLis Nano 0.53 m²	T1 3.0 m²	T2 2.0 m²
Seeding density (cells/cm²)	12000		
Temperature (°C)	37		
Stirring linear velocity during seeding (cm/s)	2.0		
Medium volume during seeding (mL)	850	1000	850
pH	7.0–7.2		
Airflow rate (mL/min)	30	45	45
DO （%）	60		
Stirring linear velocity during culture (cm/s)	1.0		
Circulation bottle volume (L)	1.0	1.5	1.0
Total culture volume (L)	1.85	2.50	1.85
Medium volume-to-surface area ratio (mL/cm²)	0.35	0.08	0.09
Supernatant harvest after 3 days (L)	1.0	1.5	1.0
Every other day harvest since day 3 (L)	1.0	1.5	1.0
Daily harvest since day 14	—		
Terminated at day 16 and harvest	—		

Purification

iCELLis Nano, initial volume (F1) was 7200 mL, concentrated approximately 9.5 to 10.5-fold, and washed in PBS 10 times (TFF1). The exosome protection solution was exchanged 10 times (DS), then the final collected volume was 810 mL, and the sterile filtration volume was 800 mL.

T1, initial volume (F1) was 12 000 mL, concentrated approximately 12.5 to 13.5-fold, and washed in PBS 10 times (TFF1). The exosome protection solution was exchanged 10 times (DS), then the final collected volume was 930 mL, and the sterile filtration volume was 920 mL.

T2, initial volume (F1) was 4800 mL, concentrated approximately 6.5 to 7.5-fold, and washed in PBS 10 times (TFF1). The exosome protection solution was exchanged 10 times (DS), then the final collected volume was 700 mL, and the sterile filtration volume was 690 mL.

Parameters	iCELLis Nano 0.53 m²	T1 3.0 m²	T2 2.0 m²
Initial volume (F1) (mL)	7200	12000	4800
Concentrated times	9.5–10.5	12.5–13.5	6.5–7.5
PBS wash times	10		
Number of exosome protection solution exchange times	10		
Final collected volume (mL)	810	930	700
Sterile filtration volume (mL)	800	920	690

Analysis and detection:

Particle size analysis: Flow NanoAnalyzer (NanoFCM)

CD63/CD81/CD90 positivity detection: Flow NanoAnalyzer (NanoFCM)

IL6% and IL27% inhibitory activity detection: ELISA

Results

Cell culture

The glucose concentration in all three bioreactors decreased in a stepwise manner from approximately 1.0 g/L to 0.2 g/L (Fig 1).

Lactate concentrations in all three bioreactors increased from about 0.1 g/L to a maximum of 0.8 g/L. The lactate curve of iCELLis Nano and T2 bioreactors was similar in the first 11 d, rising from 0.1 g/L to 0.6 g/L (Fig 2).

LDH (lactate dehydrogenase) concentrations in all three bioreactors increased from about 40 U/L to a maximum of 120 U/L, consistent with the lactate curve. Starting on Day 12, the LDH level in iCELLis Nano remained lower than in T1 and T2 bioreactors (Fig 3).

NH_4^+ concentrations increased from about 0.5 mmol/L to a maximum of 1.5 mmol/L, with iCELLis Nano bioreactor exhibiting the lowest level (Fig 4).

Terminated at Day 16 and harvest, iCELLis Nano collected 7.2 L of exosome supernatant with a total of 1.32×10^{14} particles, T1 collected 12 L with 8.56×10^{14} particles, and T2 collected 7.2 L with 9.50×10^{13} particles. The exosome yield per unit area for iCELLis Nano bioreactor was 2.50×10^{10} particles/cm², close to T1 and 5.26 times greater than T2 bioreactors (Fig 5).

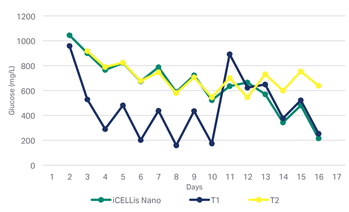


Fig 1. Glucose concentration.

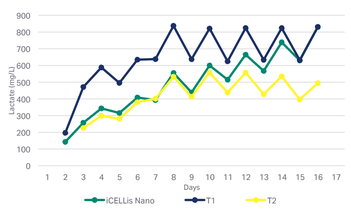


Fig 2. Lactate concentration.

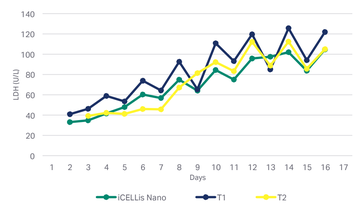


Fig 3. LDH concentration.

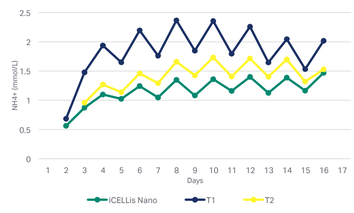


Fig 4. NH_4^+ concentration.

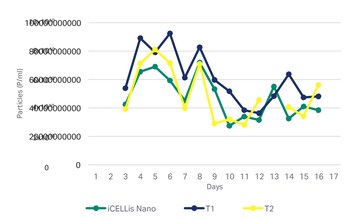


Fig 5. Exosome expression levels.

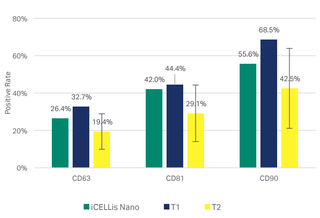


Fig 8. Exosome positivity rates for CD63, CD81, and CD90 expression.

Purification

Following TFF steps, the final particle recovery rates for iCELLis Nano, T1, and T2 bioreactors were 67%, 35%, and 41% to 79%, respectively. The variation in recovery rate is likely due to initial sample concentration and membrane fouling. T1 had a higher initial exosome concentration (7.13×10^{10} particles/mL), which may have led to greater membrane blockage during concentration, reducing recovery efficiency (Fig 6). The purity of iCELLis Nano samples (particles/BCA ratio) was the highest (2.73×10^{11}particles/mg), indicating improved purity compared to the other bioreactors (Fig 7).

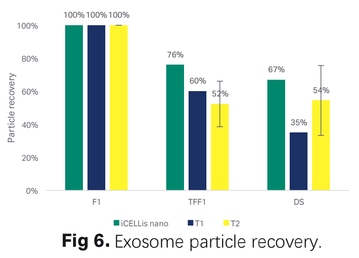


Fig 6. Exosome particle recovery.

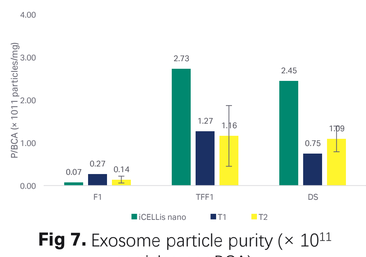


Fig 7. Exosome particle purity (× 10^{11} particles per BCA).

Exosome characterization and quality control

The CD63 expression rate in iCELLis Nano (26.40%) was slightly lower than in T1 (32.70%), while T2 showed a range of 10% to 32.7%. These variations may be related to exosome heterogeneity during the culture process. Considering this study and preliminary experiments, the CD63/81/90 expression rates of the iCELLis Nano samples were within the range of historical data of MSC-EVs (Fig 8).

IL-6 and IL-27 assays demonstrated that exosomes derived from iCELLis Nano fixed-bed cultures and purified via TFF exhibited anti-inflammatory activity comparable to the positive control. In contrast, exosomes from T1 and T2 fixed-bed systems showed lower anti-inflammatory activity than the positive control (Fig 9 and 10).

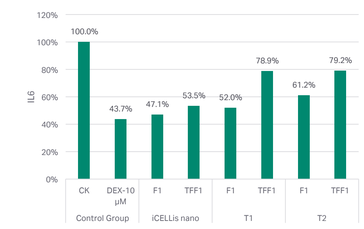


Fig 9. IL6 activity.

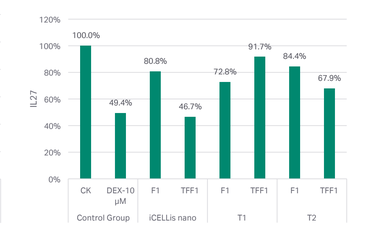


Fig 10. IL27 activity.

Conclusions

Overall, this study confirms that the iCELLis Nano bioreactor provides higher exosome yields with improved purity and anti-inflammatory activity compared to the T1 and T2 bioreactors. The iCELLis Nano bioreactor has excellent performance in unit area yield, leading to high purification efficiency. More efficient nutrient utilization and higher total exosome production make iCELLis Nano bioreactor a good choice for large-scale exosome production.

References

1. Ng CY, Kee LT, Al-Masawa ME, et al. Scalable production of extracellular vesicles and its therapeutic values: A Review. *Int J Mol Sci.* 2022;23(14):7986. Published 2022 Jul 20. doi:10.3390/ijms23147986

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