Tangential Flow Filtration-Based Isolation of UC-MSC Exosome: The Effect of Flowrate on Physical Properties

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Abstract. Exosomes derived from mesenchymal stem cells (MSCs) have emerged as promising candidates in regenerative medicine due to their immunomodulatory properties. Tangential flow filtration (TFF) offers a scalable and GMP-complaint approach for exosome isolation, yet critical process parameters such as flowrate remain poorly understood. This study aimed to investigate the impact of TFF flowrate on the physical characteristics of UC-MSC Exosomes. UC-MSCs were cultured, condition media was collected and proceeded using TFF at two flowrates: low (10 mL/min) and high (30 mL/min) with a 500 kDa cutoff membrane. Physical characterization revealed that both groups produce exosomes within the expected size range (30-200 nm), but those isolated at low flowrate exhibited better monodispersity (PDI = 0.428 ± 0.01 vs. 0.520 ± 0.01) and greater colloidal stability, as indicated by more negative zeta potential (-27.9 ± 1.16 mV vs 18.4 ± 1.57 mV). These findings underscore the importance of TFF flowarate as critical parameters for maintaining exosome quality. Further study will be continued to quantify exosome particles, characterizing marker expression through flow cytometry (FACS), characterizing morphology through Cryo TEM and evaluating anti-inflammatory efficacy using in vitro assays.

Keywords: Characterization, Exosome, Flowrate, UC-MSC, TFF.

1 Introduction

The immunomodulatory properties of Mesenchymal stem cells (MSCs) which are crucial for tissue repair and alleviating inflammation, have made them a major focus in

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regenerative medicine [1] One of the key mechanisms by which MSCs exert their therapeutic effects is through their paracrine mechanism, particularly via the secretion of extracellular vesicles, such as exosomes [2,3]. Exosomes are nanosized vesicles (30–200 nm) that carry a rich cargo of bioactive molecules, including proteins, lipids, nucleic acids such as microRNAs (miRNAs) and play a role in intercellular communication by sending signals to other cells [4,5]. Exosomes from particular cell type contain a distinct set of biomolecules reflective of their cell of origin. Consequently, exosomes are often described as "miniature versions" of their parent's cell which inherit similar therapeutic effect [6]. These attributes position exosomes as potential cell-free therapeutic treatments in regenerative medicine for various applications, including immune diseases, cancer, neurological, cardiovascular and respiratory disease [7].

As exosomes are explored as cell-free alternatives to MSC therapy, the development of scalable, reproducible, and GMP-compatible methods for their isolation becomes critical. The 2014 and 2018 updates of the Minimal information for studies of extracellular vesicles (MISEV) recommendation stated that there is no single optimal separation/isolation method of extracellular vesicles; rather, the optimal approach should be chosen based on the intended scientific application [8] Among available techniques, TFF is emerging as a promising method for exosome isolation, offering higher scalability, yield, and reproducibility compared to other method such as ultracentrifugation or precipitation methods [9]. Given the prospective translational application of exosome as cell-free therapy, TFF was selected for the isolation of UC-MSC Exosomes due to its scalability and adaptability with GMP-compliant workflows. Building on the limited understanding of how flowrate influence exosome quality, this study aimed to compare low and high flowrate conditions using a 500 kDa cutoff membrane to evaluate their effect on the physical characteristic of UC-MSC exosomes. These findings provide a foundational step towards developing robust exosome production protocols, aligning with GMP-compliant biomanufacturing and translational needs for exosome-based cell-free therapies.

2 Methods

2.1 Isolation of UC-MSC Exosome

Conditioned media were collected from UC-MSC cultures at passage 8 and centrifuged at 1300 rpm for 6 minutes to remove cell debris. The supernatant was then filtered through a 0.22 μm PES membrane bottle-top filter (NALGENE®, Thermo Scientific, USA) to eliminate residual cellular debris and large non-exosomal particles. Exosome isolation was performed using a Tangential Flow Filtration (TFF) system (Merck Millipore, Germany) equipped with a 500 kDa molecular weight cut-off membrane and filtration area 50 cm^2 (0.0050 m^2). Two different feed flows were applied: low (10 mL/min) and high (30 mL/min). Following isolation, the exosomes underwent diafiltration with an appropriate volume of PBS to remove non-exosomal proteins, nutrients, and cellular waste products. The purified exosomes were aliquoted and stored at -80°C for subsequent analyses [10,11].

2.2 Physical and Structural Characterization UC-MSC Exosome

The particle size, distribution, and zeta potential of exosomes were analyzed using a Malvern Zetasizer 2000 (Malvern Instruments) after the samples were diluted in 1 mL of distilled water. The Zetasizer was used to determine the size distribution of the exosome population in nanometers. The homogeneity (or polydispersity) of the samples was determined. Measurements were performed in triplicate, and the results are presented as mean ± standard deviation [12]

2.3 Protein Quantification of UC-MSC Exosome

Exosome protein quantification/concentration was performed using the BCA protein assay kit according to the protocol in the manual. The albumin standard solution was diluted (BSA) into several clean vials using the same solvent as the sample. The working reagent was prepared by mixing 50 parts of reagent A BCA with 1 part of reagent B BCA (50:1, Reagent A). The 25 μL of sample and standard solutions were added to each well of a 96-well microplate. The 200 μL of working reagent was added to each well, mixed by shaking for 30 seconds. The 96-well microplate was covered and incubated at 37°C for 30 minutes. The plate was equilibrated to room temperature. Absorbance was measured at or near 562 nm on a plate reader.

2.4 Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Group comparisons were evaluated using Student's *t*-test, assuming equal variances. Statistical significance was defined at $p < 0.05$. Both equal variance and Welch's unequal variance approaches were initially considered; results were consistent in direction, and equal variance tests are reported here for clarity. Given the limited number of replicates, findings are interpreted as preliminary.

3 Results and Discussion

3.1 Rationale for Membrane Selection

In line with the study aim, UC-MSC exosomes were isolated using 500 kDa molecular weight cut-off (MWCO) PES (polyethersulfone)-based membrane cassette, selected due to their low protein binding affinity and robust physicochemical properties [13]. Previous study demonstrated that 500 kDa membranes yielded higher purity compared to 300 kDa during UC-MSC Exosome isolation, with similar surface marker profiles [14]. Furthermore, another study reported that 500 kDa PES membrane achieved superior purity and productivity compared to 100 kDa and 300 kDa membranes of the same material[15]. This aligns with our rationale for selecting 500 kDa PES membrane for UC-MSC exosome isolation in the present study.

3.2 Exosome Characterization by DLS

The UC-MSC-derived exosomes were characterized using Dynamic Light Scattering (DLS) to evaluate their size distribution, polydispersity, and stability. DLS also provides qualitative conformation of submicrometric particles and potential aggregates that might be present in EV/Exosome samples [16]. The findings demonstrated that exosomes derived from UC-MSCs in both conditions fell within the expected exosomal size range (30-200 nm), showing 153.2 ± 3.34 nm for the low flowrate group and 139.9 ± 0.59 nm for the high flowrate group. Notably, exosomes isolated under low flowrate condition exhibited a lower PDI, indicating a more monodisperse population. Furthermore, the UC-MSC exosome isolated under low flowrate exhibited more negative zeta potential (-27.87 ± 1.16 mV) compared to those from high flowrate group (-18.4 ± 1.57 mV), indicating better colloidal stability which is essential for maintaining the structural integrity and functionality of the exosomes. The observed zeta potential in low flowrate indicates sufficient electrostatic repulsion between particles, reducing aggregation and ability to maintain stability during storage and handling. A zeta potential value above ±25 mV is generally considered indicative of good colloidal stability, suggesting that the isolated exosomes can remain well-dispersed in suspension without forming aggregates over time [17]. Additionally, the moderate zeta potential observed in this study may also play a crucial role in the cellular uptake efficiency of the exosomes [18].

Table 1. The Summary of Dynamic Light Scattering Results

Sample Name	Size (nm)	PDI	Zeta Potential (mV)
Exosome Low flowrate (10mL/min)	153.2 ± 3.34	0.428 ± 0.01	- 27.9 ± 1.16
Exosome High flowrate (30mL/min)	139.9 ± 0.59	0.520 ± 0.01	-18.4 ± 1.57

Exosomes isolated under low flowrate exhibited significantly larger mean size ($p < 0.05$), lower PDI ($p < 0.01$), and more negative zeta potential ($p < 0.05$) compared to those isolated at high flowrate. These findings indicate that low flowrate favors the production of exosomes that are larger, more monodisperse, and more colloidally stable. Although experiments were conducted in triplicate, the limited sample size warrants cautious interpretation of statistical outcomes.

3.3 Protein Quantification

The protein content of the UC-MSC-derived exosomes was measured using the Bicinchoninic Acid (BCA) assay, yielding a concentration of 0.369 mg/ml at low flowrate and 0.652 mg/ml at high flowrate. Protein quantification serves as an important parameter for evaluating the yield and quality of exosome preparations, as proteins such as tetraspanins (CD9, CD63, and CD81) and heat shock proteins are commonly associated with exosomal membranes. The BCA protein assay kit, one of the most widely used

methods for extracellular vesicle/exosome quantification, was employed for this analysis[19]. While high flowrate increased protein yield, the improved stability and homogeneity observed at low flowrate highlight the need to balance yield and quality when optimizing TFF parameters for clinical-grade exosome production.

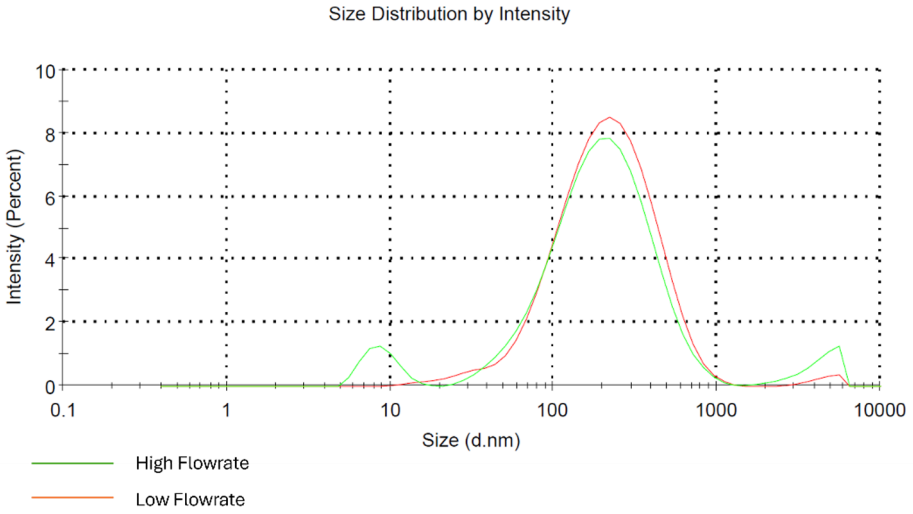

Fig. 1. Size distribution by intensity of UC-MSC Exosome.

4 Conclusion

This study shows that flowrate during TFF significantly influences UC-MSC exosome quality. Low flowrate produced exosomes with lower polydispersity, and more negative zeta potential, indicating superior stability, while high flowrate increased protein yield. These findings highlight the need to balance yield and quality when optimizing exosome production for clinical applications. Future studies with larger sample sizes and extended characterization are needed to validate these findings and refine process parameters for scalable therapeutic applications.

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Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

References

1. Andrzejewska A, Lukomska B, Janowski M. Concise Review: Mesenchymal Stem Cells: From Roots to Boost. Stem Cells2019;37:855–64.

2. Liang X, Ding Y, Zhang Y, Tse HF, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: Current status and perspectives. Cell Transplant2014;23:1045–59.
3. Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Res Ther2016;7.
4. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells2019;8.
5. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Communication and Signaling2021;19.
6. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci2017;18.
7. Nooshabadi VT, Mardpour S, Yousefi-Ahmadipour A, Allahverdi A, Izadpanah M, Daneshimehr F, et al. The extracellular vesicles-derived from mesenchymal stromal cells: A new therapeutic option in regenerative medicine. J Cell Biochem 2018;119:8048–73.
8. Royo F, Théry C, Falcón-Pérez JM, Nieuwland R, Witwer KW. Methods for Separation and Characterization of Extracellular Vesicles: Results of a Worldwide Survey Performed by the ISEV Rigor and Standardization Subcommittee. Cells 2020;9.
9. Haraszti RA, Miller R, Stoppato M, Sere YY, Coles A, Didiot MC, et al. Exosomes Produced from 3D Cultures of MSCs by Tangential Flow Filtration Show Higher Yield and Improved Activity. Molecular Therapy 2018;26:2838–47.
10. Lee JH, Ha DH, Go HK, Youn J, Kim HK, Jin RC, et al. Reproducible large-scale isolation of exosomes from adipose tissue-derived mesenchymal stem/stromal cells and their application in acute kidney injury. Int J Mol Sci 2020;21:1–16.
11. Kim JY, Rhim WK, Seo HJ, Lee JY, Park CG, Han DK. Comparative Analysis of MSC-Derived Exosomes Depending on Cell Culture Media for Regenerative Bioactivity. Tissue Eng Regen Med 2021;18:355–67.
12. Villatoro AJ, Martín-Astorga MDC, Alcoholado C, Sánchez-Martín MDM, Becerra J. Proteomic analysis of the secretome and exosomes of feline adipose-derived mesenchymal stem cells. Animals 2021;11:1–15.
13. Ma Z, Lan Z, Matsuura T, Ramakrishna S. Electrospun polyethersulfone affinity membrane: Membrane preparation and performance evaluation. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:3686–94.
14. Kim JY, Rhim WK, Yoo YI, Kim DS, Ko KW, Heo Y, et al. Defined MSC exosome with high yield and purity to improve regenerative activity. J Tissue Eng 2021;12.
15. Lei R, Ren S, Ye H, Cui Z. Purification of mesenchymal stromal cell-derived small extracellular vesicles using ultrafiltration. Journal of Extracellular Biology 2025;4.

16. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles 2024;13.
17. Midekessa G, Godakumara K, Ord J, Viil J, Lättekivi F, Dissanayake K, et al. Zeta Potential of Extracellular Vesicles: Toward Understanding the Attributes that Determine Colloidal Stability. ACS Omega 2020;5:16701–10.
18. Fröhlich E. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. Int J Nanomedicine2012;7:5577–91.
19. Franquesa M, Hoogduijn MJ, Ripoll E, Luk F, Salih M, Betjes MGH, et al. Update on controls for isolation and quantification methodology of extracellular vesicles derived from adipose tissue mesenchymal stem cells. Front Immunol 2014;5.