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Value from Innovation

Development of a method for large-scale purification of Extracellular Vesicles using the PS Affinity Method

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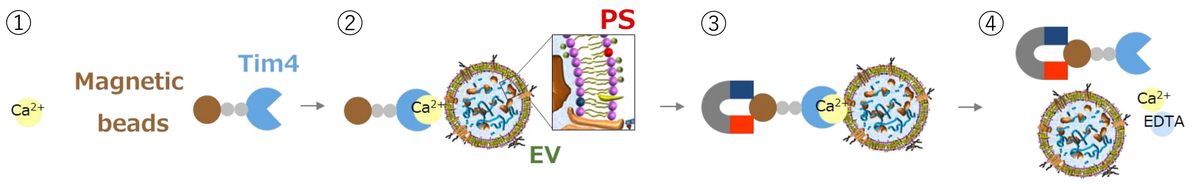
Abstract & Introduction

Since extracellular vesicles (EVs) derived from some stem cells, such as mesenchymal stem cells (MSCs), have therapeutic potential for various diseases, the use of EVs as therapeutic agents in medicine has shown great promise. Consequently, multiple methods, such as tangential flow filtration (TFF), size-exclusion chromatography (SEC), anion exchange chromatography (AEX), and their combinations, have been developed for large-scale EV purification. In this study, we developed a scalable and reproducible method for the affinity purification of EVs using Tim4-immobilized carriers (PS affinity). First, we found that Tim4 specifically binds phosphatidylserine(PS) on EVs and shows stronger affinity to EVs in comparison to other EV binding molecules, such as Annexin V, antibody, indicating that Tim4 is optimal molecule for EV affinity purification. We next demonstrated that this method enables the purification of EVs from MSC-derived culture supernatant with higher purity, yield, and anti-inflammatory activity than other commonly used methods. Lastly, we confirmed that the PS-affinity method is scalable, consistent, and reproducible by showing that EVs purified from different scales of culture supernatant, different purification formats, or through a repetitive purification process exhibited similar characteristics, such as particle size, marker/membrane protein expression, and anti-inflammatory activity. In summary, this study indicates that the PS-affinity method is applicable for the large-scale purification of therapeutic EVs for pharmaceutical purposes.

PS Affinity Method

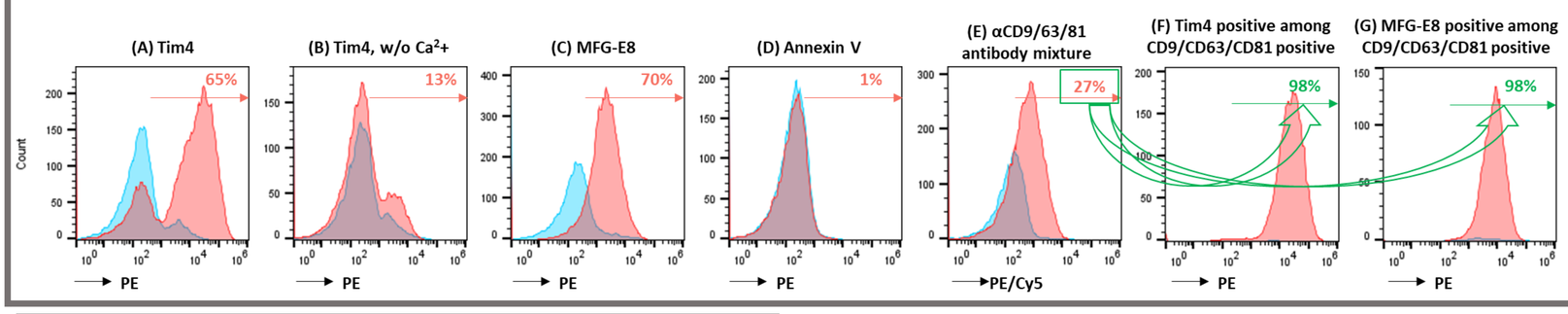
Principle of the PS Affinity Method

Utilizes Tim4 protein that binds to Phosphatidylserine(PS) in a Ca2+-dependent manner



Tim4's high affinity and detection sensitivity for EVs

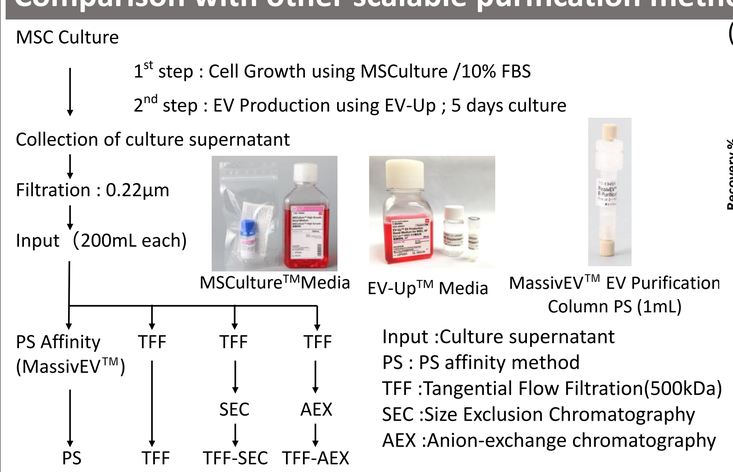
Purified EVs are detected by FACS (Sony SA3800) using Tim4, MFG-E8, Annexin V, and antibodies



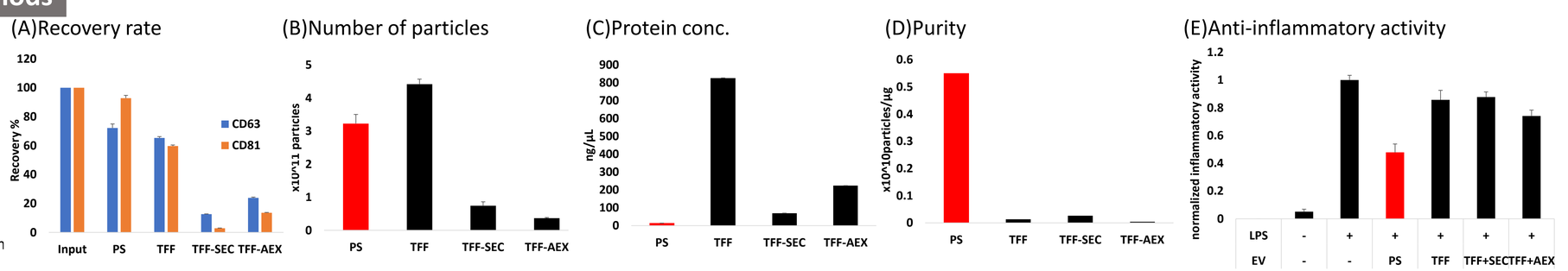
Analysis of purified EVs by FACS. (A)-(E) Purified EVs are labeled with Tim4 (A), Tim4 in buffer without Ca2+ (B), MFG-E8 (C), Annexin V (D), and an αCD9/63/81 antibody mixture (E), and then analyzed by FACS. (F), (G) Detection of Tim4-positive (F) or MFG-E8-positive (G) EVs among αCD9/63/81 antibody mixture-positive EVs.

Tim4 specifically and strongly binds to PS on EVs, and EV marker-positive EVs are 98% positive for Tim4 or MFG-E8, indicating that Tim4 is an optimal molecule for capturing EVs.

Comparison with other scalable purification methods



Purified EVs by each method are analyzed by ELISA, BCA and NTA



Analysis of EVs purified by various purification methods. (A) Recovery rate of purified EVs measured by αCD63/αCD63 and αCD81/αCD81 sandwich ELISA . (B) Number of particles of purified EVs analyzed by Nano Tracking Analysis (NTA). (C) Total protein amount of purified EVs measured by BCA method. (D) Purity of purified EVs (number of particles analyzed by NTA per 1 μg of protein). (E)Anti-inflammatory activity analyzed by cell-based assay (EV concentration=2.8 × 10^9 particles/mL).

Compared to other methods, PS affinity method using MassivEV™ EV Purification Column PS was able to purify EVs with higher purity and recovery efficiency, and the purified EVs showed higher anti-inflammatory activity.

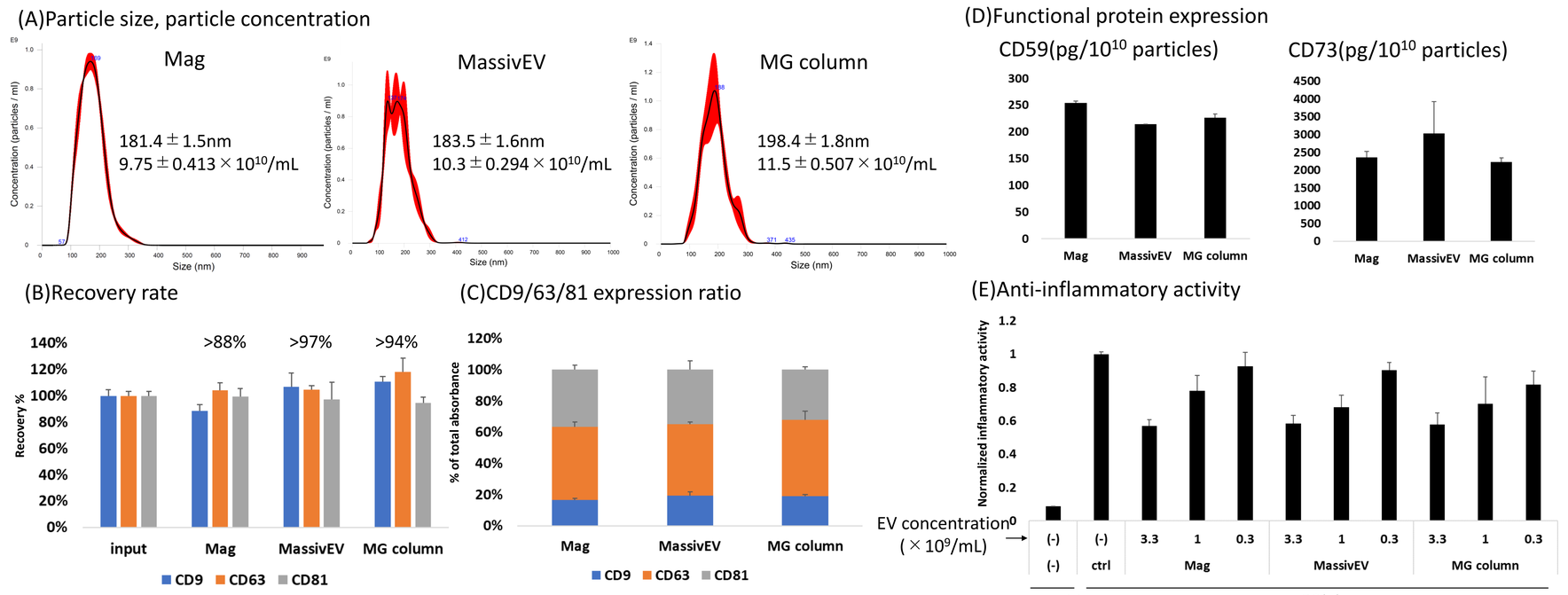
Scalability, consistency and reproducibility

PS affinity purification at different scales/formats

EVs are purified from different scales of MSC-derived culture supernatant and their characteristics are analyzed.

Sample	Purification method		Vol. of sup
Mag	MagCapture™ Exosome Isolation Kit PS Ver.2		5mL
MassivEV	MassivEV™ EV Purification Column PS (1mL)		200mL
MG column	Manufacturing grade Tim4 resin column(5mL)*		1L

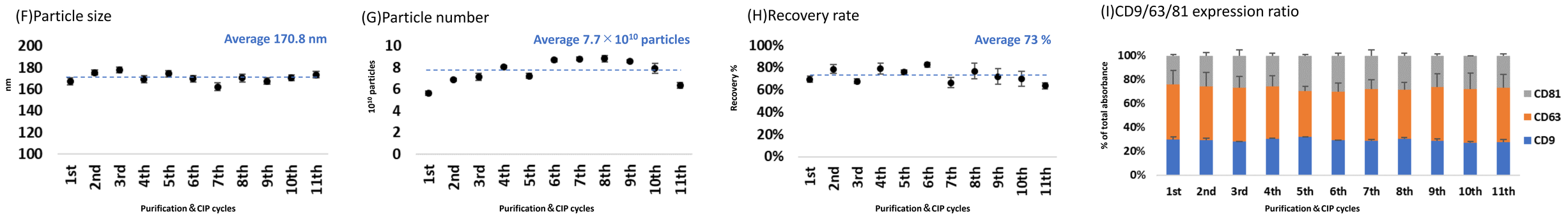
*under development



Analysis of EVs purified from different scales of culture supernatant using different purification formats. (A) Number and size of particles of purified EVs analyzed by Nano Tracking Analysis (NTA). (B) Recovery rate of purified EVs measured by Tim4/CD9, CD63, CD81 sandwich ELISA (PS Capture™ Exosome ELISA Kit). (C)CD expression ratio measured by Tim4/CD9, CD63, CD81 sandwich ELISA (PS Capture™ Exosome ELISA Kit). (D) CD59 and CD73 protein amounts of purified EVs measured by sandwich ELISA. (E) Anti-inflammatory activity analyzed by a cell-based assay.

Repetitive purification by PS affinity method

EVs are repeatedly purified from MSC-derived culture sup using Tim4-resin loaded column



Analysis of purified EVs after 11 repeated purification and CIP cycles. (F) Particle size of purified EVs analyzed by NTA. (G) Particle number of purified EVs analyzed by NTA. (H) Recovery rate of purified EVs measured by Tim4/CD9, CD63, CD81 sandwich ELISA (PS Capture™ Exosome ELISA Kit). (I) CD expression ratio measured by Tim4/CD9, CD63, CD81 sandwich ELISA (PS Capture™ Exosome ELISA Kit).

EVs purified from different scales of culture supernatant, different purification formats, or through a repetitive purification process exhibited similar characteristics, such as particle size, marker/functional protein expression, and dose-dependency of anti-inflammatory activity. These data indicate that homogeneous EVs can be reproducibly purified across different formats, scales, or through repeated purification by using PS affinity method.

Conclusion & Outlook

- Tim4 is optimal for EV affinity purification due to its high affinity for EVs and Ca2+ dependency.
- Compared to other commonly used methods, Tim4-based PS affinity purification enables the isolation of EVs with higher purity and yield, and the purified EVs exhibit higher biological activity.
- PS affinity is a scalable, consistent, and reproducible method for EV purification.
- PS affinity has the potential to be used in the manufacturing process of therapeutic EVs.
- Manufacturing grade Tim4-resin column for biopharmaceuticals is under development.
- Fujifilm Wako obtained the Certificate of Compliance for Regenerative Medicine Product Materials for Tim4-resin from PMDA.

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