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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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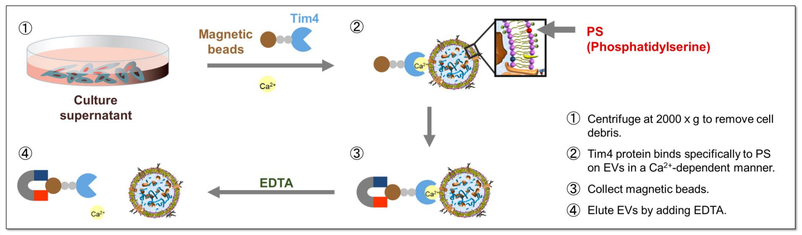
Abstract ＆Introduction

Since extracellular vesicles (EVs) derived from some stem cells, such as mesenchymal stem cells (MSCs), have therapeutic potential on various diseases, the use of EVs as therapeutic agents in medicine has been promising. Consequently, multiple methods, such as tangential flow filtration (TFF), size-exclusion chromatography (SEC), anion exchange chromatography (AEX), and their combination have been developed for a larger scale EV purification. In this study, we have developed a scalable and reproducible method for affinity purification of EVs by using Tim4-immobilized resin. We demonstrated that this method enables purification of EVs from litter-scale culture supernatant with higher purity and yield than other commonly used methods. We also confirmed that Tim4-resin purified EVs showed higher anti-inflammatory activity than the other methods.

PS Affinity Method

Principle of the PS Affinity Method

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



Ex.)EV purification using Tim4-immobilized on magnetic beads

MagCapture™ Exosome Isolation Kit PS Ver.2

Features

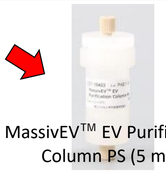
- High recovery rate
- High purity
- Intact EV purification
- High reproducibility

Development of a scalable PS Affinity Method

Scalable purification by using Tim4-immobilized resin,MassivEV EV Purification Column PS

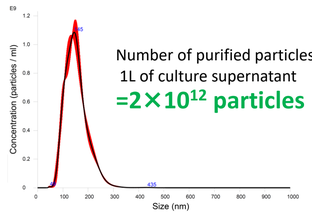
Sample: 1 L of MSC-derived culture supernatant➡MassivEV EV Purification Column PS (5 mL)

➡Analysis by NTA and ELISA



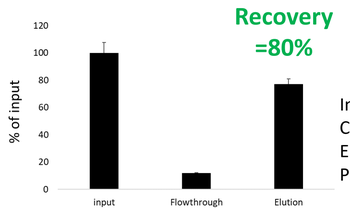
MassivEV™ EV Purification Column PS (5 mL)

(A) NTA



Number of purified particles from 1L of culture supernatant
=2×10¹² particles

(B) ELISA



Recovery =80%

Input: Culture sup
Elution: Purified EV

Analysis of EVs purified by PS affinity method using MassivEV™ EV Purification Column PS. (A) Particle size distribution and number of particles analyzed by Nano Tracking Analysis (NTA). (B) Recovery rate of purified EVs measured by Tim4/αCD63 sandwich ELISA.

Approximately 80% of EVs (=2×10¹² particles) could be recovered from 1 L culture supernatant.

Comparison with other scalable purification methods

MSC Culture

1st step : Cell Growth using MSCulture /10% FBS

2nd step : EV Production using EV-Up ; 5 days culture

Collection of culture supernatant

Filtration : 0.22µm

Input (200mL each)

MSCulture™Media

EV-Up™ Media

MassivEV™ EV Purification Column PS (1mL)

PS Affinity (MassivEV™) | TFF | TFF | TFF

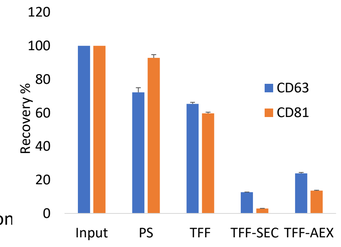
SEC | AEX

PS | TFF | TFF-SEC | TFF-AEX

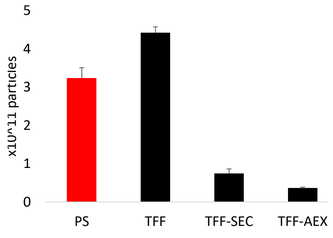
Input :Culture supernatant
PS : PS affinity method
TFF :Tangential Flow Filtration(500kDa)
SEC :Size Exclusion Chromatography
AEX :Anion-exchange chromatography

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

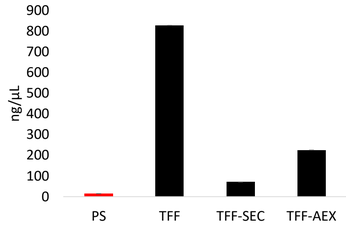
(A)Recovery rate



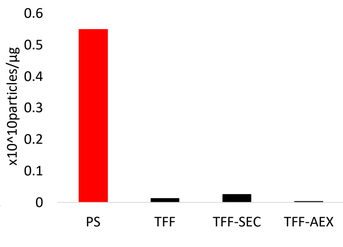
(B)Number of particles



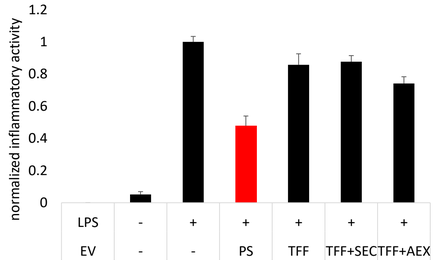
(C)Protein conc.



(D)Purity



(E)Anti-inflammatory activity



Analysis of EVs purified by various purification methods. (A) Recovery rate of purified EVs measured by αCD63/αCD63, 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 (inflammation was induced by adding LPS in presence or absence of 2.8 × 10⁸ purified EVs).

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

Conclusion & Outlook

- We developed a scalable purification method for EVs using Tim4-immobilized resin.
- Compared to other commonly used methods, our method enables purification of EVs with higher purity and yield, besides the purified EVs showed higher biological activity.
- Our purification method has the potential for the use in the manufacturing process of therapeutic EVs.
- We are under development of Tim4-resin for large-scale manufacture of biopharmaceuticals.

Tim4-resin prepacked column for RUO (MassivEV™ EV Purification Column PS) is currently available. Sample requests are welcome!