

Development of a method for large-scale purification of extracellular vesicles using the PS Affinity Method

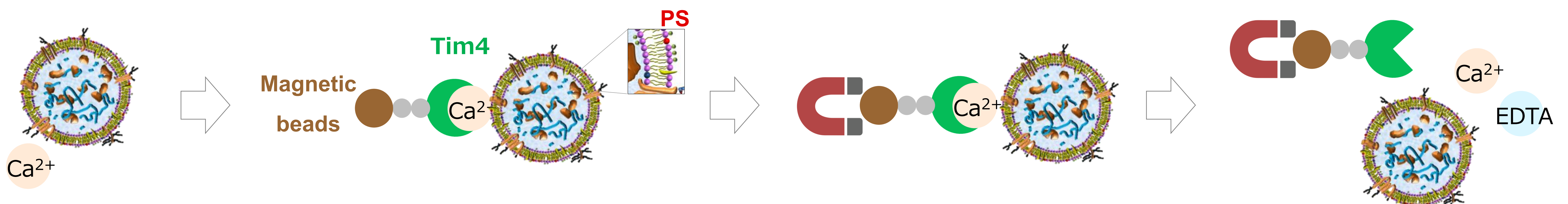
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This Study's Highlights

- We developed a Tim4-based PS-affinity purification column for scalable EV manufacturing.
- PS-affinity purification achieved higher purity, recovery, and anti-inflammatory activity than conventional methods.
- EV characteristics and biological activity were maintained across different scales and purification formats.
- We obtained the certification through PMDA Material Suitability Consultation for Regenerative Medical Products.
- PS-affinity purification may contribute to robust, scalable, and reproducible therapeutic EV production.

What is PS-affinity Purification?

EV affinity purification which utilizes **Tim4**, a protein that binds **phosphatidylserine (PS)** in a calcium-dependent manner.

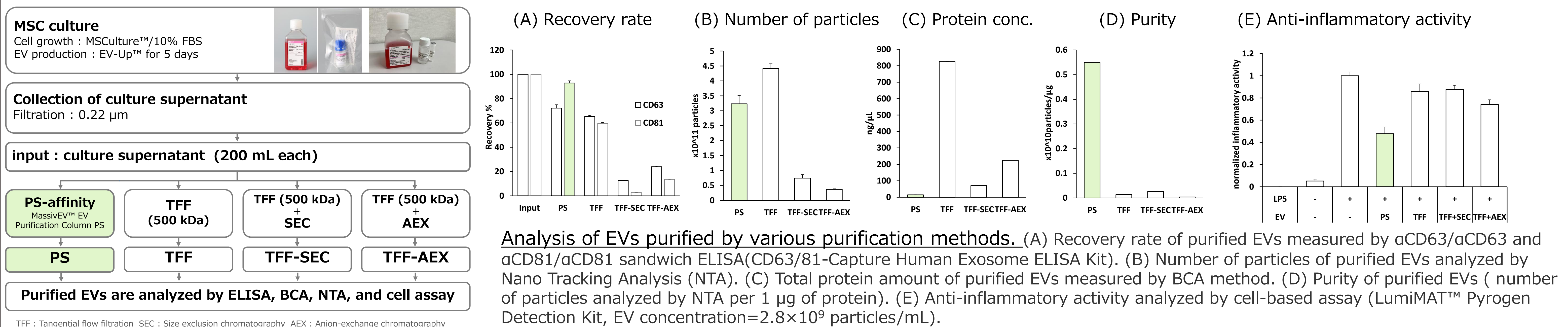


Overview of PS-affinity EV purification using Tim4-immobilized magnetic beads

Tim4 captures EVs through calcium-dependent binding to PS on the EV membrane. Captured EVs are gently released by chelating calcium with EDTA.

Comparison with Other Conventional Purification Methods

PS-affinity achieved **higher purity, recovery, and anti-inflammatory activity** than conventional methods

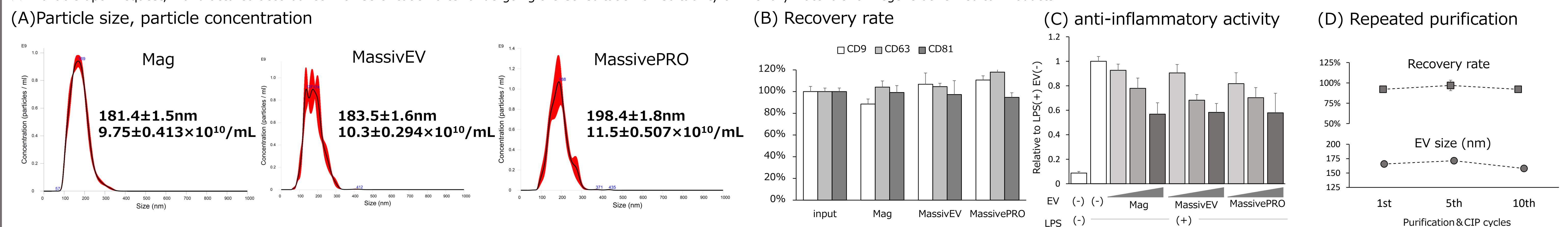


Scalability and Consistency of PS-affinity

EV characteristics, biological activity were **maintained across different scales, formats and through repeated purification.**

Sample	Purification	Vol. of cuture sup	How to purify
Mag	MagCapture™ Exosome Isolation Kit PS Ver.2	5mL	Manual
MassivEV	MassivEV™ EV Purification Column PS (1mL)	200mL	Peristaltic pump
MassivePRO	MassivePRO™ EV Purification Column PS (※) (5mL)	1L	Chromatography system (e.g., ÄKTA™)

※ Available upon request, manufactured accordance with Certification after undergoing the Consultation on Suitability of Ancillary Materials for Regenerative Medical Products



Analysis of EVs purified under different conditions. EVs are purified from MSC culture supernatant by using different scales or 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) Anti-inflammatory activity analyzed by cell-based assay (LumiMAT™ Pyrogen Detection Kit, EV concentration=0.3, 1.0, 3.3×10⁹ particles/mL). (D) MassivePRO™ EV Purification Column PS was subjected to up to 10 repeated purification/CIP cycles. The recovery rate of purified EVs was determined by Tim4/CD63 sandwich ELISA, while EV size was measured by NTA.