FUJIFILM
Value from Innovation

Wako

Larger Scale Isolation of Intact Extracellular Vesicles via Phosphatidylserine Affinity Method

Naomi Tsurutani[1], Takamasa Ishidome[2], Yuki Marutani[2], Masayuki Yamane[2], Kodai Sasamoto[2],
Naoko Imawaka[2], Ryo Ukekawa[2], and Takahiro Nishibu[2]

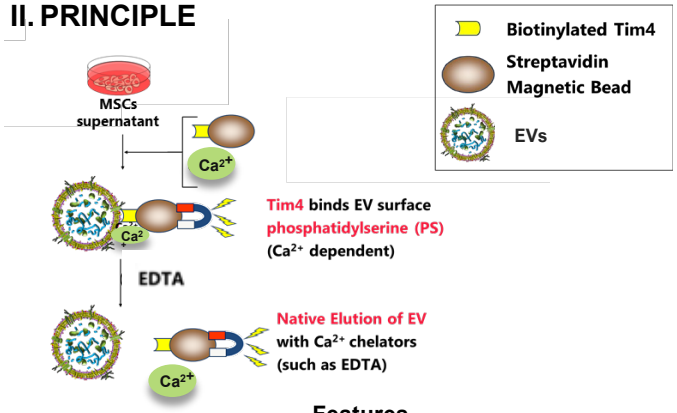
[1]FUJIFILM Wako Chemicals USA, [2]Bio Science & Engineering Laboratory, FUJIFILM Corporation

I. INTRODUCTION

Extracellular vesicles (EVs) and microvesicles serve as messengers of the intercellular networks, allowing the exchange of cellular components between cells. EVs carry lipids, proteins, and nucleic acids derived from their producing cells, and have potential as biomarkers specific to cell types and cellular states.

Various EV isolation methods such as ultracentrifugation, density gradient centrifugation, and antibody affinity method are available. We developed the phosphatidylserine (PS) affinity method using the Tim4 protein which specifically binds to PS on the surface of EVs in a Ca2+-dependent manner. This EV isolation method has better recovery efficiency, purity, collection of intact vesicles, and reproducibility than conventional methods. Furthermore, the PS affinity method can be scaled-up for large amounts of EVs.

II. PRINCIPLE



Features

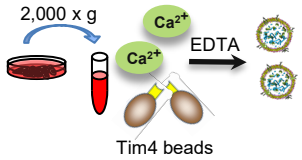
- **Simple and easy method using beads**
- **Capable of purifying exosomes from various samples such as cell cultures and body fluids (plasma, serum, etc.).**
- **Maximize recovery of intact exosomes by native elution buffer**

III. MATERIALS AND METHODS

①Cell culture

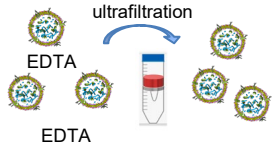
Human BM-derived MSCs are cultured to 90% confluence, replaced with DMEM/10% EV-depleted FBS, and cultured for 120 hours.

②EVs isolation



After centrifuging the cell culture supernatant at 2,000 x g, and then EVs are isolated.

③Buffer exchange



To remove EDTA, the buffer is exchanged with D-PBS using a 100K ultrafiltration column.

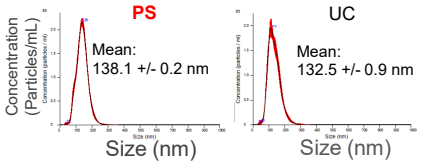
④EVs treatment and qRT-PCR

Human fetal lung diploid fibroblast cells (TIG3 cells) are treated with EVs and determined a gene expression amount using RT-PCR.

IV. RESULTS

1. Characterization of BM-MSC-derived EVs

A. NanoSight



B. Western blot

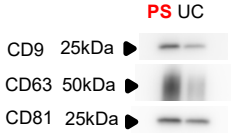


Figure 1 (A) Particle size distribution analysis using NanoSight. (B) Western blot analysis on the expression of the exosome markers CD9, CD63, and CD81. **PS**: Phosphatidylserine. US: Ultracentrifugation.

2. BM-MSC-derived EVs decrease the expression of fibrosis markers in TIG3 cells.

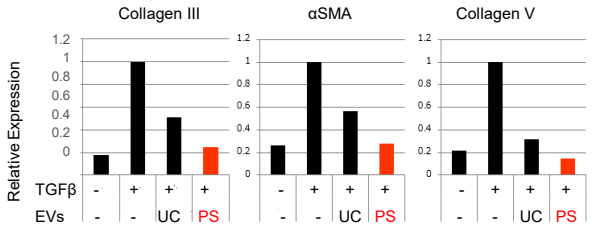
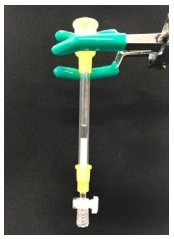


Figure 2. The relative expression levels of the fibrocystic genes in TIG3 cells were evaluated by qRT-PCR after stimulation by TGFβ and MSC-EVs.

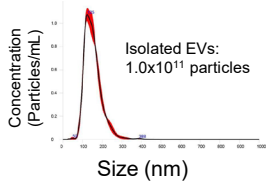
3. Larger scale EVs Isolation

A. Tim4-immobilized agarose beads (500 µl)



Sample: MSC culture sup (10 mL)

B. NanoSight



C. Recovery rate (CD63 ELISA)

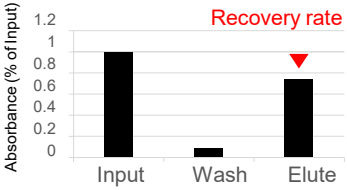


Figure 3. 1.0 x 10^{11} particles were isolated from 10 mL of MSC culture supernatant with 500 µL of Tim4-immbobilaized agarose beads. Applicable for treatment in humans if scaled up about 10 times.

V. CONCLUSION AND DISCUSSION

- The PS affinity method can isolate intact MSC-derived EVs with high recovery efficiency while maintain the high activity of them.
- Application of Column for larger scale isolation of EVs is underway.

MagCapture™ Exosome Isolation Kit PS Ver.2

#294-84101 2 tests & #290-84103 10 tests

For Research Use Only. Not for use in diagnosticprocedures.

FUJIFILM Wako Chemicals USA ・1600 Bellwood Rd ・Richmond, VA 23237 ・ wkuslabchem@fujifilm.com e-reagent.com