Demeter EVPrep™ Automated Extracellular Vesicle Isolation: Superior Yield and Efficiency

Authors: Veronika Shevchenko, Alanna Chan, Catherine Millar-Haskell, Jordan Curtis, Han-Mei Chen, Haejin An, Brian Chin, Daniel Heineck, Janet Anna, and Amber Murray.

Introduction

Isolation of extracellular vesicles (EVs) from other components of biological media/fluids is a critical first step prior to EV characterization and analysis. Many isolation techniques exist today, each with their own well-documented benefits and limitations. Techniques that isolate EVs based on a single physical characteristic, such as size or density, force a tradeoff between purity and recovery of isolated EVs[1]. This is because these complex environments contain particles that share the same physical properties as EVs[2].

Dielectrophoresis is a fundamental physical effect that has been used for particle separation in a variety of fields, including the semiconductor and biomedical industries[3]. Dielectrophoresis results from the interaction between a non-uniform electric field and a difference in the polarizability between a particle and the media surrounding it[4]. Particles that polarize have a dipole moment (p) defined by the equation $q \cdot d = p$, where d is the distance between the charges and q is the total charge[5]. This charge separation within a particle results in an imbalance in coulombic forces on the particle, and thus a net force[6]. Polarized particles will experience force toward high or low electrical field gradients, enabling their separation[4]. By leveraging frequency and voltage, a dielectric field can be tuned to separate particles of interest based on their unique dipole moments.

Because dielectrophoresis acts upon dipole moments, defined by both the size and composition (accessible mobile charge) of polarized particles, two physical properties are leveraged at once, ensuring both recovery and specificity. Therefore, this novel application of dielectrophoresis to isolate EVs does not suffer from a tradeoff between purity and recovery.

Demeter EVPrep is an automated EV isolation system consisting of an instrument, consumable cartridge, and software, which leverages dielectrophoresis for the isolation of EVs. Compared to size exclusion chromatography, Demeter EVPrep demonstrates superior EV recovery as demonstrated by Western Blot and ExoView analysis of EV-specific biomarkers.

[1] Cocozza F, Grisard E, Martin-Jaular L, Mathieu M, Théry C. SnapShot: Extracellular Vesicles. Cell. 2020 Jul 9;182(1):262-262.e1. doi: 10.1016/j.cell.2020.04.054.

[2] Simonsen, Jens B. What Are We Looking At? Extracellular Vesicles, Lipoproteins, or Both? Circulation Research. 2017 Sep 29; 121(8):920-922. doi:10.1161/CIRCRESAHA.117.311767

[3] Pethig R. Review article-dielectrophoresis: status of the theory, technology, and applications. Biomicrofluidics. 2010 Jun 29;4(2):022811. doi: 10.1063/1.3456626.

[4] O'Donnell MC, Kepper M, Pesch GR. A brief history and future directions of dielectrophoretic filtration: A review. Electrophoresis. 2024; 1-21. https://doi.org/10.1002/elps.202400116

[5] https://en.wikipedia.org/wiki/Electric_dipole_moment, accessed May 5, 2025

[6] Gascoyne PR, Vykoukal J. Particle separation by dielectrophoresis. Electrophoresis. 2002 Jul;23(13):1973-83. doi: 10.1002/1522-2683(200207)23:13<1973::AID-ELPS1973>3.0.CO;2-1. PMID: 12210248; PMCID: PMC2726256.

Materials and Methods

Sample Production

H1975 cells were grown in a monolayer in RPMI media supplemented with 10% FBS and 1% penicillin/streptomycin. At 80% confluency, media was replaced with 20 mL serum-free RPMI without antibiotics. Cells were incubated for 24 hours under serum-starvation conditions, at which point conditioned media was collected, clarified, and concentrated 100X with 100 kDa MWCO spin concentrators (Figure 1, left side).

Extracellular Vesicle Isolation

For size exclusion chromatography (SEC) isolation, three independent isolations were performed using iZON qEV 70 columns. Following column equilibration, 500 µL of concentrated conditioned media was added to each SEC column, fractions were collected via gravity flow, and fractions 5-9 were pooled and concentrated, per manufacturer's instructions. Each isolation yielded 250 µL isolated EVs (Figure 1, top right).

For Demeter EVPrep isolation, three independent isolations were performed. 200 µL of concentrated conditioned media (100X from 20 mL conditioned media) was added to each of three cartridges. EVs underwent automated, chip-based isolation using dielectrophoresis, yielding 50 µL isolated EVs per sample (Figure 1, bottom right).

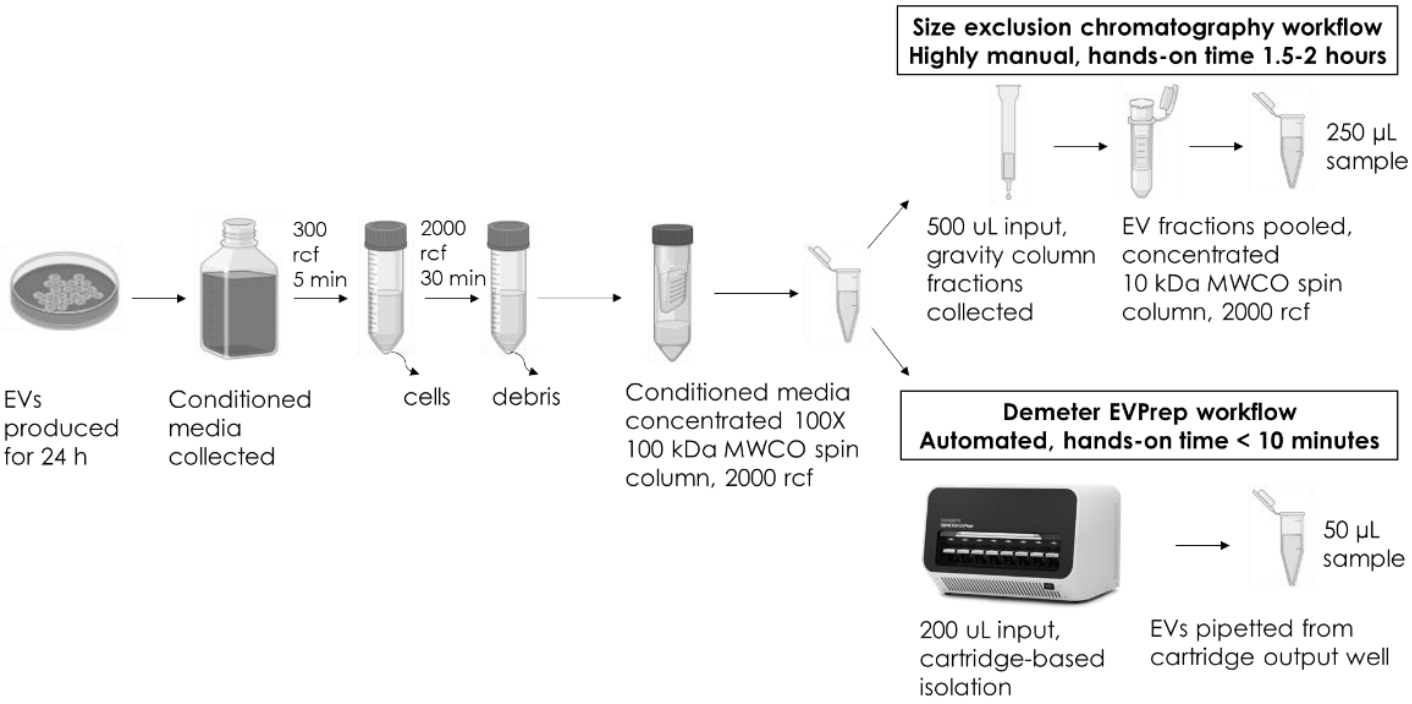


Figure 1. Conditioned media production and EV isolation workflows.

Analytical Methods

For Western blotting, samples were boiled in reducing (TSG101) or non-reducing (CD9, CD81) SDS-PAGE sample buffer and run on pre-cast Invitrogen gradient gels. Proteins were transferred to nitrocellulose membranes via Bio-Rad Turbo-transfer. Immunochemistry was performed with marker-specific antibodies, and bands were visualized using SuperSignal West Femto substrate on a Bio-Rad Chemi-Doc. Lanes were loaded to normalize to conditioned media input for each isolation: 20 mL input → 50 µL output for Demeter EVPrep (400X concentration), 3.8 µL per lane; 50 mL input → 250 µL output for SEC (200X concentration), 7.5 µL per lane.

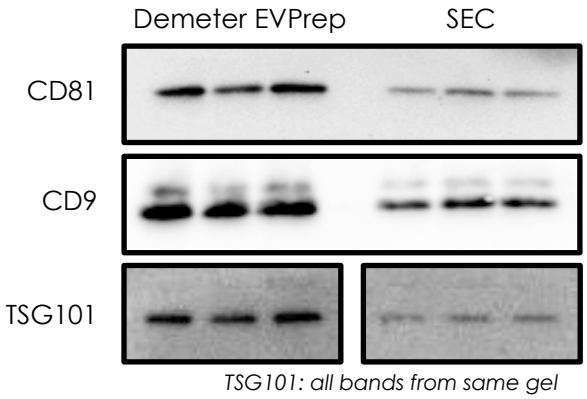
For tetraspanin-positive particle counting, samples were processed, scanned, and analyzed on an ExoView R100 per manufacturer's instructions at the Children's Hospital Los Angeles EV Core facility.

For EV characterization via transmission electron microscopy, carbon-coated 400 mesh copper grids were glow discharged, floated on drops of sample for 5 min, washed, and negatively stained with 2% uranyl acetate. Grids were analyzed with a JOEL 1400 TEM at 120 kV and imaged with a Gatan Ultrascan 1000 CCD camera at the University of California San Diego EM Core facility. EV diameters were measured with calibration using ImageJ.

Results

Isolated Extracellular Vesicle Biomarker Quantification

By Western blot, Demeter EVPrep isolates contained significantly higher amounts of CD81 and CD9 (EV-specific tetraspanin surface markers) than SEC isolates (Figure 2). Demeter EVPrep isolates also contained significantly higher amounts of TSG101 (internal marker essential for ESCRT-dependent EV release from cells) than SEC isolates (Figure 2).



TSG101: all bands from same gel

Figure 2. Western blots of EV-specific markers for Demeter EVPrep and for SEC isolations.

ExoView particle counting confirmed that both Exokeryx's Demeter EVPrep and SEC yielded CD63 positive EVs, CD81 positive EVs, and CD9 positive EVs (Figure 3). Demeter EVPrep recovered significantly more tetraspanin-positive EVs than SEC per unit volume of conditioned media input (Figure 3).

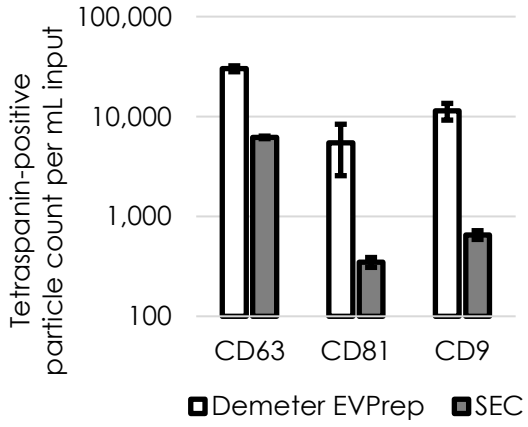


Figure 3. ExoView analysis of tetraspanin-positive particle counts per mL of conditioned media input for Demeter EVPrep (white bars) and SEC (gray bars) isolation methods. Mean +/- SD of three technical replicates for each bar.

Isolated Extracellular Vesicle Morphology

Extracellular vesicles ranging from < 50 nm to ~300 nm in diameter, with expected staining and morphology, were present in both Demeter EVPrep and size exclusion chromatography isolates (Figure 4).

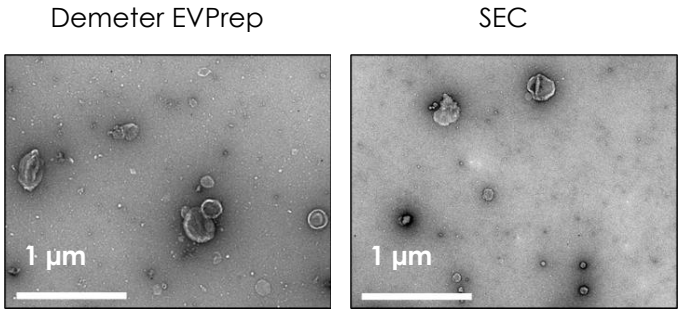


Figure 4. Transmission electron microscopy of extracellular vesicles isolated by Demeter EVPrep and size exclusion chromatography. Representative images for each isolation workflow.

Discussion

Size exclusion chromatography exhibits significantly poorer EV yield than Demeter EVPrep by multiple measurement techniques, including bulk EV marker analysis (Western blotting), tetraspanin-positive particle counting (ExoView), and transmission electron microscopy.

A fundamental limitation of size exclusion chromatography is loss of EVs due to adsorption onto various surfaces. Both the sizing column itself and the spin concentration of pooled eluted fractions expose clean, dilute samples to extensive resin, tube, and membrane surfaces. These EVs remain stuck to the column or tube surfaces and are therefore absent from the output samples.

Another contributing factor to poor yields for size exclusion chromatography is its inability to separate smaller EVs from similarly sized proteins and particles. Thus, there is a trade-off between recovery of smaller EVs and contamination with non-EV proteins. EVs smaller than ~50 nm can co-elute with protein fractions, and these elution fractions are typically avoided to prevent protein contamination. Demeter EVPrep has no such limitation, as EV dipole moments are significantly different than protein dipole moments, allowing high yield isolations of EVs ranging from ~30 to 300 nm without risk of protein contamination.

Size exclusion chromatography also requires significantly more steps and hands-on time than Demeter EVPrep, during which columns are equilibrated (with care that they do not run dry), samples are loaded manually onto columns, fractions are collected using gravity flow, and eluates are pooled and spin-concentrated. In contrast, Demeter EVPrep is fully automated, with less than 10 minutes of hands-on time and no step more complex than pipetting samples into and out of cartridge wells.

The success of extracellular vesicles in basic research, biomarker discovery, diagnostics, and therapeutics depends on reproducible and scalable isolation methods. Current methods for EV isolation are time-consuming, complex, and labor-intensive. In contrast, Demeter EVPrep is a reproducible, automated EV isolation tool that ensures reliable results and significantly speeds up discoveries.