SCE 0607 Public Evidence Appendix

EV separation methods

Literature review date: September 8, 2026

Controlling principle. EV separation methods enrich particles according to operational physical or biochemical properties; no separation method alone establishes EV identity, purity, or exosome biogenesis.

Evidence map

Method	Primary separation principle	Key limitation
Differential centrifugation	Sequential sedimentation at increasing centrifugal force	Pellets can contain mixed EVs, debris, organelles, aggregates, and contaminants.
Ultracentrifugation	Very-high-force sedimentation of small extracellular particles	Protein aggregates, lipoproteins, and nonvesicular particles may co-sediment.
Density gradients	Separation according to buoyant density	Relevant density ranges can overlap and recovery may decrease.
Size-exclusion chromatography	Separation according to hydrodynamic size through a porous stationary phase	Similarly sized lipoproteins/nanoparticles can co-elute; output is often dilute.
Ultrafiltration	Membrane retention according to nominal size or molecular-weight cutoff	Adsorption, clogging, shear, and concentration polarization can affect recovery.
Tangential-flow filtration	Cross-flow membrane separation, concentration, and diafiltration	Membrane properties and process variables influence recovery and quality.
Precipitation	Reduced solubility, often using polyethylene glycol	Frequent co-precipitation of proteins, lipoproteins, nucleic acids, and polymers.
Immunoaffinity capture	Binding to a selected surface epitope	Captures only marker-positive subsets and may not represent the whole EV population.

Interpretive conclusions

Separation is not identity. Centrifugation, filtration, chromatography, precipitation, and affinity capture define operationally enriched fractions rather than biological identity.

Recovery and purity trade off. Methods that maximize particle recovery may also recover more contaminants; more stringent workflows can reduce yield or exclude relevant subpopulations.

Combination workflows are common. Complementary methods can improve selected quality attributes but add processing steps and opportunities for particle loss.

Manufacturing relevance is method-specific. Clinical-scale separation requires reproducibility, scalability, process controls, and compatibility with the intended sterility and quality strategy.

Principal references

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