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Supplementary Materials

The Microfluidic Toolbox for Analyzing Exosome Biomarkers of Aging

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Table S1. Comparison of conventional techniques for exosome isolation, detection and characterization.

Isolation Technique	Basis of Isolation	Advantages	Limitations	Citations
Ultracentrifugation or differential centrifugation	Isolation based on size, shape, and particle density	Most used; standardized speeds; Vesicle concentration and enrichment; High sample purity; Subtype vesicle populations isolated with given speeds	Extensive assay time (<16 hrs); Low yield; Vesicle aggregation from high speeds; Potential contamination with soluble proteins and other macromolecules; Requires large and expensive equipment	[1-2]
Filtration or ultrafiltration	Isolation based on size and molecular weight (MWCO)	Yields higher concentration of exosomes; High sample purity; High intact exosome cargo (proteins and miRNA); Simple set up and equipment; Less time	Yields dilute samples; Potential loss of exosomes in membrane filter pores/clogging; Still requires a high-speed centrifuge	[3-4][3,4]
Size-exclusion chromatography (SEC)	Isolation based on size with gel-based chromatography	Yields highly pure samples; More uniform population size range; Ideal for small scale isolation; Pore sizes can match different exosome size ranges; Typically needs only low speed centrifugation step	Long extraction times; Difficult for large volumes; Dilute samples; Potentially low vesicle yield	[4-5]
Polymer-based precipitation	Isolation based on size with mesh-like polymeric precipitation	Exosomes pelleted at low centrifuge speeds; Uniform population size	Low purity of sample; Polymer precipitation excludes larger exosomes in sample; Single Exoquick kits are expensive; Limited to only a small number of samples	[4-5][4,5]
Immunoaffinity	Isolation based on immunoassays on beads or other surfaces	Higher target specificity; Secures exosome integrity; Techniques are simple and easy to use; Ability to isolate subpopulations with specific surface markers	Strong antibody-exosome interactions make elution difficult; Immuno-isolation devices require more expertise; Antibodies are costly; Other extracellular vesicles may have same surface markers	[1, 4]

Characterization Technique	Basis of Characterization	Advantages	Limitations	Citations
Size-based characterization				
Dynamic light scattering	Measure of scattered light upon incidence with particles of varying size	No sample preparation; Determines average size in monodisperse sample; Some software's can find zeta potential; Retain sample	Not suitable for polydisperse or heterogeneous samples; Only provides size	[6]
Nanoparticle tracking analysis	Particles scatter laser beam upon incidence and motion is recorded by a CCD camera	Quick sample dilution prep; Determines size and concentration; Dynamic range within standard 10^{7}-10^{11}; Analysis of fluorescently labeled vesicles possible	Expertise for accurate optimization of camera and analysis settings required; No biochemical information of vesicles; Loss of sample in analysis	[6]
Resistance pulse sensing	Detection of single EV by transient decrease of ionic current	Tunable nanopores optimized for detection of different nanometer size ranges	Less widely used; Passing of electrical current in presence of vesicles; Lower LOD 100 nm	[6-8]
Flow cytometry	EVs on beads illuminated by lasers in a flow chamber with hydrodynamic focusing	Small EVs (<500nm) detected collectively with swarm effect; High powered laser with specific modifications (CCD camera) for detection of smaller ~ 100 nm EVs *Current development of single suspension and cytometry	Single EV detection only at above 500 nm; identify subpopulations of large EVs with specific fluorescent antigen fluorophores	[9-10]
Morphology-based identification				

Transmission electron microscopy	Uses electrons that pass-through sample for detection for 2D image of EVs	Images based on transparency of EV giving information about inner structures	Fixation, drying and vacuuming of sample (Very complicated); Electron beam can damage EVs	[10-11][10,11]
Scanning electron microscopy	Electron beam with detection of secondary electrons emitted by atoms in the area	Topography of EV surface; Size and morphology of EVs; Single EV morphology	Fixation, drying and vacuuming of sample (Very complicated); Electron beam can damage EVs	[10-11]
Cryo-electron microscopy	Imaging of ultra-thin vitrified film from flash freezing EVs in liquid nitrogen	High resolution imaging of EVs in native state without drying or vacuuming; Identification of specific subsets of EVs with immunogold labelling; Most accurate size determination; 3D tomography images possible	Can have high background noise; Equipment availability	[10-11][10,11]
Atomic force microscopy	EV interaction with tip of cantilever nanoprobe	Native EV sensing with minimal sample prep; Real 3D image of surface topography; Size and structural information of EVs; Gives info of mechanical EV properties like stiffness and elasticity	High resolution images require EVs be attached to atomically flat surface; EVs may change shape once bound to flat surface; Low through-put; Equipment availability	[10-11]
Biochemical EV Analysis				
Bradford assay or micro-bicinchoninic acid (BCA) assay	Total protein concentration colorimetric assays	Easy to use; Simple standard colorimetric assay; Conventionally used as an estimation of EV concentration	General; Need highly pure EV sample; Protein contaminants can affect accuracy	[10][10]
Immunosorbent Assays (ELISA)	Use of specific EV antibodies to capture EVs on a supporting surface	Commonly done through ELISA with CD63, CD9 or CD81; Strong EV enrichment with capture; Capture antibodies can give subpopulations	Antibodies can be expensive and have different cross-reactivity; Typically for surface antigens unless do lysing step prior	[10]

Immunoblotting	Lysing of purified EVs and direct spotting on membrane (dot blot assay) or SDS-PAGE separation of proteins as in Western blot assay)	Commonly done through ELISA with CD63, CD9 or CD81 or cargo proteins ALIX, TSG101	Only semi quantitative and limited to bulk assays; Does not provide on heterogeneity of sample; Requires large volumes and extensive sample processing	[10][10]

Table S2. Comparative analysis and specifications of microfluidic devices for exosome isolation and detection.

Device name	Sample Type	Input Volume	Method	Data Output	Limit of Detection	Sensitivity	Yield	Time	Citation
Exosome Isolation									
Field-Based Isolation									
Acoustofluidics	Human blood	100 μL	Acoustics	Separation of primary human trophoblast-derived EVs and MVs; isolation of >150 nm particles	N/A	N/A	~82%	25 min	[12]
Asymmetric flow field-flow fractionation (AF4)	Melanoma-derived EVs	N/A	Separation based on particle density and hydrodynamic properties	Separation of large (90-120 nm) and small (60-80 nm) exosomes and ~35 nm nanoparticles. real-time dynamic light scattering (DLS) measurements	N/A	N/A	N/A	A few hours	[13]
Thakur et al. microfluidic device	A-549 cells, SH-SY5Y cells, blood serum, urine from a lung cancer model	N/A	Localized surface plasmon resonance	Detection and distinguishment of exosomes from multivesicular vesicles	0.194 μg/mL	0.01793 μg/mL	N/A	30 min	[14]
RInSE	Cell culture supernatant	~ 20 uL	Rapid inertial solution exchange + immunolabeling on magnetic beads	Characterization of EPCAM cancer marker; Exosome diameter; RNA fluorescence measurements	N/A	N/A	99%	4-5 hrs	[15]
Eletrophoretic system	Plasma	1000 μL/h (500 μL for tests)	electrical migration and size exclusion	Zeta potential shift; diameter size	N/A	N/A	65%	30 min	[16]
Surface functionalized Isolation									
Xia et al. microfluidic device	Serum from breast cancer patients	N/A	Carbon nanotubes with anti-CD63; Colorimetric assay	Colorimetric detection	$5.2x10^5$ particles/ μL	N/A	N/A	40 min	[17]
Zn-O Chip	Blood serum	100 μL	Surface functionalization w/ anti-CD63 Ab; TMB-based colorimetric assay	Colorimetric detection	$2.2x10^4$ particles/ μL	$2.2x10^5$ - $2.4x10^7$ particles/ μL	N/A	N/A	[18]
BAF-TiN Biosensor	Serum-derived exosomes	N/A	Immunocapture	Raman investigation of biotin direct adsorption on TiN film. And atomic	4.29 × 10−3 μg mL−1	0.005-500 μg/mL	N/A	2200s (36.7min)	[19]

	from mice			force microscopy to detect anti-CD63 antibody.	for CD63, an exosome marker, and 2.75 × 10−3 μg mL−1 for epidermal growth factor receptor variant-III,				
gFET biosensor	Lyophilized exosomes	10 uL	gFET surface functionalization w/ anti-CD63 Ab	Real-time current and voltage measurements; exosome concentration	0.1 ug/ml	5000 exosomes/ μL	N/A	30 min	[20]
Doldan et al. microfluidic device	N/A	1.5 uL	Surface functionalization + electrochemical sensing	Real-time current measurements; exosome concentration	2x10^2 particles/uL	N/A	N/A	N/A	[21]
An integrated double-filtration microfluidic device	Urine of bladder cancer patients	10 μL of plasma per marker	Size-exclusion filtration	ELISA	N/A	81.3%	74.2%	N/A	[22]
Integrated Magneto−Electrochemical exosome (iMEX) platform	Plasma or serum	10 μL	magnetic enrichment and enzymatic amplification,	Current change over time	3 × 104 exosomes per sample (10 μL)	~10^5 vesicles with 10 μL of samples	N/A	1 hour	[23]
Surface Plasmon Resonance Platform	Serum and exosomes from BT474 breast cancer cell line.	N/A	Surface plasmon resonance	Spectral shift analysis of captured exosomes	2070 exosomes/ μL	2.07x10^3 to 3.3x10^4 exosomes/uL	N/A	N/A	[24]
Alternating Current Electrokinetic (ACE) Microarray Chip	Undiluted plasma spiked with glioblastoma exosomes. Also whole blood	30−50 μL	Dielectrophoretic separation force; immunofluorescence	Flourescence analysis of exosomes, EVs, and RNA and immunoflourescence analysis of CD 63 and TSG101 proteins	N/A	N/A	N/A	>30 min	[25]

ExoTENPO	Murine and clinical cohort exosomes in serum and plasma	N/A	Magnetically trapping and sorting magnetically labeled exosomes	Dynamic light scattering; exosome concentration; RNA; machine learning	N/A	N/A	N/A	10mL/h	[26]
Liu et al. microfluidic device	Plasma; Cell culture supernatant	50 uL	Surface plasmon resonance w/ gold film	Retractive index; Exosome concentration with specific antibody binding	2 × 10^10 exosomes/mL	9.258 x 10^3 %/RIU	N/A	N/A	[27]
Plasmonic interferometer array	Lung cell culture supernatant	N/A	Plasmonic interferometer array (PIA)	Refractive index unit	$3.86x10^8$ exosomes/mL	$9.72x10^9$ exosomes/mL (smartphone detection)	N/A	N/A	[28]
Oh et al. Microfluidic assay	Bovine serum cultured with neuroblastoma and cervical cancer cells.	N/A	In vitro microfluidic cell culture assay	Immunofluorescent stains, NTA with Nanosight, RT-PCR	N/A	N/A	N/A	N/A	[29]
SAW-IEM Chip	Untreated plasma	20 uL	Surface acoustic waves and ion exchange membranes	Mechanism exosome lysing; miRNA quantification	1pM	N/A	N/A	20 min	[30]

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