Supplementary Information

"A perfect pair": Extracellular vesicle-based novel strategies for cancer precise diagnosis and effective treatment

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Table. S1 Pros and cons of extracellular-vesicle extraction/isolation

Separation methods	Principle	Pros	cons	References
Differential ultracentrifugation	Steps of differential centrifugation	Simple operation; Suitable for vesicle collection of large samples	Low degree of purity and time-consuming nature	[1]
Density-gradient centrifugation	Employ prefabricated density gradient	The operation is simple; The purity is high, cross-contamination is avoided and the integrity of the sample is maintained	The operation is complex; Has a low yield	[2]
Size exclusion chromatography	Comprises ultrafiltration and SEC	Large and rapid collections;	Time-consuming; With low	[3]

		Low cost; Mild approaches	yield and low purity	
Affinity capture	Utilize antibodies for specific seizure	High specificity along with high purity	The yield is low; The cost is high; The operation is complex; And only EVs with specific markers can be separated	[4]
Ultrafiltration	The resulting EV particle size distribution is determined by the pore size of the membrane	Simple to operate; Economical and efficient; With uniform particle size	The structure of EVs is prone to change; With low purity; And the ultrafiltration membrane is liable to be blocked	[5]
Precipitation	Employ PEG to lower the solubility of EVs	Simple; Rapid; and highly productive	Low level of purity	[7]

Table. S2 Advantages and disadvantages of drug loading approaches for EVs.

Drug loading	**Suitable**	**Advantages**	**Disadvantage**	**Reference**

methods	cargoes		s	s
Electroporation	Small molecule medications; siRNAs; Nanoparticles	Universality; High loading efficacy; Simple and convenient	High voltage pulses undermine the integrity of EV membranes; Low output	[8]
Sonication	Small-molecule medications; Nanoparticulates	Simple and convenient; And suitable for loading hydrophilic drug molecules or macromolecular proteins	Damage to the integrity of EVs; It could undermine the integrity of EV membranes	[9]
Freeze and thaw cycles	Liposomes loaded with drugs; Proteins; RNAs	High yield; High loading efficiency; High encapsulation efficiency	Complex operation; Damage to EVs	[10]
Saponin treatment	Drugs with hydrophilic properties	Mild; The efficiency of drug loading is enhanced	Toxicity; Additional purification	[11]
Transfection and transduction	Proteins; RNAs	Preserve the integrity of EV membranes;	High technical complexity; High expense	[12]

		Facilitate access		
Incubation	Small molecule drugs; Nanomaterials	Easy accessibility; Universality; Simple and convenient,	Time-consuming; Cytotoxicity exists for the parent cells	[13]

Table. S3 Clinical Trials of EVs in Cancer Diagnosis and Monitoring

Cancer type	**Year**	**Enrollment**	**Sample**	**Study Type**	**Purpose**	**Treatment**	**Detection target**	**Detection method**	**Status**
Retinoblastoma (NCT04164134)	2018	378	Blood	Observational	Monitoring	N/A	EV RNA, DNA	N/A	Completed
Lymphomas and Lymphoproliferative Disorders (NCT06782854)	2022	102	Plasma and mononuclear cells	N/A	N/A	N/A	N/A	N/A	Completed

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