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REVIEW

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Methods to isolate extracellular vesicles for diagnosis

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Abstract

Extracellular vesicles (EVs) are small membrane-bound bodies that are released into extracellular space by diverse cells, and are found in body fluids like blood, urine and saliva. EVs contain RNA, DNA and proteins, which can be biomarkers for diagnosis. EVs can be obtained by minimally-invasive biopsy, so they are useful in disease diagnosis. High yield and purity contribute to precise diagnosis of disease, but damaged EVs and impurities can cause confused results. However, EV isolation methods have different yields and purities. Furthermore, the isolation method that is most suitable to maximize EV recovery efficiency depends on the experimental conditions. This review focuses on merits and demerits of several types of EV isolation methods, and provides examples of how to diagnose disease by exploiting information obtained by analysis of EVs.

Keywords: Extracellular vesicle (EV), Ultracentrifugation (UC), Immunoaffinity, Size exclusion, Polymer precipitation, Microfluidics techniques, Diagnosis

Background

Extracellular vesicles (EVs) are nanometer-to-micrometer sized vesicles that are released by cells [1]. EVs occur in several forms that differ in dimension, mechanism of production, and cellular origin (Fig. 1) [2]. According to mechanism of production, EVs are classified as exosomes, microvesicles, and apoptotic bodies [3, 4]. Exosomes arise from early endosomes [5]: as intraluminal vesicles formed by invagination of endosomal membranes, early endosomes come to have several intraluminal vesicles and become multivesicular bodies (MVBs) [4, 6]. When MVBs fuse with plasma membranes, vesicles within MVBs are released into the extracellular environment and are called exosomes [4]. Exosomes are 40–100 nm in diameter and generally bear surface markers such as tetraspanins (CD9, CD81, CD63), Alix, or TSG101 [7]. Microvesicles, sometimes called ectosomes, are generated by outward budding or evagination of plasma membranes into extracellular space; they carry cytoplasmic contents. Microvesicles do not arise from the endosomal pathway.

Characteristics of exosomes and microvesicles do not clearly distinguish them. Some studies have tried to discriminate exosome and microvesicle based on origin, size and density [8]. Microvesicles are larger (100–1000 nm) than exosomes (40–100 nm) [9]. The size and number of EVs are normally determined by nanoparticle tracking analysis (NTA) or tunable resistive pulse sensing (TRPS) [10]. Exosomes and microvesicles can be distinguished based on size, but cannot be easily distinguished based on only protein markers on the vesicle membrane. Tetraspanins, which are commonly used to define EVs, are enriched in both exosomes and microvesicles [11, 12]. Distinction between the two classes requires more-accurate clues than tetraspanin presence.

Apoptotic bodies are produced by dying cells as they disintegrate [13, 14]; the bodies are diverse in size (generally 50–5000 nm) [15]. However, this classification based on biogenesis can cause confusion, because small microvesicles that arise from evagination of plasma membranes are also called exosomes in some cases, and because different groups of EVs cannot be completely discriminated during isolation [5, 16]. Sometimes EVs are named after the cells that released them; for example,

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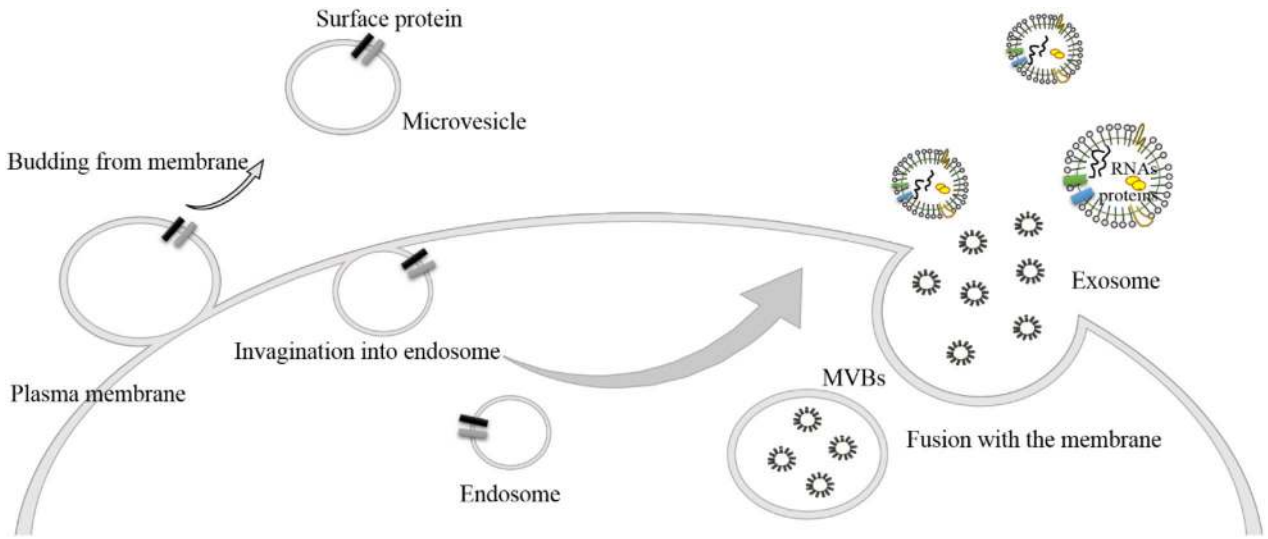

Fig. 1 Biogenesis and release of EVs. Invagination into endosome to form multi-vesicular bodies (MVBs). MVBs fuse with plasma membrane, then exosomes are secreted into extracellular space. Microvesicles are released from plasma membrane with surface protein from cells. Exosomes and microvesicles are affiliated with EVs. EVs derived from cells contribute to cell communication, cell signaling and diagnosis

vesicles released from cardiomyocytes are called cardiosomes [17], vesicles from prostate epithelial cells are called prostatosomes [18], and vesicles released from tumor cells are called oncosomes [19].

EVs contain mRNAs and small RNAs of cells that produced them [20, 21]. For example, microarray analysis has detected ~1300 mRNA species in EVs of the mouse mast cell line MC/9; detected mRNAs comprise 8% of total mRNA in cells of origin [21]. mRNA in MC/9 EVs is translated into polypeptides in vitro if translational machinery is supplied; i.e. the mRNA remains functional [21].

When an EV contacts a cell, their plasma membranes merge; as a result, the contents of the EV enter the cell, and can affect its function [21]. Proteins that are associated with EVs also have functions in the microenvironment. For example, the tetraspanins CD81 and CD9 immunoprecipitate together. EVs can contribute to tumor progression by facilitating angiogenesis while suppressing immune responses [22–24]. Functional proteins are released in association with EVs, so they are also involved in many neurological processes [25, 26].

EVs are found in most body fluids (e.g., blood, urine, saliva, amniotic fluid, semen, tears) [26–28]. An EV contains information that is related to its cell of origin, so isolating and analyzing EVs from body fluids can give important clues to diagnosis and prognosis of disease [29]. Many studies have tried to apply nucleic acids and proteins from EVs in diagnosis (Table 1). Profiles of miRNAs within serum EVs from ovarian cancer patients are significantly different than those of normal patients and of patients with benign cancer [30]. Urinary EVs may be a valuable source of diagnostically-useful miRNAs in prostate cancer [31]. Proteins in urinary EVs may provide biomarkers of acute kidney injury; for example, fetuitin-A in urinary EVs may be useful in diagnosing structural renal injury [32, 33], and decrease in the level of podocalyxin-like protein 1 (PODXL) in urinary EVs may provide a biomarker to classify renal disease [34].

However, in addition to EVs, body fluids contain diverse molecular components that can impede accurate and efficient analysis [35]. For example, plasma is widely used as a specimen, but it contains abundant proteins at concentrations of 60–80 mg/ml [36, 37]. Furthermore, EVs are not abundant in biological fluids, so their contents are not easy to analyze [38]. Conventional isolation by ultracentrifugation recovers an average $0.21–1.08 \times 10^8$ particles from 200 µl of plasma [39] and $1–2 \times 10^{12}$ particles from 150 ml of cell culture medium [40]. Furthermore, the average concentration of RNA within EVs is only 10–15 ng in 200 µl plasma [28]. Altogether, the presence of other particles, the scarcity of EVs, and their contents hinder efficient analysis of EVs and complicate subsequent procedures that use them. Accordingly, to improve the yield and purity of EVs, and to ensure that subsequent analyses are not disturbed, efficient isolation of EVs is important (Fig. 2) [38, 41].

Various methods have been developed to isolate EVs effectively from biological fluids by exploiting a characteristic of EVs to separate them from coexisting particles. The methods differ in yield, purity and size distribution of isolated EVs. Therefore, high-quality EVs must be

Table 1 Some EV applications in diagnosis

Disease	Biospecimen	Isolation method	Biomarker	Reference
Breast cancer	Serum	Ultracentrifugation	EpCAM	[54]
Acute kidney injury (AKI)	Urine	Differential centrifugation	Fetuin-A	[33]
Ovarian cancer	Serum	Density gradient centrifugation	OVCAR-3 and IGROVI	[55]
Lung cancer	Plasma	Immunoaffinity	EGFR level	[65]
Ovarian cancer	Serum	Modified magnetic activated cell sorting procedure	Overexpressed specific miRNAs	[30]
Prostate cancer	Urine	Ultrafiltration	PCA3 and TMPRSS2-ETG	[53]
Focal segmental glomerulosclerosis (FSGS)	Urine	Ultrafiltration	Podocalyxin-like protein 1 (PODXL)	[34]
Ovarian adenoma	Serum	Size exclusion chromatography	EGFRvIII	[77]
Prostate cancer	Urine	Polymer precipitation	Prostate specific membrane antigen (PSMA)	[85]
Acute coronary syndromes (ACS)	Serum	Polymer precipitation	Polygenic immunoglobulin receptors, cystatin C(cycteine proteinase) and C5a	[81]
Ovarian adenoma	Serum	Size exclusion chromatography	EGFRvIII	[77]
Glioblastoma multiforme (GBM)	Serum	Microfluidics	Isocitrate dehydrogenase (IDH-1)	[90]
Melanoma cancer	Blood	Microfluidics	MelanA	[33]

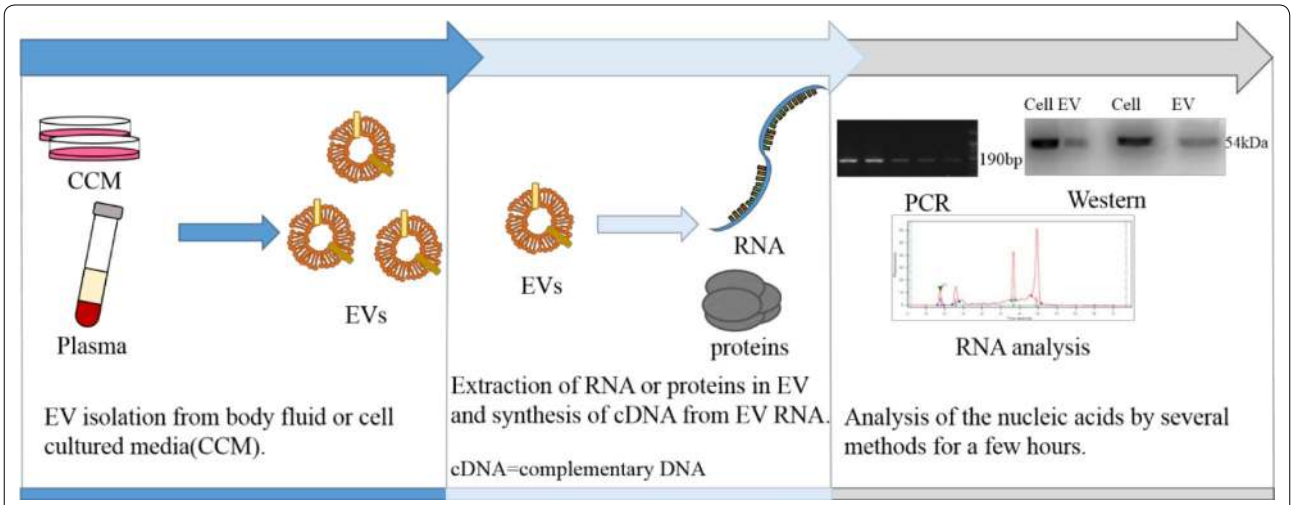

Fig. 2 Outline of the workflow for procedure for isolation and analysis of EVs. EVs are isolated from body fluid or cell culture medium (CCM). The information from EVs is obtained using PCR, western blot and RNA analysis for diagnosis

isolated using a method that is appropriate to the aim of the study, and is compatible with subsequent analyses [8, 38]. The following sections discuss the merits and demerits of various techniques to isolate EVs.

Ultracentrifugation

Ultracentrifugation (U/C) is a classical method that uses strong centrifugal force to isolate EVs [42, 43]. EVs sink due to the centrifugal force and form a pellet at the bottom in an ultracentrifuge tube [44]. U/C can be categorized based on principles of separation: (1) differential U/C and (2) density gradient U/C (Fig. 3) [45–47].

Differential U/C involves serial stepwise centrifugation to remove components other than EVs: centrifugation at (1) 300×*g* for 10 min to remove live cells, (2) at 2000×*g* for 10 min to remove dead cells; (3) at 10,000×*g* to remove cellular debris and isolate large EVs; then finally (4) at 100,000×*g* to isolate exosomes (small EVs) [27, 48, 49]. This is the most-commonly-used protocol, but must be modified when the EVs are suspended in viscous fluids such as plasma or saliva [50]. The quantity of collected EVs is affected by centrifuging speed and time, so these parameters must be optimized for each rotor type.

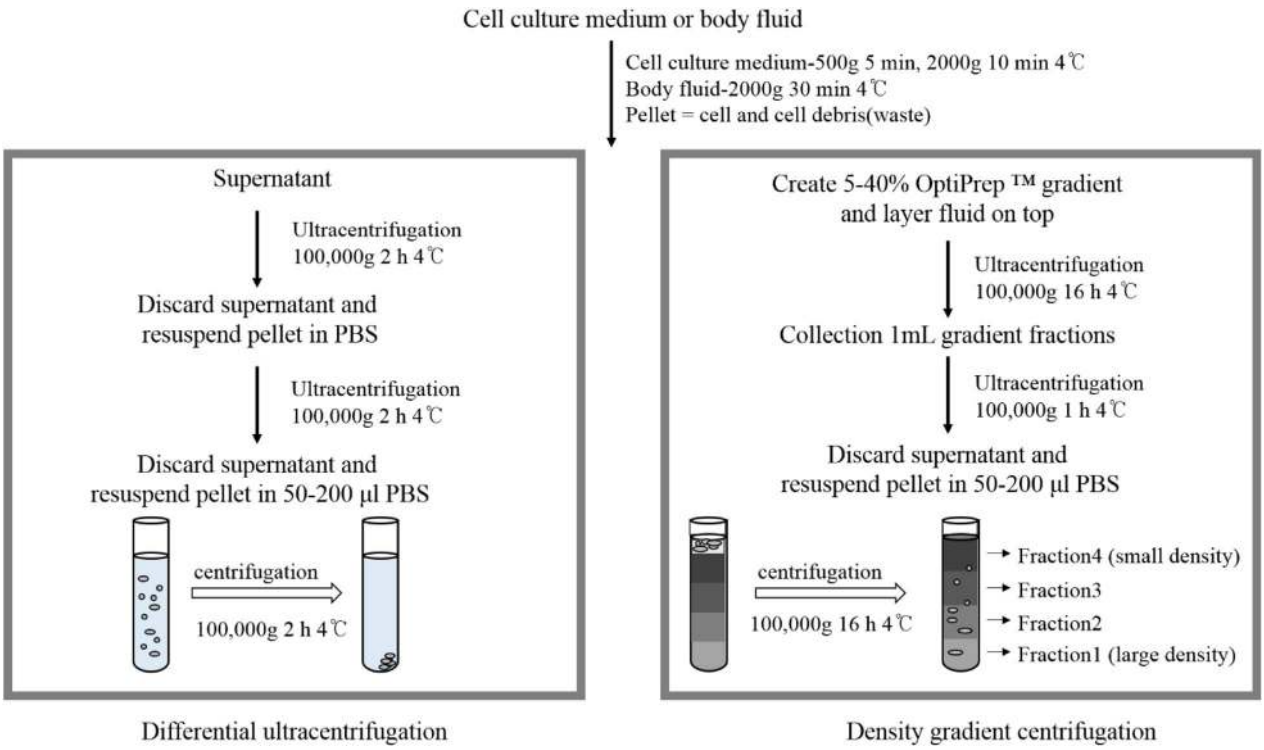

Fig. 3 Ultracentrifugation: differential ultracentrifugation and density gradient centrifugation. Ultracentrifugation is a centrifugation with high g-force. Ultracentrifugation is commonly used to isolate EVs; density gradient centrifugation isolates EVs with fewer impurities and aggregation

Density gradient U/C segregates EVs to specific layers according to their buoyancy in a gradient material such as sucrose, iohexol (Nycodenz™) or iodixanol (Optiprep™) [51]. The density fraction in which EVs accumulate is affected by gradient material [46, 52], and by the source of EVs [47]. For example, EVs from saliva accumulate in fractions with higher densities than EVs from conditioned medium [47].

EVs isolated by U/C have been assessed for their value as diagnostic clues. Two prostate cancer biomarkers, PCA3 and TMPRSS2-ERG, have been detected in urinary EVs from prostate cancer patients [53]. Also, urinary EVs are enriched with miRNAs, so isolation and analysis of urinary EVs can help to identify miRNA biomarkers for diseases such as prostate cancer [31]. Results can provide a diagnostic standard for comparison of patients with healthy individuals [54]. CD24 is present in serum EVs of breast-cancer patients, whereas EpCAM is not, so this difference may be a way to discriminate breast cancer from others [54]. The protein profiles in EVs of ovarian cancer cell lines OVCAR-3 and IGROV1 [55] were obtained by differential U/C then further purification using density gradient U/C (1.09–1.15 g/ml), and revealed that ovarian cancer EVs contain tissue-specific proteins that are related to tumorigenesis and metastasis.

Differential U/C has disadvantages. It is slow [50], and the presence of protein contaminants in the pellet of EVs frequently requires additional an U/C step called double pelleting; the second step reduces aggregation between EVs and proteins, but also decreases the amount of EVs isolated [56]. Another disadvantage is that protein contaminants co-occur with EVs isolated by this method, so use of a Bradford assay to estimate EV amount is not reliable [44, 51, 57].

Differential U/C that uses high rotation speed can isolate small size vesicles, but the process can induce aggregation of vesicles and proteins [58]. Moreover, EVs isolated at high rotation speed have different phenotype, size, and even surface proteins than those isolated at low rotation speed. Due to these problems, EV analysis can have different results depending on the g-force and k factor of differential U/C. For example, EVs that are released from HEK293 cells are more pure when low-g force is used (33,000×*g*, though 67,000×*g*) [59].

Density gradient U/C suffers less from protein contamination (only 5–25% of EV concentration) than differential U/C because proteins are accumulated into different layers of density than EVs [40, 60]. Hence, EVs isolated by density gradient centrifugation have good purity and intact morphological characteristics [56, 61]. These

disadvantages have been overcome by combining density gradient U/C with other methods like filtration and chromatography. However, isolation based on size cannot discriminate EVs from other small vesicles, or among subpopulations of similarly-sized EVs.

Immunoaffinity

Immunoaffinity exploits interactions between antibodies and surface proteins of EVs to isolate EVs. Antibodies specific to surface proteins of EVs (e.g., CD9, CD81, CD63, TSG101, Alix) are linked to chemically-modified or protein-coated beads, and capture EVs by binding to these proteins [4, 62, 63]. EVs isolated based on immunoaffinity have different characteristics than those isolated based on size. Size-based separation cannot distinguish among subpopulations of EVs. For example, if the goal is to distinguish the CD81+ subpopulation of EVs from the CD63+ subpopulation, immunoaffinity is recommended [64]. Many studies have discovered EV biogenesis and subpopulations by exploiting the interaction of surface proteins with specific antibodies. Tetraspanin-specific antibodies are common in immunoaffinity [65]; for example, CD81 is internalized more slowly than CD9 [66]. ELISA with anti-CD81 antibody has been used to quantify EGFR level in plasma EVs from lung cancer patients. The EGFR exosomal proteins are possible diagnostic biomarkers in immunoaffinity methods [65].

Immunoaffinity has been incorporated into hybrid approaches that use more than one isolation method [68–69]. EVs can be isolated using magnetic beads coated with antibodies in a minimal volume of plasma [54]. Isolation of the magnetic beads enriches EVs (Fig. 4); this is a multiple technique that combines detection of EVs and proteins in one device [67]. Surface plasmon resonance imaging (SPRi) with antibody microarrays detects specific proteins on EV membranes in tumor-cell culture medium [69]. EVs isolated by the device have positive correlation with tumor cell in metastasis and prognosis prediction.

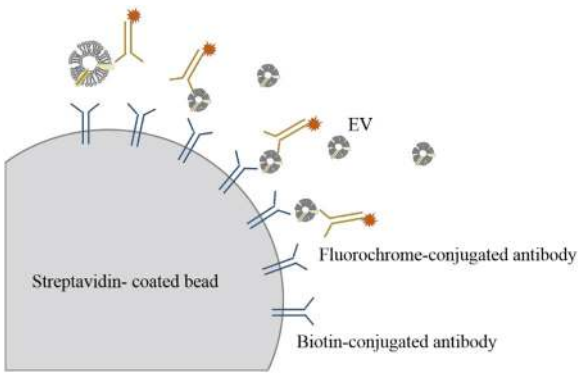

Fig. 4 Isolation method using antibody-conjugated bead. Interaction with antibody and surface protein in EV is a key factor in immunoaffinity. Immunoaffinity method enables identification of proteins including positive markers in EVs

Immunoaffinity extracts more-purified EVs that bear specific identified proteins than does U/C, and achieves higher yield and greater purity of EVs than does U/C [8, 70]. When immunoaffinity is used to isolate EVs, subpopulations of EVs can be identified by sorting them according to their specific surface proteins. For example, ~80% of A33 in EVs from cell culture medium can be captured using immunoaffinity, which achieves a yield of ~25 μg in a western blot assay [70]. However, antibodies are expensive, so this method is not appropriate for large samples. Moreover, for subsequent experiments, EVs must be displaced from the beads; this step may damage the EVs.

Recent studies have classified EV by size rather than by surface protein. Use of immunoaffinity does not identify the source of EV (exosome, ectosome, apoptotic body), so this method is not suitable for sorting by size. For example, exosomes, microvesicles and apoptotic bodies all have EpCAM on the membrane [8]; immunoaffinity detects the same exosomal marker in these vesicles, but they have different characteristics. Future studies should develop immunoaffinity methods to correlate surface protein identity with EV functions and properties.

Size exclusion

Size differences can be exploited to isolate EVs. In general, two types of size exclusion are used: filtration and chromatography (Fig. 5) [41, 71]. Filtration captures EVs on membranes, while allowing small particles like proteins to pass through them [72]. This method requires appropriate choice of pore size. Filtration is often combined with U/C or other isolation methods [21, 56].

Size exclusion can concentrate EVs by using other methods, such as pressure-driven methods or U/C [40]. The pressure-driven device is suitable for samples with volumes >400 ml because the device is suitable for large volumes; centrifugation is appropriate for samples with volume <400 ml [40]. Pore size of 0.1 μm is appropriate when a sonicating water bath is combined with size exclusion to isolate EVs from plasma [71]; this method revealed that EV level in plasma is lower in smokers than in non-smokers. The EV level in smokers is decreased by early apoptosis.

EVs can be isolated by combining electrophoretic migration processes and size exclusion (30-nm pore size) [73]; a nanoporous membrane allows small impurities to pass through into the flow, but retains EVs larger than membrane pore size. In 30 min, up to 65% of EVs were recovered, and up to 83.6% of protein was removed. This method is faster and achieves 14 times higher recovery

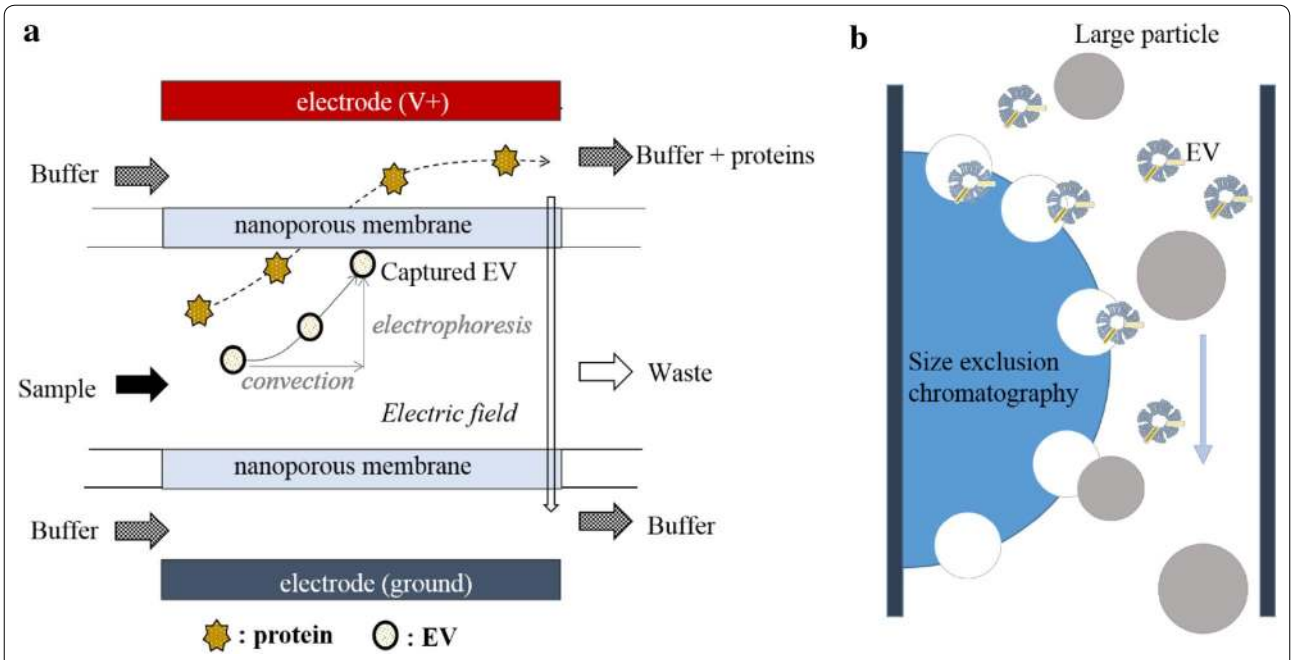

Fig. 5 Size-exclusion with (**a**) filter and (**b**) chromatography. Sorting targeted-sized EVs by size exclusion. **a** Filter-based isolation method considers pore size for isolation desired size vesicles. Filter-based isolation requires sorting proteins and EVs. The device with electrophoretic migration processes and size exclusion isolates EVs. **b** Chromatography with ultracentrifugation for isolation EVs. Small particles enter the pore and move slowly, whereas large particles pass through the column rapidly. EV-containing solution is collected

efficiency than does U/C. Size-exclusion chromatography (SEC) uses a column packed with beads that have pores smaller than EVs of concern [74]. Particles smaller than pore size enter them and move slowly, whereas particles larger than pore size pass around beads and exit the column rapidly. Fractions containing samples are eluted sequentially in order of decreasing size; the EV-containing fraction can be collected and analyzed. When SEC is used, aggregation of proteins and EVs is minimal [40, 75], and the function of EVs is usually unaffected. However, filter-based isolation and SEC separate components by their size, and therefore cannot discriminate particles that have similar size but different characteristics.

One important requirement of EV isolation is to remove high-density lipoproteins (HDL) as well as proteins. Sepharose CL-2B column in the SEC method isolates EVs of diverse size distribution in fractions [76]. EVs are highly isolated in fractions 9–12. The recovery efficiency of EVs in these fractions is (46 ± 6) % by NTA and (60 ± 10)% by resistive pulse sensing (RPS). The recovery of HDL in fractions 18–20 is (32 ± 2) % by APO A1. HDL in fractions 9–12 is just (0.65 ± 0.3) %, the fractions that are isolated HDL and EVs are different.

EVs isolated by chromatography are used in diagnosis [78–79]. Ovarian adenoma can be diagnosed in serum EVs isolated by chromatographic isolation; serum EVs are analyzed using NTA and western blotting. EGFRvIII is produced by tumors, so it is detected using western blotting [77]. SEC easily isolates EVs from concentrated body fluid in one step, and has applications in diagnosis of some diseases [78].

Precipitation

Polymer precipitation

Various methods that use polymers have been developed to ease the isolation process and reduce the isolation time [80]. The methods use polymers that can precipitate or displace EVs according to surface characteristics. Exoquick™ (System Biosciences, USA) is composed of polymers; it isolates EVs quickly and effectively by precipitation. The system just needs mixing, incubation and centrifugation; it does not require high-priced U/C or large volumes of samples [61]. Exoquick™ is efficient and is becoming adopted as an alternative to U/C, although further verification is necessary. For example, EVs are isolated by Exoquick™ from serum, then some proteins in serum EVs are analyzed using proteomics as biomarkers for acute coronary syndrome (ACS) [81]; Cystatin C and C5a in EV isolated by U/C and Exoquick™ are biomarkers associated with ACS in men. These proteins are demonstrated in presence of ACS.

The aqueous two phase system (ATPS) has been applied to isolate EVs (Fig. 6) [82]. After samples are completely mixed with aqueous two-phase solution

(polyethylene glycol (PEG) and dextran), the mixture is centrifuged at 3000×*g* for 10–30 min. EVs move toward the dextran phase, which has surface characteristics that are favorable to EVs [83]. ATPS is quick and easy, and does not requiring any incubation process [84]. In several biological fluids, ATPS shows higher recovery of EVs than does U/C and Exoquick™ [82]. However, as EVs are isolated in a dextran-containing solution, so the effect of dextran must be reduced before ATPS can be widely accepted. EVs isolated by ATPS have diagnostic markers. EVs purified using ATPS from prostate cancer patients are easily detected using prostate-specific membrane antigen (PSMA). ATPS isolates high-quality EVs in ~15 min. ATPS isolates uncontaminated EVs without aggregation complexes [85].

Protein organic solvent precipitation

Protein organic solvent precipitation (PROSPR) is an EV precipitation method that is a fast and simple process with organic solvents (acetone, chloroform, trichloroacetic acid) [86]. Biological fluids contain numerous proteins, soluble factors, and lipoproteins [37]. Removal of protein from biological fluids is an important requirement in EV analysis. Proteins that have hydrophilic and hydrophobic regions have dielectric strength in aqueous solution. The organic solvent is attracted to oppositely-charged amino acid residues and promote protein aggregation. The ion-pairing effect contributes to PROSPR's precipitation efficiency. Moreover, organic solvent with a salt improves protein removal [87].

PROSPR overcomes the disadvantages of U/C in which EVs are extracted with unwanted proteins, and can be damaged by high rotation speed. EVs isolated by PROSPR have only ~20% as much protein contamination than those isolated by U/C. Compared to ultracentrifugation-cushion, PROSPR can isolate EVs with EV-associated protein by 7.2 times [88].

Microfluidics techniques

EVs can be isolated by microfluidic devices that induce flow of liquids within micro-sized channels [38, 89]. Microfluidic devices are small but purify and separate faster than other isolation methods. Microfluidic devices require only a small sample, so the cost, processing time, and consumption of reagents are reduced. EVs isolated using microfluidic devices tend to retain their morphology. Microfluidics techniques capture EVs by using immunoaffinity, by sieving, or by trapping them in porous structures [90].

Microfluidic devices have been combined with immunoaffinity methods to detect diagnostic markers. EVs are captured by binding to specific antibodies on channels or by passing through membranes (Fig. 7a). Modified microfluidics can isolate EVs from 100–400 µl serum samples and brain tumor specimens [90]; modified surfaces are biotinylated anti-63, control IgG and anti-CD4. The antibodies bind specific EVs because EVs secreted by tumor cells have surface specific markers. Antibodies coated on the surface of device increase the efficiency of EV capture.

EVs protect RNA from RNase in body fluids, which otherwise degrade it rapidly [91]; therefore RNAs in EV are stable during RNA analysis, so RNA in EVs can be analyzed to detect biomarkers for diagnosis. RNA level

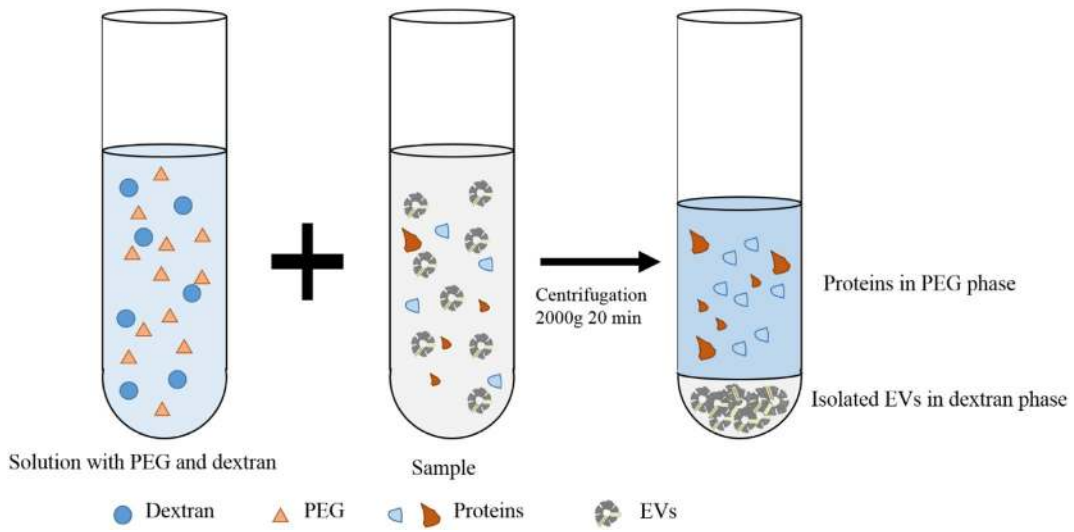

Fig. 6 Scheme of aqueous two phase system (ATPS) separation. EVs move into a dextran phase that has surface properties that are favorable to EVs. ATPS purifies EVs based on precipitating properties from sample without expensive equipment or large samples

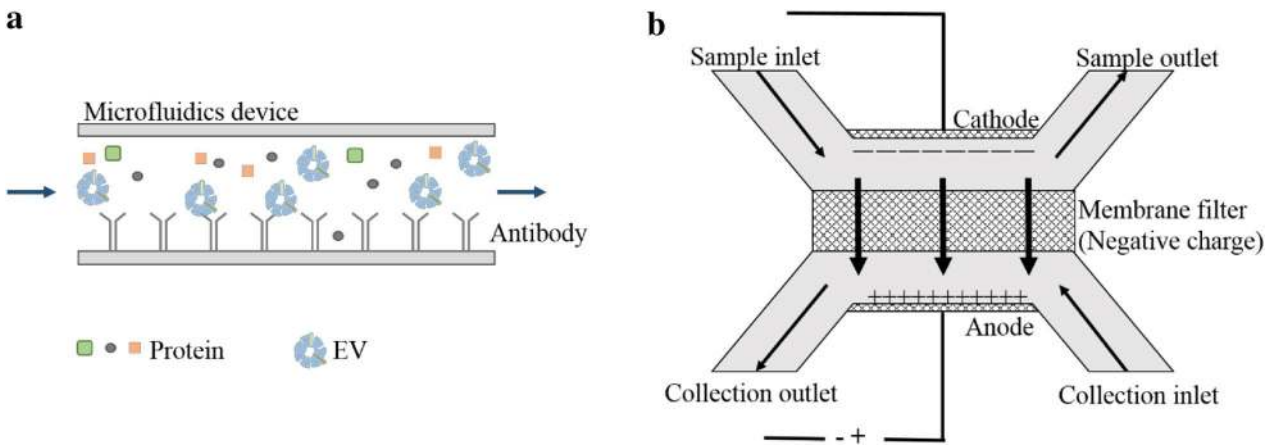

Fig. 7 Microfluidic system to isolate EVs. Microfluidics techniques developed for EV purification are classified into immunoaffinity approach, sieving EVs, and trapping EVs with porous structures. **a** Capture of EVs occurs by binding to specific antibodies on channels. The device with antibody increases EV-capture efficiency. **b** Microfluidics with filter pore size ~500 nm isolate EVs without aggregation. EVs have negatively charged phospholipid membranes and therefore move across filter membranes

in EVs differs between glioblastoma multiforme (GBM) patients and healthy individuals; real-time polymerase chain reaction (RT-PCR) and RNA sequencing of mRNA in EVs from GBM patients detected mRNA of mutated IDH-1. The recovery of EVs was 42–94% at the RNA level [90]. Microfluidic devices can sort specific EVs, and enable can diagnoses disease from a small sample in a single step [92].

Microfluidic devices with filter membranes can purify EVs like small vesicles (Fig. 7b) [93]. A microfluidics device that includes a filter membrane that uses porous polymer monoliths (PPM) can isolate EVs from mouse blood; the membrane removes cells and cell debris and allows passage of small vesicles like EVs for isolation and diagnosis.

Filtration can be classified as pressure-driven or electrophoresis-driven [93]. Pressure-driven filtration uses a syringe pump; filter membranes with 500-nm pores are usually used because EVs can clog the filter if pores are smaller than this. Electrophoresis-driven filtration exploits the negatively-charged phospholipid membranes of EVs to move them across filter membranes, whereas proteins and other molecules have different charges due to side chains of amino acids. EVs cross the filter membrane and are isolated into a collection solution. Electrophoresis-driven filtration is not blocked by any molecule, but the flow is sometimes hindered by gas bubbles that form at output >0.7 V/cm.

A microfluidics device with ciliated micropillars purifies EVs without contamination [94]. Ciliated micropillars on the wall filter lipid vesicles like EVs on several scales. Intact EVs are trapped in nanowires, whereas large vesicles like cells and cell debris, and small molecules like proteins are passed in a continuous flow. Soaking micropillars in PBS for 24 h releases the trapped EVs by dissolving the vesicles. A quantum dot (QD) directly visualizes the trapped EVs on micropillars; the QD is not dissolved in PBS and not carried with EVs.

EV purification using microfluidic systems is still in its early stages of development, but many microfluidic devices have demonstrated that it is an effective method to isolate EVs. Microfluidic devices can damage EVs due to shear stress, and require macro-scale samples [92]. Microfluidics engineers should collaborate with biologists and clinicians to develop techniques that are suitable for EV recovery. Microfluidic devices to isolate EVs could be used in clinics.

Conclusion

Although EVs have potential applications in diagnosis and therapy of diseases, points for improvement should be noted. According to purpose, the choice of a suitable isolation method increases the effectiveness of isolation. For example, immunoaffinity can detect specific EVs but is not appropriate for large-volume samples [50]. In acute clinical usage, the method must be fast and inexpensive for use in emergencies. Also, a purification process must isolate intact EVs. The genetic information in EVs is generally unstable [95], so damaged EVs may not be useful in precise experiments.

Many studies have used EVs in disease diagnosis, disease monitoring and predictive tracking [96, 97]. The advantage of diagnosis using EVs is that they can be isolated from body fluids by minimally invasive biopsy [65]. Minimalized invasive diagnosis is preferable to traditional invasive diagnosis which causes bleeding and pain

[98]. Moreover, EVs also can be used as vehicles for drug delivery in disease therapy [99]. Stem cell-derived EVs can induce tissue regeneration as a cell-free approach. Taken together, clinical application of EV analysis will be expanded, but much research remains to be completed. EVs focus on individual biologics to strengthen the knowledge in the field. Continued search will identify EV-based diagnostic and prognostic biomarkers, and will improve understanding of the mechanisms of disease pathogenesis.

Authors' contributions

HK and JK summarized previous works and wrote the manuscript. JP provided advice for the manuscript. All authors read and approved the final manuscript.

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Competing interests

We confirm that I have read SpringerOpen's guidance on competing interests and have included a statement indicating that none of the authors have any competing interests in the manuscript.

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